Dimethomorph

Document M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

[redacted]

Representative:

[redacted]

Tel.No.: [redacted]
Fax No:
Mobile No:
Contact person:
e-Mail:

CA 1.2 Producer

Manufacturer of dimethomorph (legal entity):

[redacted]

Contact person: Please refer to CA 1.1 Applicant.

Location of manufacturing sites for Dimethomorph

CONFIDENTIAL information - data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Dimethomorph

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

CA 1.4.1 IUPAC nomenclature

(E,Z) 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine

CA 1.4.2 1.4.2 CA nomenclature

(E,Z) 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxy-phenyl)-1-oxo-2-propenyl]-morpholine

CA 1.5 Producer's Development Code Numbers

AC 336379, CL 336379, CME 151, SAG 151, WL 127294, BAS 550 F

CA 1.6 CAS, EC and CIPAC Numbers

CAS No.:	110488-70-5
CIPAC No.:	483
EINECS No.:	404-200-2
ELINCS No.:	Not assigned

CA 1.7 Molecular and Structural Formula, Molar Mass

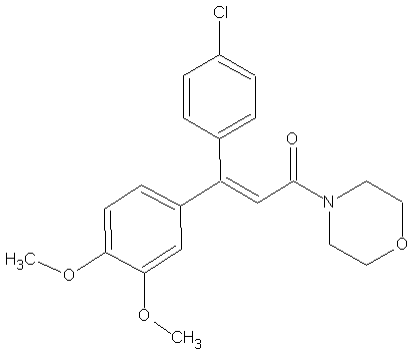
CA 1.7.1 Molecular formula

$C_{21}H_{22}ClNO_4$

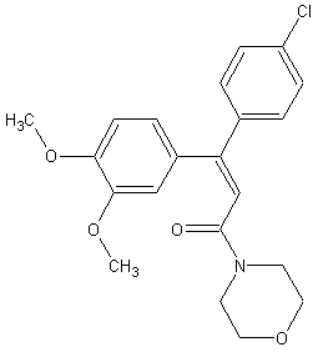
CA 1.7.2 Structural formulae

Figure 1.7.2/1

E isomer



Z isomer



CA 1.7.3 Molar mass

387.9 g/mol

CA 1.8 Method of Manufacture (synthesis pathway) of the active substance

CONFIDENTIAL information - data provided separately (Document J)

CA 1.9 Specification of Purity of the Active Substance in g/kg

Minimum purity: 965 g/kg (E/Z ratio: 40/60 - 50/50 % w/w)

CA 1.10 Identity and Content of Additives (such as Stabilisers) and impurities

CA 1.10.1 Additives

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.2 Significant impurities

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.3 Relevant impurities

Dimethomorph does not contain impurities of toxicological, eco-toxicological or environmental relevance.

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

Dimethomorph

Document M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
23/03/2016	MCA – Section 2 – Information on GLP status, test material purity and test method, author and study report is added.	MCA Section 2 Version 1 BASF DocID 2016/1000206

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point	EEC A1 DSC (differential scanning colorimeter)	Pure a.i. 99.1%	Melting point E/Z mixture: 125.2 – 149.2 °C (99.1 %, E/Z 48/52) E isomer: 136.8 - 138.3 °C (99.1 %) Z isomer: 166.3 - 168.5 °C (99.1 %) Boiling point not applicable (decomposition at 280 °C, both E and Z isomer) Information already reported, peer-reviewed and accepted previously.	N[1]	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Allman & Henke, 1989, DK-303-001
CA 2.2 Vapour pressure, volatility	OECD 104 Gas saturation method Calculation	Pure a.i. 99.1%	Vapor pressure $9.7 \cdot 10^{-7}$ Pa (E); $1 \cdot 10^{-6}$ Pa (Z), 25 °C Henry's law constant $5.4 \cdot 10^{-6}$ Pa m^3 mol^{-1} (E); $2.5 \cdot 10^{-5}$ Pa m^3 mol^{-1} (Z) Information already reported, peer-reviewed and accepted previously.	N[1]	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Rech & Henke, 1989, DK-306-004
CA 2.3 Appearance (Physical state, colour)	Visual assessment	Pure a.i. 98.8%	White crystalline solid (98.8%) Information already reported, peer-reviewed and accepted previously.	N[2]	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Cevasco, 1999, DK-301-007

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr>
<td>CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td>
<td></td>
<td>Pure a.i.
98.8%</td>
<td>
UV/Vis

λ_{max} [nm] ε
200 4.5 · 104
205 3.0 · 104
221 1.6 · 104
242 2.0 · 104
286 9.1 · 103
312 4.5 · 103

IR
<table>
<tr><th>IR Band Wavelength (cm-1)</th><th>Assignment</th></tr>
<tr><td>1626</td><td>C=O strecth of amide group</td></tr>
<tr><td>1610, 1512</td><td>Aromatic ring C=C stretch</td></tr>
<tr><td>1454</td><td></td></tr>
<tr><td>1428</td><td>amide C-N stretch</td></tr>
<tr><td>1253</td><td>Asymmetric C-O-C stretch</td></tr>
<tr><td>1228</td><td>Asymmetric C-O-C stretch</td></tr>
<tr><td>1040</td><td>Symmetric C-O-C stretch</td></tr>
<tr><td>800-760</td><td>Aromatic CH OOP bend</td></tr>
<tr><td></td><td></td></tr>
</table>

^{1}H-NMR
<table>
<tr><th>H</th><th>Chemical shift [ppm]</th><th>Coupling</th></tr>
<tr><td>18-21</td><td>3.0-3.4</td><td>Multiplets</td></tr>
<tr><td>15, 16</td><td>3.6-3.8</td><td>Singlets</td></tr>
<tr><td>1</td><td>6.4-6.5</td><td>Singlet</td></tr>
<tr><td>10</td><td>6.6</td><td>Doublet of doublets</td></tr>
<tr><td>14</td><td>6.7-7.0</td><td>Doublet</td></tr>
<tr><td>11</td><td>6.9-7.0</td><td>Doublet</td></tr>
<tr><td>4, 5, 7, 8</td><td>7.1-7.4</td><td>doublets</td></tr>
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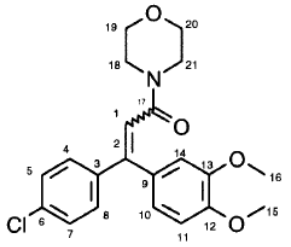
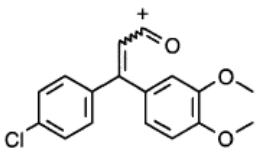
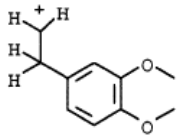
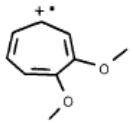
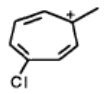
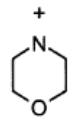

</td>
<td>Y</td>
<td>EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints

Jones, 1995, DK-360-007</td>
</tr>
</table>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			MS Ion (m/z) / Assignment / Molecular Formula 387 / $[M]^{+\cdot}$ / $C_{21}H_{22}NO_4Cl$ 352 / $[M]^{+\cdot}$ - Cl / $C_{21}H_{22}NO_4$ 301 /  / $C_{17}H_{14}O_3Cl$ 165 /  / $C_{10}H_{13}O_2$ 152 /  / $C_9H_{12}O_2$ 139 /  / C_8H_8Cl 125 /  / C_7H_6Cl 86 /  / C_4H_8NO IR, NMR and MS spectra are consistent with given structure of Dimethomorph.		Draft Assessment Report (DAR), Vol. 3, Annex B

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			Information already reported, peer-reviewed and accepted previously.		2, May 2004
CA 2.5 Solubility in water	EEC A6 (shake flask method)	E-isomer purity of 98.9% Z-isomer purity of 96.3%	20 °C [g/L]. E-isomer: 0.0472 Z-isomer: 0.0107 Information already reported, peer-reviewed and accepted previously.	Y	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Akkari K., 2002, DK-311-007
CA 2.6 Solubility in organic solvents	OECD 105	Pure a.i. 99.1%	Individual isomers E(Z) [g/L; 20 °C] CH_2Cl_2: 296 (165) Acetone: 84.1 (16.3) Ethyl acetate: 39.9 (8.4) Toluene: 39.0 (10.5) Methanol: 31.5 (7.5) n-Hexane: 0.076 (0.036) 1,2-Dichloroethane: 182.5 (92.5) Xylene: 22.2 (6.4) Heptane: 0.120 (0.053) Information already reported, peer-reviewed and accepted previously.	N[1]	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Grimm & henke, 1989, DK-312-001

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.7 Partition co-efficient n-octanol/water	EEC A6 (HPLC method)	Pure a.i. 99.1%	2.63 (E) 2.73 (Z), 20 °C, Milli Q water Information already reported, peer-reviewed and accepted previously.	N	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Rech & Henke, 1989, DK-315-001
CA 2.8 Dissociation in water - dissociation constant(s) (pKa values) - identity of dissociated species - dissociation constant(s) (pKa values) of the active principle	Calculation	Not applicable	Dissociation constant - 1.3 (calculation)	N	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Martin C., 2002, DK-390-059
CA 2.9 Flammability and self-heating	EEC A10 EEC A16	TGAI 98.6% TGAI 98.5%	Not flammable. The test substance is not self-igniting according to EEC method A16 Information already reported, peer-reviewed and accepted previously.	Y Y	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Van Helvoirt, 1989, DK-330-001 and DK-330-002

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.10 Flash point			Not applicable (melting point > 40 °C) Information already reported, peer-reviewed and accepted previously.		Draft Assessment Report (DAR), Vol. 3, Annex B 2, May 2004
CA 2.11 Explosive properties	EEC A14	TGAI 98.6%	Not explosive. Information already reported, peer-reviewed and accepted previously.	Y	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Cardinaals, 1989, DK-334-001
CA 2.12 Surface Tension	EEC A5	TGAI 98.6%	60.8 mN/m (20 °C, 90% saturated aqueous solution) Information already reported, peer-reviewed and accepted previously.	Y	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Werle, 1999, DK-340-002

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.13 Oxidising properties	EEC A17	TGAI 98.6%	Not oxidizing. Information already reported, peer-reviewed and accepted previously.	Y	EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph Appendix 1 – list of endpoints Van Hervoirt, 1991, DK-356-002
CA 2.14 Other studies					

[1] At the time the study was conducted GLP was not compulsory. However the study was conducted according to the principles of Good Laboratory Practices.
[2] Study not subject to GLP regulations

Dimethomorph

Document M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Dimethomorph belongs to the fungicide group of the cinnamic acid amides (CAA) and is the first representative of this fungicide group (dimethomorph, benthiavalicarb, iprovalicarb and mandipropamid). It exhibits activity against all members of the family *Peronosporaceae* (downy mildews), but also against the genus *Phytophthora* in *Pythiaceae*. It disrupts fungal cell wall biosynthesis, causing cell wall lysis and subsequent death of the fungal cell. Foliar applications of dimethomorph give good protectant, curative and antisporulant activity and provide long residual protection. Dimethomorph is systemically translocated in the plant when applied to the roots and is translaminar with local systemicity when applied to the foliage.

CA 3.2 Function

Dimethomorph is used as a fungicide to control harmful diseases, which are members of the family Peronosporaceae (downy mildews) and genus *Phytophthora* in *Pythiaceae*. Dimethomorph is a key active ingredient in grapes as well as in nearly all commercially relevant vegetables. Different formulations containing dimethomorph are used around the world to protect those high value specialty crops.

CA 3.3 Effects on Harmful Organisms

Dimethomorph disrupts fungal cell wall biosynthesis, causing cell wall lysis and subsequent death of the fungal cell. It is active against all stages in the fungal life cycle other than the formation of zoospores and zoospores motility since these structures lack cell wall. Despite this, the natural zoospore encystment prior to the germ tube formation is inhibited which will further block the infection process. It also exhibits an excellent antisporulant activity. When applied before sporulation, almost complete inhibition can be achieved. Dimethomorph is readily translocated in the xylem, but not in the phloem. It has an excellent translaminar activity, by which it protects the opposite leaf surface. Curative activity has also been shown because dimethomorph could stop infection process 2 days after inoculation achieving also good performance when infection had already started 5 days prior to product application.

CA 3.4 Field of Use Envisaged

Agriculture

CA 3.5 Harmful Organisms Controlled and Crops or Products Protected or Treated

Dimethomorph is used to control fungal diseases such as:

- All members of the family *Peronosporaceae:*
  - *Basidiophora spp.*
  - *Benua (genus) spp.*
  - *Bremia spp.*
  - *Graminivora spp.*
  - *Hyaloperonospora spp.*
  - *Novotelnova spp.*
  - *Paraperonospora spp.*
  - *Perofascia spp.*
  - *Peronosclerospora spp.*
  - *Peronospora spp.*
  - *Plasmopara spp.*
  - *Plasmoverna spp.*
  - *Poakatesthia spp.*
  - *Protobremia spp.*
  - *Pseudoperonospora spp.*
  - *Sclerospora spp.*
  - *Viennotia spp.*

- *Pythiaceae:*
  - *Phytophthora spp.*

Dimethomorph is used in many crops, such as for example the following ones:

Perennial crops

- Berries and small fruits
  - Strawberries
  - Raspberries
  - Blackberries
- Grapevine (wine, table)

Vegetables

- Fruiting vegetables
  - Solanacea crops
  - Cucurbits
- Root and tuber vegetables
  - Potato
  - Radish
- Bulb vegetables
  - Onions
- Leafy vegetables and herbs
  - Lettuce and similar
  - Chard
  - Witloof chicory
- Brassica vegetables
  - Head cabbage
  - Leafy cabbage
  - Flowering cabbage
  - Brussel sprouts

Others

- Hops
- Oranges
- Tobacco
- Ornamentals

CA 3.6 Mode of Action

Dimethomorph belongs to the fungicide group carboxylic acid amides (CAA). Studies with *Plasmopara viticola* and *Phytophthora spp.* indicated that the compound interferes with processes involved in fungal cell wall biogenesis. In addition, other studies showed that the mode of action of CAA compounds is directly linked to the inhibition of cellulose synthesis in Oomycetes. CAA-fungicides are grouped in the MoA Group H (cell wall biosynthesis inhibitors) with the target site code H5 (cellulose synthase inhibitors) (FRAC 2015).

CA 3.7 Information on Occurrence or Possible Occurrence of the Development of Resistance and Appropriate Management Strategies

Dimethomorph has now been on the market since 1993. Intensive resistance monitoring programmes are running for *Plasmopara viticola* and *Phytophthora infestans*. The monitoring results have indicated CAA resistance in *Plasmopara viticola* in different grape vine growing regions in Europe and first cases of CAA resistance has been found for *Pseudoperonospora cubensis*. No instances of reduced sensitivity have been shown in other Oomycete pathogens, including *Phytophthora infestans*, which has received extensive monitoring.

Within the CAA fungicide group, cross resistance exists in general. However, differences of efficacy on CAA resistant isolates can be found when CAAs are applied preventively. Dimethomorph shows still some efficacy on CAA resistant isolates, while other CAAs showed lower activity. In curative applications efficacy of all CAAs was reduced on CAA resistant isolates. Dimethomorph is not cross-resistant to QoIs, phenylamides, cymoxanil, zoxamide, cyazofamid, Al-fosetyl, ametoctradin and other compounds effective on Oomycetes.

Dimethomorph is classified by FRAC as a low to medium risk compound according to the principles described in FRAC Monographs 1 and 2.

If the combined resistance risk has been assessed to be medium under unrestricted use, anti-resistance management strategies are recommended. For *Phytophthora* root and collar diseases, *Peronospora parasitica* and *Bremia lactucae* no dimethomorph resistant field isolates were found up to now. However, to prevent the occurrence of dimethomorph resistance and to maintain the sensitive situation, management strategies are also recommended.

CA 3.8 Methods and Precautions Concerning Handling, Storage, Transport or Fire

Exposure Controls / Personal Protection

Control parameters

Components with occupational exposure limits
No occupational exposure limits known.

Exposure controls

Personal protective equipment

Respiratory protection:
Respiratory protection not required.

Hand protection:
Suitable chemical resistant safety gloves (EN 374) also with prolonged, direct contact
(Recommended: Protective index 6, corresponding > 480 minutes of permeation time according to EN 374): E.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) and other

Eye protection:
Safety glasses with side-shields (frame goggles) (e.g. EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Handling and Storage

Precautions for safe handling

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:
Avoid dust formation. Dust can form an explosive mixture with air. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Conditions for safe storage, including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions: Keep away from heat. Protect against moisture. Protect from direct sunlight.

Storage stability:
Storage duration: 24 Months

Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

First-Aid Measures

Description of first aid measures

Remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air, seek medical attention.

On skin contact:
Wash thoroughly with soap and water.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

On ingestion:
Rinse mouth immediately and then drink plenty of water, induce vomiting, seek medical attention.

Most important symptoms and effects, both acute and delayed

Symptoms: No significant reaction of the human body to the product known.

Indication of any immediate medical attention and special treatment needed

Treatment: Symptomatic treatment (decontamination, vital functions).

Transport Information

Land transport

ADR

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMETHOMORPH 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number UN3077	
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMETHOMORPH 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Inland waterway transport

ADN

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMETHOMORPH 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known
Transport in inland waterway vessel:	Not evaluated

Sea transport

IMDG

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMETHOMORPH 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Marine pollutant:	YES
Special precautions for user:	None known

Air transport

IATA/ICAO

UN number: UN 3077

UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMETHOMORPH 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Fire-Fighting Measures

Extinguishing media

Suitable extinguishing media:
water spray, foam, dry powder

Unsuitable extinguishing media for safety reasons:
carbon dioxide

Special hazards arising from the substance or mixture

carbon monoxide, hydrogen chloride, Carbon dioxide, nitrogen oxides, organochloric compounds
The substances/groups of substances mentioned can be released in case of fire.

Advice for fire-fighters

Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

Further information:
Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire.

CA 3.9 Procedures for Destruction or Decontamination

Waste treatment methods

For purposes of disposal, combustion of Dimethomorph or its pesticide products in a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

Although it is possible to incinerate the product at lower temperatures, combustion at approximately 1100°C with a residence time of about 2 seconds is advised.
By doing so, i.e., operating the incinerator according to the conditions laid down in council directive 94/67/EEC resp. directive 2000/76/EC of the European Parliament, one will achieve complete combustion and minimize the formation of undesired by-products in the off-gases.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the substance/product.

CA 3.10 Emergency Measures in Case of an Accident

Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Use personal protective clothing. Avoid contact with the skin, eyes and clothing.

Environmental precautions

Do not discharge into the subsoil/soil. Do not discharge into drains/surface waters/groundwater.

Methods and material for containment and cleaning up

For small amounts: Contain with dust binding material and dispose of.
For large amounts: Sweep/shovel up.
Avoid raising dust. Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.

Dimethomorph

Document M-CA, Section 4

ANALYTICAL METHODS

Compiled by:

[redacted]

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e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
31/03/2016	CA 4.1.2/18: Information provided on delay of morpholine data (M550F021) due to delays in completion of the method validation. CA 4.2/10: Fully summary of the ILV to the water method added as finalised report available.	MCA Section 4, Version 1, BASF DocID 2016/1000208
20/06/2016	CA 4.1.2 – Table 4.1.2-1: renumbering of the studies CA 4.1.2/18: Full OECD-summary provided of validation of residue analytical method L0013/04 for the analysis of metabolite M550F021 (BASF DocID 2014/1187244).	MCA Section 4, Version 2, BASF DocID 2016/1103871
30/09/2016	CA 4.2/9: renumbering of the DocID 2014/7004121 by DocID 2016/7006199 CA 4.2/10: renumbering of the DocID 2015/1237993 by DocID 2016/1118111	
May 2017	Following the requests included in the dRAR, submission of new data and renumbering of some studies CA 4.1.2/6: Amendment 2017/1068958 to 4.1.2/5 CA 4.1.2/17: Amendment 2017/1077907 (+2017/1068700) to CA 4.1.2/16 CA 4.1.2/21-36: Submission of 16 new study report / amendments – methods used for data generation in residue trials; renumbering of subsequent summaries due to added paragraphs. CA 4.2/10: Revised report 2017/1069823 replacing 2016/1118111 CA 4.2/16: New study 2017/1068967	MCA Section 4, Version 3 BASF DocID 2016/1189857

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CA 4 ANALYTICAL METHODS

Introduction

CA 4.1 Methods used for the generation of pre-approval data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a)Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Report:	CA 4.1.1/1 Schneider S., 2014a Analytical method APL0692/01 - Determination of Dimethomorph in Dimethomorph TGAI (technical grade active ingredient) by means of HPLC 2014/1135447
Guidelines:	none
GLP:	no

Please Note: the validation study for the analytical method APL0692/01 contains confidential information, thus being moved to the confidential part (see document JCA).

Principle of the methods

This method APL0692/01 describes the determination of the content of the active ingredient Dimethomorph (BAS 550 F) in the technical grade active ingredient (TGAI). The samples are analyzed using an HPLC procedure with external standard calibration and UV detection.

Method parameters (HPLC-UV)

<table>
<tr><td>Column</td><td>Column, Hypersil Gold aQ, 50 x 2.1 mm, 1.9 µm particle size (UPLC), Thermo Scientific or comparable</td></tr>
<tr><td>Column temperature</td><td>25°C</td></tr>
<tr><td>Injection volume</td><td>5 µL</td></tr>
<tr><td>Flow rate</td><td>0.6 mL/min</td></tr>
<tr><td>Eluent</td><td>Eluent A: Acetonitrile
Eluent B: Water</td></tr>
<tr><td>Elution</td><td>Gradient elution
<table>
<tr><td>Time [min]</td><td>% A</td><td>% B</td></tr>
<tr><td>Initial</td><td>20</td><td>80</td></tr>
<tr><td>8.00</td><td>32</td><td>68</td></tr>
<tr><td>18.00</td><td>44</td><td>56</td></tr>
<tr><td>18.01</td><td>90</td><td>10</td></tr>
<tr><td>20.00</td><td>90</td><td>10</td></tr>
<tr><td>20.01</td><td>20</td><td>80</td></tr>
<tr><td>22.00</td><td>20</td><td>80</td></tr>
</table></td></tr>
<tr><td>Detection</td><td>UV/VIS: 230 nm
DAD: 200 – 250 nm (200 – 400 nm for specificity)</td></tr>
<tr><td>Analysis time</td><td>22.00 min</td></tr>
<tr><td>RT</td><td>6.7 ± 0.1 min – Reg. No. 4110868 (E)
7.5 ± 0.1 min – Reg. No. 4110869 (Z)</td></tr>
</table>

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Dimethomorph as manufactured does not contain impurities of toxicological, eco-toxicological or environmental concern.

Information on significant impurities and additives is provided in the confidential part (see Document JCA).

CA 4.1.2 Methods for risk assessment

General remarks

The order of the study summaries is different compared to the information given in the application submitted for renewal of approval. Reason is that the order of the studies had to be altered to present the residue analytical methods in accordance to the required chronology of matrices in the document M-CA 4.1.2. In case references are summarized which were not listed in the Application, or in case references listed in the application are not contained in this chapter, an additional comment will be made at the respective section.

An overview of the changes of order in chapter 4.1.2 compared to the application is given in the table below for the reviewer's convenience:

Table 4.1.2-1: Overview of changes of documents submitted compared to originally listed documents in the application

Data point in application	Data point in current dossier	DocID	Changes to application	Reason for change
4.1.2/6	4.1.2/1	DK-242-008	Renumbering/altered position	Change of order of studies
4.1.2/7	4.1.2/2	2015/7001156	Renumbering/altered position; change of DocID	Change of order of studies
N/A	4.1.2/3	2014/7001070	Not in application	Additional information on extraction efficiency
4.1.2/8	4.1.2/4	2014/7003705	Renumbering/altered position; different DocID	Change of order of studies; correct DocID assigned
N/A	4.1.2/5	2015/1237992	Not in application	An additional, lower level (LOQ) was validated in addition to the method described in 2014/7003705
	4.1.2/6	2017/1068958	Not in application	Report amendment to 2015/1237992
N/A	4.1.2/7 4.1.2/8	DK-432-002 DK-123-087	Not in application	Summary of analytical data of relevant toxicological study
N/A	4.1.2/9	DK-427-003	Not in application	Summary of analytical data of relevant toxicological study
N/A	4.1.2/10	2015/1260809	Not in application	Appendix A to DK-427-003
N/A	4.1.2/11	2015/1260810	Not in application	Appendix B to DK-427-003
N/A	4.1.2/12	DK-425-002	Not in application	Summary of analytical data of relevant toxicological study
N/A	4.1.2/13	DK-425-003	Not in application	Summary of analytical data of relevant toxicological study
N/A	4.2.1/14	2015/1260811	Not in application	Appendix A to DK-425-003
N/A	4.1.2/15	DK-245-002	Not in application	Summary of analytical data of relevant toxicological study
4.1.2/1	4.1.2/16	2005/1026082	Renumbering/altered position	Change of order of studies
N/A	4.1.2/17 4.1.2/18	2017/1077907 (+ 2017/1068700)	Not in application	Amendment to 2005/1026082
4.1.2/2	4.1.2/19	2014/7000494	Renumbering/altered position	Change of order of studies
4.1.2/3	4.1.2/20	2014/1186695	Renumbering/altered position	Change of order of studies
4.1.2/4	4.1.2/21	2014/1187244	Renumbering/altered position	Change of order of studies
	4.1.2/22	2002/5002648	Not in application	Data generation method in support of field trials
	4.1.2/23	2005/7014914	Not in application	Data generation method in support of field trials
	4.1.2/24	2002/5002982	Not in application	Data generation method in support of field trials
	4.1.2/25	DK-244-08	Not in application	Data generation method in support of field trials
	4.1.2/26	DK-244-009	Not in application	Data generation method in support of field trials
	4.1.2/27	DK-244-002	Not in application	Data generation method in support of field trials
	4.1.2/28	DK-244-013	Not in application	Data generation method in support of field trials
	4.1.2/29	DK-244-015	Not in application	Data generation method in support of field trials
	4.1.2/30	DK-244-019	Not in application	Data generation method in support of field trials
	4.1.2/31	DK-123-235	Not in application	Data generation method in support of field trials
	4.1.2/32	DK-713-039	Not in application	Data generation method in support of field trials
	4.1.2/33	DK-713-040	Not in application	Data generation method in support of field trials

	4.1.2/34	DK-713-041	Not in application	Data generation method in support of field trials
	4.1.2/35	DK-713-042	Not in application	Data generation method in support of field trials
	4.1.2/36	DK-244-025	Not in application	Data generation method in support of field trials
	4.1.2/37 4.1.2/38	DK-244-022 DK-123-225	Not in application	Data generation method in support of field trials
N/A	4.1.2/39	2015/1000643	Data generation method; change of DocID – study was allocated the DocID originally proposed for another study in the Application	Re-validation of the original data generation method FAMS 023-01 (DK-245-007), used in the available and already peer reviewed livestock feeding study, according to the recent guidelines. As a new validation was conducted, the method is allocated a new unique BASF method number.
N/A	4.1.2/40	DK-245-013	Not in Application	Assessment of extraction efficiency of FAMS 023-01 in comparison with metabolism extraction scheme
N/A	4.1.2/44	DK-245-011	Data generation method	Method FAMS 024-01 used for analysis of milk samples in feeding study (DK-705-006). As this method was not peer-reviewed during the last EU-review, the method is submitted together with the feeding study for completeness of data
N/A	4.1.2/42	DK-245-008		
N/A	4.1.2/43	DK-243-003	Data generation method	Analytical method used in support of ecotoxicological study
N/A	4.1.2/44	2005/1026675	Data generation method	Analytical method used in support of ecotoxicological study

N/A Not applicable; study was not included in the original Application.

An overview of the metabolites of relevance for the analytical methods is given together with other structures of relevance in Document N. The respective table also contains detailed information on different metabolite codes used due to historic reasons.

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

In addition to the older data generation method applied for analysis of field studies previously conducted in the USA (refer to M-CA 4.1.2/1), new residue analytical methods were developed and validated to allow for separation and individual quantitation of the *E*- and *Z*-isomers of the parent compound in the environmental matrices soil, water, and air. Any analysis necessary in sediment, can be done using the fully validated soil method. The new analytical methods are all based on highly sensitive and selective MS/MS-detection. A new method for air was developed as the peer-reviewed method from Weitzel 1995 (AIIA-4.2.1; DAR 2004) applying GC-NPD would not comply with the requirements of the updated current guidelines (specificity). The previously submitted additional method for air (Class 1999, AIIA-4.2.1) was not considered fully valid in the DAR dated from 2004, as it did not fulfill the requirements for residue analytical methods. Hence, a new method was developed as is presented in chapter M-CA 4.2 as proposed new monitoring method.

The following analytes are included in the residue analytical methods in support of pre-registration data-requirements.

Soil: BAS 550 F (dimethomorph *E*- and *Z*-isomers; Reg. No 4110868 and 4110869), metabolites M550F006 (Z67, meta desmethyl dimethomorph, Reg.No 4060806) and M550F007 (Z69, para desmethyl dimethomorph, Reg. No 4060805)

Sediment: As no aquatic field studies were conducted, no separate stand-alone method validation for the determination of unlabeled dimethomorph or any of its metabolites was required in support of any environmental fate or ecotoxicological studies. Due to similarity to soil matrix, determination of such, if required, any analysis required can be accomplished by applying the fully validated soil method, especially as the extraction solvent used for soil extraction contains an elevated amount of water which accounts for the different water contents of the matrices.

Water: BAS 550 F (dimethomorph *E*- and *Z*-isomers; Reg. No 4110868 and 4110869)

Air: BAS 550 F (dimethomorph *E*- and *Z*-isomers; Reg. No 4110868 and 4110869)

A short summary of the analytical methods can be found in the respective section of Doc N, chapter 5.1.3.

Soil

Analytical methods for the determination of dimethomorph residues in soil were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in Table 4.1.2-2 for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2004). All analytical methods on which the evaluation of the inclusion in Annex I was based on are listed as peer-reviewed. All other methods were submitted for evaluation for Annex I inclusion, but were not peer-reviewed.

Table 4.1.2-2: Summary of already peer-reviewed analytical methods for determination of dimethomorph residues in soil

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	EU reviewed
./.	DK-244-001	soil, fruit	HPLC-UV	dimethomorph	0.2 mg/kg	1987	submitted as additional method, but not peer-reviewed
./.	DK-249-005	soil, animal	GC-NPD; GC-MS	dimethomorph	0.01 mg/kg	1999	yes
./.	DK-249-004	soil (air, milk, egg)	HPLC-UV; GC-MS	dimethomorph	0.01 mg/kg	1999	yes
./.	DK-242-004	soil	HPLC-UV	dimethomorph, Z67, Z69	0.01 mg/kg	1999	submitted as additional method, but not peer-reviewed
./.	DK-242-012	soil	GC-MS	dimethomorph, Z67, Z69	0.01 mg/kg	2002	yes

Table 4.1.2-3: Summary of newly submitted analytical methods for determination of dimethomorph residues in soil

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	comment
M2656	DK-242-008	soil	GC-MS	dimethomorph	0.01 mg/kg	1998	data generation method in support of additional field and storage stability studies
L310/01	2015/7001156	soil	LC-MS/MS	dimethomorph (M550F006, M550F007	0.01 mg/kg	2015	new proposed data generation method allowing separate isomer quantitation
./.	2014/7001070	soil	14C-HPLC	dimethomorph	n.a.	2014	assessment of extraction efficiency on aged residue applying different extraction schemes

Report: CA 4.1.2/1
Babbitt B., 1998 b
CL 336,379 (Dimethomorph): Independent laboratory validation of GC method M 2656 for the determination and confirmation of CL 336379 residues in soil
DK-242-008
Guidelines: EPA PR Notice 96-1
GLP: yes
(certified by United States Environmental Protection Agency)

Remark: Although the study in entitled "independent laboratory validation", the report describes the data generation method used for the respective terrestrial field dissipation study. The original method validation was stopped and only this method developed and validated.

Principle of the method

Analytical method M 2656 was developed for the determination and confirmation of dimethomorph (CL 336379) residues in soil. Residues of dimethomorph were extracted from samples with acetonitrile-water and purified using solid phase extraction techniques. Quantitation of dimethomorph residues was accomplished by fused silica capillary gas chromatography (Rtx-1 column) equipped with a nitrogen-phosphorous detector. Results are calculated as dimethomorph by the direct comparison of peak heights to those of external standards. Residues of dimethomorph were confirmed using GC/MS.

The limit of quantitation of the method is 10 µg kg^{-1} and the limit of detection is approximately 1 µg kg^{-1}.

Recovery findings

The method was found to be satisfactory for the determination of residues of dimethomorph in soil. Fortification levels were 0.00 (control), 10, 20, 100 and 500 µg kg^{-1}. The overall mean recovery of dimethomorph from all soil samples was 86% (n=8) with a standard deviation of ± 12% (see table below). Apparent residues of dimethomorph in control soil averaged 1.1 µg kg^{-1}, hence considered as natural background observed.

Remark: A sufficiently high number of fortified samples (concurrent recoveries) was prepared with the actual field dissipation studies DK-620-302, DK-620-033, DK-620-034, DK-620-040, and DK-620-042, which are presented in chapter M-CA 7.1.2.2.1. In total n=36 procedural recoveries were prepared in each field dissipation study covering fortification levels of 10, 20, 100, 500, and 5000 µg kg^{-1}. Of each concentration level, at least five replicates or more were prepared. Recoveries were all within the valid range of 70-120% as required by the guidelines OPPTS 860.1340 and OCSSP 850.6100.

Table 4.1.2-4: Overview of recoveries of fortified samples prepared at different concentration levels.

Soil matrix	Fortification level [1] (µg kg^{-1})	Recovery (%)	Overall mean recovery (%)	Relative standard deviation (%)
Greenville	10	90	86	10
	500	84		
Tippecanoe	20	89		
	100	88		
Buelah	10	96		
	500	80		
Princeton	20	94		
Melrose	100	64		

[1]: at least duplicate samples were prepared.

Linearity

Good linearity was observed in the range of 0.05 to 0.4 µg mL^{-1}. Standards used for calibration were prepared in ethyl acetate.

Specificity

Two soil extracts from soil type Buelah (one control sample and one control sample fortified at 10 µg kg^{-1}) were analyzed by GC/MS. The results confirm that the method is suitable for the specific determination of dimethomorph residues and that GC-NPD yield. The mass spectra obtained unequivocally confirm the presence of the analyte of interest in the fortified samples and absence of the analyte in the untreated control sample.

Limit of quantitation

The validated sensitivity (LOQ, limit of quantitation) of the method is 10 µg kg^{-1} (10 ppb).

Limit of detection

The limit of detection (LOD) of the method is 1 µg kg^{-1} (1 ppb).

Repeatability

As at least duplicates of each sample per soil was investigated in this study, only a limited statement can be made about the repeatability of the results. As over the entre concentration range and n=8 samples prepare from different soil types, repeatability was fully confirmed by the relative standard deviation of 12%. However, in the field dissipation studies DK-620-302, DK-620-033, DK-620-034, DK-620-040, and DK-620-042, which are presented in chapter M-CA 7.1.2.2.1, a total of n=36 procedural recoveries were prepared in each field dissipation study covering concentrations of 10, 20, 100, 500, and 5000 µg kg^{-1}. Of each concentration, at least 5 replicates or more were prepared. Recoveries were within the valid range of 70-120% as stated in OPPTS 860.1340 and OCSSP 850.6100. Hence, repeatability was assessed and confirmed to be sufficiently good.

Standard stability Results from the standard storage stability showed the peak height responses did not vary by more than 10% confirming that dimethomorph prepared in ethyl acetate is stable for at least one month.

Reproducibility Reproducibility of the method was not determined within this validation study as no ILV was conducted. An ILV is not required as this method's sole purpose is pre-registration data generation.

Conclusion **GC method M 2656 for the determination and confirmation of dimethomorph (CL 336379) residues in soil uses fused silica capillary gas chromatography equipped with a nitrogen-phosphorous detector for final determination with a limit of quantitation of 10 µg kg^{1}. As confirmatory technique GC-MS was applied.**
The method was found to be satisfactory for the determination of residues of dimethomorph in soil with an LOQ of 10 µgkg^{-1} and that GC-NPD can be used for unequivocal quantitation of dimethomorph.

Report:	CA 4.1.2/2 Gordon B., 2015 b Validation of Method D1509 (L0310/01): Method for the determination of Dimethomorph (BAS 550 F, Reg. No. 247723) and its metabolites M550F006 (Reg. No. 4060806) and M550F007 (Reg. No. 4060805) in soil by LC-MS/MS 2015/7001156
Guidelines:	EPA 850.6100, EPA 830.1800, EPA PR Notice 2011-3, SANCO/3029/99 rev. 4 2000 (pre-registration residue methods), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Remark: The study was announced in the Application with DocID 2014/7002565; the same study was allocated a different DocID: 2015/7001156.

Principle of the method

BASF analytical method No. D1509 (L0310/01) was developed for the determination of residues of the geometric isomers of dimethomorph (BAS 550 F), *E*-dimethomorph and *Z*-dimethomorph, and its metabolites M550F006 and M550F007 in soil. The study was performed by BASF, North Carolina, USA.
5 g soil samples are sequentially extracted using acetonitrile, acetonitrile:water (80:20, v/v), and acetonitrile:water (70:30, v/v). Residues in the combined extracts are brought to volume with water and then an aliquot is centrifuged, filtered and diluted with water. The residues are determined by high performance liquid chromatography (HPLC) with detection by positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI), monitoring two ion transitions for each analyte. The geometric isomers of dimethomorph are separated by their retention times on the HPLC column. The results are calculated by direct comparison of the sample peak responses to those of external standards.

The limit of quantitation (LOQ) of the method is 0.01 mg kg^{-1} and the limit of detection (LOD) is 0.002 mg kg^{-1} for all four analytes.

Recovery findings

The method is suitable to determine residues of dimethomorph and its metabolites M550F006 and M550F007 in soil.
Samples were fortified with the analytes at the limit of quantitation of 0.01 mg kg^{-1} and 10 times higher (0.1 mg kg^{-1}).
Mean recovery values (mean of five replicates per fortification level and analyte) were between 76% and 98% for all four analytes (see table below). Apparent residues of each analyte were below the method limit of detection (<0.002 mg/kg) in all of the control soil samples.

Table 4.1.2-5: Recoveries for dimethomorph (*E*- and *Z*-dimethomorph) and its metabolites in soil

Soil Matrix	Analyte	Mass transition	Fortification Level [mg/kg]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Clay loam	*E*-Dimethomorph	388→301	0.01	5	94	3	**93**	3
			0.1	5	92	3		
		388→165	0.01	5	95	3	**93**	3
			0.1	5	92	3		
	Z-Dimethomorph	388→301	0.01	5	94	1	**92**	3
			0.1	5	90	3		
		388→165	0.01	5	98	3	**95**	4
			0.1	5	93	3		
	M550F006	374→287	0.01	5	89	1	**86**	3
			0.1	5	84	1		
		374→151	0.01	5	92	5	**90**	4
			0.1	5	88	2		
	M550F007	374→287	0.01	5	82	2	**79**	4
			0.1	5	76	1		
		374→199	0.01	5	83	1	**79**	5
			0.1	5	76	3		
Sand	*E*-Dimethomorph	388→301	0.01	5	91	1	**91**	2
			0.1	5	91	2		
		388→165	0.01	5	89	1	**90**	3
			0.1	5	91	4		
	Z-Dimethomorph	388→301	0.01	5	89	1	**89**	1
			0.1	5	89	1		
		388→165	0.01	5	90	3	**91**	3
			0.1	5	91	3		
	M550F006	374→287	0.01	5	89	2	**90**	3
			0.1	5	91	2		
		374→151	0.01	5	93	7	**94**	6
			0.1	5	96	4		
	M550F007	374→287	0.01	5	89	1	**89**	2
			0.1	5	89	3		
		374→199	0.01	5	89	1	**91**	2
			0.1	5	93	1		

Linearity

Good linearity (r >0.9989) was observed in the range of 0.01 ng mL^{-1} to 0.25 ng mL^{-1} for the two mass transitions of each test item using solvent-based standards (diluted in acetonitrile:water, 7.5:92.5, v/v).

Specificity

Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. As HPLC-MS/MS is regarded as a highly-specific detection method when two ion transitions have been validated, an additional confirmatory method or technique is not required.

Matrix effects It could be demonstrated that the matrix load in the samples from each soil type had no significant influence on analysis (matrix effects <20%).

Limit of quantitation The limit of quantitation (LOQ) is defined by the lowest fortification level successfully tested. LOQ is 0.01 mg kg^{-1} for each analyte which corresponds to a concentration in the final volume of 0.05 ng mL^{-1}.

Limit of detection The limit of detection (LOD) is defined as 20% of the LOQ; equivalent to 0.002 mg/kg for each analyte, which corresponds to a concentration in the final volume of 0.01 ng mL^{-1}.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Standard stability The stability of each analyte in standard solutions has been determined. In a previous study, dimethomorph was shown to be stable in methanol, the solvent used for preparation of stock solutions, for at least 3 months, under refrigeration. In conjunction with this study, M550F006 and M550F007 were shown to be stable in stock solutions prepared in methanol for at least 1 month (44 days) when stored under refrigeration. In addition, each analyte was demonstrated to be stable in fortification and calibration solutions prepared in acetonitrile and acetonitrile:water (7.5:92.5, v/v), respectively, for 44-57 days when stored under refrigeration. During the course of this study, the test/reference substance solutions were stored in a refrigerator and all solutions were used within the demonstrated time period of stability.

Extract stability The method validation fortification sample extracts were analysed within one day of extraction. The acceptable method recoveries obtained during analysis demonstrate the storage stability of residues of dimethomorph in the extracts prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the initial extracts stored under refrigeration and HPLC final volume held at room temperature, indicated that each analyte, for the representative matrix tested (clay loam soil) is stable in final extracts for at least the time period tested, 8 to 10 days, sufficient to support the storage intervals and conditions incurred by the extracts in the subject study.

Reproducibility Reproducibility of the method was not determined within this validation study.

Conclusion

BASF analytical method No. D1509 (L0310/01) for analysis of geometric isomers of dimethomorph (BAS 550 F), *E*-dimethomorph and *Z*-dimethomorph, and its metabolites M550F006 and M550F007 in soil uses LC-MS/MS for final determination, with a limit of quantitation of 0.01 mg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly and accurately determine residues of dimethomorph in soil.

Remark: Extraction efficiency was not assessed for the residue analytical method described in M-CA 4.1.2/2 (2015/7001156), as the extraction scheme proposed in the aerobic metabolism study was applied for the residue analytical method. Solely the last of the four extraction steps, applying an aqueous solution of methanol, was not applied as it was shown that negligible amounts of radioactivity were extractable with methanol-water-mixtures as fourth extraction step. However, extraction efficiency of different solvents and aqueous mixtures of such were evaluated using soil samples generated during aerobic soil metabolism. This was conducted prior to establishing the residue analytical method. Aim was to assess the suitability of different solvent prior to development and validation of the residue analytical method for soil. The findings and conclusions are described below (M-CA 4.1.2/3).

Report:	CA 4.1.2/3 Keenan D.,Brusky M., 2014 a Comparative analysis of three extraction procedures on [14C]Dimethomorph aerobic soil metabolism study samples 2014/7001070
Guidelines:	OECD 307 (2002), EPA 835.4100
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the different methods

The purpose of the study was, to compare the extraction method used in the aerobic soil metabolism study (chapter CA 7.1.1.1; BASF DocID 2014/7001068) with two other extraction procedures used or planned to be used as part of a residue analytical method. Therefore, three extra soil samples additionally prepared during the aerobic soil metabolism study (Lufa 5M, sandy loam) were used for assessment of extraction efficiency of extraction procedures differing form the one applied in the metabolism study.
During the metabolism study, soil was treated with [^{14}C]-dimethomorph at a rate of 1.2 µg kg^{-1} and incubated under aerobic conditions.
Analytical determination of dimethomorph and its degradation products in the soil extracts was accomplished by HPLC (Phenomenex Gemini C18 column, mobile phases: 0.1% trifluoroacetic acid and acetonitrile) followed by UV/VIS detector and radioactive flow detector; total radioactivity in the extracts was determined by liquid scintillation counting.

Extraction Procedure 1 *(from aerobic soil metabolism study):*
The soil was sequentially extracted at a ratio of 1/2 m/v with: (1) acetonitrile, (2) 80:20 acetonitrile:water, (3) 70:30 acetonitrile:water, and (4) 80:20 methanol:water (v/v).

Extraction Procedure 2: Soil was extracted twice with 70:30 acetone:0.1 M HCl (v/v) at a soil to liquid ration of 1 to 2 (m/v); extracts were pooled for analysis.

Extraction Procedure 3: Soil was extracted once with a mixture of methanol/water (2 N HCl (70/25/5, v/v/v).

Limit of detection

LSC Detection Limit: The limit of detection is equivalent to 38 dpm and considering the specific activity of [^{14}C]-dimethomorph corresponds to 0.17 ng g^{-1} (solid samples) or 0.1 ng mL^{-1} (liquid samples).

HPLC Detection Limit: A detection limit for HPLC analysis of two times the background response in the vicinity of [^{14}C]-dimethomorph permitted detection of radioactivity in samples of at least 0.02 µg mL^{-1}.

Extractability

The three extraction procedures were consistent with the results from the aerobic soil metabolism study (chapter CA 7.1.1.1; BASF DocID 2014/7001068), with the third extraction procedure providing the highest efficiency due to the acidic conditions: the amount of extractable radioactivity for all three extraction methods was 47.5%, 52.0% and 73.1% of applied radioactivity (AR), respectively (Table 4.1.2-6 to Table 4.1.2-8), hence extraction recoveries of 110.6% and 153.9% were obtained.

HPLC-analysis of Soil extracts

Recoveries of radioactivity following sample concentration and reconstitution in water were acceptable, ranging from 84.8% to a maximum of 93.6% across the three samples. Therefore, quantitation of dimethomorph was not corrected for any losses of radioactivity that might have occurred during sample preparation.

It can be concluded from the chromatographic profiles that [^{14}C]-dimethomorph is stable under the extraction conditions analysed and that conversion from *E*-dimethomorph to *Z*-dimethomorph can be forced by concentrating acidic samples without neutralization prior to evaporation of the solvent.

The HPLC results of the soil extracts are presented in Table 4.1.2-6 to Table 4.1.2-8.

Table 4.1.2-6: Extraction Method 1: Extractability and HPLC distribution of radioactivity in soil treated with [^{14}C]-dimethomorph

Extraction Method 1 - metabolism extraction scheme *(applied in CA 7.1.1.1/2; BASF DocID 2014/7001068)*					
Applied dpm:	**28061200**				
LSC					
Extract	**Solution**	**Volume (mL)**	**dpm/mL**	**Total dpm**	**% AR**
1	ACN	83	61196	5079268	18.10
2	80:20 ACN:Water	99	59682	5908518	21.06
3	70:30 ACN:Water	100	17967	1796700	6.40
4	80:20 MeOH:Water	98	5584	547232	1.95
Pooled (1-3)		282	47196	13309272	47.43
			Total Recovery =	**13331718**	**47.51**

HPLC			
Retention Time (min)	**% Area by HPLC**	**% of Applied**	**Name**
33.31	10.56	5.01	E-Dimethomorph
33.71	89.44	42.42	Z- Dimethomorph

note: Extract 4 was not pooled, contained <5% of applied radioactivity (AR)

note: Total recovery is calculated from the sum of total dpm recovered from extracts 1-4

AR = applied radioactivity

Table 4.1.2-7: Extraction Method 2: Extractability and HPLC distribution of radioactivity in soil treated with [^{14}C]-dimethomorph

Extraction Method 2					
Applied dpm:	**28061200**				
Extractions					
Extract	**Solution**	**Volume (mL)**	**dpm/mL**	**Total dpm**	**% AR**
1	70:30 Acetone: 0.1 M HCl	80	148216	11857280	42.26
2	70:30 Acetone: 0.1 M HCl	93	29398	2734014	9.74
Pooled (1-2)	200 mL H_2O+ 20 mL Saturated NaCl	370	35554	13154980	46.88
			Total Recovery = 14591294		**52.00**
HPLC					
Retention Time (min)	**% Area by HPLC**	**% of Applied**	**Name**		
33.25	11.66	5.47	E-Dimethomorph		
33.65	88.34	41.41	Z- Dimethomorph		

note: Total recovery is calculated from the sum of total dpm recovered from extracts 1 and 2
AR = applied radioactivity

Table 4.1.2-8: Extraction Method 3: Extractability and HPLC distribution of radioactivity in soil treated with [^{14}C]-dimethomorph

Extraction Method 3					
Applied dpm:	**28061200**				
LSC					
Extract	**Solution**	**Volume (mL)**	**dpm/mL**	**Total dpm**	**% AR**
1	70:25:5 MeOH:Water:2N HCl	93	22055	2051115	**73.09**
HPLC					
Retention Time (min)	**% Area by HPLC**	**% of Applied**	**Name**		
33.24	25.40	18.57	E-Dimethomorph		
33.64	74.60	54.53	Z- Dimethomorph		

AR = applied radioactivity

Table 4.1.2-9: Comparison of the three different extraction schemes applied.

Extraction Method	Extractable radioactivity in % applied as determined by LSC	% of metabolism extraction scheme	HPLC-pattern in % applied (E/Z)
Metabolism scheme: ACN, ACN/H_2O (80/20), ACN/H_2O (70/30), MeOH/H_2O (80/20)	**47.51**	**100**	**5.01 / 42.42**
2 x Acetone/ 0.1 M HCl (70/30)	**52.00**	**109.5**	**5.47 / 41.41**
MeOH/Water/2N HCl (70/25/5)	**73.09**	**153.8**	**18.57 / 54.53**

MeOH = methanol; ACN = acetonitrile

Conclusion **The study demonstrates that the extraction efficiency of dimethomorph from an aerobic soil system can be increased under acidic conditions. The study confirmed that the extractability results generated from extraction procedures two and three were consistent or even higher than the extraction procedure applied in the aerobic soil metabolism study. In addition, the study concluded that concentration of dimethomorph extracts containing acid without preceding neutralization can enhance the conversion of the dimethomorph *E*-isomer to the dimethomorph *Z*-isomer. However, it has to be taken into consideration that applying an acidic extraction scheme, soil structure is soil organic matter in form of fulvic acids and low molecular humic acids is removed from the soil matrix. Hence, those extraction procedures, if applied, would partially destroy the original matrix. As a consequence those extraction procedures applying strong acidic conditions are not considered suitable to be used as data generation method as the amount of extractable, hence bioavailable, residues would be overestimated.**

Remark: As the data generation method (refer to M-CA 4.1.2/2) applies an extraction scheme identical to the aerobic soil metabolism study, no further extraction efficiency for this residue analytical methods needed to be assessed. The last extraction step of the metabolism extraction scheme, applying a methanol-water-mixture, was not applied in the residue analytical procedure as negligible amounts of radioactivity were extracted.

Water

Analytical methods for the determination of dimethomorph residues in water were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in Table 4.1.2 for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2004). A new method for water was developed as the original detection based on HPLC-UV would not be considered as selective according to the most recent guideline, unless a confirmatory technique would be applied. To fulfill this requirement, highly sensitive and selective MS/MS-detection is applied for the newly developed residue analytical method.

Although the methods described below (M-CA 4.1.2/4 and 4.1.2/5) were not used as direct data generation method for water analysis in support of any environmental fate studies, the method is presented under chapter M-CA 4.1.2 as it is a suitable data generation method. However, its full suitability for enforcement and monitoring purposes has been successfully confirmed by an independent laboratory validation, which is presented in chapter M-CA 4.2.

Table 4.1.2-10: Summary of already peer-reviewed analytical methods for determination of dimethomorph residues in water

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	EU reviewed
RU 151/35/90	DK-243-011	water	HPLC-UV	dimethomorph	0.05µg/L	1999	yes
RU 151/35/90	DK-243-012	drinking water	HPLC-UV	dimethomorph	0.05µg/L	2001	yes
RU 151/35/90	2001/1014995	drinking water	HPLC-UV	dimethomorph	0.05µg/L	2001	yes

Table 4.1.2-11: Summary of newly submitted analytical methods for determination of dimethomorph residues in water

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	comment
D1410 (L0257/01)	2014/7003705	water	LC-MS/MS	dimethomorph	0.05 µg/L per isomer	2015	proposed data generation and monitoring method allowing the separated quantitation of dimethomorph
ILV to D1410 (L0257/01)	2014/7004121	water	LC-MS/MS	dimethomorph	0.05 µg/L per isomer	2015	ILV to 2014/7003705 for enforcement purposes
D1410 (L0257/01	2015/1237992 2017/1068958	water	LC-MS/MS	dimethomorph	0.015 µg/L per isomer	2015	additional validation of lower LoQ for monitoring purposes as additional information
ILV to D1410 (L0257/01	~~2015/1237993~~ 2017/1069823	water	LC-MS/MS	dimethomorph	0.015 µg/L per isomer	2015	ILV to 2015/1237992 as additional information

Report: CA 4.1.2/4
Gordon B., 2015 a
Validation of method D1410 (L0257/01): Method for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water at LOQ of 0.05 ppb using LC/MS/MS
2014/7003705

Guidelines: EPA 850.6100, EPA 830.1800, EPA PR Notice 2011-3, SANCO/3029/99 rev. 4 2000 (pre-registration residue methods), SANCO/825/00 rev. 8.1 (16 November 2010)

GLP: yes
(certified by United States Environmental Protection Agency)

Principle of the method

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method No. 01410 (L0257/01) for the determination of residues of the geometric isomers of dimethomorph (BAS 550 F), *E*-dimethomorph (Reg. No. 4110868) and *Z*-dimethomorph (Reg. No. 4110869), in drinking (tap) water and surface (pond) water.
The residues of dimethomorph in water samples were analyzed by direct injection onto a high performance liquid chromatography (Atlantis T3) column (mobile phases: water/formic acid and methanol/formic acid) with detection by positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+), monitoring ion transitions at m/z 388→301 (proposed as the primary transition for quantitation) and m/z 388→165 (proposed for confirmatory purposes) for both isomers. The isomers were fully separated, enabling separate quantitation of each isomer. The results were calculated by direct comparison of the sample peak responses to those of external standards.

Recovery findings

The method validation was successfully performed for each water type and the LC-MS/MS ion transitions (primary and secondary): Mean overall recoveries of *E* and *Z*-dimethomorph from drinking and surface water samples fortified with each analyte at 0.05 and 0.5 µg L^{-1} ranged from 94 to 96%, with 2 to 3% relative standard deviations (RSD), considering results obtained using both the primary and secondary transitions. Apparent residues of *E* and *Z*-dimethomorph were below the method limit of detection (< 0.01 µg L^{-1}) in all of the untreated control samples of drinking and surface water. A summary of the recoveries is shown in Table 4.1.2-12.

Table 4.1.2-12: Recoveries of Dimethomorph (*E*- and *Z*-isomers) from Drinking and Surface Water using Solvent-Based Standards

Analyte	Matrix	Fortification Level (µg L^{-1})	N	Recovery (%)	Average Recovery (%)	Standard Deviation	% RSD [a]
E-isomer	Drinking water (tap water)	Primary Quantitation (m/z 388 → 301)					
		0.05	5	92, 93, 93, 94, 96	94	2	2
		0.5	5	95, 94, 93, 96, 96	95	1	1
		Overall	**10**	**Range, 92 - 96**	**94**	**1**	**2**
		Confirmatory Quantitation (m/z 388 → 165)					
		0.05	5	91, 98, 95, 97, 95	95	3	3
		0.5	5	96, 97, 91, 96, 96	95	2	3
		Overall	**10**	**Range, 91 - 98**	**95**	**2**	**3**
	Surface water (pond water)	Primary Quantitation (m/z 388 → 301)					
		0.05	5	94, 94, 95, 92, 92	93	1	1
		0.5	5	97, 96, 98, 98, 97	97	1	1
		Overall	**10**	**Range, 92 - 98**	**95**	**2**	**2**
		Confirmatory Quantitation (m/z 388 → 165)					
		0.05	5	97, 98, 94, 93, 90	94	3	3
		0.5	5	96,97, 100,97, 97	97	2	2
		Overall	**10**	**Range, 90 - 100**	**96**	**3**	**3**
Z-isomer	Drinking water (tap water)	Primary Quantitation (m/z 388 → 301)					
		0.05	5	94, 94, 95, 94, 97	95	1	1
		0.5	5	95, 94, 93, 98, 97	95	2	2
		Overall	**10**	**Range, 93 - 98**	**95**	**2**	**2**
		Confirmatory Quantitation (m/z 388 → 165)					
		0.05	5	93, 95, 94, 93, 97	94	2	2
		0.5	5	98, 94, 93, 98, 94	95	2	3
		Overall	**10**	**Range, 93 - 98**	**95**	**2**	**2**
	Surface water (pond water)	Primary Quantitation (m/z 388 → 301)					
		0.05	5	97, 97, 94, 93, 92	95	2	2
		0.5	5	99, 97, 97, 97, 99	98	1	1
		Overall	**10**	**Range, 92 - 99**	**96**	**2**	**2**
		Confirmatory Quantitation (m/z 388 → 165)					
		0.05	5	94, 96, 96, 93, 90	94	2	3
		0.5	5	98, 96, 98, 95, 97	97	1	1
		Overall	**10**	**Range, 90 - 98**	**95**	**2**	**3**

a - Relative Standard Deviation = (Standard Deviation / Average Recovery) x 100

Linearity	Good linearity was observed for the calibration range of both mass transitions for each analyte. Regression coefficients were at least 0.99 (r >0.99). Linearity was confirmed over a concentration range over 0.01 to 0.25 ng mL^{-1} using calibration prepared in water. Per calibration curve 5 different concentrations were prepared.
Specificity	The *E* and *Z*-dimethomorph residues are determined by high performance liquid chromatography (HPLC) and positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+), monitoring two ion transitions for both isomers. The isomers were fully separated, enabling quantitation of the contribution of each analyte. No interfering peaks were observed at the retention times of both isomers and both mass transitions. As HPLC-MS/MS is regarded as a highly-specific detection method. With two specific mass transitions being validated, an additional confirmatory technique is not required.
Matrix effects	Matrix-matched standards were analyzed to evaluate any potential matrix effects. The results confirm that the matrix load in the samples from each natural water type has no significant influence on analysis (matrix effects <20%); therefore, the validation samples were analyzed against calibration standard solutions prepared in HPLC-grade water.
Limit of quantitation	The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of dimethomorph in water is 0.05 µg L^{-1}, which corresponds to a concentration in the final volume of 0.05 µg L^{-1}.
Limit of detection	The LOD for each analyte in water was set at 20% of the LOQ, or 0.01 µg L^{-1}, which corresponds to 0.01 µg L^{-1} in the final volume.
Repeatability	Mean overall recoveries of *E*- and *Z*-dimethomorph from drinking and surface water ranged from 94% to 96% with a relative standard deviation of 2% to 3% (values of both fortification levels considered).
Standard stability	Dimethomorph was shown to be stable in methanol, the solvent used for preparation of stock and intermediate standard solutions, for at least 3 months under refrigeration. Additionally, both isomers of dimethomorph were shown to be stable in calibration standards prepared in water for at least 11 days when stored refrigerated in the dark. During the course of this study, all solutions were used within the demonstrated time period of stability.

Extract stability As the method relies on the direct injection of the water samples onto the HPLC column, there were no extracts; however, based on the results obtained, each analyte was stable over a period of at least 11 days when stored refrigerated.

Reproducibility Reproducibility residue analytical method was confirmed successfully in an independent laboratory validation, which is presented in detail in chapter M-CA 4.2.

Conclusion **The results of this method validation study demonstrate that BASF Analytical Method D 01410 (L0257/01) fulfils the requirements with regard to specificity, repeatability, limit of quantitation, and recoveries and is, therefore, applicable to correctly determine residues of dimethomorph (E- and Z-isomer) in drinking (tap) water and surface (pond) water.**

Report:	CA 4.1.2/5 Schelling D., 2015 a Validation of a lower LOQ (15 ng/L) of method D1410 (L0257/01) for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water using LC/MS/MS 2015/1237992
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.6100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.1.2/6 Schelling D., 2017 a Validation of a lower LOQ (15 ng/L) of method D1410 (L0257/01) for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water using LC/MS/MS 2017/1068958
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.6100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Remark: The method used is identical to the one described under M-CA 4.1.2/4, however, a lower limit of quantitation of 15 ng/L for each isomer was established to cover a wider concentration range for monitoring purposes. However, the originally developed analytical method (N-CA 4.1.2/6) covers the required LOQ of 50 ng/L per isomer.

Principle of the method

The purpose of this study was the validation of a lower limit of quantitation of analytical method D1410 (L0257/01) for the determination of the geometric isomers of dimethomorph (BAS 550 F), E-dimethomorph (Reg. No. 4110868) and Z-dimethomorph (Reg. No. 4110869) in surface water and groundwater using LC-MS/MS. The validation was performed at BASF, Limburgerhof, Germany.
The residues of dimethomorph in water samples were analyzed by direct injection of the water samples including 1% methanol onto a high performance liquid chromatography column with detection by electrospray ionization tandem mass spectrometry (MS/MS-ESI+), monitoring ion transitions at m/z 388--->301 and m/z 388--->165 for both isomers. Analysis was accomplished using an Atlantis T3 column and a methanol water gradient with formic acid as modifier. The two isomers of dimethomorph were individually quantified. The results were calculated by direct comparison of the sample peak responses to those of external standards.

The method has a limit of quantitation of 0.015 µg L^{-1} for each, the *E*- and *Z*-i*somer* of dimethomorph.

Recovery findings

The method validation was successfully performed for both water types examined. Water samples were fortified with E- and Z-dimethomorph at the limit of quantitation of 0.015 µg L^{-1} and 10 times higher (0.15 µg L^{-1}) of each isomer. Mean recovery values (mean of five replicates per fortification level and analyte) ranged from 84% to 108% for both isomers of dimethomorph (see table below).

Table 4.1.2-13: Method recoveries of dimethomorph (*E* and *Z*-i*somer*) from surface water and groundwater using matrix-matched standards

Analyte	Matrix	Fortification Level (µg L^{-1})	n	Recovery (%)	Mean Recovery (%)	% RSD
E-dimethomorph	Surface Water	Primary Quantitation (m/z 388 → 301)				
		0.015	5	86, 88, 81, 80, 83	84	4.0
		0.15	5	94, 94, 114, 92, 91	97	10.0
		Overall	**10**	**Range, 80 - 114**	**90**	**10.8**
		Confirmatory Quantitation (m/z 388 → 165)				
		0.015	5	88, 85, 85, 81, 85	85	2.6
		0.15	5	95, 94, 115, 93, 90	97	10.1
		Overall	**10**	**Range, 81 - 115**	**91**	**10.4**
	Groundwater	Primary Quantitation (m/z 388 → 301)				
		0.015	5	104, 103, 88, 91, 105	98	8.1
		0.15	5	101, 101, 92, 90, 81	93	9.0
		Overall	**10**	**Range, 81 - 105**	**96**	**8.5**
		Confirmatory Quantitation (m/z 388 → 165)				
		0.015	5	112, 108, 92, 92, 101	101	9.3
		0.15	5	101, 101, 92, 90, 82	93	8.5
		Overall	**10**	**Range, 82 - 112**	**97**	**9.4**
Z-dimethomorph	Surface Water	Primary Quantitation (m/z 388 → 301)				
		0.015	5	92, 89, 81, 77, 83	85	7.2
		0.15	5	96, 96, 118, 94, 92	99	10.5
		Overall	**10**	**Range, 77 - 118**	**92**	**12.1**
		Confirmatory Quantitation (m/z 388 → 165)				
		0.015	5	89, 87, 83, 85, 88	86	2.7
		0.15	5	96, 95, 118, 94, 94	99	10.4
		Overall	**10**	**Range, 83 - 118**	**93**	**10.5**
	Groundwater	Primary Quantitation (m/z 388 → 301)				
		0.015	5	101, 97, 93, 95, 105,	98	4.8
		0.15	5	104, 103, 95, 92, 87	96	7.4
		Overall	**10**	**Range, 87 - 105**	**97**	**6.0**
		Confirmatory Quantitation (m/z 388 → 165)				
		0.015	5	112, 114, 103, 99, 110	108	6.0
		0.15	5	105, 105, 94, 94, 84	96	9.2
		Overall	**10**	**Range, 84 - 114**	**102**	**9.3**

RSD = Relative Standard Deviation

Linearity	Good linearity was observed for the standard range and the two mass transitions tested for both isomers of dimethomorph: The method-detector response was linear over the 0.004 to 0.25 ng mL^{-1} range ($r > 0.995$) using matrix-matched standards. At least seven calibration points were used for quantitation.
Specificity	The method allows the specific determination of E-dimethomorph and Z-dimethomorph in surface water and groundwater by using LC-MS/MS. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered. As HPLC-MS/MS is regarded as a highly-specific detection method when two ion transitions have been validated, an additional confirmatory technique is not required.
Matrix effects	Solvent-based as well as matrix-matched standards were analysed to assess potential matrix effects at the lower concentration range compared to the original method. The calculated overall mean response factor (response factor of solvent-based standards set to 100%) for *E*- and *Z*-dimethomorph was 127% obtained from surface water matrix-matched standards and 80% obtained from groundwater matrix-matched standards. Therefore, matrix-matched standards were used for the quantitation of the validation samples.
Limit of quantitation	The limit of quantitation (LOQ) of the method is defined by the lowest fortification level successfully tested. The validated LOQ of this method for residues of dimethomorph in water is 0.015 µg L^{-1}, for each, *E*-dimethomorph and *Z*-dimethomorph, respectively.
Limit of detection	The limit of detection (LOD) is defined as the lowest calibration level tested. The LOD of this method for *E*-dimethomorph and *Z*-dimethomorph is 0.004 µg L^{-1} in water samples (approx. 27% of LOQ).
Repeatability	The relative standard deviation (RSD, %) for all fortification levels was below 11%.
Standard stability	The standard stability was proved in the previous validation of this

Extract stability As the method relies on the direct injection of the water samples including 1% methanol onto the HPLC column, there are no extracts; however, based on the method validation results obtained in this study, each analyte was stable in water prior to analysis.

Reproducibility The reproducibility of the method was determined within an independent laboratory validation study (CA 4.2/11, BASF DocID 2015/1237993).

Conclusion **The analytical method D1410 (L0257/01) for analysis of *E*-dimethomorph (Reg. No. 4110868) and *Z*-dimethomorph (Reg. No. 4110869) in water uses LC-MS/MS for the final determination, with a limit of quantitation of 0.015 µg L^{-1}.**
It could be demonstrated that method L0257/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of dimethomorph (*E*-i*somer* and *Z*-isomer) in surface water and groundwater.

Air

Analytical methods for the determination of dimethomorph residues in soil were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2004). All analytical methods on which the evaluation of the inclusion in Annex I was based on are listed as peer-reviewed. All other methods were submitted for evaluation for Annex I inclusion, but were not peer-reviewed.

The overview comprises data generation as well as monitoring methods and this overview is not listed again in chapter M-CA 4.2.

Table 4.1.2-14: Summary of already peer-reviewed analytical methods for determination of dimethomorph residues in air

Method No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	EU reviewed
./.	DK-241-003	air		dimethomorph	83 µg/m^3	1995	yes
./.	DK-249-004	air (soil, milk, egg)	HPLC-UV; GC-MS	dimethomorph	10 µg/m^3	1999	submitted as additional method, but not peer-reviewed

Table 4.1.2-15: Summary of newly submitted analytical methods for determination of dimethomorph residues in air

Method No.	DocID	Matrix	Method principle	Target analytes	LOQ	year	comment
./.	2015/1192602	air	LC-MS/MS	dimethomorph	3 µg/m^3 (sum of isomers)	2015	new proposed monitoring method (refer M-CA 4.2)

No methods for air were required for risk assessment in support of environmental fate studies. An analytical method for post-approval and monitoring purposes is described in detail in chapter M-CA 4.2, as this method was not used for any data generation purposes.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

No stand-alone validation of analytical methods was required in support of efficacy studies.

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Analytical methods used in support of dietary and gavage studies (non-radiolabeled) have been established and validated either in separate study reports or were reported as analytical phase reports included in the original study report; both are described in detail in this section. If the methodology / analytical report was part of the study, the summary of the results is summarised under the DocID of the relevant toxicological study.

Report: CA 4.1.2/7
[redacted], 1989 a
SAG 151 - Oral (gavage) teratogenicity study in the rat
DK-432-002
Guidelines: EPA 83-3, OECD 414
GLP: yes
(certified by Der Regierungspraesident Muenster, Muenster, Germany)

Report: CA 4.1.2/8
[redacted] 1996 a
SAG 151: Oral (gavage) teratogenicity study in the rat
DK-123-087
Guidelines: EPA 83-3, OECD 414
GLP: yes
(certified by Der Regierungspraesident Muenster, Muenster, Germany)

Remark: The study identifier DK-432-002 is the one of the relevant toxicological study however only the analytical phase is summarised below.

Principle of the method

Samples of the aqueous suspensions of the test article were well mixed. Then, aliquots of 1 mL were mixed with 9 mL of acetonitrile for exactly 30 seconds. Dimethomorph was analysed by HPLC-UV at a wavelength of 254 nm. The chromatography was performed on a Hyperchrome RP Nucleosil-100 C18 column at a flow rate of 1.5 mL/min. As mobile phase a mixture of 40% monopotassium phosphate buffer (0.05 M) and 60% acetonitrile, pH 2.3, adjusted with approximately 6 mL o-phosphoric acid, was used.
Concentrations of dimethomorph in the formulation samples were calculated by direct comparison with standard response.

Recovery findings

The recoveries of dimethomorph in aqueous suspension are summarised in Table 4.1.2-16. The results obtained were within the valid recovery range after fortification with 20 mg/mL, 60 mg/mL, and 160 mg/mL dimethomorph, with 94% and 101%

Table 4.1.2-16: Recovery results of dimethomorph in aqueous suspension

Matrix	Fortification level [mg/mL]	n	Recovery[1] [%]	Average recovery [%]	Relative standard deviation [RSD, %]
Aqueous suspension	20	9	90, 93, 93, 88, 90, 93, 100, 103, 98	94	5.4
	60	9	93, 101, 96, 100, 93, 96, 103, 103, 101	98	4.1
	160	9	91, 109, 91, 116, 116, 91, 97, 97, 103	101	10.2
	Overall Recovery	**27**		**98**	**7.5**

[1] inclusive the results of a repeat analysis

Linearity	Regression is r >0.99 for a concentration range from 0 to 200 mg/L. (Remark: This information was derived from the chromatograms given in the study report).
Specificity	The UV-wavelength chosen is specific for the analyte dimethomorph. The identification and quantitation were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in the aqueous suspension. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary. No significant matrix interferences were observed in the investigated control extract.
Matrix effects	As no interference at the elution time of the analyte of interest was observed in the UV-trace at 254 nm, no adverse effects of any matrix occurred.
Interference	No interfering signal was observed at the elution time of the analyte of interest in the control sample.
Limit of quantitation	The limit of quantitation of the method, defined by the lowest fortification level is 20 mg/mL.
Limit of detection	The limit of detection is the level at which a signal to noise ratio of 1 to 3 is still given.
Repeatability	The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different dose levels were between 5.4% and 10.2%. The detailed values are shown in Table 4.1.2-16.
Reproducibility	Reproducibility of the method was not determined within the validation study. No independent laboratory validation needs to be conducted for a data generation method / method for concentration control.

Stability Analyses of stored samples indicated that dimethomorph is stable for at least 24 hours at -20°C. The detailed values are shown in Table 4.1.2-17.

Table 4.1.2-17: Stability results of dimethomorph in aqueous suspension samples

Matrix	Storage period [hours]	Approx. fortification level [mg/kg]	n	Recovery[1] [%]	Mean recovery [%]	Relative standard deviation [RSD, %]
Aqueous suspension	0	20	3	93, 98, 105	102	7.0
		60	3	96, 98, 104		
		160	3	113, 113, 103		
	4	20	2	93, 100	104	8.5
		60	2	100, 101		
		160	2	116, 113		
	24	20	2	88, 95	101	7.6
		60	2	105, 103		
		160	2	103, 109		

[1] inclusive the results of a repeat analysis

Conclusion: **The described method is considered fully suitable for the quantitative analysis of dimethomorph (BAS 550 F) in aqueous suspension.**

Report:	CA 4.1.2/9 [redacted], 1988 a SAG 151 - 52 week dietary toxicity study in dogs DK-427-003
Guidelines:	OECD 409, EPA 83-1
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Report:	CA 4.1.2/10 Anonymous, 2015 a Appendix A: Excerpt of raw data on quality control samples / procedural recoveries prepared during diet analysis of study DK-427-003 2015/1260809
Guidelines:	none
GLP:	no

Report:	CA 4.1.2/11 Anonymous, 1986 a Appendix B: Excerpt of raw data on methodology of blood analysis used in study DK-427-003 2015/1260810
Guidelines:	none
GLP:	no

Remark: The analytical phase report titled "Measurement of concentrations of SAG-151 in whole blood samples withdrawn from dogs on 52 week toxicity study ([redacted] Project No. 636876" was not assigned an individual DocID, but is reported as part of the toxicological study DK-427-003. Results are comprised in the summary given below which also addressed verification of homogeneity of the test diets. The analysis of the diet was conducted in [redacted] Study No. 335504, using method M3506 ([redacted] Project No. 335065) however these reports were not issued a separate final report, but was reported as part of DK-427-003 or its respective raw data. The required information obtained is presented as Appendices to study DK-427-003 which are assigned separate DocIDs to facilitate assignment of presented information. Where information was taken directly from the study's raw data (Appendices), this is highlighted by an appropriate comment with reference to the relevant study number / DocID.

Executive summary of the diet analysis:

Principle of the method A high performance liquid chromatographic method (HPLC) has been established for the of rodent and dog diets. Diet samples (ca. 5 g) were analysed, after addition of an internal standard (2,6-dichlorophenol) and extraction using acetonitrile. Quantitation was accomplished by comparison to external standards by adding known amounts of the test item to test substance free diet and treating those in the same way. Three quality control samples were also analysed with each group of diet samples to validate each analysis *(Information from Appendix A; DocID 2015/1260809)*. Extracts were diluted after preparation with the HPLC mobile phase and subsequently subjected to HPLC analysis. Analysis was conducted on an ODS Hypersil column using an acetonitrile water gradient with UV detection at 230 nm.

Recovery findings With each set of analysis, a set of at least 3 procedural recoveries was analysed. A total of n=117 procedural recoveries (quality control samples) were analysed covering a concentration range from 70 µg/g to 7700 µg/g. At the lowest level n=15 was prepared, at the highest level n=12 *(information from Appendix A; DocID 2015/1260809; recovery was calculated based on the values given in the original document - Appendix A DocID 2015/1260809)*

Linearity Calibration standards of known concentrations were prepared in an identical way than the diet samples by addition of a known amount of analyte. Quantitation was done by comparison of the unknown sample to the respective standard of the expected concentration range. Hence a linear regression is not applicable *(information from Appendix A; 2015/1260809)*.

Specificity The UV-wavelength chosen is specific for the analyte dimethomorph. The identification and quantitation were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in the aqueous suspension. No significant matrix interferences were observed in the investigated control extract.

Matrix effects Calibration standards were prepared in an identical manner than the unknown samples.

Interference No interference was observed at the elution time of the analyte or the internal standard.

Limit of quantitation	The lowest level of fortification, hence 70 µg/g, can be considered as LOQ.
Limit of detection	The limit of detection is defined as the concentration where a good signal to noise ratio of at least 1 to 3 was observed.
Repeatability	The relative standard deviation (RSD, expressed in percent) over the entire concentration range was 12% with respect to recoveries following fortifications at the different dose levels.
Reproducibility	No independent laboratory validation is required for a data generation method.
Stability	Stability of diet was considered to be satisfactory, hence analysis was repeated after 14 days of preparation. Preparation were considered chemically stable if mean concentrations immediately after mixing and 14 days agreed within ±10% *(information from Appendix A; 2015/1260810)*.
.	
Homogeneity of diet	Analysis of the test diets showed that during the 52 week study the test diets were well within the tolerance limits (± 10%) in terms of deviation from the nominal concentration. Only the high dose indicated a deviation of -11.1% compared to the nominal dose. Further analysis undertaken in week 24 and subsequently during the study period, confirmed that all formulations were within 10% of nominal. The slight deviation at week 22 is no considered to have any impact on the study results. The low dose was calculated to be 5 mg/kg and the high dose 45 mg/kg; the intermediate concentration was 15 mg/kg. The grouped mean values of the achieved dosage (mg/kg/day) during the duration of 52 weeks was 4.9-5 mg/kg/day, 14.7-15.7 mg/kg/day, and 44.6-47.0 mg/kg/kg. It can thus be concluded that homogeneity of the diet was ensured throughout the entire duration of the study. The results of diet analysis in due course of the study are shown in the Table below in Table 4.1.2-18.

Table 4.1.2-18: Analysis of test diets – confirmation of correct dose

Study Week	Theoretical concentration	Mean concentration found	n	Deviation from nominal [%]	Coefficient of variation [CV, %]
1	0	0	3	0	0
	150	139	3	-7.3	1.9
	450	419	3	-6.9	3.8
	1350	1262	3	-6.5	2.0
4	0	0	3	0	0
	150	147	3	-2.0	2.0
	450	423	3	-6.0	2.7
	1350	1270	3	-5.9	2.7
9	0	0	3	0	0
	150	146	3	-2.7	1.7
	450	436	2	-3.1	3.1
	1350	1268	3	-6.4	1.7
22	0	0	3	0	0
	150	152	2	+1	0.5
	450	413	3	--8.2	5.2
	1350	1200	3	-11.1	6.4
24	1350	1319	3	-2.25	1.9
35	0	0	3	0	0
	150	138	3	-8.0	1.4
	450	427	3	-5.2	0.4
	1350	1372	3	+1.6	1.0
49	0	0	3	0	0
	150	147	3	-2.0	4.4
	450	443	3	-1.6	3.4
	1350	1304	3	-3.4	0.3

Conclusion: **The described method is suitable for the determination of dimethomorph in dog diet. Homogeneity of the test item in the dog diet was confirmed.**

In addition to the test diets, whole blood samples were analysed to confirm uptake of the substance by the animal after gavage (positive control).

Executive summary of the blood analysis:

As part of study DK-427-003, blood was taken from 2 animals per dog group during week 1, 3, and 52. These samples were analysed for both *E* and *Z*-isomers. The analytical results were reported in [redacted] Project No. 336755 which is part of DK-427-003 (Appendix 23 of the GLP report).

The blood analysis / analytical method is presented for completeness, as the toxicological endpoints are not derived from the analyte level in the blood. This analysis serves as a mere positive control that the test item was present in the animals' blood stream.

Principle of the method

Blood samples were analysed by HPLC-UV at 242 nm after addition of internal standard solution followed by extraction with t-butyl ether. The samples were shaken for 15 min and then centrifuged at 3000 rpm for 10 min. The organic layer was removed and evaporated under a stream of nitrogen; the residue was re-constituted in the mobile phase of the HPLC-system, centrifuged for 5 min at 3000 rpm, and after filtration through a 0.45 µm filter subjected to HPLC analysis. Chromatography was performed on a Hypersil ODS column using a mobile phase of acetonitrile:methanol:water (40/20/40 v/v/v) at a flow rate of 2 mL/min at ambient temperature. The injection volume was 50 µL. Concentrations of dimethomorph in the formulation samples were calculated by direct comparison with standard response.

Linearity

Calibration standard solutions were prepared from the methanolic stock solution by dilution in distilled water. Final calibration standards were prepared by adding known amounts of the E and Z isomer (internal standard) to the blank control of dog blood to cover a concentration range from 0 to 5 µg/mL.
Calibration curves were generated by plotting the sum of the E and Z isomer against the blood concentration of SAG-151. Linear regression was chosen as function of best fit. Satisfactory linearity >0.998 was observed over the chosen concentration range of 0-5 µg/mL for both, the *E* and *Z*-isomer.

Specificity

The UV-wavelength chosen is specific for the analyte dimethomorph. The identification and quantitation were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in the aqueous suspension. No significant matrix interferences were observed in the investigated control extract.

Matrix effects

As no interference at the elution time of the analyte of interest was observed in the UV-trace at 242 nm, no adverse effects of any matrix occurred *(refer to Appendix A DocID 2015/1260811 or DK-245-002).*

Interference	No interfering signal was observed at the elution time of the analyte of interest in the control sample.
Limit of quantitation	No limit of quantitation was determined by preparation of fortified samples. All concentrations above the LOD were quantified at time of analysis.
Limit of detection	Limit of reliable determination of 0.14 µg/mL and 0.15 µg/mL for the *E* and *Z*-isomer respectively.
Reproducibility	Reproducibility of the method was not determined within the validation study. No independent laboratory validation needs to be conducted for a data generation method / method for concentration control.
Stability	Stability of diet was considered to be satisfactory.
Conclusion	**The described method is suitable for the determination of dimethomorph for verification of the presence of dimethomorph in blood samples.**

Report: CA 4.1.2/12
[redacted] 1986 a
CME 151: 13 week dietary toxicity study in dogs
DK-425-002
Guidelines: OECD 409, EPA 82-1
GLP: yes
(certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Report: CA 4.1.2/13
Henderson M.S., 1987 a
Measurement of concentrations of CME-151 in whole blood samples withdrawn from dogs in 13 week toxicity study [redacted] project No. 635212)
DK-425-003 ([redacted] Project Number 335573)
Guidelines: none
GLP: yes

Report: CA 4.1.2/14
Anonymous, 1987 a
Appendix A: Excerpt of raw data - Chromatograms of blanks obtained from blood samples analysed for study DK-425-003
2015/1260811
Guidelines: none
GLP: no

Report: CA 4.1.2/15
Henderson M.S., 1988 a
Establishment of analytical methods for the determination of CME 151 and CME 152 in blood from the animals on the toxicity testing programme
DK-245-002
Guidelines: none
GLP: yes

Remark: Homogeneity of the dog diet was assessed and reported in DK-427-03; analysis of blood samples is addressed and reported in DK-425-03. To facilitate the overview of the results, the summary below comprises the information of both studies with an appropriate remark when data from the respective study is addressed.

Executive summary of study DK-425-002:
Representative 100 g samples of the diet preparations from all treatment groups, including controls, were taken from the freshly mixed diets at the beginning of each study week for archival purposes. For analysis of the test material 3 x 50 g samples of the diet mix from all treatment groups, including Controls, were also taken at the start of treatment and during Weeks 2, 7, 9 and 13 of the study. All assays yielded values within 10% deviation from nominal concentration and were within acceptable limits.

A detailed summary of the diet analysis is already described under M-CA 4.1.2/9 (DK-427-003).

Stability

Stability of diet was considered to be satisfactory, hence analysis was repeated after 14 days of preparation. Preparation were considered chemically stable if mean concentrations immediately after mixing and 14 days agreed within ±10% *(Appendix B, DocID 2015/1260810)*.

Homogeneity of diet

The grouped mean values of the achieved dosage (mg/kg/day) during the duration of 52 weeks was 4.9-5 mg/kg/day, 14.7-15.7 mg/kg/day, and 44.6-47.0 mg/kg/kg. It can thus be concluded that homogeneity of the diet was ensured throughout the entire duration of the study. The results of diet analysis in due course of the study are shown in the Table 4.1.2-19.

Table 4.1.2-19: Analysis of test diets – confirmation of correct dose

Study Week	Theoretical concentration	Mean calculated concentration [ppm]	Deviation from theoretical [%]	Coefficient of variation [CV, %]
1	0	0	0	0
	150	14	-6.0	1.5
	450	430	-4.4	1.7
	1350	1329	-1.6	2.6
2	0	0	0	0
	15	136	-9.3	3.4
	450	409 (414)	-9.1 –(8.0)	2.2 (1.1)
	1350	1340	-0.7	2.1
7	0	0	0	0
	15	142	-5.3	1.1
	450	431	-4.2	4.2
	1350	1328	-1.6	1.2
9	0	0	0	0
	15	149	-0.7	4.1
	450	449	-0.2	0.5
	1350	1377	+2.0	0.5
13	0	0	0	0
	15	150	0	0
	450	431	-4.2	-4.2
	1350	1351	+0.1	+0.1

Values in brackets are from repeat assays due to suspected quality control samples

Conclusion **The described method is suitable for the determination of dimethomorph in dog diet Homogeneity of the test item in the dog diet was confirmed.**

In addition to the test diets, whole blood samples were analyzed to confirm uptake of the analyte into the animals' blood stream (positive control).

Executive summary of the analytical report DK-425-003 and DK-245-002:

Blood samples were withdrawn from one male and one female dog in each of the four dose groups at 6 and 12 weeks after commencement of the study.

The blood analysis / analytical method is presented for completeness only, as the toxicological endpoints are not derived from the analyte level in the blood. This analysis serves as positive control that the test item was present in the animals' blood stream.

Principle of the method The whole blood samples (10 mL) was diluted to 20 mL with distilled water, applied to an Extrelut column and left for 20min. The column was eluted with ethyl acetate, which was reduced under vacuum to dryness. The residue was re-constituted in methanol which was evaporated to dryness under a stream of nitrogen. The final residue was re-constituted in tetrahydrofuran which is subjected to further clean-up one the Extrelut column. The eluate is evaporated to dryness and the residue dissolved in methanol. The entire procedure was conducted under exclusion of day light. Analysis was done on a Hypersil ODS in a mobile phase of acetonitrile/methanol/water using UV-detection at ambient temperature.

Recovery findings The recovery of CME-151 from whole blood samples was estimated by comparison of the theoretical concentrations of the spike samples with the values calculated from non-extracted standard curve. The mean recoveries are summarised in the table below (mean values and RSD were calculated based on the data reported):

Table 4.1.2-20: Recoveries of fortified blood samples (CME-151); results for the sum of isomers are presented (from DK-245-002)

Amount nominal [ng]	Amount measured [ng]	Mean calculated concentration [ppm]	percent recovery [%]	RSD [%]	n
1584	1247.9	n.a	n.a	1	1
792	624.5	78.9	13	6	6
396	285.8	n.a	n.a	1	1
198	161.7	n.a	n.a	1	1
39.6	52.5	n.a	n.a	1	1

Linearity	At least 6 different calibration standards covered a concentration range from 0 to 40 µg/mL. Linearity was satisfactory with linear correlation coefficients >0.99 (*DK-425-003*). The method itself was established to cover a wider concentration range of 0-3960 ng. Good linearity was observed (r>0.999) (*DK-245-002*). Both concentration ranges showed satisfactory linearity.
Specificity	The UV-wavelength chosen is specific for the analyte dimethomorph. The identification and quantitation were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in the aqueous suspension.
Matrix effects	As no interference at the elution time of the analyte of interest was observed in the UV-trace at 240 nm, no adverse effects of any matrix occurred (*DK-245-002*).
Interference	No interfering signal was observed at the elution time of the analyte of interest in the control sample *(DK-245-002).*
Limit of quantitation	No limit of quantitation was determined by preparation of fortified samples. All concentrations above the LOD were quantified at time of analysis.
Limit of detection	Limit of reliable determination of 0.14 µg/mL and 0.15 µg/mL for the E and Z isomer respectively.
Reproducibility	Reproducibility of the method was not determined within the validation study. No independent laboratory validation needs to be conducted for a data generation method / method for concentration control.
Conclusion	**The described methods are suitable for the determination of dimethomorph as well as for the verification of the presence of Dimethomorph in blood samples.**

(d)Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No stand-alone validated data generation methods for the determination dimethomorph were required for exposure studies. Where necessary, analytical method parameters were addressed within the respective study.

(e)Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

The included data generation methods used for analysis of samples of plant and animal origin cover the following compounds:

Plant: BAS 550 F (dimethomorph *E*- and *Z*-isomers; Reg. No 4110868 and 4110869), metabolites M550F002 (Reg. No 4060806) and M550F007 (Z69, para desmethyl dimethomorph, Reg. No 4060805) and metabolite M550F021 (morpholine, Reg. No 21322),.

Animal: BAS 550 F (dimethomorph *E*- and *Z*-isomers; Reg. No. 4110868 and 4110869); metabolites M550F006 (Z67, meta desmethyl dimethomorph, Reg. No 4060806) and M550F007 (Z69, para desmethyl dimethomorph, Reg. No 4060805), and metabolite M550F008 (Z89, CUR 7117, Reg. No 4108146 for *E*/*Z* mixture) in milk and milk products only

Plant matrices:

Analytical methods for the determination of dimethomorph residues in plant matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in the table below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2004). No differentiation has been made with regards to data generation or enforcement methods as both are summarized in Table 4.1.2-21. All analytical methods on which the evaluation of the inclusion in Annex I was based on are listed as peer-reviewed. All other methods were submitted for evaluation for Annex I inclusion, but were not peer-reviewed.

Table 4.1.2-21: Summary of already peer-reviewed analytical methods for determination of dimethomorph residues in plant matrices

Method. No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	EU reviewed
./.	DK-249-005	Wine, grapes, onions, hops, rapeseed	GC-NPD	Dimethomorph	0.02 mg/kg	1999	Yes
./.	DK-240-004	White grapes, hops	GC-NPD	Dimethomorph	0.02 mg/kg, 0.2 mg/kg	1999	Submitted as additional method, but not peer-reviewed
./.	DK-244-001	Potatoes, grapes, soil	HPLC-UV	Dimethomorph	0.01-0.05 mg/kg	1987	Submitted as additional method, but not peer-reviewed
./.	DK-244-004	White grapes, onions, hops, rapeseed	GC-NPD	Dimethomorph	0.01 mg/kg	1999	Yes
./.	DK-244-026	White grapes, hops	GC-NPD	Dimethomorph	0.02 mg/kg, 0.2 mg/kg	1999	Submitted as additional method, but not peer-reviewed
FAMS 022-02	DK-244-008	Potatoes, grape, wine	HPLC-UV	Dimethomorph	0.05 mg/kg	1991	Submitted as additional method, but not peer-reviewed
./.	DK-244-011	Potatoes	HPLC-UV; GC-NPD	Dimethomorph	0.01 mg/kg or 0.02 mg/kg	1995	Submitted as additional method, but not peer-reviewed
./.	DK-244-015	Potatoes grapes, grape waste, raisins, fruit juice, wine	HPLC-UV; GC-NPD	Dimethomorph	0.01 mg/kg	1995	Submitted as additional method, but not peer-reviewed
FAMS 073-03	DK-244-020	Dried hop cones	GC-NPD	Dimethomorph	0.2 mg/kg	1997(a)	Submitted as additional method, but not peer-reviewed
FAMS 022-02	DK-244-032	Grapes, grape juice	GC-NPD	Dimethomorph	0.05 mg/kg	2001	Yes
RLA 12654.00V	DK-244-033	Potatoes, grapes, wine	GC-NPD, LC-MS/MS	Dimethomorph	0.05 mg/kg	2002	Yes
RLA 12654.00V	DK-244-034	Potatoes, grape, wine	LC-MS/MS	Dimethomorph	0.05 mg/kg	2001	Yes
./.	DK-724-026	Potatoes	HPLC-UV	Dimethomorph	0.01 mg/kg	1995	Submitted as additional method, but not peer-reviewed

Table 4.1.2-22: Summary of newly submitted analytical methods for determination of dimethomorph residues in plant matrices

Method. No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	Comment
DFG S19 (L0321/01)	2014/1186696	Lettuce, onion, grape, potato, hops, dried bean, oilseed rape seed	LC-MS/MS	Dimethomorph	0.05 mg/kg per isomer	2015	Proposed new enforcement method
ILV to DFG S19	2015/1204836	Lettuce, onion, grape, potato, hops, dried bean, oilseed rape seed	LC-MS/MS	Dimethomorph	0.05 mg/kg per isomer	2015	ILV to enforcement method
575/0 (L0013/01)	2005/1026082	Lettuce, tomato, pea, onion, potato, grapes, hops	LC-MS/MS	Dimethomorph	0.01 mg/kg	2005	Data generation method in support of field trials
L0013/02	2014/700494	Lentil, soy bean seed, barley, lettuce, orange, soya bean oil, maize stover	LC-MS/MS	Dimethomorph (*E* and *Z*-isomer)	0.01 mg/kg	2014	New proposed data generation method allowing separate quantitation of isomers
L0013/03	2014/1186695	Lettuce, strawberry, dried peas, oilseed rape seed, potato	LC-MS/MS	M550F002, M550F007	0.01 mg/kg	2015	Data generation method for metabolites
L0013/04	2014/1187244	Lettuce, strawberry, dried peas, oilseed rape seed, potato	LC-MS/MS	M550F021 (morpholine)	0.01 mg/kg	2016	Data generation method for metabolites
M3463	2002/5002648 2002/7014914	Canola seed, sunflower seed	LC-MS/MS	Dimethomorph	0.05 mg/kg	2002	Data generation method for residues in canola seed and sunflower seed
M3502	2002/5002982	Broccoli, spinach, wheat grain, wheat straw	LC-MS/MS	Dimethomorph	0.05 mg/kg	2002	Data generation method
FAMS 02-02	DK-244-008, DK 244-009, DK-244-002, DK-244-013, DK-244-015	Raisins, grape waste material	LC-UV	Dimethomorph	0.05 mg/kg	1991, 1995	Data generation method
FAMS 076-01	DK-244-019, DK-123-235	Raisins, grape waste material	LC-UV	Dimethomorph	0.02 mg/kg	1996	Data generation method
SOP MR 029	DK-713-039, DK-713-040, DK-713-041, DK-713-042			Dimethomorph			Data generation method
FAMS 098-02	DK-244-025	Oilseed rape seeds	GC-MS	Dimethomorph	0.01 mg/kg	1999	Data generation method
M2577	DK-244-022, DK-123-225	Tomato, processing commodities	GC-MS	Dimethomorph	0.05 mg/kg 0.01 mg/kg (juice)	1997	Data generation method

In addition to the current data generation method 575/0, a new data generation method allowing the separation and individual quantitation of each isomer. Furthermore, new methods for the analysis of metabolites as listed above were developed.

Remarks to extraction efficiency – plant matrices:
Comparison of extraction recoveries of residue analytical methods with the extraction scheme used in plant metabolism is addressed in the respective metabolism studies. For plant matrices this is addressed in chapter M-CA 6.2.1 within the following studies: 2014/1093386 (grape) and 2015/100060 (lettuce).

Grape (DocID 2014/1093386): In order to compare the extraction efficiency, the amount of parent compound released with the different residue methods was set in proportion to the results from the metabolism study (extraction with methanol and water). For grapes, comparable amounts of dimethomorph were extracted with the residue analytical methods (yielding extraction efficiencies of 93.2% to 97.5% of the metabolism extraction scheme). For leaves, the investigated methods showed comparable extractabilities (residue method 575/0: 89.2% of the metabolism study, DFG S19: 99.0% of the metabolism extraction scheme) with the exception of the multimethod QuEChERS, which resulted in a lower extractability (71.4% of the metabolism extraction scheme). Based on the data obtained, DFG S19 was opted for as the more suitable approach for establishing an enforcement method. A detailed description of the data obtained can be found in chapter M-CA 6.2.1, DocID 2014/1093386.

Lettuce (DocID 2015/1000601: In addition to the methanol, water and acetone extractions used in the metabolism investigations (see Section 3.2), samples were extracted with acetone: water (2:1 v/v) (multimethod approach according to DFG S19), and with methanol: water: 2M HCl (75/25/5 v/v/v) (data generation method 575/0). The aim was to show that BAS 550 F (*E* and *Z*-isomers) as the only main residue in lettuce was efficiently extracted with the solvents used in these analytical residue methods. In comparison to the metabolism method, the DFG S19 multimethod and residue method 575/0 extracted 98.3 and 102.7%, respectively, of the BAS 550 F at the immature harvest and 92.4 and 99.1%, respectively, at the m*a*ture harvest. The sum of the parent compound isomers was similar for all extraction methods, thus both methods proved to be fully suitable for reliable determination of BAS 550 F in leafy matrices. A detailed description of the data obtained can be found in chapter M-CA 6.2.1, DocID 2015/1000601.

It should be noted that all data generation methods ("old" data generation method 575/0=L0013/01, the newly developed data generation method L0013/02 for the separation of *E*/*Z*-isomers as well as method L0013/03 for the determination of M550F002 and M550F007 in plant matrices) use the identical extraction solvent mixture, thus the extraction efficiency data generated within the metabolism studies for method 575/0 is also valid for all the other data generation methods. Hence, no further assessment of the newly developed data generation methods has been conducted as full suitability of the extraction solvent used had been confirmed for method 575/0 = L013/01.

The following recovery and repeatability criteria are required according to the OECD Guidance document on analytical methods (ENV/JM/MONO(2007)17), depending on the fortification levels:

≤1 µg/kg	50 - 120 ± 35%
>1 µg/kg ≤0.01 mg/kg	60 - 120 ± 30%
>0.01 mg/kg ≤0.1 mg/kg	70 - 120 ± 20%
>0.1 mg/kg ≤1.0 mg/kg	70 - 110 ± 15%
>1 mg/kg	70 - 110 ± 10%

Report:	CA 4.1.2/16 Lehmann A.,Mackenroth C., 2005 a Validation of the analytical method 575/0: Method for the determination of BAS 550 F (Dimethomorph) in plant matrices 2005/1026082
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), EEC 6/46, SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.1.2/17 Orozco A.G., 2017 a Amendment No. 1 - Validation of the analytical method 575/0: Method for the determination of BAS 550 F (Dimethomorph) in plant matrices 2017/1077907
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), EEC 6/46, SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

[see KCA 4.1.2/18 2017/1068700]

General Remark: Method 575/0 (L0013/01) is the current data generation method but was not validated for individual quantitation of the two isomers. Validation was based on the sum of *E*- and *Z*-isomers, although isomers have been separated during analysis. This method is superseded by the newly developed residue analytical method L0013 (DocID 2014/7000494). The latter allows the individual quantitation of both, the *E*- and *Z*-isomer.

Principle of the method Dimethomorph (BAS 550 F) is extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of dimethomorph is performed by HPLC-MS/MS. Analysis was accomplished using a Betasil C_{18} column and a methanol-pure water gradient with formic acid as modifier. Detection was accomplished in ESI+ mode at mass transitions 388 → 301 for quantitation and 388 → 165 for confirmation.

Recovery findings In all matrices tested, the mean recovery values was between 70% and 110%. The detailed results are given in the table below.

Table 4.1.2-23: Validation results of method 575/0: dimethomorph (BAS 550 F) in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph	Lettuce	0.01	5	76	79	1.7	2.6
		0.1	5	73	74	2.0	2.4
	Tomato	0.01	5	73	73	3.1	1.3
		0.1	5	73	73	4.8	4.4
	Pea	0.01	5	95	96	5.2	8.3
		0.1	5	93	94	5.8	4.9
	Onion	0.01	5	88	93	2.2	2.2
		0.1	5	77	77	5.5	5.6
	Potato	0.01	5	80	80	1.1	3.5
		0.1	5	79	79	2.7	2.1
	Grape	0.01	5	80	79	2.5	2.1
		0.1	5	78	77	1.0	1.0
	Hops	0.01	5	76	79	5.9	4.9
		0.1	5	79	79	1.3	1.8

Linearity	Good linearity was observed over the concentration range tested. Linear correlations with coefficients >0.99 were obtained for dimethomorph. Nine calibration points distributed over a concentration range of 0.025 to 10 ng/mL were used. Calibration standards were prepared in a mixture of methanol and pure water (50/50 v/v).
Specificity	LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required as two different, highly specific mass transitions are used for quantitation and qualification.
Matrix effects	~~Since the sensitivity of the analytes is influenced by matrix (peak enhancement), matrix matched standards (containing at least 90% matrix load) have to be used for quantitation. Only for the matrices peas and onion, the matrix effect is <20%.~~ Matrix effects have been assessed in each analytical sequence by analysis of matrix-matched standards. Recoveries show that matrix effects were ≤ 20% for all matrices analyzed (2017/1077907).
Interference	No significant interference were observed at elution times of the analytes of interest (interference <30% LOQ).
Limit of quantitation	The limit of quantitation, expressed as the sum of both isomers, was 0.01 mg/kg in all matrices.

Limit of detection The lowest calibration standard of 0.025 ng/mL was defined as limit of detection of the analytical method.

Repeatability The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. The detailed values are presented in Table 4.1.2-23.

Stability of solutions Dimethomorph was proven to be stable in extracts for a time period of 11 to 29 days, if stored under refrigerator conditions in the dark (see Table 4.1.2-24). The final volume is stable for a time period of 4 to 8 days, if stored under refrigerator conditions in the dark (see Table 4.1.2-25). Standards solutions were stable for 91 days stored under refrigerator conditions in the dark (see Table 4.1.2-26).

Table 4.1.2-24: Extract stability of dimethomorph (BAS 550 F) in plant matrices after 11 to 29 days of storage (refrigerated and dark)

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph	Lettuce	0.01	5	68	68	7.6	8.9
		0.1	5	73	73	0.6	1.2
	Tomato	0.01	5	70	69	6.8	5.7
		0.1	5	72	72	2.9	2.4
	Onion	0.01	5	84	89	1.5	1.9
		0.1	5	77	79	2.6	3.3
	Potato	0.01	5	79	79	2.4	1.8
		0.1	5	78	77	1.5	1.5
	Grape	0.01	5	77	78	2.1	1.6
		0.1	5	77	77	1.4	1.0
	Hops	0.01	5	79	79	4.0	4.2
		0.1	5	78	79	1.7	1.5

Table 4.1.2-25: Final volume stability of dimethomorph (BAS 550 F) in plant matrices after 4 to 8 days of storage (refrigerated and dark)

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph	Lettuce	0.01	5	67	69	8.0	8.9
		0.1	5	78	77	7.0	6.3
	Tomato	0.01	5	71	71	11.0	10.9
		0.1	5	79	77	4.9	5.1
	Onion	0.01	5	82	83	7.5	9.9
		0.1	5	79	79	3.4	3.8
	Potato	0.01	5	77	78	2.6	2.7
		0.1	5	78	78	1.8	2.3
	Grape	0.01	5	74	76	3.7	1.9
		0.1	5	76	76	1.9	1.4
	Hops	0.01	5	73	74	8.3	10.4
		0.1	5	77	77	0.9	1.0

Table 4.1.2-26: Standard stability of dimethomorph (BAS 550 F) after 91 days of storage (refrigerated and dark)

Test substance	Standard	Concentration Ng/ml	Average recovery (%)	
Transition			388 → 301	388 → 165
Dimethomorph	1	0.025	99	102
	2	0.05	75	83
	3	0.1	96	94
	4	0.25	108	105
	5	0.5	106	106
	6	1	129	126
	7	2.5	104	103
	8	5	107	105
	9	10	105	103
	10	100	109	106
	11	1000	118	114
	13	10000	110	104
	14	10000	117	112
	15	1000000	108	103

Conclusion: **Residue analytical method 575/0 (L0013/01) is fully suitable for the analysis of dimethomorph with a limit of quantitation of 0.01 mg/kg in crop commodities of high water (lettuce, tomatoes, onions), high starch (potatoes), high acid (grapes), and in difficult matrices (hops) using LC-MS/MS after an extraction of the plant material using mixture of methanol, water and hydrochloric acid.**

Report:	CA 4.1.2/19 Gooding R.,Andrews R.S., 2014 a Validation of BASF analytical method L0013: Method for the determination of the geometric isomers of BAS 550 F (Reg.No. 4110868, 4110869) in plant matrices at LOQ of 0.005 mg/kg using LC/MS/MS 2014/7000494
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by United States Environmental Protection Agency)

General Remark: Method L0013/02 is the updated data generation method allowing the separation and individual quantitation of the *E* and *Z*-isomer. The limit of quantitation of 0.005 mg/kg is related to each isomer. The method uses the same extraction solvent mixture as the data generation method 575/0 (L0013/01).

Principle of the method Residues of dimethomorph (*E*- and *Z*-isomers) are extracted from lentils, soya bean (seed and oil), orange, lettuce, barley (grain) and maize (stover) using an acidified methanol/water solution. After sample extraction and clean-up by partitioning against cyclohexane, residues are determined by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Analysis was accomplished using a Unison UK C_{18} column and a methanol-water gradient with formic acid as modifier. Detection was accomplished in ESI+ mode at mass transitions 388 → 301 for quantitation and 388 → 165 for confirmation.

Recovery findings In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.1.2-27: Validation results of method L0013: dimethomorph (BAS 550 F) isomers in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
E-dimethomorph	Lentil	0.005	5	83	109	10	6
		0.5	5	85	96	7	7
	Soya bean seed	0.005	5	94	97	7	17
		0.5	5	82	92	8	16[1]
	Barley	0.005	5	104	108	4	5
		0.5	5	88	98	8	7
	Lettuce	0.005	5	98	97	14	10
		0.5	5	85	87	7	5
	Orange	0.005	5	93	97	9	12
		0.5	5	90	90	11	18[2]
	Soya bean oil	0.005	5	86	93	3	4
		0.5	5	78	92	6	10
	Maize stover	0.005	5	75	78	15	23
		0.5	5	82	80	11	10
Z-dimethomorph	Lentil	0.005	5	90	100	5	6
		0.5	5	90	101	3	8
	Soya bean seed	0.005	5	86	97	11	16
		0.5	5	78	100	17	22[3]
	Barley	0.005	5	100	106	6	11
		0.5	5	93	96	6	8
	Lettuce	0.005	5	93	104	18	14
		0.5	5	91	89	4	14
	Orange	0.005	5	89	107	8	6
		0.5	5	92	99	13	15
	Soya bean oil	0.005	5	96	97	6	8
		0.5	5	85	81	7	11
	Maize stover	0.005	5	73	73	17	15
		0.5	5	81	82	13	14

1 Overall recovery over both fortification levels was 16%
2 Overall recovery over both fortification levels was 15%
3 Overall recovery over both fortification levels was 18%

Linearity

Good linearity was observed over a concentration range of 0.02 to 0.5 ng/mL and of 0.05 to 1 ng/mL (5 concentration levels) for dimethomorph (external reference standard) with correlation coefficients ≥ 0.99 for both transitions. Calibration standards were prepared in methanol.

Specificity	LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required as two different, highly specific mass transitions are used for quantitation and qualification.
Matrix effects	Matrix matched standards were compared to standard solutions. Matrix effects were below 20%, accordingly quantitation was accomplished using solvent-based standard solutions.
Interference	No significant interference were observed at elution times of the analytes of interest (interference <30% LOQ).
Limit of quantitation	The limit of quantitation was 0.005 mg/kg for each analyte in all matrices.
Limit of detection	The LOD set at 20% of the LOQ was 0.001 mg/kg for each analyte.
Repeatability	The relative standard deviations (RSD, %) for all commodities were <30% when fortified with 0.005 mg/kg and ≤15% when fortified with 0.5 mg/kg. Exceptions were at the confirmatory transition for soya bean seed at 0.5 mg/kg fortification level, where the RSD were 16% (*E*-isomer) and 22% (*Z*-isomer) and for orange at 0.5 mg/kg fortification, where the RSD of the *E*-isomer was 18%. The detailed values are shown in Table 4.1.2-27.
Reproducibility	No independent laboratory validation was conducted, as the methods purpose is pre-registration data generation.
Stability of solutions	The stability of solution was not assessed during the course of this study.
Conclusion	**Residue analytical method L0013/02 is considered suitable for the analysis and individual quantitation of the *E* and *Z*-isomers of dimethomorph in lentil, soya bean seed, barley, lettuce, orange, soybean oil, and maize stover with an LOQ of 0.005 mg/kg using LC-MS/MS.**

Report:	CA 4.1.2/20 Jooss S.,Tussetschlaeger S., 2015 a Validation of the BASF L0013/03 residue method for the determination of M550F002 (Reg.No. 4581886) and M550F007 (Reg.No. 4060805) in various plant matrices, using LC/MS/MS 2014/1186695
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340, OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

This method was developed to generate residue data for metabolite M550F002, which was found to be the most prevalent metabolite of dimethomorph in the new grape metabolism and confined rotational crop study, and for its aglycon M550F007. The method uses the same extraction solvent mixture as the data generation method 575/0 (L0013/01). The method was allocated a unique BASF residue method number, L0013/03.

Principle of the method Residues of M550F002 (Reg. No 4581886) and M550F007 (Reg. No 4060805) were extracted from lettuce, strawberry fruit, dried pea, oilseed rape seed and potato tuber using a mixture of methanol / water / 2N HCl (70/25/5). After sample extraction and liquid/liquid partition with dichloromethane the extracts were dried to dryness and redissolved in methanol / water (1/1, v/v). The final determination was performed by HPLC-MS/MS. Analysis was accomplished using a Waters Acquity BEH C_{18} column and a methanol-water gradient with formic acid as modifier. Detection was accomplished in ESI+ mode at mass transitions 536 → 374 (M550F002) and 374 → 287 (M550F007) for quantitation and 536 → 287 (M550F002) and 374 → 199 (M550F007) for confirmation.

Recovery findings In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.1.2-28: Validation results of method L0013/03: M550F002 in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				536 → 374	536 → 287	536 → 374	536 → 287
M500F002	Lettuce	0.01	5	72.5	73.1	7.1	7.9
		0.10	5	71.8	73.5	18	18
	Strawberry	0.01	5	75.7	71.4	9.2	12
		0.10	5	74.2	71.4	11	10
	Dried Pea	0.01	5	72.7	73.2	12	12
		0.10	5	74.6	76.0	11	13
	Oilseed Rape Seed	0.01	5	71.9	72.0	6.6	6.1
		0.10	5	70.8	70.3	5.1	3.4
	Potato Tuber	0.01	5	77.0	78.7	6.4	6.6
		0.10	5	79.3	80.1	5.3	5.5

Table 4.1.2-29: Validation results of method L0013/03: M550F007 in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				374 → 287	374 → 199	374 → 287	388 → 165
M500F007	Lettuce	0.01	5	88.5	87.2	4.6	3.8
		0.10	5	89.3	89.0	9.1	9.0
	Strawberry	0.01	5	83.6	84.9	3.0	3.0
		0.10	5	89.2	89.4	4.6	4.8
	Dried Pea	0.01	5	81.5	80.0	14	15
		0.10	5	96.3	94.8	8.6	8.6
	Oilseed Rape Seed	0.01	5	74.3	72.0	6.4	6.0
		0.10	5	76.7	75.5	5.4	5.3
	Potato Tuber	0.01	5	99.3	98.5	3.4	4.9
		0.10	5	101	101	4.1	4.8

Linearity

Good linearity was observed over a concentration range of 0.040 to 5.0 ng/mL for M550F002 and M550F007 (external reference standard) with correlation coefficients >0.997 for both transitions. Calibration standards were prepared in a mixture of methanol-water (50/50 v/v).

Specificity

LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required as two different, highly specific mass transitions are used for quantitation and qualification.

Matrix effects	Matrix matched standards were compared to standard solutions. Matrix effects were below 20% for lettuce, strawberry and potato tuber for both analytes and for dried pea for M550F002. Dried pea (for M550F002) and oilseed rape seed (both metabolites) showed a matrix effect >20%. Consequently, matrix matched standards were used for evaluation of the results in all matrices.
Interference	No significant interference were observed at elution times of the analytes of interest (interference <30% LOQ).
Limit of quantitation	The limit of quantitation (LOQ) defined by the lowest fortification level successfully tested was 0.010 mg/kg for each analyte.
Limit of detection	The LOD set at 20% of the LOQ was 0.002 mg/kg for each analyte.
Repeatability	The relative standard deviations (RSD, %) for all commodities and all fortification levels were ≤ 20% for both metabolites. The detailed values are shown in Table 4.1.2-28 and Table 4.1.2-29.
Reproducibility	No independent laboratory validation was conducted as the methods purpose is pre-registration data generation.
Standard stability	In this study, M550F002 and M550F007 were shown to be stable in methanol (stock solutions, fortification solutions) and in a methanol/water mixture (1/1 v/v) (calibration solutions) for up to 12 days, when stored refrigerated.
Extract stability	Sample extracts of M550F002 and M550F007 in methanol/water (1/1, v/v) were re-injected after at least 5 days of storage under refrigerated conditions. Sample extracts of lettuce, strawberry fruit, dried pea, oilseed rape seed and potato tuber were found to be stable.
Conclusion	**It could be demonstrated that the method L0013/03 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is therefore applicable and suitable to correctly determine residues of the dimethomorph metabolites M550F002 and M550F007 in plant matrices of high water content (lettuce), high acid (strawberries), dried commodities of high protein content (dried peas), high oil content (oilseed rape seed) and root and tuber vegetables of high starch content (potato tuber).**

Report:	CA 4.1.2/21 Richter S.,Schmiedt S., 2016 a Validation of BASF Method L0013/04 to be used for the determination of Morpholine (M550F021, Reg.No. 21322) in various crop matrices, using LC-MS/MS 2014/1187244
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

~~Remark:~~ ~~At time of submission, the methodology has been fully established but validation is still ongoing. The fully validated residue analytical method proposed for data generation purposes of morpholine in plant matrices will be provided by the applicant together with the data package ready for shipment in Q1/2016.~~

~~At the time of the current dossier update, the method validation for metabolite M550F021 has not yet been completed. Elevated blank values in untreated plant material used as control matrix has led to an unforeseen delay of the anticipated completion date of the validation study. The observed elevated blank values are anticipated to have its origin in the widespread use of this chemical structure (morpholine) in industrial products. As a consequence of this unforeseen delay, the final study report is no available for the current dossier update. The data is available in September (Q3-early Q4 2016) to be presented to the Rapporteur Member State.~~

This method was developed to generate residue data for metabolite M550F021 (morpholine). The method was allocated the unique BASF residue method number L0013/04.

Principle of the method	Residues of morpholine (M550F021, Reg. No 21322) were extracted from homogenized plant material with acidified methanol. Prior to extraction, the water content of the plant material was adjusted and internal standard was added. Plant matrices investigated were adjusted samples of lettuce, strawberry fruit, dried pea, oilseed rape seed and potato tuber. The extract was filtrated and the filtrate further diluted prior to analysis by HPLC-MS/Mt. Analysis was accomplished using a Thermo Acclaim Trinity P1 column and an acetonitrile-water gradient with 50 mM ammonium formate (pH4) as modifier. Detection was accomplished in ESI+ mode at mass transitions *m/z* 88 → 70 for quantitation and *m/z* 88 → 44 for confirmation. As mass transition of the internal standard morpholine-d8 *m/z* 96 → 78 was used.

Recovery findings In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.1.2-30: Validation results of method L0013/04: Morpholine in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Mass transition *m/z*				88 → 70	88 → 44	88 → 70	88 → 44
M500F021 (morpholine)	Lettuce	0.02	5	110	106	1.8	7.0
		0.20	5	93.7	95.7	3.7	2.0
	Strawberry	0.02	5	90.6	98.7	4.9	7.6
		0.20	5	103	106	2.2	2.4
	Potato Tuber	0.02	5	99.9	102	5.6	9.1
		0.20	5	101	102	1.9	1.7
	Oilseed Rape Seed	0.02	5	99.6	96.3	7.6	12
		0.20	5	103	101	3.3	4.6
	Dried Pea	0.02	5	102	96.9	7.3	10
		0.20	5	104	104	4.6	5.7

Linearity Good linearity ($r > 0.99$) was observed using 8 standards in the range of 0.050 ng/mL to 7.0 ng/mL corresponding to 0.004 mg/kg to 0.56 mg/kg for the two mass transitions of morpholine. Calibration standards were prepared in a mixture of methanol and water (50/50 v/v) containing an internal standard.

Specificity LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required.

Matrix effects The matrix effect was found to be significant (suppression $\leq \pm 20$ %) for all matrices except of dried peas. Nevertheless, internal calibration standards in solvent were used for the evaluation of the results, since the internal standard compensates these matrix effects.

Interference The interferences/residues of the analytes measured at the retention time in the control samples were below 30 % of the limit of quantitation (LOQ) for each matrix and each mass transition.

Limit of quantitation The limit of quantitation (LOQ) defined by the lowest fortification level successfully tested was 0.020 mg/kg, corresponding to a concentration in the extract of 0.25 ng/mL.

Limit of detection The LOD is set at 20% of the LOQ, hence equivalent to 0.004 mg/kg, corresponding to a concentration in the extract of 0.050 ng/mL.

Repeatability	The relative standard deviation (RSD, %) for all commodities analysed and all fortification levels was ≤ 10%. Detailed values are shown in Table 4.1.2-30.
Reproducibility	No independent laboratory validation was conducted as the methods purpose is data generation for pre-registration purposes.
Standard stability	Morpholine indicated sufficient stability (less than 10% difference) in stock and fortification solutions, both prepared in methanol, for 46 days, as well as in calibration solutions prepared in a mixture of methanol and water (50/50, v/v) for at least 11 days when stored refrigerated in the dark.
Extract stability	The final sample extracts in methanol/water (50/50, v/v) were re-injected after 12 days of storage under refrigerated conditions. For strawberry and the confirmation transition of dried peas recoveries at LOQ level were slightly below 80% (74 to 78% of initial value) although an internal standard was used. Thus it is recommended to inject final extracts of strawberry and dried peas as soon as possible after their preparation. No significant decrease (84 to 107 % of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared internal calibration solutions in solvent for all other matrices and transitions. Stability of final extracts of lettuce, potato tuber and oilseed rape seed is considered sufficiently proven for 12 days under refrigerated storage conditions.
Conclusion	**It could be demonstrated that the method L0013/04 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is therefore applicable and suitable to correctly determine residues of morpholine in plant matrices of high water content (lettuce), high acid (strawberries), dried commodities of high protein content (dried peas), high oil content (oilseed rape seed) and root and tuber vegetables of high starch content (potato tuber).**

Report:	CA 4.1.2/22 Stewart J., 2002 a Independent Method Validation of BASF Analytical Method M3463 entitled BAS 550 F (dimethomorph): LC/MS/MS method for the determination of BAS 550 F in canola seed and sunflower seed 2002/5002648
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 4.1.2/23 Dacunha A., 2002 a BAS 550 F (Dimethomorph): Laboratory validation of LC/MS/MS method M 3463.02 for the determination of BAS 550 F (CL 336379) residues in canola seed and sunflower seed 2002/7014914
Guidelines:	SANCO/825/00 rev. 6 (20 June 2000), EPA 860.1340 (1996)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method	Residues of dimethomorph are extracted from the samples with acetone and purified by liquid/liquid partition. Then, the extracts are subjected to cleanup with C18 solid phase extraction followed by silica solid phased extraction. The measurements of dimethomorph are accomplished by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using positive ion electrospray ionization.
	The chromatography was performed using a Xterra MS C18 column at a flow rate of 0.25 mL/min. As mobile phase a water-methanol gradient with 1% acetic acid as modifier was used. Quantification was accomplished with mass transition 388→301 for dimethomorph. The results were calculated by direct comparison of peak areas to an external calibration curve. The results of Z- and E-isomers of dimethomorph were summed for quantification.
Recovery findings	The recoveries of dimethomorph in canola and sunflower seed are summarized in Table 4.1.2-31. The mean recoveries were within the valid range between 77% and 91% after fortification with 0.05 mg/kg (LOQ) and 5.0 mg/kg (100x LOQ) dimethomorph.

Table 4.1.2-31: Recovery results of dimethomorph in canola seed and sunflower seed

Matrix	Fortification level [mg/kg]	n	Recovery [%]	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
Canola seed	0.05	5	71, 82, 84, 77, 70	76.8	8.2	77.4	8.7
	5.0	5	88, 78, 83, 69, 72	78.0	10		
Sunflower seed	0.05	5	83, 79, 88, 84, 89	84.6	4.8	90.9	11
	5.0	5	106, 102, 106, 83, 89	97.2	11		

RSD = Relative standard deviation

Linearity Good linearity was observed for the calibration range tested with correlation coefficients $r \geq 0.999$. Four calibration points distributed over a concentration range of 0.5 ng/mL to 8.0 ng/mL were used. Calibration standards were prepared in methanol/water (1/1, v/v).

Specificity The method allows the determination of dimethomorph using LC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required.

Matrix effects As no interferences at the elution times of the analytes of interest (E- and Z-isomers) were observed, no adverse effects of any matrix occurred.

Interference No residues above the limit of detection were detected in the control samples.

Standard stability Standard solutions of dimethomorph in methanol and methanol/water (1/1, v/v) were stable for 1 month when stored refrigerated (RES 02-001).

Limit of quantification The limit of quantification (LOQ) of the method, defined by the lowest fortification level, is 0.05 mg/kg.

Limit of detection The limit of detection (LOD) of the method is 0.01 mg/kg.

Repeatability The relative standard deviations (RSD, %) with respect to recoveries following fortifications at two different fortification levels were ≤ 11% for dimethomorph.

Reproducibility Reproducibility of the method was determined within the study BASF DocID 2002/5002648 (CA 4.1.2/21).

Conclusion: **The described analytical method M3463 is considered suitable for the quantitative analysis of dimethomorph in canola and sunflower seed.**

Report:	CA 4.1.2/24 Stewart J., 2002 b Independent method validation of BASF analytical method M3502 entitled: BAS 550 F (Dimethomorph): LC/MS/MS method for the determination of BAS 550 F in broccoli, celery, spinach, and wheat (grain, hay, straw, forage) 2002/5002982
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method	Residues of dimethomorph are extracted from the samples with acetone. Then, the extracts are subjected to cleanup with silica solid phase extraction followed by C18 solid phase extraction. The measurements of dimethomorph are accomplished by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using positive ion electrospray ionization. The chromatography was performed using a Xterra MS C18 column at a flow rate of 0.25 mL/min. As mobile phase a water-methanol gradient with 1% acetic acid as modifier was used. Quantification was accomplished with mass transition 388→301 for dimethomorph. The results were calculated by direct comparison of peak areas to an external calibration curve. The results of Z- and E-isomers of dimethomorph were summed for quantification.
Recovery findings	The recoveries of dimethomorph in broccoli, spinach, wheat grain and wheat straw are summarized in Table 4.1.2-16. The mean recoveries were within the valid range between 87% and 104% after fortification with 0.05 mg/kg (LOQ) and 5.0 mg/kg (100x LOQ) dimethomorph.

Table 4.1.2-32: Recovery results of dimethomorph in broccoli, spinach, wheat grain and wheat straw

Matrix	Fortification level [mg/kg]	n	Recovery [%]	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
Broccoli	0.05	5	94, 101, 102, 103, 103	101	4	100	4
	5.0	5	95, 96, 101, 103, 105	100	4		
Spinach	0.05	5	98, 98, 102, 104, 104	101	3	99	5
	5.0	5	89, 93, 95, 103, 104	97	6		
Wheat grain	0.05	5	100, 101, 104, 107, 108	104	3	102	6
	5.0	5	90, 95, 100, 104, 107	99	7		
Wheat straw	0.05	5	88, 89, 91, 91, 93	90	2	89	4
	5.0	5	82, 86, 87, 87, 94	87	5		

RSD = Relative standard deviation

Linearity	Good linearity was observed for the calibration range tested with correlation coefficients r ≥ 0.998. Four calibration points distributed over a concentration range of 0.05 ng/mL to 0.8 ng/mL were used. Calibration standards were prepared in methanol/water (1/1, v/v).
Specificity	The method allows the determination of dimethomorph using LC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required.
Matrix effects	As no interferences at the elution times of the analytes of interest (E- and Z-isomers) were observed, no adverse effects of any matrix occurred.
Interference	No residues above the limit of detection were detected in the control samples.
Standard stability	Standard solutions of dimethomorph in methanol and methanol/water (1/1, v/v) were stable for 1 month when stored refrigerated.
Limit of quantification	The limit of quantification (LOQ) of the method, defined by the lowest fortification level, is 0.05 mg/kg.
Limit of detection	The limit of detection (LOD) of the method is 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) with respect to recoveries following fortifications at two different fortification levels were ≤ 7% for dimethomorph.
Reproducibility	Reproducibility of the method was not determined within the study.
Conclusion:	**The described analytical method M3502 is considered suitable for the quantitative analysis of dimethomorph in broccoli, spinach, wheat grain and wheat straw.**

Report:	CA 4.1.2/25 Mirbach M.J.,Huber H.P., 1991 b Confirmatory validation of an analytical method for the determination of residues of Dimethomorph in plant material (FAMS 002-02) DK-244-008
Guidelines:	EPA 171-4, US EPA PR Notrice 88-5 Tolerance Enforcement Methods Independent Laboratory Confirmation, EPA Subdivision O of the Pesticide Assessment Guidelines
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Report:	CA 4.1.2/26 Mirbach M.J., 1991 a First amendment to report No. 308722: Confirmatory validation of an analytical method for the determination of the residues of Dimethomorph in plant material (FAMS 002-02) DK-244-009
Guidelines:	EPA 171-4, US EPA PR Notrice 88-5 Tolerance Enforcement Methods Independent Laboratory Confirmation, EPA Subdivision O of the Pesticide Assessment Guidelines
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Report:	CA 4.1.2/27 Eichler D., 1989 a Method for the determination of residues of Dimethomorph (CME 151) in potatoes, tomatoes, grapes (including grape waste material, raisins, fruit juice, wine) DK-244-002
Guidelines:	none
GLP:	no

Remark: This document is a non-GLP technical procedure describing the technical details of the analytical method.

Report:	CA 4.1.2/28 Weitzel R., 1995 a Dimethomorph CL 183 776, CL 336 379: Method for the determination of residues of active ingredient in potatoes, tomatoes and grapes (including grape waste material, raisins, fruit juice, wine) DK-244-013
Guidelines:	none
GLP:	no
	Remark: This document contains additional information to the analytical procedure FAMS 002-02.

Report:	CA 4.1.2/29 Weitzel R., 1995 b Dimethomorph (CL 183 776, CL 336 379): Method for the determination of residues of Dimethomorph in potatoes, tomatoes and grapes (including grape waste material, raisins, fruit juice, wine) DK-244-015
Guidelines:	none
GLP:	no
	Remark: This document contains additional information to the analytical procedure FAMS 002-02 and FAMS 002-003.

Remark: The analytical method FAMS 002-02 can be used for the determination of dimethomorph in potatoes, tomatoes, grapes and its processing products. The matrices grapes and grape juice were selected for the confirmatory validation experiments, as they were thought to be two of the most difficult matrices.

Principle of the method	Residues of dimethomorph are extracted from the homogenized sample with acetone (grapes) or dichloromethane using an Extrelut cartridge (grape juice). For the analysis, a partition in dichloromethane is followed. Then, after evaporation of the solvent, the residues are taken up in methanol and further purified by gel chromatography followed by silica gel chromatography.
	The measurements of dimethomorph are accomplished by liquid chromatography with UV-detection at 240 nm. The chromatography is performed using a Superspher 100-RP18 column at a flow rate of 1.0 mL/min. As mobile phase a solvent mixture of acetonitrile/methanol/water (40/20/40, v/v/v) was used.

Recovery findings	The recoveries of dimethomorph in grapes and grape juice are summarized in Table 4.1.2-16. The overall recoveries were within the valid range amounting to 101.8% and 107.4% after fortification with 0.05 mg/kg (LOQ) and 0.25 mg/kg (5x LOQ) dimethomorph.

Table 4.1.2-33: Recovery results of dimethomorph in grapes and grape juice

Matrix	Fortification level [mg/kg]	n	Recovery [%]	Mean recovery [%]	RSD [%]
Grapes	0.05	2	99.8, 103.9	107.4	6.4
	0.25	2	111.0, 115.0		
Grape juice	0.05	2	98.4, 100.1	101.8	3.0
	0.25	2	103.8, 104.9		

RSD = Relative standard deviation

Linearity	The correlation coefficient (R^2) of all calibration curves was ≥ 0.999. Five calibration points distributed over a concentration range from 0.1 µg/mL to 2.0 µg/mL were used. Calibration standards were prepared in methanol.
Specificity	The UV-wavelength chosen is specific for dimethomorph. The identification and quantification of the analyte was based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in grapes and grape juice.
Matrix effects	As no interference at the elution time of the analyte of interest was observed, no adverse effects of any matrix occurred.
Interference	No residues above the limit of detection were detected in untreated control samples.
Stability of solutions	The substance solutions were stored refrigerated and were stable over a period of 5 days.
Limit of quantification	The limit of quantification (LOQ) of the method, defined by the lowest fortification level, is 0.05 mg/kg.
Limit of detection	The limit of detection (LOD) of the method is 0.02 mg/kg, corresponding to the lowest calibration concentration of 0.1 µg/mL.
Repeatability	The relative standard deviations (RSD, %) with respect to recoveries following fortifications at three different fortification levels were < 7% for dimethomorph.
Reproducibility	Reproducibility of the method was not determined within the study.
Conclusion:	**The described analytical method FAMS 002-02 is considered suitable for the quantitative analysis of dimethomorph in grapes and grape juice.**

Report:	CA 4.1.2/30 Weitzel R., 1997 a Dimethomorph (CL 336379): Validation of analytical method FAMS 076-01 for the determination of active ingredient in raisins and grape waste material (Germany, 1996) DK-244-019
Guidelines:	EEC 91/414, BBA I 1-2, DFG Method Series for Pesticide Residue Analysis V and VIII (1991), IVA-Leitlinie Rueckstandsversuche Teil I (1992)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz, Mainz, Germany)

Report:	CA 4.1.2/31 Weitzel R., 1996 a Dimethomorph (CL 336,379): Method for the determination of the active ingredient in raisins and grape waste material DK-123-235
Guidelines:	EEC 91/414,BBA I 1-2,DFG Method Series for Pesticide Residue Analysis V and VIII (1991),IVA-Leitlinie Rueckstandsversuche Teil I (1992)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz, Mainz, Germany)

Principle of the method	Residues of dimethomorph are extracted from the homogenized matrix with acetone/water (raisins) or acetone (grape waste material) followed by liquid/liquid partition with dichloromethane. Clean-up is carried out by chromatography using a Florisil column and subsequent gel permeation chromatography (GPC). The measurements of dimethomorph are accomplished by liquid chromatography with UV-detection at 240 nm. The chromatography was performed using a Nucleosil 100-phenyl column at a flow rate of 1.0 mL/min. As mobile phase a solvent mixture of acetonitrile/water (60/40, v/v) was used.
Recovery findings	The recoveries of dimethomorph in raisins and grape waste material are summarized in Table 4.1.2-16. The overall recoveries were within the valid range amounting to 94% and 97% after fortification with 0.02 mg/kg (LOQ), 0.2 mg/kg (10x LOQ) and 2.0 mg/kg (100x LOQ) dimethomorph.

Table 4.1.2-34: Recovery results of dimethomorph in raisins and grape waste material

Matrix	Fortification level [mg/kg]	n	Recovery [%]	Overall recovery [%]	RSD [%]
Raisins	0.02	2	100, 97	94	4.4
	0.2	2	96, 93		
	2.0	2	89, 91		
Grape waste material	0.02	2	94, 97	97	4.4
	0.2	2	105, 94		
	2.0	2	95, 94		

RSD = Relative standard deviation

Linearity The correlation coefficient (r^2) of all calibration curves was ≥ 0.999. Five calibration points distributed over a concentration range of 0.1 µg/mL to 2.0 µg/mL were used. Calibration standards were prepared in methanol.

Specificity The UV-wavelength chosen is specific for dimethomorph. The identification and quantification of the analyte was based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in raisins and grape waste material.

Matrix effects As no interference at the elution time of the analyte of interest was observed, no adverse effects of any matrix occurred.

Interference Blank signals or unspecific interferences in untreated control samples were not detected.

Stability of solutions The substance solutions were store refrigerated at 6°C ± 4°C and were stable over a period of at least 2 month.

Limit of quantification The limit of quantification (LOQ) of the method, defined by the lowest fortification level, is 0.02 mg/kg.

Limit of detection The limit of detection (LOD) of the method is 0.002 mg/kg.

Repeatability The relative standard deviations (RSD, %) with respect to recoveries following fortifications at three different fortification levels were < 5% for dimethomorph.

Reproducibility Reproducibility of the method was not determined within the study.

Conclusion: **The described analytical method FAMS 076-01 is considered suitable for the quantitative analysis of dimethomorph in raisins and grape waste material.**

Report:	CA 4.1.2/32 Roland L., 1993 a Dosage de residus de Dimethomorph dans des fraises (Essai: 1991-1992 - Opzoekingsstation van Gorsem) DK-713-039
Guidelines:	none
GLP:	no

Report:	CA 4.1.2/33 Roland L., 1994 a Residus de Dimethomorph sur fraises - Essai 1993-1994 - Melsele DK-713-040
Guidelines:	none
GLP:	no

Report:	CA 4.1.2/34 Roland L., 1994 b Residus de Dimethomorph sur fraises - Essai 1993-1994 - Zwijndrecht DK-713-041
Guidelines:	none
GLP:	no

Report:	CA 4.1.2/35 Roland L., 1994 c Residus de Dimethomorph sur fraises - Essai 1993-1994 - Stekene DK-713-042
Guidelines:	none
GLP:	no

Principle of the method	Residues of dimethomorph are extracted from the sample material with a mixture of dichloromethane/acetone (50/50, v/v), followed by the addition of water and saturated sodium chloride and subsequent liquid/liquid partitioning. The organic extracts are evaporated to dryness and re-dissolved in a mixture on hexane/acetone (90/10, v/v). Those extracts are further purified over silica gel columns.
	The measurements of dimethomorph are accomplished by liquid chromatography with UV-detection at 240 nm. The chromatography is performed using a Nucleosil C18 column at a flow rate of 1.0 mL/min. As mobile phase a solvent mixture of acetonitrile/0.1% phosphoric acid (35/65, v/v) is used.

Recovery findings	The recoveries of dimethomorph in strawberries are summarized in Table 4.1.2-16. The recoveries were in an acceptable range > 90% after fortification with 0.01 mg/kg (LOQ), 0.02mg/kg (2x LOQ) and 0.05 mg/kg (5x LOQ) dimethomorph.

Table 4.1.2-35: Recovery results of dimethomorph in strawberries

DocID	Concentration level [mg/kg]	Recovery [%]
DK-713-039	0.01	> 90
	0.02	
	0.05	
DK-713-040	0.01	> 90
	0.05	
DK-713-041	0.01	> 90
	0.05	
DK-713-042	0.01	> 90
	0.05	

Linearity	Good linearity was observed for the calibration range of 0.1 µg/mL to 2.0 µg/mL (DK-713-039 = 4.0 µg/mL) with correlation coefficients $r \geq 0.999$.
Specificity	The UV-wavelength chosen is specific for dimethomorph. The identification and quantification of the analyte was based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph in strawberries.
Interference	No residues above the limit of quantification of 0.01 mg/kg were detected.
Limit of quantification	The limit of quantification (LOQ) of the method is 0.01 mg/kg.
Limit of detection	Good detectability is achieved at a signal to noise ratio of 3:1, which is defined as the limit of detection (LOD).
Reproducibility	Reproducibility was not tested.
Conclusion:	**The described SOP MR 029 is considered suitable for the quantitative analysis of dimethomorph in strawberries.**

Report:	CA 4.1.2/36 Weitzel R., 1999 a Dimethomorph (CL 336379): Validation of method FAMS 098-02 for the determination of residues of the active ingredient in oil seed rape (Germany, 1998)
	DK-244-025
Guidelines:	BBA I 1-2, IVA-Leitlinie Rueckstandsversuche Teil I (1992), DFG Method Series for Pesticide Residue Analysis V and VIII (1991), EEC 91/414
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Principle of the method	The residues are extracted from the oilseed rape seeds with acetonitrile. This is followed by liquid-liquid partition with n-hexane to remove fatty impurities. Then, the extract is subjected to further cleanup by gel permeation chromatography followed by silica gel column chromatography. The collected main fraction was evaporated to dryness, reconstituted with cyclohexanone and subjected to GC-MSD equipped with an fused silica capillary column, liquid phase: methyl silicone (film thickness 0.33 µm, 12.5 m 0.2 mm i.d.) monitoring 2 ions 387.1 and 389.1 (electron impact ionization). Helium (0.85 mL/min) was used as carrier gas.
Recovery findings	The recoveries of dimethomorph in oilseed rape seeds are summarized in the table below. The mean recoveries were within the valid range from 92% to 109% after fortification with 0.01 mg/kg (LOQ), 0.02 mg/kg (2x LOQ) and 0.20 mg/kg (20x LOQ) dimethomorph.

Table 4.1.2-36: Validation results of method FAMS 098-02 for dimethomorph in oilseed rape seeds

Matrix	Fortification level [mg/kg]	n	Mean recovery [%]	RSD [%]	Overall mean recovery [%]	Overall RSD [%]
Oilseed rape seeds	0.01	5	109	18	100	13
	0.02	5	92	6.3		
	0.20	5	100	0.9		

RSD = Relative standard deviation

Linearity	The evaluation is carried out using separate calibration curves for the E-isomer and the Z-isomer. Five calibration standards in the range of 0.05 to 1 µg/mL were used, resulting in correlation coefficients (r^2) of > 0.999. Calibration standards were prepared in cyclohexanone.

Specificity	The method allows the determination of dimethomorph using GC-MSD. The identification and quantification of the analytes (E- and Z-isomers) was based on the selected ions and the retention times. Under the described conditions the method is specific for the determination of dimethomorph in oilseed rape seeds.
Interference	Blank signals or unspecific interferences in untreated control samples were not detected.
Matrix effect	As no interferences at the elution times of the analytes of interest (E- and Z-isomers) were observed, no adverse effects of any matrix occurred.
Limit of quantitation	The limit of quantitation, defined by the lowest fortification level successfully tested was 0.01 mg/kg for dimethomorph in oilseed rape seeds.
Limit of detection	The lowest calibration standard of 0.05 µg/mL was defined as limit of detection of the analytical method.
Repeatability	The relative standard deviation (RSD, %) for all fortification levels were below 20%.
Reproducibility	Reproducibility of the method was not determined within this validation.
Standard stability	No stability investigations were performed.
Conclusion	**The GC-MSD method FAMS 098-02 can be used for the analysis of dimethomorph in oilseed rape seeds.**

Report:	CA 4.1.2/37 Zeng M., 1997 a CL 336379 (Dimethomorph): Independent laboratory validation of GC and GC/MS confirmatory method M 2577 for the determination of CL 336379 residues in tomato fruit and various tomato processing comodities (tomato dry pomace, wet pomace, puree, paste, catsup and juice)
	DK-244-022
Guidelines:	EPA PR Notice 96-1
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 4.1.2/38 Zeng M., 1998 a CL 336379 (Dimethomorph): Independent laboratory validation of GC and GC/MS confirmatory method M 2577 for the determination of CL 336379 residues in tomato fruit and various tomato processing comodities (tomato dry pomace, wet pomace, puree, paste, catsup and juice)
	DK-123-225
Guidelines:	EPA PR Notice 96-1
GLP:	no

Principle of the method	Analytical method M 2577 was developed for the determination and confirmation of dimethomorph (CL 336379) residues in plant matrices. Residues of dimethomorph are extracted from samples with acetone and purified using solid phase extraction techniques. Quantitation of dimethomorph residues is accomplished by fused silica capillary gas chromatography (Rtx-1 column) equipped with a nitrogen-phosphorous detector. Helium was used as carrier gas. Results are calculated as dimethomorph by the direct comparison of peak heights to those of external standards. Residues of dimethomorph are confirmed using GC/MS (DB-5 column, m/z = 387→301).
Recovery findings	The method was found to be satisfactory for the determination of residues of dimethomorph in tomato commodities. Fortification levels 0.05, 0.1 and 0.5 mg/kg except for tomato juice with fortification levels of 0.01, 0.02 and 0.05 mg/kg.
	In the storage stability study DK-723-040 concurrent recoveries at a fortification level of 0.25 mg/kg were analyzed. Although not a full set of recoveries can be provided, the overall mean recovery values between 88.6% and 99.5% of dimethomorph with a relative standard deviation of < 20% (see table below) show that the method is fit for purpose.

Table 4.1.2-37: Recovery results of dimethomorph in tomato fruit and processed tomato commodities

Matrix	Fortification level [mg/kg]	Recovery [%]	Overall mean recovery [%]	Relative standard deviation [%]
Tomato fruit	0.05	64, 85	88.6	14
	0.1	102, 104		
	0.25 [a]	80, 98, 97, 84		
	0.5	84, 78		
Tomato wet pomace	0.05	76, 86	94.8	19
	0.1	130, 95		
	0.5	93, 89		
Tomato juice	0.01	88, 117	90.9	15
	0.02	99, 100		
	0.25 [a]	80, 88		
	0.05	74, 81		
Tomato puree	0.05	93, 97	96.5	10
	0.1	105, 95		
	0.25 [a]	106, 95		
	0.5	76, 105		
Tomato paste	0.05	79, 68	88.9	17
	0.1	95, 100		
	0.25 [a]	107, 106		
	0.5	84, 72		
Tomato catsup	0.05	106, 105	99.5	9.3
	0.1	82, 101		
	0.5	106, 97		
Tomato dry pomace	0.05	110, 98	92.1	15
	0.1	66, 80		
	0.25 [a]	92, 90		
	0.5	96, 105		

[a] Concurrent recoveries from storage stability study DK-723-040 (Babbitt B., 1998)

Linearity Linearity was tested using at least 4 different standard concentrations in the range of 0.05 to 0.4 µg/mL. Standards used for calibration were prepared in ethyl acetate.

Specificity Eight extracts of processed tomato commodities (four control samples, three control samples of tomato fruit, tomato dry pomace and tomato paste fortified at 0.05 mg/kg, respectively and one control sample of tomato juice fortified at 0.01 mg/kg) were analyzed by GC/MS. The results confirm that the method is suitable for the specific determination of dimethomorph residues and that GC-NPD yield. The mass spectra obtained unequivocally confirm the presence of the analyte of interest in the fortified samples and absence of the analyte in the untreated control sample.

Matrix effects	As the untreated control sample did not show any interference, absence of matrix effects can be concluded. In addition, the extensive sample clean-up was applied to eliminate any matrix effects. No matrix effect was investigated.
Interference	No interference > 30% of the LOQ was observed in the untreated control samples at the retention time of interest.
Limit of quantitation	The limit of quantitation of the method, defined by the lowest fortification level is 0.05 mg/kg for tomato commodities except for tomato juice with 0.01 mg/kg.
Limit of detection	The lowest calibration standard of 0.05 µg/mL was defined as limit of detection of the analytical method.
Repeatability	The relative standard deviation (RSD, %) for all fortification levels were below 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Standard stability	No stability investigations were performed.
Conclusion	**GC method M 2577 for the determination and confirmation of dimethomorph (CL 336379) residues in tomato commodities uses fused silica capillary gas chromatography equipped with a nitrogen-phosphorous detector for final determination with a limit of quantitation of 0.05 mg/kg for tomato commodities except for tomato juice with 0.01 mg/kg. As confirmatory technique GC-MS was applied.** **The method was found to be satisfactory for the determination of residues of dimethomorph in tomato fruit and processed tomato commodities.**

Animal matrices

Analytical methods for the determination of dimethomorph residues in animal matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in the table below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2004). No differentiation has been made with regards to data generation or enforcement methods as both are summarized in Table 4.1.2-38. All analytical methods on which the evaluation of the inclusion in Annex I was based on are listed as peer-reviewed. All other methods were submitted for evaluation for Annex I inclusion, but were not peer-reviewed.

Table 4.1.2-38: Summary of already peer-reviewed analytical methods for determination of dimethomorph residues in animal matrices

Method. No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	EU reviewed
./.	DK-240-004	Muscle, fat, milk, egg	GC-NPD	Dimethomorph	0.02 mg/kg	1999	Submitted as additional method, but not peer-reviewed
./.	DK-244-004	Muscle, fat, milk, egg	GC-NPD, GC-MS	Dimethomorph	0.01 mg/kg	1999	Yes
./.	DK-249-004	Milk, egg	GC-MS	Dimethomorph	0.01 mg/kg	1999	Yes
./.	DK-249-005	Milk, meat, fat	GC-NPD	Dimethomorph	0.01 mg/kg	1999	Yes
FAMS 024-01	DK-245-011 & DK-245-008	Milk and milk products	HPLC-UV	Dimethomorph; Z67, Z69, CUR7117	0.01 and 0.02 mg/kg	1991	Used as data generation method in livestock feeding study
FAMS 023-01	DK-245-010	Muscle, kidney, liver	GC-NPD	Dimethomorph; Z67, Z69	0.05 mg/kg	1991	Submitted as additional method, but not peer-reviewed

Table 4.1.2-39: Summary of newly submitted analytical methods for determination of dimethomorph residues in animal matrices

Method. No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	Comment
L0326/01 (FAMS 023-01)	2015/1000643	Muscle, kidney, liver, fat	GC-MS	Dimethomorph, Z67, Z69	0.1 mg/kg	2015	New validation of FAMS 023-014 according to the most recent guidelines
FAMS 023-01	DK-245-013	Liver, kidney	TLC, GC (^{14}C)	Dimethomorph, Z67, Z69	./.	1993	Assessment of extraction efficiency comparing acetonitrile extraction with metabolism scheme (methanol)
L0138/01	2009/1051339	Egg, fat, kidney, liver, milk, muscle	LC-MS/MS	Dimethomorph	0.01 mg/kg	2009	Proposed enforcement method
ILV to L0138/01	2009/1078429	Egg, fat, kidney, liver, milk, muscle	LC-MS/MS	Dimethomorph	0.01 mg/kg	2009	ILV to enforcement method

Although the analytical method FAMS 024-01 was already submitted in the previous dossier in the context of the inclusion in Annex I of Directive 91/414/EEC, this method is described again in detail for the reviewer's convenience, as the data generated in the livestock feeding study (DK 705-007) will be relied upon in this evaluation. The data generation method FAMS 023-01 (DK 245-010) was not considered as fully valid in the last review (DAR 2004, Volume 3 B5), as it did not fulfill the requirements for residue analytical methods, if an MRL for food of animal would be established. Reason for this was, that only two determinations per fortification level were prepared, instead of the required five replicates per level. The analytical method FAMS 023-01 has been re-validated again, however not using GC-NPD, but highly sensitive and selective MS-detection to comply with the most recent guidelines. The new validation confirms that the methodology used is fully suitable for data generation purposes in animal matrices.

General remarks to extraction efficiency – animal matrices:

Extraction efficiency of data generation method FAMS023-01 using acetonitrile as extraction solvent compared to the already peer-reviewed metabolism studies using methanol as extraction solvent confirmed comparability of acetonitrile and methanol as extraction solvents. A detailed description of this assessment is given in M-CA 4.1.2-15. Furthermore, the newly submitted metabolism studies in goat and poultry apply acetonitrile as extraction liquid for exhaustive extraction of residues.

For the newly developed and submitted residue analytical methods, extraction efficiency was not addressed in a separate study as in the proposed analytical method, extraction was accomplished using the same extraction solvent, methanol, as in the metabolism studies. Already peer-reviewed metabolism studies (DK-440-005 and DK440-003) in goat and hen used methanol and aqueous solutions of such, as extraction solvent, similar to the proposed method for enforcement and monitoring purposes (M-CA 4.2/6, DocID 2009/1051339). Consequently, no further assessment was required as suitability of the extraction solvent methanol has been confirmed by satisfactory removal of residue during the metabolism studies.

The following recovery and repeatability criteria are required according to the OECD Guidance document on analytical methods (ENV/JM/MONO(2007)17), depending on the fortification levels:

≤1 µg/kg	50 - 120 ± 35%
>1 µg/kg ≤0.01 mg/kg	60 - 120 ± 30%
>0.01 mg/kg ≤0.1 mg/kg	70 - 120 ± 20%
>0.1 mg/kg ≤1.0 mg/kg	70 - 110 ± 15%
>1 mg/kg	70 - 110 ± 10%

Report: CA 4.1.2/39
Bending P., 2015 a
Validation of the BASF analytical method L0326/01 for the determination of Dimethomorph (BAS 550 F) and its metabolites Reg.No. 4060805 and Reg.No. 4060806 in animal matrices
2015/1000643

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Remark: Residue analytical methods FAMS 023-01 was used for analysis of samples originating from

Principle of the method

Method L0326/01 / FAMS 023-01

The analytical approach was adapted from Shell Forschung method FAMS 023-01. However, the extraction and clean-up method was not changed. Only the GPC collection window was adapted to the instrumentation used in this study. The method extracts animal matrices (30 g) with acetonitrile (100 mL) using an Ultra-Turrax. Further clean-up was performed as follows:
After centrifugation the extract was decanted and washed by partitioning with n-hexane. The washed acetonitrile layer was added with water and aqueous sodium chloride and the analytes were partitioned twice into dichloromethane. The combined dichloromethane extracts were evaporated just to dryness. This residue was reconstituted with methanol and an aliquot of it was purified with gel permeation chromatography (GPC). The collected target GPC fraction was evaporated to just dryness, reconstituted with cyclohexanone and subjected to GC-MS quipped with an Aglilent DB-17 MS column (30m 0.25mm) monitoring 1 fragment ion listed in the original method for quantitation and of 2 GC-MS/MS mass transitions (SRMs) for confirmation in the E+ mode.

Recovery findings

In all matrices tested, the mean recovery values of all three analytes in the fragment ion and both mass transitions at both fortification levels was between 70 % and 110 %. The detailed values are given in the tables below.

Table 4.1.2-40: Validation results of method L0326-01 for dimethomorph in animal matrices

Matrix	Mass transition	Fortification level (mg/kg)	n	Average recovery (%)	Relative standard deviation (%)	Overall mean recovery (%)	Overall relative standard deviation (%)
Fat	301	0.01	5	88.6	2.2	90.3	6.2
		0.1	5	92.0	8.4		
	301 → 165 m/z	0.01	5	92.0	3.0	89.3	7.5
		0.1	5	86.5	10		
	303 → 165 m/z	0.01	5	97.6	3.2	93.4	6.1
		0.1	5	89.2	4.9		
Kidney	301	0.01	5	85.6	14	87.4	10
		0.1	5	89.2	5.8		
	301 → 165 m/z	0.01	5	85.2	12	86.5	8.8
		0.1	5	87.8	5.9		
	303→ 165 m/z	0.01	5	85.4	12	87.0	9.7
		0.1	5	88.6	7.6		
Liver	301	0.01	5	89.9	14	83.7	14
		0.1	5	77.5	7.0		
	301 → 165 m/z	0.01	5	86.3	13	82.3	11
		0.1	5	78.3	7.0		
	303 → 165 m/z	0.01	5	84.7	14	80.2	13
		0.1	5	75.7	8.9		
Muscle	301	0.01	5	88.8	15	86.3	12
		0.1	5	83.9	8.4		
	301 → 165 m/z	0.01	5	87.3	16	84.4	14
		0.1	5	81.4	11		
	303→ 165 m/z	0.01	5	87.4	17	84.6	14
		0.1	5	81.7	8.4		

Table 4.1.2-41: Validation results of method L0326-01 for M550F007 in animal matrices

Matrix	Mass transition	Fortification level (mg/kg)	n	Average recovery (%)	Relative standard deviation (%)	Overall mean recovery (%)	Overall relative standard deviation (%)
Fat	287	0.01	5	94.2	5.0	88.1	9.8
		0.1	5	82.0	9.0		
	287 → 151 m/z	0.01	5	92.2	6.0	88.5	9.3
		0.1	5	84.8	11		
	287 → 227 m/z	0.01	5	96.5	9.6	93.3	11
		0.1	5	90.2	12		
Kidney	287	0.01	5	88.2	12	94.8	14
		0.1	5	101	14		
	287 → 151 m/z	0.01	5	86.7	12	92.7	10
		0.1	5	98.6	3.9		
	287 → 227 m/z	0.01	5	87.7	12	92.8	10
		0.1	5	98.0	5.1		
Liver	287	0.01	5	102	5.0	94.9	11
		0.1	5	87.6	12		
	287 → 151 m/z	0.01	5	92.5	12	88.9	11
		0.1	5	85.4	11		
	287 → 227 m/z	0.01	5	102	7.0	95.6	11
		0.1	5	89.3	11		
Muscle	287	0.01	5	91.2	12	90.7	9.5
		0.1	5	90.2	6.7		
	287 → 151 m/z	0.01	5	93.4	12	93.0	12
		0.1	5	92.6	14		
	287 → 227 m/z	0.01	5	93.3	12	91.9	10
		0.1	5	90.4	7.9		

Table 4.1.2-42: Validation results of method L0326-01 for M550F006 in animal matrices

Matrix	Mass / Mass Transition	Fortification level (mg/kg)	n	Average recovery (%)	Relative standard deviation (%)	Overall mean recovery (%)	Overall relative standard deviation (%)
Fat	287	0.01	5	89.2	8.3	81.4	15
		0.1	5	73.5	16		
	287 -> 151 m/z	0.01	5	86.2	5.5	81.9	12
		0.1	5	77.7	16		
	287-> 227 m/z	0.01	5	90.3	4.6	85.8	10
		0.1	5	81.3	12		
Kidney	287	0.01	5	83.6	8.1	87.5	7.6
		0.1	5	91.5	4.3		
	287 -> 151 m/z	0.01	5	85.1	8.8	93.8	12
		0.1	5	102	6.7		
	287-> 227 m/z	0.01	5	87.4	12	90.7	10
		0.1	5	94.0	8.6		
Liver	287	0.01	5	91.9	12	85.4	14
		0.1	5	78.9	12		
	287 -> 151 m/z	0.01	5	96.7	13	92.9	13
		0.1	5	89.0	14		
	287-> 227 m/z	0.01	5	86.0	10	87.6	10
		0.1	5	89.1	11		
Muscle	287	0.01	5	85.9	14	87.9	13
		0.1	5	89.9	13		
	287 -> 151 m/z	0.01	5	90.2	12	91.5	12
		0.1	5	92.8	14		
	287-> 227 m/z	0.01	5	94.4	12	92.1	12
		0.1	5	89.9	14		

Linearity

Good linearity was observed over the concentration range tested for the GC-MS(/MS) detectors. Linear correlations with coefficients >0.99 were obtained for all analytes. At least six calibration points distributed over a concentration range of 50 to 5000 ng/mL were used. Calibration standards were prepared in cycolhexanone.

Specificity

The highly selective and sensitive GC-MS(/MS) method was used for determination of dimethomorph and two of its metabolites monitoring one characteristic fragment ion for quantitation and simultaneously two GC-MS/MS mass transitions for confirmation. Therefore a confirmatory technique is not required (SANCO/825/00).

Interference

No significant interference was observed at elution times of the analytes of interest (interference <20% LOQ).

Matrix effect	No significant matrix effects were observed (deviation of matrix matched standards from standards prepared in cyclohexanone were <20%).
Limit of quantitation	The limit of quantitation, defined by the lowest fortification level successfully tested was 0.01 mg/kg for each analyte and all matrices.
Limit of detection	The limit of detection (LOD) in animal commodities set at 25% of the LOQ was or 0.0025 mg/kg for each analyte and all matrices.
Repeatability	The relative standard deviation (RSD, %) for all commodities and all fortification levels were well below 20%. The detailed values are shown in Table 4.1.2-40 to Table 4.1.2-42.
Reproducibility	Reproducibility of the method was not determined within this validation study as no independent laboratory validation is required for a data generation method.
Standard stability	All analytes indicated sufficient stability in stock (acetonitrile) as well as in calibration solutions (cyclohexanone) for at least 35 days when stored frozen in the dark.
Extract stability	The final sample extracts in cyclohexanone were injected after at least 15 days of storage under frozen conditions. No decrease in stability in the stored extracts could be observed. Re-injections of final extracts resulted in recoveries within the acceptable range of 70 to 110 %.
Conclusion	**It could be demonstrated that method L0326/01 fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of dimethomorph and its metabolites M550F007 and M550F006 in various animal matrices**

Report: CA 4.1.2/40
Dijk A. Van, 1993 a
14C-Dimethomorph (CME 151): Evaluation of the method of analysis for residues of Dimethomorph (CME 151) and metabolites Z 67 and Z 69 in liver and kidney of cows
DK-245-013
Guidelines: EPA 171-4, EPA 540/9-82-023
GLP: yes
(certified by Eidgenoessisches Departement des Innern, Bern, Schweiz)

Principle of the methods

The purpose of the study was to compare the extraction method used in the lactating goat metabolism study (chapter CA 6.2; BASF DocID DK-440-005) with the extraction procedures used in residue analytical method FAMS 023-01. Therefore, liver and kidney homogenates originating from the metabolism study were used for assessment of extraction efficiency.
Analytical determination of dimethomorph and its degradation products in liver and kidney extracts was accomplished by GC (Capillary DB-1 column, carrier gas: Helium; 1.0 bar) followed by NPD detector with and integrator (HP 3396 A).Total radioactivity in the extracts was determined by liquid scintillation counting.

Extraction procedure 1 *(from lactating ruminant metabolism study):*

Liver and kidney homogenates were extracted 3 times with methanol/water at a ratio of 8/2 (v/v), followed by an exhaustive extraction with methanol. After air-drying of the remaining tissue, the non-extractable were determined by combustion. The aqueous phase of combined and concentrated extract was diluted with acetonitrile (1/1, v/v). Thereafter the aqueous phase was partitioned twice with hexane (1/1, v/v), twice with dichloromethane (1/2, v/v) and, after acidifying with HCl, twice with ethyl acetate. All organic extracts were dried and the radioactivity was determined by LSC. The dichloromethane/ethyl acetate extracts were combined, concentrated and adjusted with methanol to final volume before analysis by TLC. Additionally, liver extract was purified by GPC and analyzed by GC-NPD.

Extraction procedure 2 (FAMS 023-01):

Liver and kidney homogenates were extracted with acetonitrile and partitioned with hexane. The acetonitrile phase was mixed with bi-distilled water and saturated sodium chloride solution before partitioning twice with dichloromethane. All extracts were measured by LSC. The dichloromethane extracts were concentrated, re-dissolved in methanol before purification by GPC. Final analysis was performed by GC-NPD.

Limit of detection

GC detection limit: A detection limit for GC analysis based on the lowest standard was 0.025 mg/kg and 0.03 mg/kg for liver and kidney, respectively.

Extractability

The metabolism method extracted 89.3% and 94.0% of the radioactivity from liver and kidney, respectively, which determined before by combustion. Method FAMS 023-01 extracted 75.7% and 74.0% of the radioactivity from liver and kidney respectively (see Table 4.1.2-6).

Analysis of extracts

Using the metabolism method 72.6% of the radioactivity in liver was identified by TLC, 64.7% was identified with GC (mean of both 68.7%). With method FAMS 029-01 75.4% of the radioactivity in liver was identified by GC.

In kidney a total of 16.9% of the radioactivity was identified by TLC. With method FAMS 12.5% of the radioactivity in liver was identified by GC.

Detailed results are presented in Table 4.1.2-45.

Table 4.1.2-43: Radioactive residues in goat homogenates treated with [^{14}C]-dimethomorph

Residual radioactive residue *(applied in CA 6.2;* DocID DK-440-005*)*		
Applied:	11.53 µCi/mg (15 days)	
	Residual radio-active residue* (mg/kg)	
Extract	**Present study**	**Metabolism study**
Liver	7.247	7.718
Kidney (goat 1)	0.295	0.289
Kidney (goat 2)		0.270

* Determined by combustion including background correction

Table 4.1.2-44: Extractability of radioactivity in liver and kidney homogenates treated with [^{14}C]-dimethomorph measured by LSC

	Metabolism method		**FAMS 023-01**		**Deviation***
Extract	**Extracted residue (mg/kg)**	**Extracted residue (% of combustion)**	**Extracted residue (mg/kg)**	**Extracted residue (% of combustion)**	**(%**
Liver	6.472	89.3	5.486	75.7	15.2
Kidney	0.277	94.0	0.218	74.0	21.3

* Deviation of FAMS 023-01 compared to metabolism method (100%) based on mg/kg values.

Table 4.1.2-45: Comparison of methods quality

	Liver								Kidney			
Method	**Metabolism**						**FAMS 023-01**		**Metabolism**		**FAMS 023-01**	
Analyses	**TLC (mg/kg)**	**[%]***	**GC (mg/kg)**	**[%]***	**TLC/GC (mg/kg)**	**[%]***	**GC (mg/kg)**	**[%]***	**TLC (mg/kg)**	**[%]***	**GC (mg/kg)**	**[%]***
BAS 550 F	5.18	71.5	4.64	64.0	4.91	67.8	5.39	74.4	0.032	10.8	0.029	9.8
M550F007	0.080	1.1	0.054	0.7	0.067	0.9	0.070	1.0	0.018	6.1	0.008	2.7
Sum	5.26	72.6	4.7	64.7	5.0	68.7	5.46	75.4	0.05	16.9	0.037	12.5

* Percentage of the determined residue (mg/kg) compared to the residue determined by combustion

Conclusion **The study proves that method FAMS 023-01 is highly efficient for extracting residues of dimethomorph and its metabolites M550F006 (Z67) and M550F007 (Z69) from animal matrices. The amounts of dimethomorph and metabolite M550F007 extracted by FAMS 023-01 (with its acetonitrile based extraction) were within 10% of the values achieved by methanol based extraction in the goat metabolism study.**

Report:	CA 4.1.2/41 Mirbach M.J.,Huber H.P., 1991 a Confirmatory validation of an analytical method for the determination of the residues of Dimethomorph and its metabolites in milk (FAMS 024-01) DK-245-011
Guidelines:	EPA 171-4
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 4.1.2/42 Weitzel R., 1991 a Method for determining of Dimethomorph (CME 151) and its metabolites Z67, Z69 and CUR 7117 in milk and milk products DK-245-008
Guidelines:	none
GLP:	no

Remark: DK-245-008 is the method description for analysis of milk samples. DK-245-011 is the validation study of residue analytical methods FAMS 024-01. In the summary below, information from both studies are summarized for the reviewer's convenience in one comprehensive overview. In addition to the data presented for the validation procedure of the method, additional concurrent procedural recoveries from feeding study DK-705-007 are summarized as well for completeness of the data set.

Principle of the method

Method FAMS 024-01

Samples of milk and milk products were extracted by shaking with acetone. The extract was centrifuged and the acetone was distilled off and the aqueous residue was distributed into ethyl acetate and evaporated just to dryness. In case of cream an additional distribution between acetonitrile and n-hexane was performed. After a clean-up step with gel-permeation chromatography, the residues were analyzed by HPLC-UV at 240 nm.

The chromatography was performed on a Nucleosil 100-Phenyl 7 µm column at a flow rate of 1.0 mL/min. As mobile phase acetonitrile/0.01 mol/L phosphoric acid (50/50, v/v) was used.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110%, except for M550F008 in skimmed milk and acid whey. The detailed results are given below.

Table 4.1.2-46: Validation results of method FAMS 024-01 in milk and milk products (DK-245-008)

Matrix	Test substance	Fortification level (mg/kg)	Average recovery (%)	Relative standard deviation (%)
Milk	Dimethomorph (CME 151)	0.01 – 0.1	90	3
	M550F006 (Z67)	0.02 – 0.1	84	15
	M550F007 (Z69)	0.02 – 0.1	91	12
	M550F008 (CUR 7117)	0.01 – 0.1	79	13
Cream	Dimethomorph (CME 151)	0.01 – 0.1	91	7
	M550F006 (Z67)	0.02 – 0.1	75	7
	M550F007 (Z69)	0.02 – 0.1	90	1
	M550F008 (CUR 7117)	0.01 – 0.1	81	5
De-creamed milk	Dimethomorph (CME 151)	0.01 – 0.1	87	8
	M550F006 (Z67)	0.02 – 0.1	73	5
	M550F007 (Z69)	0.02 – 0.1	75	8
	M550F008 (CUR 7117)	0.01 – 0.1	90	12

Table 4.1.2-47: Validation results of method FAMS 024-01 in milk and milk products (DK-245-011)

Matrix	Test substance	Fortification level (mg/kg)	Average recovery (%)[2]	Relative standard deviation (%)[2]
Milk	Dimethomorph (CME 151)	0.05-0.25	89	11
	M550F006 (Z67)[1]	0.05-0.25	82	5
	M550F007 (Z69)[1]			
	M550F008 (CUR 7117)	0.05-0.25	81	10

1 Z67 and Z69 were recovered together as one compound
2 Mean values and RSD calculated from the values provided in the study report

Table 4.1.2-48: Validation results of method FAMS 024-01 in milk and milk products; procedural recoveries determined during the cow feedings study (DK-705-007)

Matrix	Test substance	Fortification level (mg/kg)	Average recovery (%)	Relative standard deviation (%)
Milk	Dimethomorph (CME 151)	0.02 0.05 0.10	95 92 91	19 (n=10) 6 (n = 9) 7 (n = 10)
	M550F006 (Z67) / M550F007 (Z69)	0.02 + 0.02	89	13 (n = 26)
	M550F008 (CUR 7117)	0.02 0.05 0.10	82 94 90	13 (n = 5) 8 (n = 5) 7 (n = 6)
Pasteurized milk	Dimethomorph (CME 151)	0.01 0.05 0.10	90 92 95	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F006 (Z67) / M550F007 (Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	95 100 95	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	92 99 91	N/A (n=1) N/A (n=1) N/A (n=1)
Skimmed milk	Dimethomorph (CME 151)	0.01 0.05 0.10	105 93 91	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F006 (Z67) / M550F007 (Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	88 94 91	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	126 94 87	N/A (n=1) N/A (n=1) N/A (n=1)
Acid whey	Dimethomorph (CME 151)	0.01 0.05 0.10	101 94 95	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F006 (Z67) / M550F007 (Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	91 96 84	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	124 110 94	N/A (n=1) N/A (n=1) N/A (n=1)
Cream	Dimethomorph (CME 151)	0.01 0.05 0.10	104 95 99	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F006 (Z67) / M550F007 (Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	105 98 99	N/A (n=1) N/A (n=1) N/A (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	101 90 89	N/A (n=1) N/A (n=1) N/A (n=1)

RSD Relative standard deviation

N/A Not applicable

Linearity	Good linearity was observed over a concentration range of 0.1 to 2.0 µg/mL with at least 5 concentrations (external reference standard) with correlation coefficients >0.998. Calibration standards were prepared in methanol.
Specificity	The identification and quantitation were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of dimethomorph and its metabolites in milk and cream. No significant matrix interferences were observed in the investigated control extract.
Matrix effects	As no interference at the elution time of the analyte of interest was observed in the UV-trace at 240 nm, no adverse effects of any matrix occurred.
Interference	No significant interference were observed at elution times of the analytes of interest (interference <30% LOQ) for dimethomorph (CME151) and M550F007 (Z69). The blank of M550F008 (CUR 7117) in milk and decreamed milk is <LOQ, as well as the blank of M550F006 (Z67) in cream.
Limit of quantitation	The limit of quantitaion (LOQ) defined by the lowest fortification level successfully tested was 0.01 mg/kg for dimethomorph and M550F008 (CUR 7117) and 0.02 mg/kg for M550F006 (Z67) and M550F007 (Z69).
Limit of detection	The LOD for each analyte was set at 0.002 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all commodities and all fortification levels were ≤ 20% for all metabolites. The detailed values are shown in Table 4.1.2-46 and Table 4.1.2-48.
Reproducibility	No independent laboratory validation is required for data generation methods.
Conclusion	**It could be demonstrated that the method FAMS 024-01 fulfills the requirements with regard to linearity, repeatability, limit of quantitation, and recoveries and is therefore applicable and suitable to correctly determine residues of dimethomorph and its metabolites M550F006, M550F007 and M550F008 in milk and milk products.**

(f)Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

No stand-alone analytical methods were validated in support of ecotoxicological studies. Methods for the determination of concentration of dimethomorph, whenever necessary, are reported along with the respective ecotoxicological study. The detailed results were reported in the analytical phase report of the respective study. Relevant information on bee related matrices and their analysis can be found in the Analytical Phase Reports of the following studies: 2014/1000183, 2015/1000404, and 2015/1000386 in chapters M-CA 8.3 and M-CP 10.3. A brief executive summary of the results are given below:

- 2014/1000183: In addition to the full validation (5 replicates per fortification level), 3 to 6 procedural recoveries were prepared additionally. The analytical method is fully suitable to determined dimethomorph at an LOQ of 0.01 mg/kg (LOD of 0.003 mg/kg) in pollen, nectar, and inflorescences with of 88% to 98% with a relative standard deviation of 5.8%. Values were obtained by use of matrix-matched standards by HPLC-MS/MS.
- 2015/1000404: The analytical method is fully suitable for determination of dimethomorph in nectar, pollen and flowers at an LOQ of 0.01 mg/kg and a limit of detection of 0.003 mg/kg using LC-MS/MS. Average recoveries of the five replicates per matrix types ranged from 81% to 92% with a maximum relative standard deviation of 14%. As no significant matrix effects of >20% were observed, calibration was done using solvent-based standards.
- 2015/1000386: The analytical method is fully suitable for determination of dimethomorph in nectar, pollen and flowers at an LOQ of 0.01 mg/kg and a limit of detection of 0.003 mg/kg using LC-MS/MS Average recoveries of the five replicates per matrix types ranged from 82% to 97% with a maximum relative standard deviation of 15%. As matrix effects of up to 25% were observed, calibration was done using matrix-matched-based standards.

Report: CA 4.1.2/43
Kellner G., 1995 a
Validation of a HPLC method for the determination of Dimethomorph residues in aquatic test medium
DK-243-003
Guidelines: none
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie und Bundesangelegenheiten, Wiesbaden)

Principle of the method

The purpose of this study was to perform a laboratory validation of method RU 151/35/90 (American Cyanamid method) for the determination of dimethomorph (BAS 550 F) residues in aquatic test medium of an algae growth inhibition test and a prolonged fish toxicity test. The validation was conducted at RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany.
The aquatic test samples are extracted on a SPE cartridge and eluted with methanol. The samples are filtered (0.45 µm) into HPLC vials and analyzed without further clean up on an analytical reversed phase HPLC column (Nucleosil RP 18) and with acetronitrile/water (6/4, v/v) as mobile phase. The quantitation is accomplished by UV absorbance detection at 240 nm and the external standard technique.

The limit of quantitation of the method is 0.004 mg L^{-1} for *E*-dimethomorph and *Z*-dimethomorph.

Recovery findings

Amounts of dissolved dimethomorph in methanol (mixed solution of *E* and *Z*-isomer) were added to the test medium. Samples were fortified with 0.004, 0.008, 0.08 and 0.1 mg L^{-1} dimethomorph (E/Z). Mean recovery values (mean of two replicates per fortification level and isomer) are between 86% and 103% of the nominal values in water and between 96% and 107% of the nominal values in OECD medium (see table below).

Table 4.1.2-49: Method Recoveries of dimethomorph (*E* and *Z*-isomer) in two ecotoxicological test media using solvent-based standards

Matrix	Analyte	Fortification level (mg L^{-1})	n	Recovery (%)	Mean recovery (%)	RSD (%)
water (fish test)	*E*-dimethomorph	0.004	2	87.3, 88.8	88	1.2
		0.008	2	92.5, 92.8	93	0.2
		0.08	2	99.3, 102.7	101	2.4
		0.1	2	101.7, 97.7	100	2.8
		Overall	**8**	**Range, 87 - 103**	**95**	**6.1**
	Z-dimethomorph	0.004	2	85.8, 88.2	87	2.0
		0.008	2	91.0, 91.3	91	0.2
		0.08	2	98.4, 102.0	100	2.5
		0.1	2	100.6, 101.9	101	0.9
		Overall	**10**	**Range, 86 - 102**	**95**	**6.9**
OECD medium (algae test)	*E*-dimethomorph	0.004	2	95.5, 98.5	97	2.2
		0.008	2	99.8, 101.6	101	1.3
		0.08	2	106.3, 107.2	107	0.6
		0.1	2	105.9, 105.1	106	0.5
		Overall	**8**	**Range, 96 - 107**	**103**	**4.2**
	Z-dimethomorph	0.004	2	96.5, 99.5	98	2.2
		0.008	2	99.5, 102.5	101	2.1
		0.08	2	105.6, 105.9	106	0.2
		0.1	2	104.3, 103.8	104	0.3
		Overall	**10**	**Range, 97 - 106**	**102**	**3.3**

RSD Relative standard deviation

Linearity

Good linearity (r >0.99) was observed in the range of 0.2 mg L^{-1} to 40 mg L^{-1} for both i*somer*s of dimethomorph. Standards used for calibration curves were prepared in methanol.

Specificity

The identity of the test items was proved by coincidence of their retention times with the retention times of the authentic reference item peaks. It was also checked by UV-detection. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is required.

Matrix effects

In OECD medium blank values were detected. They do not result from dimethomorph residues in the medium because different amounts of *E* and *Z*-isomer were found. So, it is expected that the blank values detected result from interferences of the OECD medium. Interferences are furthermore not considered significant as the interference is factor 6.5 lower than the lowest calibration standard (LOD), hence 15% of the LOD of the method.

Limit of quantitation	The limit of quantitation (LOQ) is defined by the lowest fortification level successfully tested. The LOQ of the method is 0.004 mg L^{-1} for *E*- and *Z*-dimethomorph.
Limit of detection	The limit of detection (LOD) of the method is defined by the lowest standard analyzed and corresponds to 0.001 mg L^{-1} of *E* and *Z*-dimethomorph in the water samples (equal to 0.2 µg mL^{-1} in the final volume).
Repeatability	The relative standard deviations (RSD, %) for all fortification levels tested were below 10%.
Standard stability	Chromatographic standard solutions were freshly prepared daily from the stock solution.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The results of the study show that analytical method RU 151/35/90 is suitable for the determination of dimethomorph in aquatic test medium.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.**

Report: CA 4.1.2/44
Obermann M., 2005 a
Validation of analytical method APL0500/01: Determination of pesticides in water by HPLC/MS
2005/1026675
Guidelines: SANCO/825/00 rev. 7 (17 March 2004)
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Remark: Analytical method APL0500/03 was used in support of ecotoxicological studies. Reason for the different numbering of the method number is the fact that the method is a multi-compound method using identical analytical conditions; however, validation of dimethomorph was done in the method version APL0500/01.

Principle of the method

Analytical method APL0500/01 was validated for the analysis of several pesticides in aqueous matrices by LC-MS, to support ecotoxicological studies for dose verification. The study was performed at BASF, Limburgerhof, Germany.
The aqueous samples are diluted with acetonitrile/water, acidified with formic acid and are directly injected in the chromatographic system. The analysis of dimethomorph (BAS 550 F) is accomplished using a reversed phase HPLC column (YMC Pro C_{18} column) and a water acetonitrile gradient with formic acid as modifier. Detection is accomplished in ESI+ mode at the mass-to-charge ratio of m/z 388.

The limit of quantitation of the method is 0.001 mg L^{-1}.

Recovery findings

The accuracy of the analytical method for determination of dimethomorph in water was determined by analyzing five fortified samples at two different concentration levels (0.001 mg L^{-1} and 0.1 mg L^{-1}) in mix-water from Frankenthal (Ft-Mix-water). Additionally, the accuracy was checked for the water types AAP-, M4- and OECD-Medium at the limit of quantitation (0.001 mg L^{-1}). Mean recovery values are between 92% and 103% of the nominal values for all tested matrices (see table below).

Table 4.1.2-50: Results of the method validation for the determination of BAS 550 F in water

Matrix	Analyte	Fortification level [mg L^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ft-Mix Water (Tap-Water)	Dimethomorph	0.001	5	94	4.9	98	6.7
		0.1	5	103	5.2		
AAP-Water		0.001	1	92	--	--	--
M4-Water		0.001	1	97	--	--	--
OECD-Water		0.001	1	99	--	--	--

RSD = Relative standard deviation
Ft-Mix Water = mix-water from Frankenthal

Linearity The method-detector response was linear within the range of 0.0005 to 0.13 mg L^{-1} ($r > 0.995$) using solvent-based standards (diluted in water/acetonitrile/formic acid, 800:200:1, v/v/v).

Specificity The identity of the test items was proved by coincidence of their retention times with the retention times of the authentic reference item peaks. lt was also checked by MS-detection. No significant co-elution of dimethomorph peaks with an unknown component was observed. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is required.

Matrix effects As the method does not require any no sample extraction or clean-up, matrix effect is directly assessed by preparation of procedural recoveries as they also serve for assessment of matric effects in the different media compared to solvent-based calibration standard solutions. No matrix effect >20% were observed.

Limit of quantitation The limit of quantitation (LOQ) is defined by the lowest fortification level successfully tested. The method has a limit of quantitation of 0.001 mg L^{-1}. However, as analysis is accomplished by direct injection of the water samples after dilution, correct quantitation of possible as low as the lowest standard of the calibration curve.

Limit of detection The limit of detection (LOD) is defined by the lowest standard solution, equivalent to 0.0005 mg L^{-1}.

Repeatability The relative standard deviations (RSD, %) for the fortification levels tested were below 20%.

Standard stability	Standard stability in aqueous mixtures of acetonitrile were assessed and are reported under M-CA 4.1.2/2. Stability was confirmed for storage times of at least 44 days.
Reproducibility	Reproducibility of the method was not determined within this validation study as no ILV is required for a data generation method.
Conclusion	**The results of the study show that analytical method APL0500/01 is suitable for the determination of dimethomorph in water.** **It could be demonstrated that the method fulfills the requirements with regard to accuracy, repeatability, specificity, linearity and limit of quantitation.**

Information available from public literature:

There is also some information available from the scientific literature on the possibility of determination of dimethomorph in honey. An executive summary of this additional information is provided in chapter M-CA 4.2/8 as this method could potentially also be used for monitoring purposes.

(g)Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Where necessary, these methods / results are reported along with the respective studies. No separate study reports have been generated to address this topic.

CA 4.2 Methods for post-approval control and monitoring purposes

General remarks

The order of the study summaries is different compared to the information given in the application submitted for renewal of approval. Reason is that the order of the studies had to be altered to present the residue analytical methods in accordance to the required chronology of matrices in the document M-CA 4.1.2. In case references are summarized which were not listed in the application, or in case references listed in the application are not contained in this chapter, an additional comment will be made at the respective section.

An overview of the changes of order in chapter 4.1.2 compared to the application is given in the table below for the reviewer's convenience:

Table 4.2-1: Overview of changes of documents submitted compared to originally listed documents in the application

Data point in application	Data point in current dossier	DocID	Changes to application	Reason for change
4.2/1	4.2./1	2014/1186696	./.	./.
4.2/2	./.	2014/1186698	not submitted	Method for enforcement purposes including metabolites not required as residue definition is parent only for animal matrices
./.	4.2./2	2015/1204836	new study	ILV to plant multi-method conducted as DFG S19 used and not sufficient data available on CRL data pool
4.2/3	./.	2015/1000643	not submitted	No ILV required as no new enforcement method was required
N/A	4.2/3	2008/1103056	additional information	Additional information form scientific literature
N/A	4.2/4	2014/1327433	additional information	Additional information form scientific literature
4.2/4	4.2/5	2009/1051339	change of position	Renumbering due to changed order
4.2/5	4.2/6	2009/1078429	change of position	Renumbering due to changed order
N/A	4.2/7	2013/1420620	additional information	Additional information form scientific literature
4.2/6	4.2/8	2014/1327434	additional information	Additional information form scientific literature
4.2/6	4.2/9	~~2014/7004121~~ 2016/7006199	change of position	Renumbering due to changed order and other DocID than in Application
./.	4.2/10	~~2015/1237993~~ ~~2016/1118111~~ 2017/1069823	new study	ILV to 2015/1237992 (lower LoQ)
./.	4.2/11	2015/1192602	new study	New method for air monitoring
./.	4.2/12	2014/1327432	additional information	Additional information form scientific literature
./.	4.2/13	2011/1297951	additional information	Additional information form scientific literature
./.	4.2/14	2010/1233392	additional information	Additional information form scientific literature
./.	4.2/15	2007/1071145	additional information	Additional information form scientific literature
./.	4.2/16	2017/1068967	new study	New method for analysis of body fluids

N/A Not applicable; study was not included in the original application.

An overview of the metabolites of relevance for the analytical methods is given together with other structures of relevance in Document N3. The respective table also contains detailed information on different metabolite codes used due to historic reasons.

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Based on the current residue definition for MRL setting and enforcement, residue analytical methods are required for the parent molecule dimethomorph in food of plant origin.

For a general overview of analytical methods already peer-reviewed, please refer chapter M-CA 4.1.2. Newly developed methods which were not yet peer-reviewed are summarized below.

The already peer-reviewed multi-methods for plant matrices based on the DFG S19 approach (Weeren and Pelz, 1999, DK-249-005; Class T, 1999, DK-244-04) were considered fully valid for enforcement purposes. However, a new monitoring and enforcement method for plant matrices was developed to have a new enforcement method available, according to the most recent guidelines, in all relevant crop commodities, also additionally allowing to individually detect and quantify the *E*- and *Z*-isomers of dimethomorph with a limit of quantitation of 0.005 mg/kg per isomer. This newly developed method was successfully confirmed by an independent laboratory validation. Both methods are submitted in this dossier. DFG S19 was chosen as multi-method approach as assessment of extractability conducted during metabolism (refer to 2014/1093386) showed better extraction efficiency using acetone (DFG S19) instead of acetonitrile (QuEChERS). An overview of the available QuEChERS methods in the CRL data pool is given as additional information, as well as information found in the scientific literature, further below in this chapter.

For food of animal origin, the residue definition is defined as dimethomorph. The previous enforcement method based on DFG S19 (Weeren and Pelz, 1999, DK-249-005; Class, 1999, DK-249-004) were considered fully valid and suitable for enforcement purpose during the last evaluation for Annex I inclusion; however upon request from Germany during a national product registration, a new monitoring and enforcement method was developed and validated for all relevant animal matrices. This method has been successfully validated in an independent laboratory validation. Both methods are submitted in this dossier and are summarized in chapter M-CA 4.2.

General remarks

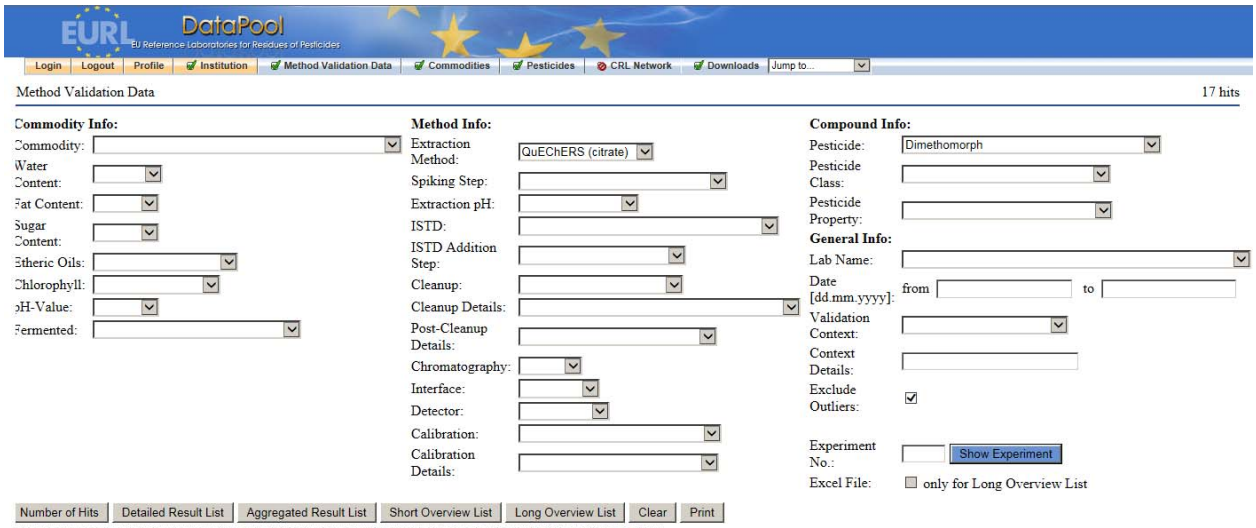
The following recovery and repeatability criteria are required according to the OECD Guidance document on analytical methods (ENV/JM/MONO(2007)17), depending on the fortification levels:

≤1 µg/kg	50 - 120 ± 35%
>1 µg/kg ≤0.01 mg/kg	60 - 120 ± 30%
>0.01 mg/kg ≤0.1 mg/kg	70 - 120 ± 20%
>0.1 mg/kg ≤1.0 mg/kg	70 - 110 ± 15%
>1 mg/kg	70 - 110 ± 10%

Food of plant origin

To cover the requirements of the new guidelines in force as well as to allow separation and individual quantification of the *E*- and *Z*-isomers, a new monitoring method for plant matrices was developed and independently validated. The new method is based on the originally chosen approach of DFG S19, as assessment of extractability pointed to better recoveries using acetone instead of acetonitrile. Nevertheless, QuEChERS is a widely used multi-method approach for enforcement purposes, so additional information from the CRL data pool is presented as well. Sufficient confirmatory information is provided by the CRL data pool. The CRL data pool is accessible via internet (http://www.eurl-pesticides-datapool.eu/). An overview of the validation data available for the different crop commodities in the CRL data pool is given in below.

Figure 4.2-1: Overview of validation data available for QuEChERS in the CRL data pool



Long Overview List

Pesticide	Chr	Matrix Type	Level min	Level max	Rec Median	Rec Mean	CV [%]	# of rec	% Rec (70-120%)	# of Labs
Dimethomorph			0,002	10	**99**	99	9	1562	**99**	11
	GC	Acidic	0,005	0,2	**101**	101	7,5	101	**99**	2
	GC	Dry (cereals, dry pulses)	0,01	0,2	**103**	104	7,6	86	**100**	1
	GC	Sugar containing	0,01	0,2	**103**	102	9,1	82	**96**	2
	GC	Water containing	0,002	1	**100**	99	6	166	**100**	4
	GC	Water containing, extract rich	0,05	10	**101**	104	8,9	4	**100**	1
	LC		0,02	0,02		102		1	**100**	1
	LC	Acidic	0,01	0,1	**97**	97	9,8	317	**100**	8
	LC	Dry (cereals, dry pulses)	0,01	0,1	**98**	98	8,8	125	**100**	6
	LC	Dry (spices, herbs, tea)	0,01	0,1	**104**	107	9,3	12	**92**	2
	LC	Fatty (oils)	0,02	0,02		111		1	**100**	1
	LC	Fatty, dry (oil seeds, nuts)	0,01	0,1	**106**	108	7,2	11	**91**	2
	LC	Fatty, wet (oily fruits)	0,01	0,1	**98**	99	6,2	10	**100**	1
	LC	Other	0,02	0,025	**95**	98	13,7	5	**100**	2
	LC	Sugar containing	0,01	0,1	**98**	99	9,1	138	**99**	8
	LC	Water containing	0,01	0,2	**99**	99	9,1	495	**100**	10
	LC	Water containing, extract rich	0,02	0,05	**110**	108	8,3	8	**100**	2

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Report:	CA 4.2/1 Richter S.,Asekunowo J., 2015 a Validation of a multi-residue method DFG S19 (BASF method No. L0321/01) for the determination of Dimethomorph (BAS 550 F) in various crop types 2014/1186696
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Remark: Based on the results obtained from assessment of extraction efficiency, DFG S19 approach was chosen as it extraction efficiency of incurred residues was better using DFG S19 approach compared to QuEChERS approach.

Principle of the method

Residues of dimethomorph (BAS 550 F) are extracted from homogenized lettuce, onion, grape, potato tuber, hop cone, dried bean and oilseed rape seed samples according to extraction modules E1 (lettuce, onion, potato tuber), E2 (dried bean, hop cone), E3 (grape) and E7 (oilseed rape), respectively, to validate the DFG method S19. After extraction of potato tuber, hop cone, dried bean and oilseed rape seed, the specimen extracts were cleaned up by gel permeation chromatography.
All samples were analyzed by LC-MS/MS at mass transition 388 → 301 for quantitation and 388 → 165 for confirmation (ESI+). Analysis was accomplished on a Thermo Betasil C18 column applying a methanol-pure water gradient using 0.1% formic acid as modifier.

Recovery findings

In all matrices tested, the mean recovery values of *E*- and *Z*-dimethomorph were between 70% and 110%. Detailed results are given in table below.

Table 4.2-2: Validation results of method L0321/01 (DFG S19): dimethomorph (BAS 550 F) in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				**388 → 301**	**388 → 165**	**388 → 301**	**388 → 165**
E-dimethomorph (BAS 550 F)	Lettuce	0.005	5	96	95	10	9.0
		0.05	5	110	108	3.6	5.0
	Onion	0.005	5	105	104	4.3	2.6
		0.05	5	96	96	7.4	6.7
	Grape	0.005	5	100	96	9.5	12
		0.05	5	106	106	6.0	4.4
	Potato tuber	0.005	5	82	82	7.5	6.9
		0.05	5	94	92	9.2	8.7
	Hop cone	0.005	5	74	75	14	12
		0.05	5	83	83	3.2	4.6
	Dried bean	0.005	5	110	108	1.7	2.7
		0.05	5	100	98	5.8	5.3
	Oilseed rape (OSR) seed	0.005	5	87	87	6.0	6.0
		0.05	5	96	97	4.7	4.8
Transition				**388 → 301**	**388 → 165**	**388 → 301**	**388 → 165**
Z-dimethomorph (BAS 550 F)	Lettuce	0.005	5	102	98	5.6	10
		0.05	5	106	105	4.5	4.6
	Onion	0.005	5	103	106	3.9	5.1
		0.05	5	97	98	7.7	7.5
	Grape	0.005	5	108	106	7.2	8.4
		0.05	5	108	110	5.5	6.0
	Potato tuber	0.005	5	106	108	6.0	6.0
		0.05	5	109	110	8.6	9.3
	Hop cone	0.005	5	80	72	14	10
		0.05	5	84	83	1.1	2.6
	Dried bean	0.005	5	103	105	5.4	5.4
		0.05	5	101	100	5.4	5.1
	Oilseed rape (OSR) seed	0.005	5	106	106	1.7	3.5
		0.05	5	110	110	3.9	3.7

Linearity	Good linearity was observed in the range tested (0.035-5.0 ng/mL). Linear correlations with coefficients ≥0.994 were obtained. Eight calibration points distributed over the range given above were used. Calibration standards were prepared in an aqueous mixture of methanol and pure water (50/50, v/v).
Specificity	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis was fully validated for two different mass transitions.
Matrix effects	No significant matrix effects on LC-MS/MS response were observed for lettuce, grape, potato tuber, dry bean and oilseed rape seed; and significant matrix effects (i.e. >20% suppression or enhancement) were observed for onion and hop. Thus, calibration solutions in matrix were used for evaluation of the results.
Interference	The interferences/residues of the analytes measured in the control samples were below 20% of the LOQ for each matrix and each mass transition. Only in the dried bean control samples residues of Z-dimethomorph (both transitions) of 0.0015 mg/kg and 0.0014 mg/kg (corresponding to 30% of LOQ) were detected
Limit of quantitation	The limit of quantitation (LOQ) defined by the lowest fortification level successfully tested was 0.005 mg/kg for all matrices tested
Limit of detection	The limit of detection determined as 20% of the LOQ is 0.001 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. The detailed values are shown in Table 4.2-2.
Reproducibility	An independent laboratory validation has been successfully conducted and is reported under CA 4.2/2 (DocID 2015/1204836).

Stability of solutions

Both isomers of dimethomorph were stable in stock (methanol) and fortification solutions (acetone) as well as in calibration solution (S1) for at least 24 days when stored frozen in the dark.

The final sample extracts in methanol/water (1/1, v/v) were re-injected after 9-19 days of storage under frozen conditions. No significant decrease in stability in the stored extracts could be observed when the results were evaluated with freshly prepared calibration solutions in solvent for all matrices except for onion and hop cone, where an significant increase of both isomers was observed (>120% of initial recovery). For onion and hop cone matrix matched standards were used. Re-injection of final extracts resulted in recoveries within the acceptable valid range of 70-120%, except for the mentioned matrices onion and hop cone.

Conclusion

Analytical method L0321/01 (DFG method S19) is considered fully suitable for the analysis of both isomers of dimethomorph in different plant matrices (high water, high starch, high protein, high acid and high oil content) for enforcement purposes.

Remark: The originally in the application listed proposed enforcement method for animal matrices is not required as no additional analytes were included for monitoring purposes. Hence, no additional method was developed and no corresponding ILV is required. Details about the changes in M-CA points are given in Table 4.2-1. The already developed enforcement method for animal matrices and its ILV addressing dimethomorph, are presented below in M-CA 4.2/3 and 4.2/4.

Report:	CA 4.2/2 Taoudi M., 2015 a Independent laboratory validation for BASF method number L0321/01: DFG S19 Method for the determination of BAS 550 F residues in plant matrices using HPLC-MS/MS 2015/1204836
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340, OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

Residues of dimethomorph (BAS 550 F) are extracted from homogenized lettuce, onion, grape, potato tuber, hop cone, dried bean and oilseed rape seed samples according to extraction modules E1 (lettuce, onion, potato tuber), E2 (dried bean, hop cone), E3 (grape) and E7 (oilseed rape), respectively, to independently validate the DFG method S19 for *E*- and *Z*-dimethomorph. After extraction of potato tuber, hop cone, dried bean and oilseed rape seed, the specimen extracts were cleaned up by gel permeation chromatography.
All samples were analyzed by LC-MS/MS at mass transition 388 → 301 for quantitation and 388 → 165 for confirmation (ESI+). Analysis was accomplished on a Thermo Betasil C18 column applying a methanol-pure water gradient using 0.1% formic acid as modifier.

Recovery findings

In all matrices tested, the mean recovery values of *E*- and *Z*-dimetomorph were between 70% and 110%. Detailed results are given in table below.

Table 4.2-3: Validation results of method L0321/01 (DFG S19): dimethomorph (BAS 550 F) in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				**388 → 301**	**388 → 165**	**388 → 301**	**388 → 165**
E-dimethomorph (BAS 550 F)	Lettuce	0.005	6	97.5	102	7.4	5.6
		0.05	6	77.9	79.7	6.9	6.6
	Onion	0.005	6	107	108	4.4	4.8
		0.05	6	86.8	86.4	7.9	9.4
	Grape	0.005	6	97.8	97.3	2.6	3.4
		0.05	6	95.2	94.4	11	10
	Potato tuber	0.005	6	82.1	80.9	3.9	3.4
		0.05	6	80.0	79.0	13	14
	Hop cone	0.005	6	76.0	81.5	5.9	5.0
		0.05	6	83.4	83.5	8.2	8.4
	Dried bean	0.005	6	88.0	86.5	11	13
		0.05	6	89.5	89.4	8.4	9.7
	Oilseed rape (OSR) seed	0.005	5	78.0	76.8	10	10
		0.05	6	79.7	80.3	9.2	11
Transition				**388 → 301**	**388 → 165**	**388 → 301**	**388 → 165**
Z-dimethomorph (BAS 550 F)	Lettuce	0.005	6	105	108	2.9	2.1
		0.05	6	83.3	84.4	5.9	7.3
	Onion	0.005	6	103	102	5.8	6.0
		0.05	6	84.6	86.2	8.8	9.2
	Grape	0.005	6	91.5	91.3	3.4	3.5
		0.05	6	93.2	92.2	10	12
	Potato tuber	0.005	6	86.5	86.2	3.7	3.0
		0.05	6	82.1	81.8	14	13
	Hop cone	0.005	5	81.2	81.2	9.2	11
		0.05	6	86.0	85.8	8.9	9.0
	Dried bean	0.005	6	84.9	87.8	11	13
		0.05	6	82.5	83.3	8.8	9.0
	Oilseed rape (OSR) seed	0.005	6	80.0	80.1	9.1	12
		0.05	6	82.8	82.2	8.8	9.6

Linearity

Good linearity was observed in the range tested (0.035-5.0 ng/mL). Linear correlations with coefficients ≥0.996 were obtained. Eight calibration points distributed over the range given above were used. Calibration standards were prepared in an aqueous mixture of methanol and pure water (50/50, v/v).

Specificity

LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis was fully validated for two different mass transitions.

Matrix effects	No significant matrix effects (i.e. >20% suppression or enhancement) on LC-MS/MS response were except for dried bean and hop (*E*-dimethomorph) and in dried bean, hop and potato matrix (*Z*-dimethomorph). However, with regards to dried bean, hop and potato matrix matched standards were used for the evaluation.
Interference	No significant interference was observed. At elution time of the signals of interest, interference was below the required limit of 30% LOQ in all cases, as no signal was observed at elution times of the analytes of interest.
Limit of quantitation	The limit of quantitation (LOQ), defined by the lowest fortification level successfully tested was 0.005 mg/kg for all matrices.
Repeatability	The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. The detailed values are shown in Table 4.2-3.
Reproducibility	These results of the independent laboratory validation confirm the good results of the validation study reported above (DocID 2014/1186696).
Stability of solutions	The stability of the solutions was investigated in the appropriate validation study reported above (DocID 2014/1186696).
Conclusion	**Analytical method L0321/01 (DFG method S19) is considered fully suitable for the analysis of both isomers of dimethomorph in different plant matrices (high water, high starch, high protein, high acid and high oil content) for enforcement purposes.**

Information on the determination of dimethomorph in difficult matrices, such as for example tobacco and tea is given below. The scientific literature was considered reliable and the information provided was considered as useful additional information to the validated methodology provided by the Applicant.

Report: CA 4.2/3
Mayer-Helm B. et al., 2007 a
Method development for the determination of selected pesticides on tobacco by high-performance liquid chromatography-electrospray ionisation-tandem mass spectrometry
2008/1103056
Guidelines: none
GLP: no

Principle of the method: Residues of dimethomorph were extracted from water-soaked tobacco with methanol. After centrifugation, the supernatant was diluted with aqueous sodium chloride. This solution was applied to a ChemElute cartridge. After equilibration, dimethomorph was eluted with cyclohexane/toluene. The solvent was evaporated and the residue reconstituted in methanol/aqueous ammonium acetate solution. After filtration, residues were determined by HPLC-MS/MS in ESI+ mode. Ion transitions were m/z 388 → 301 for quantitation and 301 → 165 for confirmation. Analysis was accomplished using a Phenomenex Synergi 4 µm Hydro-RP column and a water/methanol gradient with ammonium acetate as modifier.

Recovery findings: In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.2-4: Validation results of HPLC-MS/MS method: dimethomorph (BAS 550 F) in tobacco matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average* recovery (%)		Standard deviation (%)	
Transition				388 → 301	301 → 165	388 → 301	301 → 165
Dimethomorph (BAS 550 F)	Burley	0.5-3.0	12	93	n.r.	3	n.r.
	Orient	0.5-3.0	12	79	n.r.	3	n.r.
	Virginia	0.5-3.0	12	93	n.r.	5	n.r.
	Snuff	0.5-3.0	12	90	n.r.	1	n.r.
	Cigarette	0.5-3.0	12	95	n.r.	4	n.r.

n.r. Not reported
* Mean values of three determinations on three different days

Linearity Good linearity was observed in the range tested (0.4-4.0 mg/kg). Linear correlations with coefficients ≥0.999 were obtained. Three calibration points distributed over the range given above were used. Calibration standards were prepared in acetonitrile.

Specificity LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.

Matrix effects A stringent sample purification step was used to eliminate matrix effects. Partition of methanolic extracts of tobacco on Chem Elut extraction cartridges and subsequent selective elution with a nonpolar solvent mixture (cyclohexane/toluene) provide clean elutes. Furthermore, matrix-matched standards were used.

Limit of quantitation The limit of quantitation defined as lowest fortification level successfully tested was 0.05 mg/kg for dimethomorph. However the publication suggests a pseudo-LOQ based on the signal to noise ratio of 0.05 mg/kg to 0.2 mg/kg, depending on the tobacco type.

Repeatability The relative standard deviations (RSD, %) for all commodities and fortification levels were <10%. The detailed values are shown in Table 4.2-4.

Reproducibility The inter-day accuracy test on Orient tobacco fortified at three levels (0.5, 1.5, 3 µg/kg) showed recoveries of 106-109% with standard deviations of 1-9% (see Table 4.2-5).

Table 4.2-5: Reproducibility of dimethomorph (BAS 550 F) residues on 3 different days in tobacco

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average* recovery (%)		Standard deviation (%)	
Transition				388 → 301	301 → 165	388 → 301	301 → 165
Dimethomorph (BAS 550 F)	Orient	0.5	3	109	n.r.	9	n.r.
		1.5	3	106	n.r.	6	n.r.
		3.0	3	107	n.r.	1	n.r.

n.r. Not reported
* Mean values of three determinations on three different days

Stability of solutions The tobacco extracts (solutions ready for injection) were stable for at least three days at 8°C.

Conclusion **The HPLC-MS/MS multi-residue analytical method can be used for the analysis of dimethomorph in difficult matrices (i.e. tobacco) for enforcement purposes.**

Report: CA 4.2/4
Wang J. et al., 2013 a
Determination of pesticide residue transfer rates (percent) from dried tea leaves to brewed tea
2014/1327433
Guidelines: none
GLP: no

Principle of the method Residues of dimethomorph were extracted from dried tea leaves and brewed tea with acetonitrile containing 1% acetic acid, manganese sulfate and sodium acetate. After concentration, reconstitution and filtration, extracts were diluted in case of tea leaves prior to UHPLC-MS/MS analysis in ESI+ mode. Ion transitions were m/z 388 → 301 for quantitation and 388 → 165 for confirmation. Analysis was accomplished using a Phenomenex Kinetex C_{18} column and an acetonitrile/aqueous acetonitrile gradient with ammonium acetate as modifier.

Recovery findings In brewed tea, the mean recovery value over both mass transitions was between 70% and 110%. The detailed results are given below.

Table 4.2-6: Validation results of QuEChERS method: dimethomorph (BAS 550 F) in tea matrices

Test substance	Matrix	Fortification level (µg/L)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph (BAS 550 F)	Brewed tea (Earl Grey, oolong, green tea, herbal tea, orange pekoe)	4.0	n.r.	97.2		14.7	
		12.0	n.r.				
		20.0	n.r.				
		32.0	n.r.				

n.r. Not reported

Linearity Calibration standards were prepared in methanol. Calibration was performed using 6 standards in the range of 0.4-40 µg/L. Information on linearity was not given.

Specificity LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.

Matrix effects	Graphitized black carbon helped to remove some pigment such as chlorophyll and catechins from dry tea leaves during sample clean-up. Matrix effects in brewed tea were evaluated by comparing the responses of pesticides in sample extracts to those pesticide standards prepared in solvent buffer at the same concentration. Between 21.4 and 49.7% of the pesticides in brewed tea showed ion suppression of <30% or enhancement ≤10%. Thus, matrix-matched standards were used.
Limit of quantitation	The limit of quantitation was not mentioned.
Repeatability	The relative standard deviation (RSD, %) over both mass transitions was <20% for dimethomorph. The detailed values are shown in Table 4.2-6.
Reproducibility	The measurement uncertainty was 29.6%, i.e. ≤50%, which was a recommended default value in SANCO/12495/2011 for pesticide analysis and enforcement decisions (MRL exceedances).
Conclusion	**The QuEChERS multi-residue analytical method can be used for the analysis of dimethomorph in difficult matrices (i.e. tea) for enforcement purposes.**

Report:	CA 4.2/5 Strobl M., 2009 a Validation of BASF method L0138/01 - Method for the determination of Dimethomorph (BAS 550 F) in animal matrices 2009/1051339
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Remark: BASF method L0138/01 is the current proposed enforcement and monitoring method for animal matrices.

Principle of the method

Dimethomorph (BAS 550 F, Reg. No 247723) is extracted with methanol/water/hydrochloric acid. A defined aliquot is partitioned twice against cyclohexane at alkaline conditions (1 mL 0.2 N sodium hydroxide). The combined cyclohexane extracts are evaporated to dryness and dissolved in a methanol/water mixture. After liquid-liquid partitioning the final determination of dimethomorph is performed by HPLC-MS/MS at two transitions. Analysis was accomplished using a Betasil C_{18} column and a methanol-pure water gradient with formic acid as modifier. Detection was accomplished in ESI+ mode at mass transitions 388 → 301 for quantitation and 388 → 165 for confirmation. Both, the *E*- and *Z*-isomer are separated by the chromatographic system; however, quantitation was accomplished as the sum of both signals.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-7: Validation results of method L0138/01: dimethomorph (BAS 550 F) in animal matrices

Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Egg	Dimethomorph (m/z 388 → 301)	0.01 0.1	100 101	2 2	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	98 101	2 2	5 5
Fat	Dimethomorph (m/z 388 → 301)	0.01 0.1	95 105	1 3	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	97 105	3 2	5 5
Kidney	Dimethomorph (m/z 388 → 301)	0.01 0.1	100 98	4 3	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	98 99	4 4	5 5
Liver	Dimethomorph (m/z 388 → 301)	0.01 0.1	97 99	5 3	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	98 99	2 3	5 5
Milk	Dimethomorph (m/z 388 → 301)	0.01 0.1	104 101	3 5	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	105 103	3 5	5 5
Muscle	Dimethomorph (m/z 388 → 301)	0.01 0.1	102 97	3 5	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	103 99	3 5	5 5

Linearity Good linearity was observed over the concentration range tested. Linear correlations with coefficients >0.99 were obtained for dimethomorph. Seven calibration points distributed over a concentration range of 0.01 to 1 ng/mL were used. Calibration standards were prepared in methanol.

Specificity LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required as two different, highly specific mass transitions are used for quantitation and qualification.

Matrix effects No matrix effects are reported.

Interference No significant interference were reported at elution times of the analytes of interest (interference <30% LOQ).

Limit of quantitation The limit of quantitation was 0.01 mg/kg in all matrices tested.

Limit of detection The limit of detection was 0.001 mg/kg for dimethomorph in milk, egg, muscle and liver, based on the lowest calibration standard of 0.01 ng/mL.

Repeatability The relative standard deviations for dimethomorph (BAS 550 F) obtained from fortified egg, fat, kidney, liver, milk and muscle samples were below 20% at both fortification levels.

Reproducibility An independent laboratory validation has successfully been conducted and is reported below (DocID 2009/1078429).

Stability of solutions Dimethomorph was proven to be stable in extracts from animal matrices for at least 9 days, if stored under refrigerator conditions (see Table 4.2-8). The final volume is stable for at least 10 days, if stored under refrigerator conditions (see Table 4.2-9).

Table 4.2-8: Extract stability of dimethomorph (BAS 550 F) in animal matrices fortified at 0.1 mg/kg stored refrigerated (5°C).

Test substance	Matrix	Time interval (days)	No of tests	Average recovery (%)	Relative standard deviation (%)
Dimethomorph	Egg	0	3	102	2.4
		3	3	101	0
		7	3	106	2.7
		9	3	102	3.8
	Liver	0	3	98	3.6
		3	3	101	2.1
		7	3	93	6.3
		9	3	95	2.3

Table 4.2-9: Final volume stability of dimethomorph (BAS 550 F) in animal matrices fortified at 0.1 mg/kg stored refrigerated (5°C).

Test substance	Matrix	Time interval (days)	No of tests	Average recovery (%)	Relative standard deviation (%)
Dimethomorph	Egg	0	3	102	2.4
		3	3	99	3.1
		7	3	101	2.2
		10	3	99	2.2
	Liver	0	3	98	3.6
		3	3	98	1.1
		7-8	3	96	1.8
		10	3	93	5.6

Conclusion **The data generated during the laboratory validation confirmed that the residue analytical method L0138/01 provides a reliable, sensitive and efficient determination of dimethomorph in animal matrices by LC-MS/MS with a limit of quantitation of 0.01 mg/kg. This method is proposed as enforcement method**.

Report: CA 4.2/6
Wolf S., 2009 a
Independent laboratory validation (ILV) of an analytical method for the determination of Dimethomorph (BAS 550 F) in animal tissues (milk, egg, muscle and liver)
2009/1078429

Guidelines: EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Principle of the method Purpose of this study was to independently validate the proposed enforcement method L0138/01 for animal matrices. Dimethomorph (BAS 550 F) was extracted with a mixture of methanol/water/hydrochloric acid. An aliquot of the extract was centrifuged and partitioned twice against cyclohexane at alkaline conditions. The combined cyclohexane extracts were evaporated to dryness and dissolved in a methanol/water mixture. The final determination of dimethomorph was performed by LC-MS/MS at two transitions. Analysis was accomplished using a BETASIL C18-colum and an elution gradient of water and methanol with 0.1% formic acid as modifier. Detection was done in ESI+ mode [$M+H^+$]. Transition m/z 388 → 301 is proposed as target transition for quantitation and transition m/z 388 → 165 for confirmatory purposes.

Recovery findings In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-10: Recovery results from independent method validation of dimethomorph (BAS 550 F) in animal matrices

Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Milk	Dimethomorph (m/z 388 → 301)	0.01 0.1	98 93	9 4	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	97 94	7 2	5 5
Egg	Dimethomorph (m/z 388 → 301)	0.01 0.1	98 98	3 3	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	96 98	7 2	5 5
Muscle	Dimethomorph (m/z 388 → 301)	0.01 0.1	91 92	8 6	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	87 90	7 5	5 5
Liver	Dimethomorph (m/z 388 → 301)	0.01 0.1	98 98	3 4	5 5
	Dimethomorph (m/z 388 → 165)	0.01 0.1	95 96	4 3	5 5

Linearity	Good linearity was observed over the concentration range tested. Linear correlations with coefficients >0.99 were obtained for dimethomorph. Seven calibration points distributed over a concentration range of 0.01 to 1 ng/mL were used. Calibration standards were prepared in a mixture of methanol and pure water (50/50 v/v).
Specificity	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.
Interference	No interferences above 30% of the LOQ at the retention times of the two dimethomorph peaks were detected in the untreated control samples.
Matrix effects	No matrix effects are reported.
Limit of quantitation	The limit of quantitation was 0.01 mg/kg in all matrices tested.
Limit of detection	The limit of detection was 0.001 mg/kg for dimethomorph in milk, egg, muscle and liver, based on the lowest calibration standard of 0.01 ng/mL.
Repeatability	The overall relative standard deviations for dimethomorph (BAS 550 F) obtained from fortified egg, liver, milk and muscle samples were below 20% at both fortification levels.
Reproducibility	These results of the independent laboratory validation confirm the good results of the validation study reported above (DocID 2009/1051339).
Conclusion	**For dimethomorph the analytical method L0138/01 was successfully independently validated in animal tissues (milk, egg, muscle and liver) by analyzing two blank control samples, five replicates fortified at LOQ (0.01 mg/kg) and five replicates fortified at 10 times LOQ (0.10 mg/kg).**

Information on determination of dimethomorph in animal matrices. The scientific literature was considered reliable and the information provided was considered as useful additional information to the validated methodology provided by the Applicant as information on the feasibility of a multimethod approach for analysis of animal and ecotoxicological matrices is presented (M-CA 4.2./7 and 8).

Report: CA 4.2/7
Taylor M.J. et al., 2012 a
A liquid chromatography-electrospray tandem mass spectrometry method for the determination of multiple pesticide residues involved in suspected poisoning of non-target vertebrate wildlife, livestock and pets
2013/1420620
Guidelines: none
GLP: no

Principle of the method Residues of dimethomorph were extracted from homogenized samples with ethyl acetate. After filtration, the extract was evaporated and diluted in cyclohexane and ethyl acetate. An aliquot was diluted in methanol, evaporated again and diluted with methanol containing ammonium acetate. After filtration, analysis was performed by LC-MS/MS in ESI+ mode. Ion transitions were m/z 388 → 301 for quantitation and 388 → 165 for confirmation. Analysis was accomplished using a Hypersil Gold C_{18} BDS column and a methanol/aqueous methanol gradient with ammonium acetate as modifier.

Recovery findings In both matrices tested, the mean recovery values were between 70% and 110% at both fortification levels. The detailed results are given below.

Table 4.2-11: Validation results of LC-MS/MS method: dimethomorph (BAS 550 F) in chicken matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph (BAS 550 F)	Muscle	0.1	6	81	n.r.	4.2	n.r.
		1	6	72	n.r.	5.6	n.r.
	Liver	0.1	5	78	n.r.	3.3	n.r.
		1	6	86	n.r.	15.7	n.r.

n.r. Not reported

Linearity	Good linearity was observed over the concentration range tested. Linear correlations with coefficients ≥0.96 were. Four calibration points distributed over a concentration range of 0.025 to 0.5 µg/mL were used. Calibration standards were prepared in methanol.
Specificity	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.
Matrix effects	The matrix-matched standards used were 0.025 mg/ml; 0.1 mg/ml; 0.2 mg/ml; 0.5 mg/ml. Signal:noise value was >5:1.
Limit of quantitation	The limit of quantitation defined by the lowest fortification level successfully tested was 0.1 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for both matrices were <20% for the fortification level of 0.1 mg/kg and below 15% for the level of 1 mg/kg, except in liver. The detailed values are shown in Table 4.2-11.
Reproducibility	The method was found also suitable for the analysis of digestive tract content and blood, vomit and fecal specimens. In addition, the method is routinely and successfully applied to the analysis of carcasses, meat or eggs.
Stability of solutions	Extract stability was assessed for some analytes not including dimethomorph for a period of 7 days at 5°C.
Conclusions	**The LC-MS/MS multi-residue analytical method can be used for the analysis of dimethomorph in animal matrices for enforcement purposes.**

Report: CA 4.2/8
Kujawski M.W. et al., 2013 a
Determining pesticide contamination in honey by LC-ESI-MS/MS - Comparison of pesticide recoveries of two liquid-liquid extraction based approaches
2014/1327434
Guidelines: none
GLP: no

Principle of the method Two extraction methods were compared; 1) solid supported liquid-liquid extraction (SLE) and 2) QuEChERS extraction method. In the first approach honey sample preparation was performed using SLE with a modified agitation time.
In the QuEChERS approach, residues of dimethomorph were extracted from homogenized honey samples with acetonitrile and 4 µg/kg triphenyl phosphate and several salts. After liquid-liquid partition with n-hexane, the acetonitrile phase was filtered.
Analysis was performed by LC-MS/MS in ESI+ mode. Ion transitions were m/z 388 → 301 for quantitation and 388 → 165 for confirmation. Analysis was accomplished using a Phenomenex Kinetex C_{18} column and a water/methanol gradient with ammonium acetate as modifier.

Recovery findings In honey, the mean recovery values were between 70% and 120% at the 0.0375 mg/kg fortification level and 59-66% at the 0.5 mg/kg fortification level. The detailed results are given below.

Table 4.2-12: Validation results of QuEChERS method: dimethomorph (BAS 550 F) in honey

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 301	388 → 165	388 → 301	388 → 165
Dimethomorph (BAS 550 F)	Honey	0.0375[1]	5	70	n.r.	16	n.r.
		0.0375[2]	5	71	n.r.	8.4	n.r.
		0.050[1]	5	59	n.r.	14	n.r.
		0.050[2]	5	66	n.r.	12	n.r.

n.r. Not reported
[1] QuEChERS extraction
[2] SLE extraction

Linearity	Good linearity was observed in the range tested (0.00387 or 0.00346-0.2 mg/kg). Linear correlations with coefficients ≥0.9916 were obtained.
Specificity	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.
Matrix effects	Matrix-matched calibration standards were used, compensating for changes in ionization efficiency of analytes caused by matrix constituents.
Limit of quantitation	The limit of quantitation defined by the lowest fortification level successfully tested was 0.0375 mg/kg. However, a pseudo-LOQ based on the signal to noise ratio of 0.00387 mg/kg with QuEChERS and 0.00346 mg/kg with SLE was suggested.
Limit of detection	The limit of detection was 0.00118 mg/kg with QuEChERS and 0.00105 mg/kg with SLE.
Repeatability	The relative standard deviations (RSD, %) for both methods and fortification levels were <20%. The detailed values are shown in Table 4.2-12.
Conclusion	**The LC-MS/MS multi-residue analytical method can be used for the analysis of dimethomorph in honey for enforcement purposes if deemed necessary.** **Modified QuEChERS approach seems to have an advantage over the SLE method, because of smaller volumes of less toxic extraction solvent used in the sample preparation step, and also in regard to time consumption, as the procedure does not require evaporation to dryness.**

(b) Methods for the analysis in soil and water

~~**Report:** CA 4.2/9~~
~~Davis P.P., 2015 a~~
~~Independent laboratory validation of BASF analytical method L0257/01 (D1410): Method for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water at LOQ of 0.05 ppb using LC/MS/MS~~
~~2014/7004121~~
~~**Guidelines:** EPA 850.6100, EPA 835.6200, SANCO/825/00 rev. 8.1 (16 November 2010)~~
~~**GLP:** yes~~
~~(certified by United States Environmental Protection Agency)~~

Report: CA 4.2/9
Guo D. Davis P.P., 2016 a
Independent laboratory validation of BASF analytical method L0257/01 (D1410): Method for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water at LOQ of 0.05 ppb using LC/MS/MS
2016/7006199
Guidelines: EPA 850.6100, EPA 835.6200, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP: yes
(certified by United States Environmental Protection Agency)

Principle of the method

BASF Analytical Method L0257/01 (01410) was developed to determine the residues of dimethomorph (BAS 550 F), *E*-dimethomorph (Reg. No. 4110868) and *Z*-dimethomorph (Reg. No. 4110869) in drinking and surface water.
Residues of dimethomorph in water matrices are determined by direct injection analysis using HPLC (Atlantis T3 column, mobile phases: water/formic acid and methanol/formic acid) with MS/MS-ESI+ detection. Ion transitions were m/z 388 → 301 for quantitation and 388 → 165 for confirmation.

Recovery findings

In both matrices tested, the mean recovery values were between 70% and 110% at both fortification levels and for both mass transitions. Detailed results are presented in the tables below.

Table 4.2-13: Recoveries for BAS 550 F (E) and BAS 550 F (Z) in Drinking Water

Matrix	Fortification levels (µg L^{-1})	n	Recovery (%)	Mean recovery (%)	Standard deviation	RSD[a] (%)
Drinking water	BAS 550 F (E) : Primary transition (m/z 388 → 301)					
	0.05	5	102.0, 102.0, 103.2, 101.6, 108.8	103.5	3.0	2.9
	0.5	5	96.4, 96.4, 96.8, 97.2, 102.0	97.8	2.4	2.4
	Overall	**10**	**Range: 96.4 – 108.8**	**100.6**	**4.0**	**3.9**
	BAS 550 F (E) : Confirmative transition (m/z 388 → 165)					
	0.05	5	104.0, 101.8, 103.0, 101.8, 109.2	**104.0**	**3.1**	**3.0**
	0.5	5	96.4, 96.2, 96.6, 98.6, 102.6	**98.1**	**2.7**	**2.8**
	Overall	**10**	**Range: 96.2 – 109.2**	**101.0**	**4.1**	**4.1**
	BAS 550 F (Z) : Primary transition (m/z 388 → 301)					
	0.05	5	103.0, 103.2, 104.2, 103.8, 107.8	104.4	2.0	1.9
	0.5	5	97.8, 98.0, 99.0, 99.0, 103.2	99.4	2.2	2.2
	Overall	**10**	**Range: 97.8 – 107.8**	**101.9**	**3.3**	**3.2**
	BAS 550 F (Z) : Confirmative transition (m/z 388 → 165)					
	0.05	5	100.4, 101.8, 103.2, 103.8, 107.0	103.2	2.5	2.4
	0.5	5	97.0, 98.2, 98.0, 98.8, 102.8	99.0	2.2	2.3
	Overall	**10**	**Range: 97.0 – 107.0**	**101.1**	**3.2**	**3.1**

[a] Relative Standard Deviation = (Standard Deviation ÷ Mean Recovery) × 100

Table 4.2-14: Recoveries for BAS 550 F (E) and BAS 550 F (Z) in Surface (Pond) Water

Matrix	Fortification levels (µg L^{-1})	n	Recovery (%)	Mean recovery (%)	Standard deviation	RSD[a] (%)
Surface water	BAS 550 F (E) : Primary transition (m/z 388 → 301)					
	0.05	5	104.2, 104.4, 104.0, 105.6, 106.0	104.8	0.9	0.9
	0.5	5	102.6, 101.8, 102.8, 101.6, 103.8	102.5	0.9	0.9
	Overall	**10**	**Range: 101.6 – 106.0**	**103.7**	**1.5**	**1.4**
	BAS 550 F (E) : Confirmative transition (m/z 388 → 165)					
	0.05	5	106.6, 104.2, 103.6, 103.6, 103.6	104.3	1.3	1.2
	0.5	5	103.0, 102.2, 102.0, 102.2, 103.8	102.6	0.8	0.7
	Overall	**10**	**Range: 102.0 – 106.6**	**103.5**	**1.3**	**1.3**
	BAS 550 F (Z) : Primary transition (m/z 388 → 301)					
	0.05	5	104.8, 104.6, 105.8, 106.6, 106.6	105.7	1.0	0.9
	0.5	5	104.2, 102.2, 103.0, 102.6, 104.0	103.2	0.9	0.8
	Overall	**10**	**Range: 102.2 – 106.6**	**104.4**	**1.6**	**1.5**
	BAS 550 F (Z) : Confirmative transition (m/z 388 → 165)					
	0.05	5	106.8, 106.6, 105.6, 106.8, 108.4	106.8	1.0	0.9
	0.5	5	103.8, 102.2, 103.4, 102.8, 105.2	103.5	1.1	1.1
	Overall	**10**	**Range: 102.2 – 108.4**	**105.2**	**2.0**	**1.9**

[a] Relative Standard Deviation = (Standard Deviation ÷ Mean Recovery) × 100

Linearity	Good linearity ($r^2 > 0.99$) was observed in the range of 0.01 ng mL ^{1} to 1 ng mL^{-1} using solvent-based standards (prepared in water).
Specificity	Method L0257/01 (D1410) determines residues of BAS 550 F (E) and BAS 550 F (Z) in surface and drinking water. No interfering peaks were found at the retention times of each analyte. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Analysis is possible at two different mass transitions.
Matrix effects	Since the method requires no sample extraction or clean-up, the method validation analyses serves to evaluate the effect of matrix load on the analysis. The sample recoveries show that the matrix load in the samples has no influence on analysis; therefore, the validation samples are analyzed using solvent-based calibration standard solutions.
Limit of quantitation	The limit of quantitation (LOQ) was defined as the lowest fortification level tested. For this method, the LOQ was 0.05 µg L^{-1} for both analytes in both matrices.
Limit of detection	The limit of detection (LOD) was set at the lowest calibration standard, which was 0.01 µg L^{-1} (20% of LOQ).
Repeatability	The overall relative standard deviation (RSD, %) for all fortification levels was less than 5%.
Standard stability	Not analysed in this ILV as already addressed in the validation report.

Extract stability Not analysed in this ILV as already addressed in the validation report.

Reproducibility Inter run reproducibility of the method was not determined within this validation study.

Conclusion

This ILV was completed successfully on the first trial for the determination of residues of dimethomorph (BAS 550 F), *E*-dimethomorph (Reg. No. 4110868) and *Z*-dimethomorph (Reg. No. 4110869), in water matrices (surface and drinking water). All mean recoveries were within the acceptable range (70-120%).

The results of this method validation study demonstrate that BASF Analytical Method No L0257/01 (D1410) fulfils the requirements with regard to specificity, repeatability, limit of quantitation, and recoveries and is, therefore, applicable to correctly determine residues of dimethomorph (*E*-dimethomorph and *Z*-dimethomorph) in water matrices (surface and drinking water).

~~**Report:**~~	~~CA 4.2/10~~ ~~Woessner A., 2015 a~~ ~~Validation of a lower LOQ (15 ng/L) of Method D1410 (L0257/01) for the determination of the geometric isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in water using LC/MS/MS~~ ~~2015/1237993~~
~~**Guidelines:**~~	~~none~~ ~~EC 1107/2009 of the European Parliament, SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100 (2012),EPA 860.1340~~
~~**GLP:**~~	~~yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)~~

~~**Report:**~~	~~CA 4.2/10~~ ~~Woessner A., 2016 a~~ ~~Indendent laboratory validation of the analytical method for the determination of BAS 550 F in surface water and ground water~~ ~~2016/1118111~~
~~**Guidelines:**~~	~~EC 1107/2009 of the European Parliament, SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100 (2012), EPA 860.1340~~
~~**GLP:**~~	~~yes~~ ~~(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)~~

Report:	CA 4.2/10 Woessner A., 2017 a Indendent laboratory validation of the analytical method for the determination of BAS 550 F in surface water and ground water 2017/1069823
Guidelines:	EC 1107/2009 of the European Parliament, SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100 (2012), EPA 860.1340
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Remark: ~~At the time of submission of the dossier, the final report of the study has not yet been issued. However, results already available confirm successful independent laboratory validation of the lower LOQ of 15 ng/L per isomer. Available validation data is provided below. The final report will be submitted in Q1/2016. The available data at time of submission is presented below to confirm that the method has been successfully confirmed by an independent laboratory validation.~~

~~No matrix effect were observed so calibration was done using solvent based standard solutions. Matrix effects observed were below 25% signal enhancement. Calibration was done using matrix matched standards. Good linearity (r>0.995) was given over a concentration range from 0.004 ng/L to 0.25 ng/mL. Stability of working solutions of extracts / water samples has not been assessed as this had already been assessed during the original method validation.~~

Remark: The revised report does not contain any altered data of analysis. The certificates of analysis of the two water matrices are corrected.

Principle of the method BASF Analytical Method L0257/01 (D1410) was developed to determine the residues of the geometric isomers of dimethomorph (BAS 550 F), *E*-dimethomorph (Reg. No. 4110868) and Z-dimethomorph (Reg. No. 4110869) in drinking and surface water. Residues of dimethomorph in water matrices are determined by direct injection analysis using HPLC (Atlantis T3 column, mobile phases: water/formic acid and methanol/formic acid) with MS/MS-ESI+ detection. Two ion transitions (for quantitation and confirmatory purposes) are observed for the detection.

Recovery findings The mean recoveries in drinking water and surface water for both geometric isomers were within the required valid range of 70-120% with a relative standard deviation of below 20% for BAS 550 F (E) and BAS 550 F (Z) at the two fortification levels tested (0.015 µg/L and 0.15 µg L^{-1}), in both water types. Detailed results are presented in the tables 4.2-15 and 4.2-16.

Table 4.2-15: Recoveries for BAS 550 F (Z) and BAS 550 F (E) in Ground (Drinking) Water

Matrix	Fortification levels (µg L^{-1})	n	Recovery (%)	Mean recovery (%)	Standard deviation	RSD[a] (%)
Ground water	BAS 550 F (Z) : Primary transition (m/z 388 → 301)					
	0.015	5	119, 119, 118, 119, 117	118	0.9	1
	0.15	5	119, 120, 119, 119, 119	119	0.4	0.4
	Overall	**10**	**Range:**		**117 to 120**	
	BAS 550 F (Z) : Secondary transition (m/z 388 → 165)					
	0.015	5	116, 120, 116, 116, 120	118	2	2
	0.15	5	118, 119,117, 115, 117	117	1	1
	Overall	**10**	**Range:**		**116 to 120**	
	BAS 550 F (E) : Primary transition (m/z 388 → 301)					
	0.015	5	115, 118, 114, 113, 114	115	2	2
	0.15	5	117, 119, 117, 115, 117	117	1	1
	Overall	**10**	**Range:**		**113 to 119**	
	BAS 550 F (E) : Secondary transition (m/z 388 → 165)					
	0.015	5	115, 121, 114, 111, 115	115	4	3
	0.15	5	119, 122, 119, 115, 118	119	3	2
	Overall	**10**	**Range:**		**115 to 121**	

[b] Relative Standard Deviation = (Standard Deviation ÷ Mean Recovery) × 100

Table 4.2-16: Recoveries for BAS 550 F (Z) and BAS 550 F (E) in Surface Water

Matrix	Fortification levels (µg L^{-1})	n	Recovery (%)	Mean recovery (%)	Standard deviation	RSD[a] (%)
Surface water	BAS 550 F (Z) : Primary transition (m/z 388 → 301)					
	0.015	5	114, 113, 116, 118, 121	116	3	3
	0.15	5	107, 110, 112, 113, 113	111	3	2
	Overall	**10**	**Range:**		**113 to 121**	
	BAS 550 F (Z) : Secondary transition (m/z 388 → 165)					
	0.015	5	113, 116, 118, 120, 119	117	3	2
	0.15	5	107, 110, 111, 113, 114	111	3	2
	Overall	**10**	**Range:**		**107 to 120**	
	BAS 550 F (E) : Primary transition (m/z 388 → 301)					
	0.015	5	111, 109, 113, 116, 117	113	3	3
	0.15	5	103, 106, 108, 109, 111	107	3	3
	Overall	**10**	**Range:**		**103 to 117**	
	BAS 550 F (E) : Secondary transition (m/z 388 → 165)					
	0.015	5	108, 109, 114, 113, 113	111	3	2
	0.15	5	104, 105, 107, 110, 110	107	3	3
	Overall	**10**	**Range:**		**104 to 114**	

[b] Relative Standard Deviation = (Standard Deviation ÷ Mean Recovery) × 100

Linearity Good linearity (r^2 > 0.9990) was observed in the range of 0.004 ng mL^{-1} to 25 ng mL^{-1} using matrix-matched standards (prepared in the respective water matrix type) for both geometric isomers and both mass transitions.

Specificity Method L0257/01 (D1410) determines residues of BAS 550 F (E) and BAS 550 F (Z) in surface and drinking water. No interfering peaks were found at the retention times of each analyte. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects Matrix effects on the detection of both geometric isomers of Dimethomorph were found to be insignificant (< 20 %) for ground water and significant for surface water (> 20 %). Hence, matrix-matched standards were used for quantification of both geometric isomers in both matrices to account for any potentially occurring matrix effect in both matrices.

Limit of quantitation The limit of quantitation (LOQ) is defined as the lowest fortification level tested. For this method, the LOQ is 0.015 µg L^{-1} for both analytes in both matrices.

Limit of detection	The limit of detection (LOD) is equivalent to 0.004 µg L^{-1} which corresponds to 27% of the LOQ.
Repeatability	The overall relative standard deviation (RSD, %) for all fortification levels was less than 5%.
Standard stability	Standard stability was assessed in study BASF DocID 2014/7003705 (CA 4.1.2/4).
Extract stability	Extract stability was assessed in study BASF DocID 2014/7003705 (CA 4.1.2/4).
Reproducibility	Inter run reproducibility of the method was not determined within this validation study.
Conclusion	**It could be demonstrated that BASF Method L0257/01 (D1410) fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is therefore applicable to correctly determine residues of Dimethomorph (BAS 550 F) in surface and ground water with a LOQ of 0.015 µg/L per Isomer (E-Isomer Reg.No. 4110868 and Z-Isomer Reg.No. 4110869). No addition or modification to the original method L0257/01 (D1410) other than the necessary optimization of instrumental parameters to set up the analytical parameters on the LC-MS/MS system was required.**

(c) Methods for the analysis in air

A new method for air was developed as the peer-reviewed method from Weitzel 1995 (AIIA-4.2.1; DAR 2004) applying GC-PND would not comply with the requirements of the updated current guidelines (specificity). The previously submitted additional method for air (Class 1999, AIIA-4.2.1) was not considered fully valid in the DAR dated 2004, as it did not fulfill the requirements for residue analytical methods. Hence, a new method was developed as is presented as proposed new monitoring method in air.

Report:	CA 4.2/11 Mende P., 2015 a BAS 550 F (Dimethomorph) - Validation of BASF analytical method for the determination in air 2015/1192602
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method for the determination of dimethomorph (BAS 550 F) in air, was validated at Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany.
The analyte dimethomorph is spiked onto the front filter of an adsorbent tube. The tubes are then placed into an acclimatized chamber without light. Air is sucked at 1 L min^{-1} for 8 hours through the tubes at 35 ± 2 C and a relative humidity of ≥ 80%. After sucking air through the tubes, dimethomorph is extracted from the adsorbent material with acetone. The residue is determined by using HPLC-MS/MS monitoring two mass transitions. Analysis is accomplished using a Waters Atlantis T3 column and a water methanol gradient with formic acid as modifier. The *E*- and the *Z*-isomer of dimethomorph are quantified separately.

The limit of quantitation of the method is 3 µg m^{-3} in air for the sum of both isomers of dimethomorph; the limit of detection is 0.3 µg m^{-3} for the sum of both isomers of dimethomorph (about 0.15 µg m^{-3} for each isomer).

Recovery findings

The method is suitable to determine dimethomorph in air. Samples are fortified with dimethomorph (sum of *E*- and *Z*-isomer) at fortification levels of 1.44 µg per tube (3 µg m^{-3} air) and 14.4 µg per tube (30 µg m^{-3} air). The mean recovery values were between 92% and 99% for BAS 550 F (sum of *E*- and *Z*-isomer). Mean recovery values for the *E*-isomer were between 85% and 91% and for the *Z*-isomer between 99% and 106% (see tables below).

Table 4.2-17: Results of method validation of *E*-isomer of dimethomorph in air

Analyte	Mass transition	Fortification level [µg m^{-3}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
E-dimethomorph	388 → 301	1.37	5	91	10	88	10
		13.7	5	85	10		
	388 → 165	1.37	5	91	10	88	10
		13.7	5	85	10		

RSD Relative standard deviation

Table 4.2-18: Results of method validation of *Z*-isomer of dimethomorph in air

Analyte	Mass transition	Fortification level [µg m^{-3}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
Z-dimethomorph	388 → 301	1.63	5	106	10	102	10
		16.3	5	99	9		
	388 → 165	1.63	5	105	10	102	10
		16.3	5	99	10		

RSD Relative standard deviation

Table 4.2-19: Results of method validation of dimethomorph (sum of *E*- and *Z*-isomer) in air

Analyte	Mass transition	Fortification level [µg m-^{3}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
dimethomorph	388 → 301	3	5	99	10	96	10
		30	5	93	10		
	388 → 165	3	5	99	10	96	10
		30	5	92	10		

RSD Relative standard deviation

Linearity

Acceptable linearity was observed for the standard range and the two mass transitions tested for both isomers of dimethomorph: The method-detector response was linear within the range of 0.25 to 5 ng mL^{-1} ($r > 0.999$) using solvent-based standards (diluted in acetonitrile/water, 1:1, v/v).

Specificity	The retention times of the two isomers of dimethomorph in extracts matched the retention times in solvent and no peak interferences occurred at the retention times of both isomers. As HPLC-MS/MS is regarded a highly-specific detection method when two ion transitions have been validated, an additional confirmatory method or technique is not required.
Matrix effects	The influence of the matrix on the analysis was studied by comparing the response of the analyte prepared in standard solutions with the analyte prepared in blank matrix extracts (matrix-matched standards). No significant matrix effects (<15%) were found.
Limit of quantitation	The limit of quantitation (LOQ) was defined by the lowest fortification level successfully tested. The LOQ of the method is 3 µg/m^3 (sum of *E*- and *Z*-isomer of dimethomorph) corresponding to a concentration of about 3 ng mL^{-1} of each isomer in the extract (if a dilution factor of 50 is applied).
Limit of detection	The limit of detection of the method is 0.3 µg m^{-3} (sum of *E*- and *Z*-isomer of dimethomorph) corresponding to a concentration of 0.5 ng mL^{-1} in the extract.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were 10%.
Standard stability	Dimethomorph dissolved in acetonitrile/water (1:1, v/v) was stable for up to 19 days of storage in a refrigerator (99% to 100% of initial value found, calculated for the sum of isomers). A significant change of the isomer ratio was observed over this period, resulting in recoveries of 82% for the *E*-isomer and 117% for the *Z*-isomer.
Extract stability	The stability in tubes and extracts was checked over a period of 19 days, and the storage of tubes in the freezer up to 29 days. Storage of tubes (at ambient temperature or in a refrigerator) and extracts (in a refrigerator or in a freezer) was possible for up to 19 days without significant loss of test item. In two of three tube samples stored deep-frozen, a change of the isomer ratio was observed, resulting in 79% recovery of the *E*-isomer and up to 125% of the *Z*-isomer, with no significant change regarding the sum of isomers (104% recovery).
Reproducibility	The reproducibility of the method was not determined as no ILV is required.

Conclusion	**The analytical method for analysis of dimethomorph in air uses HPLC-MS/MS for the final determination, with a limit of quantitation of 3 µg m^{-3} air (sum of *E*- and *Z*-isomer of dimethomorph).** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of dimethomorph in air.**

(d) Methods for the analysis in body fluids and tissues

As dimethomorph is not classified as toxic, no requirement for a stand-alone validation of an analytical method in body fluids is required according to SANCO/825/00, rev. 8.1. However, in the scientific literature some publications were found which are considered as useful additional information. Relevant hits in the literature search are summarised and listed below. Sufficient information is provided to screen body fluids for the potential presence of dimethomorph.

Report: CA 4.2/12
Hyung-seung K. et al., 2014 a
General unknown screening for pesticides in whole blood and Korean gastric contents by liquid chromatography-tandem mass spectrometry
2014/1327432
Guidelines: none
GLP: no

Principle of the method: Residues of dimethomorph were extracted from blood and gastric contents using methanol/acetonitrile (30:70, v/v) with formic acid (0.4%). Extraction buffer salt was added and the mixture was centrifuged. For cleanup, an SPE sorbent was used. The clean extract was mixed with water and filtered before UPLC-MS/MS in ESI+ mode. Analysis was accomplished using an Acquity UPLC BEH C^{18} column and a water/methanol gradient with formic acid as modifier.

Recovery findings: In blood, the mean recovery values were between 70% and 110%. In gastric contents, the mean recovery values were between 142% and 130%.

Table 4.2-20: Validation results of mini-QuEChERS method: dimethomorph (BAS 550 F) in blood and gastric contents

Test substance	Matrix	Fortification level (ng mL^{-1})	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				388 → 165	388 → 301	388 → 165	388 → 301
Dimethomorph (BAS 550 F)	Blood	50	3	106.37	n.r.	n.r.	n.r.
		100	3	93.51	n.r.	n.r.	n.r.
	Gastric contents	50	3	142.23	n.r.	n.r.	n.r.
		100	3	129.91	n.r.	n.r.	n.r.

n.r. Not reported

Linearity:	Good linearity was observed over a concentration range of 5 to 200 µg mL^{-1} (6 concentration levels) for dimethomorph with correlation coefficients ≥0.988 for the whole blood and ≥0.990 for gastric contents.
Specificity:	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.
Matrix effects:	A modified QuEChERS that uses dispersive solid phase extraction for a small amount of sample, mini-QuEChERS, was applied to blood and gastric contents to reduce matrix effect.
Limit of quantitation:	The limit of quantitation was between 2.5–50 ng mL^{-1} for all pesticides analyzed (215); details for dimethomorph were not given.
Repeatability:	The relative standard deviations (RSD, %) were not reported.
Reproducibility:	The linear range and correlation coefficient (r2) by Q/TOF mass spectrometry were not much inferior to triple quadrupole mass spectrometry.
Conclusion:	**The HPLC-MS/MS multi-residue analytical method can be used for the analysis of dimethomorph in blood if deemed necessary.**

Report: CA 4.2/13
Cazorla-Reyes R. et al., 2011 a
Single solid phase extraction method for the simultaneous analysis of polar and non-polar pesticides in urine samples by gas chromatography and ultra high pressure liquid chromatography coupled to tandem mass spectrometry
2011/1297951
Guidelines: none
GLP: no

Principle of the method: Residues of dimethomorph were extracted from urine using water and C_{18} Sep-Pak cartridges conditioned with dichloromethane, followed by water. The retained analytes were eluted with dichloromethane. The extracts were evaporated to dryness and the residue was dissolved in ethyl acetate solution.
An aliquot was directly analyzed by GC-MS/MS in the EI mode. Analysis was accomplished using a Varian Factor Four Capillary Column VF-5ms and helium as carrier and collision gas.

Recovery findings: The mean recovery values were between 70% and 110%. Details are presented in the table below.

Table 4.2-21: Validation results of GC-MS/MS method: dimethomorph (BAS 550 F) in urine

Test substance	Matrix	Fortification level (µg L^{-1})	No of tests	Average recovery (%)	Relative standard deviation (%)
Transition				**301 → 165**	**301 → 165**
Dimethomorph (BAS 550 F)	Urine	5	5	88	6
		10	5	115	5
		50	5	90	1

Linearity: Good linearity was observed over a concentration range of 0.1 to 100 µg L^{-1} (7 concentration levels) with correlation coefficients >0.996.

Specificity: GC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. However, only one mass transition was validated.

Matrix effects: Matrix-matched calibration standards were used.

Limit of quantitation: The limit of quantitation was 0.063 µg L^{-1} for dimethomorph.

Limit of detection: The limit of detection was 0.019 µg L^{-1} for dimethomorph.

Repeatability: The relative standard deviations (RSD, %) for all fortification levels were <10%. The detailed values are shown in Table 4.2-21.

Reproducibility: The inter-day precision (RSD values obtained at 5 µg/L; n = 5) was 9%.
The applicability of the method was evaluated in 14 real urine samples, showing that the method can be applied in routine analysis and monitoring programs.

Conclusion: **The GC-MS/MS multi-residue analytical method can be used for the analysis of dimethomorph in urine if deemed necessary.**

Report: CA 4.2/14
Dulaurent S. et al., 2009 b
Screening of pesticides in blood with liquid chromatography-linear ion trap mass spectrometry
2010/1233392
Guidelines: none
GLP: no

Briefly, after solid-phase extraction (SPE) from whole blood, the compounds were separated by liquid chromatography (LC) using a reversed-phase column and identified by mass spectrometry (MS). The mass spectrometer was operated in the full-scan MS mode, in the positive and negative polarities, followed by MS scanning of ions selected in data-dependent acquisition. The detection limit for dimethomorph in the dual-polarity MS mode was 0.1 mg L^{-1}. Dimethomorph was also detectable at 0.01 mg L^{-1} in the single polarity MS mode. The results obtained with the screening method were satisfactory in terms of sensitivity, selectivity and specificity for the identification of unknown pesticides, including dimethomorph, in a complex matrix such as blood.

Report: CA 4.2/15
Simonelli A. et al., 2007 b
Analytical method validation for the evaluation of cutaneous occupational exposure to different chemical classes of pesticides
2007/1071145
Guidelines: none
GLP: no

Principle of the method: Residues of dimethomorph were extracted from fiber paper pads, which were cut into small pieces, using acetonitrile. Then, samples were sonicated for 10 min. Collected solvent aliquots were dried and residues were dissolved with acetonitrile. Final analysis was performed by GC-MS/SIM. Analysis was accomplished using a J & W Scientific DB5-MS capillary column and helium as carrier gas.

Recovery findings: The mean recovery value was between 70% and 110%. Details are presented in the table below.

Table 4.2-22: Validation results of GC-MS/SIM method: dimethomorph (BAS 550 F) in fiber paper pads

Test substance	Matrix	Fortification level (mg L^{-1})	No of tests	Average recovery (%)	Standard deviation (%)
Dimethomorph (BAS 550 F)	Fiber paper pads, representing skin	0.11	n.r.	103.6	0.3
		0.33	n.r.		
		0.99	n.r.		
		2.96	n.r.		
		8.87	n.r.		
		26.6	n.r.		

n.r. Not reported

Linearity: Good linearity was observed over a concentration range of 1.1 to 816.3 ng cm^{-2} (7 concentration levels) with correlation coefficients >0.997. Standards were prepared in acetonitrile.

Specificity: GC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible for three ions (m/z 301, 303 and 387).

Matrix effects: Matrix-based calibration standards were used.

Interference: The analysis of matrix-based blank samples showed that no interfering species were present at the retention time of dimethomorph.

Limit of quantitation: The limit of quantitation was 0.9 ng cm^{-2} for dimethomorph.

Limit of detection: The limit of detection was 0.8 ng cm^{-2} for dimethomorph.

Repeatability: The relative standard deviation (RSD, %) over all fortification levels was <10%. The detailed values are shown in Table 4.2-22.

Stability of solutions: Dimethomorph was stable at room temperature for 24 h. Samples could be stored at −20°C for 1 week without a relevant loss of signal.

Conclusion: **The GC-MS/MS multi-residue analytical method can be used for the estimation of dermal exposure to dimethomorph and other active substances in open air field applications, especially by spraying.**

Report:	CA 4.2/16 Ivanov E., Bruhn F., 2017 Validation of BASF analytical method L0360/01 for the determination of BAS 550 F (Dimethomorph) and its metabolites M550F007 and M550F013 in body fluids 2017/1068967
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA OPPTS 860.1340 (August 1996)
GLP:	yes (certified by Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method	BASF method L0360/01, based on the multi-residue method QuEChERS, was validated for the determination of dimethomorph (BAS 550 F) and metabolites M550F007 and M550F013 in animal body fluids of whole blood and urine by LC-MS/MS. Samples of animal body fluids (whole blood and urine) are extracted with acetonitrile thereof addition of water by whole blood. After addition of magnesium sulfate and sodium chloride, the mixture is shaken intensively and centrifuged for liquid-liquid phase separation. No further clean-up of the acetonitrile extract by dispersive SPE with primary secondary amine (PSA) is carried out due to interaction of the acidic M550F013 with the amino-sorbent. An aliquot of the acetonitrile extract is stabilized with diluted formic acid. The measurements of dimethomorph and metabolites M550F007 and M550F013 are accomplished by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The chromatography was performed using a Ascentis Express C18 column at a flow rate of 0.7 mL/min. As mobile phase a water-acetonitrile gradient with 0.1% formic acid as modifier was used. For both matrices, detection is accomplished in ESI positive mode with mass transitions 388→301 (dimethomorph) and 374→287 (M550F007) for quantification and 388→165 (dimethomorph) and 376→289 (M550F007) for confirmation. For matrix urine, detection is accomplished in ESI positive mode for mass transition 550→287 (M550F013) for quantification and 550→374 (M550F013) for confirmation. For matrix whole blood, detection is accomplished in ESI negative mode for mass transition 548→372 (M550F013) for quantification and 548→357 (M550F013) for confirmation.
Recovery findings	The recoveries of dimethomorph and metabolites M550F007 and M550F013 in whole blood and urine specimens are summarized in Table 4.2-23. The mean recoveries were within the valid range between 79.6% and 109% after fortification with 0.01 mg/L (LOQ) and 0.10 mg/L (10x LOQ) dimethomorph and M550F007 and M550F013.

Table 4.2-23: Recovery results of dimethomorph and M550F007 and M550F013 in whole blood and urine specimens

Analyte	Matrix	m/z	Fortification level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Dimethomorph	Urine	388→301	0.01	5	107	9.1	101	9.6
			0.10	5	95.3	5.5		
		388→165	0.01	5	108	3.1	102	7.0
			0.10	5	95.5	1.3		
	Whole blood	388→301	0.01	5	104	2.6	103	4.3
			0.10	5	102	5.9		
		388→165	0.01	5	104	2.0	103	3.4
			0.10	5	102	4.6		
M550F007	Urine	374→287	0.01	5	108	1.7	103	5.0
			0.10	5	98.4	1.4		
		376→289	0.01	5	109	6.3	103	8.4
			0.10	5	96.5	4.9		
	Whole blood	374→287	0.01	5	89.2	2.2	88.5	2.0
			0.10	5	87.8	1.6		
		376→289	0.01	5	87.7	4.2	86.9	3.4
			0.10	5	86.0	2.2		
M550F013	Urine	550→287 [a]	0.01	5	100	6.4	100	5.0
			0.10	5	101	3.8		
		550→374 [a]	0.01	5	102	4.0	105	4.6
			0.10	5	108	3.7		
	Whole blood	548→357 [b]	0.01	5	80.1	3.8	79.8	5.5
			0.10	5	79.6	7.3		
		548→372 [b]	0.01	5	83.4	6.1	82.3	6.3
			0.10	5	81.2	7.0		

RSD = Relative standard deviation
[a] Positive ESI mode
[b] Negative ESI mode

Linearity

Good linearity ($R^2 > 0.980$) was observed for each analyte and both mass transitions in solvent-based calibration standards prepared in acetonitrile/0.1% formic acid (1/4, v/v). For urine specimen extracts, linearity was demonstrated by single determination of at least eight solvent calibration standards ranging from 0.10 ng/mL to 10 ng/mL. For whole blood specimens, linearity was demonstrated by single determination of at least seven solvent calibration standards ranging from 0.15 ng/mL to 10 ng/mL.

Specificity

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of dimethomorph and metabolites M550F007 and M550F013 in animal body fluids matrices (whole blood and urine) monitoring two mass transitions for each analyte.

Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects	The matrix effect on the LC-MS/MS response was assessed by comparing peak areas of matrix-matched standards (at least 90% matrix amount) with solvent standards at equivalent concentrations. Matrix effects on the detection of dimethomorph and its metabolites M550F007 and M550F013 in extracts of whole blood and urine were considered as not significant (< 20%).
Interference	Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.
Standard stability	In this study, stability tests of M550F013 were performed. The stabilities of dimethomorph and metabolite M550F007 in stock, fortification and calibration solutions were shown in earlier studies [*for details refer to CA 4.1.2/19, BASF DocID 2014/1186695; CA 6.6.2/2, BASF DocID 2015/1241720 and CA 4.1.2/16, BASF DocID 2005/1026082*]. Stability tests confirmed that metabolite M550F013 was stable in stock solutions (prepared in methanol) for at least 75 days, in fortification standard solutions (prepared in methanol) for at least 43 days and in calibration standards (prepared in acetonitrile/0.1% formic acid (1/4, v/v)) for at least 36 days, when stored under refrigeration (1°C to 10°C) in the dark.
Extract stability	Dimethomorph and its metabolites M550F007 and M550F013 were stable in raw and final extracts of whole blood and urine specimens for at least 7 days when stored at 1°C to 10°C in the dark.
Limit of quantification	The limit of quantification (LOQ) of the method, defined by the lowest fortification level, is 0.01 mg/L.
Limit of detection	The limit of detection (LOD) of the method is 0.003 mg/L for both matrices, corresponding to the lowest calibration level of 0.15 ng/mL of matrix whole blood or 30% of the LOQ.
Repeatability	The relative standard deviations (RSD, %) with respect to recoveries following fortifications at two different fortification levels were < 10% for dimethomorph and M550F007 and M550F013.
Reproducibility	Reproducibility of the method was not determined within the study.
Conclusion	**It could be demonstrated that the method L0360/01 based on the multi-residue method QuEChERS fulfills the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of dimethomorph and its metabolites M550F007 and M550F013 in animal body fluids of whole blood and urine.**

Dimethomorph

Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

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e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
31/03/2016	MCA Section 5 Submission of the missing reports CA 5.6 Submission of the report 2014/1181670 CA 5.8.1/20 Summary of the study Tong 1982/7001784 CA 5.8.3 Submission of literature study reports 5.8.3/3 : 2002/1028017 5.8.3/4 : 2011/1298391 5.8.3/5 : 2010/1233632 5.8.3/6 : 2014/1329032 5.8.3/7 : 2015/1278187 Table 5.8.3-2 update	MCA Section 5, Version 1, BASF DocID2016/1000209
30/09/2016	MCA Section 5 CA 5.6.1 DK-430-001, table 5.6.1-35 : A statistical re-evaluation has been performed for the AIR 3 dossier CA 5.6.2 DK-432-002, table 5.6.2-1 : Historical control data added & DK-432-004 : Findings added CA 5.7.1 Submission of literature study reports 5.7.1/3 2015/1278188 CA 5.8.2 Submission of literature study reports 5.8.2/2 : 2011/1296591 5.8.2/3 : 2011/1295091 5.8.2/4 : 2015/1279907	MCA Section 5, Version 2, BASF DocID2016/1103872

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Studies already peer-reviewed and presented in the original Annex I dossier:

For the determination of the toxicokinetic properties of dimethomorph, several studies are available (see table below) with the test substance radiolabelled in the chlorophenyl ring system. The study BASF DocID DK-440-001 (+ Amendment DK-440-006) investigated absorption, distribution, excretion and metabolism in rats after a single oral low dose (10 mg/kg bw), a single oral high dose (500 mg/kg bw), a multiple oral low dose (10 mg/kg bw, 14+1days) and tissue distribution after multiple oral low dosing for 7 days with radiolabeled material (10 mg/kg bw). Study BASF DocID DK-440-002 investigated the biliary excretion after a single oral low (10 mg/kg bw) and high (500 mg/kg bw) dose. A third study covers dose groups for blood pharmacokinetics after a single oral low (10 mg/kg bw) and high dose (100 mg/kg bw). Experiments with intravenous dosing were not possible due to the poor water solubility of dimethomorph. All studies have been part of the previous evaluation and are therefore not submitted again in this dossier.

Category of test	Dose groups	Results	Reference (BASF DocID)
Biokinetics and Metabolism	Single oral dose 10 mg/kg bw 500 mg/kg bw Multiple oral dose (14+1) 10 mg/kg bw (^{14}C-spike after 14 d pre-treatment with unlabelled) Multiple oral dose (7 days) 10 mg/kg bw	Rapid and almost complete absorption (at higher dose levels absorption is limited). Excretion mainly via feces (85-90%) and to a lesser extend via urine (6-15%). Rapid excretion of >90% dose within 72 hours. Extensive metabolization mainly via demethylation, to a lesser extend via oxidative ring-opening of morpholine moiety. After multiple dosing, highest residues in intestine, stomach and liver. No accumulation of residues in organs or tissues. No major differences were observed with regard to sex and dose level.	DK-440-001+ DK-440-006
Biliary excretion	Single oral dose 10 mg/kg bw 500 mg/kg bw	Low dose: rapid excretion via bile (>90% dose within 24 hours), short half-life of about 3 hours. High dose: absorption limited in both sexes (30-50% dose), indicated by increased radioactivity in the feces. Longer half-life of 6 hours (female) and 11 hours (male). Metabolization via demethylation and subsequent conjugation to glucuronides.	DK-440-002
Blood pharmacokinetics	Single oral dose 10 mg/kg bw 500 mg/kg bw	At low dose absorption is faster than at the high dose. Low dose: T_{max}: male 2.8±3.6, female 1.4±0.8 T ½: male 59.2±23.5, female 68.0±20.2 High dose: T_{max}: male 11.0±2.0, female 14.7±8.3 T ½: male 65.4±9.4, female 75.8±1.8 Radioactivity was higher in red blood cells than in plasma.	DK-452-008
Tissue distribution	Single oral dose 10 mg/kg bw 500 mg/kg bw	Low dose and high dose: highest residues at T_{max} in GI tract and contents, liver, kidney, pancreas, fat. Rapid excretion of absorbed dimethomorph. No signs of accumulation of residues in organs and tissues.	DK-440-013

For reasons of convenience, brief summaries of the respective studies were extracted from the monograph and are provided below.

[redacted] 1990, The Biokinetics and Metabolism of 14CDimethomorph in the Rat, [redacted] BASF Document No. DK-440-001
and
[redacted], 14C-Dimethomorph: Investigation on the Nature of the Metabolites Occurring in the Rat, [redacted], BASF Document No. DK-440-006 [complementary data supporting study report DK-440-001]:

Test substance: Radiolabelled dimethomorph (chlorophenyl-ring), batch 2271-040, specific activity 45.12 mCi/g with chemical purity > 98% and E/Z ratio 44-47/53-56. Unlabelled dimethomorph, batch H7879 with purity 99.2% and E/Z ratio 49.5/50.5.

Test design: Male and Female rat (strain: Sprague-Dawley) were orally dosed with the test substance as follows:

Table 5.1.1-1 Summary of dose groups and dosing parameters

Test group	No of rat	Dose, mg/kg body weight (b.w.)	Dosing type	Sampling
A	1♂, 1♀	500	Single	Expired air
B	5♂, 5♀	10	Single	Urine and faeces: 8 (urine only), 24, 72, 96, 120 and 144 hours after dosing Sacrifice at end of urine/feces collection, sampling of liver kidneys, fat and carcasse
C	5♂, 5♀	10	Multiple (15 days)	
D	5♂, 5♀	500	Single	
E	5♂, 5♀	10	Multiple (7 days)	Urine and feces: 1, 6, 24, 48, and 120 after dosing Sacrifice at end of urine/feces collection, sampling of liver, adrenals, heart, brain, spleen, thryroid, bone marrow, stomach, kidneys, fat, uterus, lungs, gonads, pancreas, muscle, bone, blood, intestine and carcasse

Findings:

Excretion balance (Groups A, B, C, D)
In all dose groups (single low, single high, multiple low dose), the total amount of radioactivity was rapidly excreted, predominantly via the faecal route: 72 hours after administration 80 - 90% of the dose were recovered from faeces and 5 - 16% from urine. No radioactivity was detectable in the exhaled air. (<0.01% dose). Already within the first 24 hours after dosing, 73 - 88% of the administered radioactivity was found in faeces and 5 - 16% in urine.

Table 5.1.1-2 Mean daily excretion of radiolabelled ^{14}C-dimethomorph expressed as % dose (Total Administered Radioactivity)

Hours	Group B (single, low dose)		Group C (multiple, low dose)		Group D (single, high dose)	
	Feces	Urine	Feces	Urine	Feces	Urine
24	75.09/72.41[1]	4.93/12.66	65.64/52.35	5.83/12.53	58.91/33.14	4.57/6.15
48	13.10/12.40	0.51/0.75	16.36/22.31	1.48/3.11	25.44/39.39	1.45/2.67
Subtotal 0-48	**88.19/84.81**	**5.44/13.41**	**82/74.66**	**7.31/15.64**	**84.35/72.53**	**6.02/8.82**
72	1.43/1.16	0.07/0.13	6.32/5.12	0.33/0.51	1.98/13.62	0.13/1.37
96	0.24/0.21	0.03/0.04	0.56/0.58	0.05/0.08	0.27/2.36	0.04/0.20
120	0.10/0.10	0.01/0.02	0.16/0.15	0.02/0.04	0.09/0.32	0.02/0.03
148	0.06/0.06	0.01/0.02	0.07/0.07	0.02/0.02	0.06/0.04	0.01/0.01
168	0.05/0.04	0.01/0.01	0.05/0.01	0.01/0.02	0.03/0.04	0.01/0.01
Total 0-168	**90.07/87.28**	**5.57/14.88**	**89.17/80.61**	**7.74/16.31**	**86.79/88.91**	**6.23/10.44**

[1] x/y = values for Male and female

Tissue distribution (Group E)

Following 7 days of dosing with dimethomorph at 10 mg/kg bw, the highest residue levels were found in the excretion organs with a decrease of about 70% after 24 hours. Except for stomach, intestine and liver, the residue level in organs and tissues were very low. Radioactivity remaining in tissues and organs 120 hours post dosing (after multiple oral low dose) was below 0.1% of the dose. The results show that neither dimethomorph nor its degradation products accumulated in any of the organs or tissues of rats.

Table 5.1.1-3 Concentration of radioactivity in the organs and tissues of rats after 7 dosing day of ^{14}C-dimethomorph at low dose level (values expressed as % Total dose in organ and tissue)

Time of sacrifice after 7-dosing day	**1h**	**6h**	**24h**	**48h**	**120h**
Liver	1.44/1.26[1]	0.43/0.57	0.27/0.26	0.15/0.10	0.09/0.06
Kidneys	0.04/0.06	0.01/0.02	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Heart	0.01/0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Lungs	0.01/0.02	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Brain	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Gonads	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Spleen	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Pancreas	0.03/0.04	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Adrenals	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Thyroid	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Uterus	-/0.01	-/<0.01	-/<0.01	-/<0.01	-/<0.01
Muscle	0.39/0.76	0.14/0.31	0.02/0.04	0.01/0.01	0.01/0.02
Fat	0.15/0.50	0.07/0.11	0.02/0.03	<0.01/0.01	<0.01/0.01
Stomach (+content)	4.17/4.23	0.07/0.08	0.05/<0.01	<0.01/<0.01	<0.01/<0.01
Intestine (+content)	8.64/10.58	11.47/12.23	1.29/1.79	0.09/0.16	0.01/0.02

[1] x/y = values for Male and female

Metabolism

Metabolite profiles/degradation patterns were determined in urine and feces from Group D (Single high dose). No differences were found between metabolic profiles of both sexes neither in urine nor in feces. From urine samples, dimethomorph (< 5% of applied dose), polar fractions formed by demethylation of the dimethoxyphenyl ring (Z67=M550F006; Z69=M550F007) and traces of a compound formed by oxidation of morpholine ring (CUR 7586 =M550F010) were identified. From feces samples, the greatest portion of radioactivity was identified as the parent compound. Traces of demethylated metabolites from dimethoxyphenyl ring was also found.

Further investigation were conducted on urine sampled 24 hours after dosing from Group B (single, low dose). Traces of metabolites CUR 7117 (M550F008), CUR 7586 (M550F010), CUR 7216 (M550F011) and Z 43 (M550F005) were found confirming the oxidation of the morpholine ring.

Conclusion

Dimethomorph is rapidly absorbed in the gastrointestinal tract following oral administration to rats. The amount absorbed is limited at high dose levels. Absorbed dimethomorph is efficiently metabolised and rapidly excreted mainly via the feces. Accumulation of dimethomorph in organs and tissues did not occur.

The main degradation pathway was found to be the demethylation of the dimethoxyphenyl ring. To a smaller extent, degradation also occurred by oxidation of the morpholine ring.

[redacted]1990: 14C-Dimethomorph: Absorption, Distribution and Excretion after Bile Cannulation and Single Oral Administration to the Rat. [redacted] BASF Document No. DK-440-002

Test substance: Radiolabeled dimethomorph (chlorophenyl-ring), batch S 1050, specific activity 23.6 mCi/g with chemical purity > 99% and E/Z ratio 50/50. Unlabelled dimethomorph, batch H7879; Chemical purity: 99.2% (E/Z ratio: 49.5/50.5)

Test design: Male and female rats (Strain: Sprague-Dawley CD) were orally administered with ^{14}C-dimethomorph at nominal dose level of 10 (low dose) and 500 (high dose) mg/ kg body weight. Bile was collected in 3-hour intervals throughout the study. Urine and feces were collected 24 and 48 hours after dosing. At sacrifice (48-h after dosing), carcass and digestive tract were sampled. Cage wash were collected at the end of the study.

Findings:

Excretion balance
At low dose, the total amount of radioactivity absorbed (bile, urine and residua; carcass) is about 100% for both male and female rats. At high dose, the total amount of radioactivity absorbed is 65.5% for male and 50% for the female.

Table 5.1.1-4 Balance of radioactivity (expressed as % of Total Administered Radioactivity) 48-hours after dosing

Distribution	**Group 1 Low dose Male**	**Group 2 Low dose Female**	**Group 3 High dose Male**	**Group 4 High dose Female**
Urine	6.1	6.9	14.8	8.6
Feces	7.6	4	21.8	3.5
Bile	95.1	92.6	49.1	31.2
Residual Carcass	0.6	1.2	1.6	10.1
Digestive tract	0.4	1.1	3.3	44.1
Cage wash	0.5	0.3	0.4	0.9
Total	110.8	106.1	91	98.4
Standard Deviation	± 5.6	± 3.6	± 4.3	± 2.7

Biliary excretion

At low dose (single administration, group 1 and 2), radioactivity was rapidly eliminated during the first 6 hours following administration.

At high dose (single administration, group 3 and 4), elimination of radioactivity via the bile was described by 1st order kinetics with a half-life of 6-11 hours considering the time interval of 6 to 24 hours.

Biliary excretion was generally high, with 93-100% of dose after administration of 10 mg/kg bw/d and lower after administration of a dose of 500 mg/kg bw/d, showing saturation effects. Biliary excretion was generally slightly lower for females.

Table 5.1.1-5 Excretion pattern via the bile after administration of a single dose (mean values expressed as % total administered radioactivity)

Time (hours)	Group 1 Low dose Male	Group 2 Low dose Female	Group 3 High dose Male	Group 4 High dose Female
0-3	65.2	64.4	11.5	12.8
6	19.9	9.9	7.3	5.4
9	8	6.2	6.9	3.4
12	3	4.4	6.5	2.5
15	1.4	3.1	4.6	1.6
18	0.9	1.5	3.5	1.3
21	1.2	1.0	3.7	1.4
24	0.3	0.6	3	1.4
27	0.2	0.4	0.3	0.8
30	0.2	0.3	0.7	0.5
33	0.2	0.2	0.3	0.4
36	0.1	0.2		0.3
39	0.1	0.2	0.5	0.2
42	0.1	0.1	0.1	-
45	0.1	0.1	0.2	-
Total	**100.9**	**92.6**	**49.1**	**31.2**

Metabolite investigations

For both, male and female rats, the degradation pattern of the untreated and treated bile were similar as shown in Table 5.1.1-6 and Table 5.1.1-7.

Table 5.1.1-6 Metabolite patterns in the untreated bile after single oral administration of ^{14}C-dimethomorph (expressed in % of the total administered radioactivity) for sampling intervals 0-27h after dosing

Fraction	Group 1 Low dose Male	Group 2 Low dose Female	Group 3 High dose Male	Group 4 High dose Female
B1, unknown	1.2	0.1	ND	ND
B2, Z67/69 (not confirmed)	0.5	0.5	ND	ND
B3, unknown	0.2	9.1	ND	0.8
B4, unknown	10.1		3.3	1.7
B5, unknown	13.6	8.9	10.9	8.2
B6, unknown	ND	ND		
B7, unknown	44.4	57.2	16.4	12.7
B8, unknown	3.8	ND	2.2	1.8
B9, unknown	17.3	10.7	11.2	1.3
B10, unknown	4.6	3.3	2.3	3.1
B11, unknown	4.2	1.3	0.7	0.2

ND=not detectable

Table 5.1.1-7 Metabolite patterns in the treated bile[1] after single oral administration of ^{14}C-dimethomorph (expressed in % of the total administered radioactivity)

Fraction	Group 1/2 Low dose Male/Female	Group 3/4 High dose Male/Female	Group 1/2 Low dose Male/Female	Group 3/4 High dose Male/Female
Treated bile by	***HCl, in ethyl acetate partition***		***Glucuronidase, in ethyl acetate partition***	
B4, unknown	14.9/8.9	4.8/2.5	18.3/18.3	9.2/6.5
B12 (=B1)	7.1/5.7	2.4/2.0		
B13 (=B1)		ND		
B14, Z67/69	22.7/34.1	11.5/11.3	28.0/46.6	21.0/19.4
B15, unknown	9.5/6.6	2.2/3.3		
B16, unknown	3.2/4.3	1.0/0.9		
B17, unknown			2.1/1.0	1.5/0.6
B18, unknown			14.1/8.2	3.0/ND
B19, unknown			6.9/ND	2.2/ND
B20, unknown			5.5/0.6	2.3/ND
B21, unknown			5.8/3.8	1.6/1.1

Conclusion:
Dimethomorph is efficiently absorbed and metabolised in the rat. Biliary excretion was generally high, showing that it is a main route of elimination. Dimethomorph is mainly metabolized via conjugation to glucuronides. The main aglyca were identified as Z67 (M550F006) and/or Z69 (M550F007), i.e. the demethylated metabolites.

[1] HCL treated bile: summary data for 0-6 hour sample for group 1 and 2, and 0-12 hour sample for group 3 and 4; Glucuronidase treated bile: summary data for 0-15 hour sample for group 1 and 2, and 0-27 hour sample for group 3 and 4.

[redacted]. 1995; Dimethomorph (CL 336,379): Blood Pharmacokinetics of C-14 CL 336,379 Derived Residues in the Rat; [redacted] BASF Document No. DK-452-008

Test substance: ^{14}C-dimethomorph; Chlorophenyl-ring Labeled:Lot Number: AC 10011:71, radiochemical purity: > 96%, (E/Z ratio: about 50/50), specific activity: 25.3 µCi/mg Dimethomorph, unlabelled:Lot No.: AC 7467.004, Chemical purity: 98.8% (E/Z ratio: about 50/50)

Test design: Adult male and female rats (strain: Sprague-Dawley, Crl:CD BR) (4 animals/dose/sex) were orally administered single dose of test substance at 10 (low dose) and 500 (high dose) mg/kg body weight. Blood was sampled at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 72, 96, 120 and 144 hours after dosing. At 168 hours post-dosing, rats were sacrificed and blood collected for analysis. Radioactivity levels were determined in plasma and red blood cell (RBC).

Findings:

Pharmacokinetic parameters and concentration versus time profile
At low dose, radioactivity was rapidly absorbed and eliminated within 72 hours as indicated by the pharmacokinetic parameters (see Table 5.1.1-8). At high dose, absorption occurred later than at low dose.

Table 5.1.1-8 Pharmacokinetic parameters and concentration versus time profile

Test group		T_{max} [hour]	C_{max} [µg/g]	$T_{1/2}$ [hour]	$AUC_{0-\infty}$
Group A Low dose	Male	2.8±3.6	0.76±0.56	59.2±23.5	10.56±1.36
	Female	1.4±0.8	0.96±0.26	68±20.2	14.96±6.24
Group B High dose	Male	11.0±2.0	25.02±4.35	75.8±1.8	673.56±89.1
	Female	14.7±8.3	39.46±8.4	75.6±1.8	1211.4±35.25

Total radioactivity residue in plasma and Red Blood Cells (RBC) at 168 hours
The total radioactivity residue was higher in RBCs than in plasma in both groups, low and high dose.

Table 5.1.1-9 TRR in plasma and RBC at 168 hours post dosing [expressed in mg/kg]

Test group		Plasma	RBC
Group A Low dose	Male	< 0.022	<0.023
	Female	< 0.022	<0.023
Group B High dose	Male	< 0.022	0.750±0.276
	Female	< 0.027	0.939±0.208

Limit of detection: 0.022 in plasma and 0.023 in RBC ppm

Conclusions

Based on the pharmacokinetics parameters, it can be concluded that at low dose absorption is quicker than at high dose. At both dose levels, the elimination occurred within 72 hours post-dosing.

[redacted]. 1995; Dimethomorph (CL 336,379): Tissue distribution of14C-CL336,379 Derived Residues in the Rat; [redacted] BASF Document No. DK-440-013

Test substance: ^{14}C-dimethomorph; Chlorophenyl-ring Labeled:Lot Number: AC 10011:71, radiochemical purity: > 96%, (E/Z ratio: about 50/50), specific activity: 25.3 µCi/mg Dimethomorph, unlabelled:Lot No.: AC 7467.004, Chemical purity: 98.8% (E/Z ratio: about 50/50)

Test design:
Dimethomorph was administered as a single oral dose to rats ((strain: Sprague-Dawley, Crl:CD BR)) at nominal doses of 10 mg/kg bw (group A, low dose) and 500 mg/kg bw (group B, high dose). Each treated group consisted of 9 male and 9 female rats. Another 6 rats (3/sex) were dosed with dosing vehicle and served as the control group. For the treated groups, three male and three female rats were sacrificed and tissues were collected at about Tmax, 24 hour and 168 hour post dose, respectively. Control animals were sacrificed and tissues collected at 168 hour post dose. The Tmax values were determined empirically from a previously conducted study (see Afzal J, Wu D. 1995, Dimethomorph (CL 336,379): Blood Pharmacokinetics of C-14 CL 336,379 Derived Residues in the Rat, BASF Document No. DK-452-008). Tissues and organs were collected and radioactive residues determined in plasma, red blood cells, adrenals, bone marrow, brain, fat, gastrointestinal tract, gastrointestinal contents, heart, kidneys, liver, lungs, muscle, ovaries, pancreas, pituitary, spleen, testes, thymus, uterus, and residual carcass.

Findings:

For the low dose group (10 mg/kg bw), radioactivity levels at T_{max} (0.5 hour post dosing for males and 1.5 hours post-dosing for female) were highest in the gastrointestinal tract and carcass. Residues in tissues were found (in descending order) in liver, kidneys, and pancreas, plasma, fat, lung, heart and ovaries. The compound was quickly eliminated from the body. At 24 hours most of the residues were depleted to very low levels. At 168 hours, only liver had detectable residues. At the high dose (500 mg/kg bw), dimethomorph was eliminated at much slower rate, which was reflected in higher residue levels at 24 hours post dose. Female rats retained the compound longer than male rats. In the high dose group, tissues and organs showed similar patterns of residues when compared to the low dose group. At 168 hours post dose, tissues from the high dose rats showed similar patterns of depletion as those in the low dose group, i.e. only liver had detectable residues.

Table 5.1.1-10 Distribution of radiolabelled ^{14}C-dimethomorph expressed as % dose (Total administered radioactivity) in the low dose group at Tmax (0.5/1.5 h), 24h and 168h post-dosing

Distribution	Tmax	24-hours	168-hours
Plasma	0.06/0.10[1]	0.01/0.01	0.00/0.00
RBC	0.06/0.09	0.01/0.01	0.00/0.00
Adrenals	0.00/0.01	0.00/0.00	0.00/0.00
Bone	0.01/0.02	0.00/0.00	0.00/0.00
Bone marrow	0.00/0.00	0.00/0.00	0.00/0.00
Brain	0.01/0.02	0.00/0.00	0.00/0.00
Fat	0.04/0.09	0.00/0.00	0.00/0.00
GI Tract	13.91/11.87	0.43/1.06	0.00/0.00
GI Contents	67.67/67.52	6.68/9.03	0.01/0.02
Heart	0.06/0.04	0.00/0.00	0.00/0.00
Kidneys	0.25/0.20	0.02/0.02	0.00/0.00
Liver	5.36/3.29	0.09/0.75	0.10/0.07
Lungs	0.06/0.06	0.00/0.00	0.00/0.00
Muscle	0.03/0.06	0.00/0.00	0.00/0.00
Ovaries	NA[2]/0.01	NA/0.00	NA/0.00
Pancreas	0.10/0.07	0.00/0.00	0.00/0.00
Pituitary	0.00/0.00	0.00/0.00	0.00/0.00
Spleen	0.02/0.02	0.00/0.00	0.00/0.00
Testes	0.02/NA	0.00/NA	0.00/NA
Thymus	0.01/0.02	0.00/0.00	0.00/0.00
Thyroid	0.00/0.00	0.00/0.00	0.00/0.00
Uterus	NA/0.00	NA/0.00	NA/0.00
Carcass	7.47/6.89	0.33/0.26	0.04/0.01
Total	95.16/ 90.39	6.37/ 11.14	0.15/ 0.10

[1] x/y = values for male and female
[2] NA=Not Applicable

Table 5.1.1-11 Distribution of radiolabelled ^{14}C-dimethomorph expressed as % dose (Total administered radioactivity) in the high dose group at Tmax (8 h), 24h and 168h post-dosing

Distribution	Tmax	24-hours	168-hours
Plasma	0.05/0.03[1]	0.04/0.04	0.00/0.00
RBC	0.06/0.02	0.03/0.03	0.01/0.00
Adrenals	0.00/0.00	0.00/0.00	0.00/0.00
Bone	0.01/0.00	0.00/0.01	0.00/0.00
Bone marrow	0.00/0.00	0.00/0.00	0.00/0.00
Brain	0.00/0.01	0.00/0.01	0.00/0.00
Fat	0.02/0.06	0.00/0.04	0.00/0.00
GI Tract	5.30/3.87	2.19/3.35	0.00/0.00
GI Contents	74.40/72.33	22.16/21.76	0.01/0.01
Heart	0.02/0.02	0.01/0.02	0.00/0.00
Kidneys	0.08/0.07	0.04/0.08	0.00/0.00
Liver	1.14/0.82	0.85/1.05	0.08/0.04
Lungs	0.03/0.03	0.01/0.03	0.00/0.00
Muscle	0.01/0.00	0.00/0.02	0.00/0.00
Ovaries	NA[2]/0.00	NA/0.00	NA/0.00
Pancreas	0.02/0.02	0.01/0.03	0.00/0.00
Pituitary	0.00/0.00	0.00/0.00	0.00/0.00
Spleen	0.01/0.01	0.00/0.01	0.00/0.00
Testes	0.02/NA	0.01/NA	0.00/NA
Thymus	0.01/0.01	0.00/0.01	0.00/0.00
Thyroid	0.00/0.00	0.00/0.00	0.00/0.00
Uterus	NA/0.02	NA/0.01	NA/0.00
Carcass	4.61/3.62	0.96/3.86	0.04/0.04
Total	85.79/80.98	26.29/30.37	0.14/0.10

Conclusion:

Dimethomorph is rapidly absorbed in the gastrointestinal tract following oral administration to rats. Absorbed dimethomorph is rapidly excreted. Accumulation of dimethomorph in organs and tissues did not occur.

[1] x/y = values for male and female
[2] NA= Not Applicable

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

The following study has been performed in addition to the rat metabolism studies that have been peer-reviewed during the last Annex I inclusion process. The objective of the new study was to investigate the metabolic fate of dimethomorph radiolabeled in the morpholine moiety after a single oral low and high dose. A further aim was to thoroughly identify metabolites in bile and tissues, for which the previous studies provide only limited information. For a better comparability, both radiolabeled forms (chlorophenyl label and morpholine label) were investigated in parallel within the new study. This study also tries to provide information on the E and Z isomer ratio for parent and main metabolites as far as possible.

Report:	CA 5.1.1/1 [redacted], 2015 c BAS 550 F: Excretion and metabolism in the rat after oral administration 2015/1000602
Guidelines:	OECD 417, 2004/10/EC of 11 February 2004, EPA 870.7485
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Dimethomorph (BAS 550 F)
Lot/Batch #:	1068-0101 (p-chlorophenyl-U-^{14}C) 858-0101 (morpholine-2,3-^{14}C) 1070-0101 (acrolyl-^{13}C) 1071-0105 (morpholine-^{15}N) AC9978-68A (unlabeled)
Purity / E:Z isomer ratio:	96.4% (p-chlorophenyl-U-^{14}C) / 45:55 (HPLC UV), 43:57 (NMR) 98.6% (morpholine-2,3-^{14}C) / 46.5:53.5 (HPLC UV), 45:55 (NMR) 91.3% (acrolyl-^{13}C) / 37.5:62.5 (UV), 45:55 (NMR) 97.3% (morpholine-^{15}N) / 44:56 (HPLC UV) 97.6% (unlabeled) / 44:56
CAS#:	110488-70-5
Development code:	247723 (Reg. No)
Stability of test compound:	Stable during dosing period

2. Vehicle and/or positive control:

The vehicle used was 0.1% Tween 80

3. Test animals

Species:	Rat
Strain:	Sprague Dawley (Crl:CD(SD), [redacted]
Age:	Phase I: 8 weeks (males) / 11 weeks (females) Phase II: 9-11 weeks (males) / 12- >15 weeks (females) Phase III: 8-10 weeks (males) / 11- >15 weeks (females)
Sex:	Male and female
Number of animals:	52 males and 52 females in total Phase I: 16 males and 16 females Phase II: 20 males and 20 females Phase III: 16 males and 16 females
Weight at dosing:	Phase I: about 250 g Phase II: means of 294-337 g (males), means of 255-293 (females) Phase III: means of 254-320 g (males), means of 246-286 (females)
Acclimation period:	minimum 3 days
Diet:	Pelleted diet RM1 (E) SQC, Special Diets Services, Witham, Essex, UK, *ad libitum*
Water:	Tap water, *ad libitum*
Husbandry:	
Housing:	During acclimatization (all groups) and experiments (D4, D5, D9, D10) in groups of up to four animals in polypropylene cages. During experiments individually in metabolism cages (D1, D2, D3, D6, D7, D8).
Environmental conditions:	
Temperature:	19-23°C
Humidity:	45-65%
Air changes:	Air-conditioned
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Dates of work: May 09, 2013 - April 10, 2015

The excretion and metabolism of BAS 550 F (dimethomorph) was investigated in male and female rats after oral application of a single oral dose of 10, 250 and 500 mg/kg bw BAS 550 F, respectively. The study comprised three experiments:
Phase 1 (excretion balance, investigation of urine and faeces)
Phase 2 (biliary elimination; investigation of bile)
Phase 3 (tissue depletion; investigation of plasma, liver, and kidney)
Since for dimethomorph already sufficient and valid peer reviewed data on the excretion behavior after oral administration is available, the main purpose of this study was to generate sample matrices for further investigation of metabolites.

2. Dosing
For all dose groups, animals were dosed with mixtures of ^{13}C- and ^{14}C-radiolabeled, ^{15}N-labeled and unlabeled test item in order to facilitate metabolite identification by mass spectrometry and quantitative analysis using HPLC. The dose formulations for the p-chlorophenyl label were prepared by mixing non-labelled BAS 550 F, [acrolyl-^{13}C]-BAS 550 F and [p-chlorophenyl-^{14}C]-BAS 550 F in a ratio of 9:3:4 (for administration at 10 mg/kg, dose groups 2 and 4) or in a ratio of 30:10:1 (for administration at 250 or 500 mg/kg, dose groups 1, 3 and 5), leading to target specific activities of ca. 1.925 MBq/mg at 10 mg/kg and ca. 0.1878 MBq/mg at 250 or 500 mg/kg.

The dose formulations for the morpholine label were prepared by mixing non-labelled BAS 550 F, [morpholine-^{15}N]-BAS 550 F and [morpholine-^{14}C]-BAS 550 F in a ratio of 1:1:1 (for administration at 10 mg/kg, dose groups 7 and 9) or in a ratio of 22:11:1 for administration at 250 or 500 mg/kg, dose groups 6, 8 and 10), leading to target specific activities of ca. 1.913 MBq/mg at 10 mg/kg and ca. 0.1688 MBq/mg at 250 or 500 mg/kg.

Each formulation was prepared on the day of actual use by suspending/dissolving the batches of appropriately blended [^{14}C]-BAS 550 F in the required amount of 0.1% Tween 80. Animals were dosed by means of a syringe fitted with a gavage tube directly in to the stomach.

The following tables summarize the dose groups and dosing parameters.

Table 5.1.1-12 Summary of Dose Groups and Dosing Parameters

Dose group	**Phase 1 Excretion balance**		**Phase 2 Biliary excretion**				**Phase 3 Tissue depletion**			
No. of doses and route of administration	Single oral high		Single oral low		Single oral high		Single oral low		Single oral high	
Dose group designation	D1	D6	D2	D7	D3	D8	D4	D9	D5	D10
Radiolabel*	C	M	C	M	C	M	C	M	C	M
Number of animals per Label	8♂, 8♀		5♂, 5♀		5♂, 5♀		4♂, 4♀		4♂, 4♀	
Nominal dose (mg/kg bw)	250		10		250		10		500	
Sampling	**Urine** **Faeces** Cage wash Carcass Blood Kidney Liver		**Bile** Urine Faeces Cage wash Carcass Blood Stomach Gut				**Liver** **Kidney** Pancreas **Blood** **Plasma** at T_{max}=1h		**Liver** **Kidney** Pancreas **Blood** **Plasma** at T_{max}=12h	

In bold print: matrices that were further investigated for metabolites; other matrices only sampled for balance purposes
*C=Chlorophenyl label, M= Morpholine label

3. Sampling

Phase 1 (excretion balance: groups D1M, D1F, D6M and D6F)
Urine and faeces were sampled at pre-dose, 6 (urine only), 12 (urine only) and 24 hours post application and thereafter in daily intervals until 168 hours. At 168 hours, a terminal whole blood sample was collected. The liver and kidneys were removed from each carcass. Prior to radioactivity analysis, the separate sample types (except carcass) were pooled per label, gender, sample type and collection period/sampling time. Faeces pools were homogenised to a paste with water. Kidney and liver pools were homogenised. Aliquots of each sample pool were taken for radioactivity measurement.

Phase 2 (biliary elimination: groups D2M, D2F, D3M, D3F, D7M, D7F, D8M and D8F)
Bile was sampled at pre-dose, 3, 6, 12, 24, 36, 48, 60 and 72 hours (for some dose groups only) post application. Urine and faeces were sampled at pre-dose, 6 (only urine) 24, 48 and 72 hours (for some dose groups only) post application. At 48 hours (D7M, D7F, D8M and D8F) or 72 hours (D2M, D2F, D3M and D3F), a terminal whole blood sample was collected. The stomach and gut were removed from each carcass. Faeces samples were homogenised to a paste with water. Stomach, stomach contents, gut and gut contents were homogenised. Aliquots of each sample were taken for radioactivity measurement.

Phase 3 (tissue depletion: groups D4M, D4F, D5M, D5F, D9M, D9F, D10M and D10F)
A terminal whole blood sample was collected at the T_{max} (based on the data from study DK-452-008), which was at 1 hour post-dose (for the low dose groups) or at 12 hour post-dose (for the high dose groups) via cardiac puncture. The pancreas, kidney and liver were removed from each carcass. Prior to radioactivity analysis, the separate sample types were pooled per label, gender and sample type. Pancreas, kidney and liver pools were homogenised. Aliquots of each sample pool were taken for radioactivity measurement.

4. Analysis

TRR: For all sampled materials, radioactive residues were determined either by direct combustion or liquid scintillation counting.
HPLC: Selected matrices (Phase 1: urine, faeces; Phase 2 bile; Phase 3: liver, kidney, plasma) were further investigated by radio-HPLC for their metabolite patterns (using HPLC method LC02). The selection of tissues to be analyzed from phase 3 was based on the overall radioactivity content.
Extraction: For radio-HPLC measurement, faeces, plasma and tissue samples were sequentially extracted with acetonitrile, acetonitrile:water (90:10, v:v), acetonitrile:water (10:90, v:v), methanol, and 1% aqueous formic acid, if required. Urine were directly analysed without any further work-up. Bile samples were centrifuged and diluted with water, where necessary.
Non-extractable residues: Where considered necessary (the sample residue after extraction contained a suitable portion of the TRR), the sample residue after extraction was dried and treated with protease enzyme. The selection of samples depended on the overall % TRR, the % dose in the RRR, and the level of already achieved extractability (in cases of high overall recovery).
Metabolite identification: Structure elucidation of metabolites was achieved by LC-MS/MS. Extracts of selected faeces samples (0-24h and 24-48h timepoint) as well as extracts from all tissue and plasma samples were subjected to LC-MS/MS analysis. Urine and bile samples from selected timepoints were investigated by LC-MS/MS directly without further work-up. Selection criteria were the overall %dose contained in the respective time intervals, the presence of all major metabolites and the %dose in the individual peaks, with the aim to investigate all peaks >1% dose and provide identification as far as possible. The LC system used with MS/MS (LC02) was identical to the one used for generation of metabolite patterns, ensuring a good comparability.
Identification of polar region: For identification of metabolites in the polar region (unretained by LC02) observed in bile, urine, plasma and tissues, this region was isolated from those sample matrices where it contained enough radioactivity (urine, bile). The isolated regions were chromatographed on a second HPLC system (LC05), providing better retention. In order to identify free morpholine in the polar region, retention times of the isolated polar peaks were compared with the morpholine radioactive reference standard (on LC05). Moreover, MS identification of morpholine in the polar region was achieved by using a HILIC chromatographic system on a urine sample. For quantification of morpholine in the samples, the ratio of the morpholine peak to the rest of the polar fraction resulting from analysis on LC05 was employed to the amount of the entire polar region as observed in LC02.

II. RESULTS AND DISCUSSION

Excretion balance/radioactive residues

Phase 1: Excretion balance

As shown in Table 5.1.1-13, after a single oral dose application of [^{14}C]-BAS 550 F (250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female rats, the excretion of radioactivity during the 168 hours post-dose was similar for both sexes and both labels (>95% of the dose). Excretion of radioactivity was rapid (most was excreted within the first 48 hours after administration), with the majority excreted via faeces (ca. 84-92% of the dose) and smaller amounts via urine (ca. 6-12 % of the dose).
The excretion pattern was similar for both sexes with the females showing slightly higher urinary excretion than males. No difference was observed between the two radiolabelled forms.
The observed excretion pattern is in good agreement with the previous (already peer reviewed) data from study DK-440-001.

Phase 2: Biliary elimination

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female bile duct cannulated rats, the excretion of radioactivity during the 72 hours post-dose (p-chlorophenyl label) or 48 hours post dose (morpholine label) was generally similar for both sexes, both dose levels and both labels (>83 % dose in the low dose group and >70 % of the dose in the high dose group). The majority of the radioactivity was excreted via bile (ca. 61-88% of the dose for the low dose group and 46-60% of the dose for the high dose group), with smaller amounts via urine (ca. 3-5 % dose in males and 10-23 % of the dose in females) and faeces (ca. 5-7 % dose in the low dose group and 13-39% of the dose in the high dose group). The slightly lower biliary excretion in the high dose group in comparison to the low dose animals suggest a possible saturation of absorption at the high dose level (250 mg/kg).

This data clearly shows that the majority of the radioactivity excreted via faeces in the excretion balance phase (phase 1) was due to bile and that biliary excretion is an important route for the excretion of dimethomorph in rats. Biliary excretion in females was slightly less than that observed for males, while urinary excretion in females was slightly more than that observed for males, which is congruent with the results observed in the phase 1 experiments. At the higher dose level, biliary excretion was lower than observed at the low dose level suggesting a saturation of absorption. No major differences were observed between the two radiolabelled forms.
Again, these results are in good agreement with the already peer reviewed data from study DK-440-002.

Table 5.1.1-13: Excretion balance (phase 1)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D1 (250 mg/kg)		D6 (250 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose		% of the dose	
Urine	0-6	0.90	1.14	0.73	0.96
	6-12	1.34	1.67	1.53	1.54
	12-24	2.68	3.40	2.20	2.99
	24-48	1.41	4.20	0.95	4.00
	48-72	0.20	0.86	0.20	1.34
	72-96	0.13	0.22	0.12	0.31
	96-120	0.06	0.13	0.09	0.15
	120-144	0.06	0.12	0.04	0.09
	144-168	0.03	0.08	0.03	0.07
Total urine	*0-168*	***6.81***	***11.82***	***5.89***	***11.45***
Faeces	0-24	57.30	40.02	71.39	36.10
	24-48	29.63	40.12	19.03	36.58
	48-72	1.45	6.87	0.93	9.61
	72-96	0.24	1.55	0.22	1.26
	96-120	0.31	0.90	0.09	0.34
	120-144	0.06	0.07	0.16	0.09
	144-168	0.05	0.20	0.05	0.06
Total faeces	*0-168*	***89.04***	***89.73***	***91.87***	***84.04***
Total excreted	*0-168*	***95.85***	***101.55***	***97.76***	***95.49***
Cage wash	168	0.44	0.93	0.13	0.45
Whole Blood	168	0.01	0.01	0.06	0.06
Kidney	168	0.00	0.00	0.02	0.02
Liver	168	0.07	0.05	0.23	0.22
Total	**0-168**	**96.37**	**102.54**	**98.20**	**96.24**

Table 5.1.1-14: Biliary excretion (Phase 2)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D2 (10 mg/kg)	D3 (250 mg/kg)	D7 (10 mg/kg)	D8 (250 mg/kg)
		p-Chlorophenyl label		Morpholine label	
		Male/Female	Male/Female	Male/Female	Male/Female
		% of the dose		% of the dose	
Bile	0-3	55.06/40.08	8.56/8.85	34.17/30.76	12.81/11.87
	3-6	22.25/13.53	12.95/9.14	13.69/19.82	11.98/6.87
	6-12	8.97/6.05	18.00/11.20	10.16/17.48	18.81/14.98
	12-24	1.30/0.52	16.71/10.48	15.66/2.98	15.35/14.32
	24-36	0.16/0.16	2.82/4.83	0.61/0.31	0.90/7.92
	36-48	0.08/0.10	0.95/1.44	0.13/0.13	0.10/0.55
	48-60	0.06/0.04	0.19/0.33	-	-
	60-72	0.06/0.02	0.03/0.00	-	-
Total bile	*0-Terminal*	***87.94/60.50***	***60.20/46.27***	***74.41/71.47***	***59.94/51.51***
Urine	0-6	1.42/17.91	0.61/1.19	0.83/1.44	0.99/2.92
	6-24	1.68/4.86	2.18/7.60	2.47/10.83	3.93/8.97
	24-48	0.15/0.28	0.28/1.65	0.36/1.50	0.35/5.36
	48-72	0.06/0.15	0.01/0.00	-	-
Total urine	*0-Terminal*	***3.31/23.20***	***3.08/10.44***	***3.66/13.76***	***5.27/17.26***
Faeces	0-24	4.85/6.63	17.30/12.59	3.66/5.01	37.17/14.37
	24-48	0.31/0.51	3.20/0.35	1.09/0.86	2.22/7.26
	48-72	0.01/0.21	0.01/0.00	-	-
Total faeces	*0-Terminal*	***5.17/7.35***	***20.51/12.94***	***4.74/5.86***	***39.39/21.63***
Total excreted	*0-Terminal*	***96.42/91.05***	***83.79/69.64****	***82.81/91.09***	***104.60/90.40***

Terminal sampling: D2, D3 = 72 hours post dose, D7, D8 = 48 hours post dose
*for this dose group 18.96% dose was additionally recovered in stomach content

Phase 3: Tissue depletion
For the p-chlorophenyl label, the total recoveries of administered radioactivity in the kidney, liver, pancreas, blood and plasma at 1 hour following dosing were 0.30, 4.81, 0.06, 0.43 and 0.30% dose respectively for the males (D4M) and 0.29, 3.58, 0.06, 0.53 and 0.37% respectively for the females (D4F) for the low dose group.
In the high dose group, the total recoveries of administered radioactivity in the kidney, liver, pancreas, blood and plasma at 12 hours following dosing were 0.07, 0.80, 0.03, 0.17 and 0.11% dose, respectively for the males (D5M) and 0.06, 0.53, 0.02, 0.14 and 0.10% respectively for the females (D5F).

For the morpholine label low dose group, the total recoveries of administered radioactivity in the kidney, liver, pancreas, blood and plasma at 1 hour following dosing were 0.19, 4.02, 0.03, 0.33 and 0.24% dose, respectively for the males (D9M) and 0.21, 2.74, 0.05, 0.42 and 0.31% respectively for the females (D9F).
For the high dose group, total recoveries of administered radioactivity in the kidney, liver, pancreas, blood and plasma at 12 hours following dosing were 0.06, 0.86, 0.01, 0.18 and 0.13% dose, respectively for the males (D10M) and 0.05, 0.55, 0.01, 0.13 and 0.09% respectively for the females (D10F).

This data shows that the highest levels of radioactivity were found in liver, followed by blood, plasma and kidney while residue levels in pancreas were low. Residue levels in liver were marginally lower in females than males. Plasma residue levels were similar to whole blood. No differences were observed between the two radiolabelled forms.

Table 5.1.1-15: Radioactivity in blood and tissues of the low dose groups (Phase 3)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D4 (10 mg/kg)		D9 (10 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose/conc in µg/g		% of the dose/conc in µg/g	
Liver	1	4.81/11.37	3.58/10.18	4.02/8.679	2.74/7.486
Kidney	1	0.30/3.758	0.29/3.791	0.19/2.392	0.21/2.840
Pancreas	1	0.06/2.327	0.06/2.785	0.03/1.641	0.05/2.256
Whole blood	1	0.43/0.865	0.53/1.078	0.33/0.641	0.42/0.811
Plasma	1	0.30/1.105	0.37/1.398	0.24/0.836	0.31/1.114

Table 5.1.1-16: Radioactivity in blood and tissues of the high dose groups (Phase 3)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D5 (500 mg/kg)		D10 (500 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose/conc in µg/g		% of the dose/conc in µg/g	
Liver	12	0.80/130.2	0.53/87.56	0.86/149.2	0.55/87.07
Kidney	12	0.07/47.13	0.06/46.76	0.06/49.07	0.05/37.28
Pancreas	12	0.03/48.21	0.02/43.96	0.01/46.86	0.01/40.14
Whole blood	12	0.17/17.43	0.14/14.17	0.18/18.23	0.13/13.57
Plasma	12	0.11/21.53	0.10/18.54	0.13/24.93	0.09/17.81

Extractability

Faeces (Phase 1)

Pooled faeces samples from the chlorophenyl label were extracted with acetonitrile followed by acetonitrile:water (90:10, v:v). The achieved extractabilities (ERR) were 87-102% and 95-100% for male and female faeces, respectively.

Pooled faeces samples from the morpholine label were serially extracted, first with acetonitrile and then with acetonitrile:water (90:10, v:v). Some samples (with less than 90% recovery) were extracted a third time using acetonitrile:water (10:90, v:v) or a fourth time using methanol. The achieved extractabilities (ERR) were 83-94% and 90-94% for male and female faeces, respectively.

Tissues (Phase 3)

Pooled samples of plasma, liver, and kidney were sequentially extracted with acetonitrile, acetonitrile:water (90:10, v:v), acetonitrile:water (10:90, v:v), methanol, and 1% aqueous formic acid.
Achieved extractabilities ranged from 73-90% dose for the males and 78-95% dose for the females of the low dose group (chlorophenyl label), respectively. For the morpholine label low dose group, the ERR ranged from 75-99% dose for males and from 83-96% dose for females.
For the high dose group of the chlorophenyl label, extractabilities were 83-96% and 94-99% dose for males and females, respectively. For the high dose group of the morpholine label, the ERR were in the range of 63-90% dose and 77-88% dose for males and females, respectively.

The RRRs of some faeces, liver and plasma samples were treated with protease enzyme, which released between 38 and 41% of the bound radioactivity in faeces, between 39 and 54% of the bound radioactivity in liver and 30% of the bound radioactivity in plasma.

Identification of Metabolites

BAS 550 F was extensively metabolised, all analysed matrices (urine, faeces, bile, plasma, kidney and liver) showed HPLC patterns with a multitude of peaks, each often containing multiple metabolites (due to the possibility of E/Z isomerism and also because of very similar polarity of many metabolites due to their structural similarity). A detailed list of the structures of all identified metabolites with their molecular weight and structure can be found in Table 5.1.1-10.

Urine and faeces (Phase 1)
The metabolite patterns in faeces extracts were largely comparable for both sexes and both labels. BAS 550 F was a major metabolite in faeces and was observed in both isomeric forms (BAS 550 F (E): up to 3% of dose, BAS 550 F (Z): up to 26% of dose). BAS 550 F (Z) was generally the most abundant component, with the following metabolites present in significant amounts: M550F016_37.8min (up to 18% of dose), M550F007E (up to 13% of dose) and M550F006E (up to 6 % of the dose).

The metabolite patterns in urine were also complex despite being a more minor route of excretion than faeces and were largely comparable for both sexes. BAS 550 F was not observed in urine. The following metabolites were present in urine in significant amounts: M550F013_31.4min (up to 2% of dose), M550F016_37.8min (up to 1% of dose), M550F027_38.4min (up to 1% of dose), M550F028_43.1min (up to 2% of dose) and M550F007E (up to 2% of dose).

The morpholine label samples generally contained an additional polar radioactive region which contained the metabolite M550F021 (morpholine, up to 1% of the dose). This metabolite was not observed in the p-chlorophenyl label samples because of the positioning of the [^{14}C]-label.

Table 5.1.1-17: Identified metabolites in urine and faeces of rats

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
BAS550F(E)	-	1.96	-	2.83	-	2.54	-	1.66
BAS550F(Z)	-	16.87	-	26.35	-	18.14	-	15.5
M357_36.2min*	-	-	-	1.82	-	-	-	-
M357_44.9min*	-	3.55	-	5.8	-	3.78	-	4.44
M361	-	-	-	-	0.26	-	-	-
M371_51.4min*	-	16.87	-	26.35	-	18.14	-	15.5
M371_52.6min*	-	-	-		-	-	-	
M377_40.9min	-	-	-	-	0.26	-	-	-
M385*	-	-	-	3.33	-	-	-	-
M391	-	4.16	-	-	-	-	-	-
M405	-	-	-	-	0.26	-	-	-
M405	-	-	-	-	0.26	-	-	-
M419	-	3.39	-	-	-	-	-	-
M419	-	-	-	-	-	4.49	-	-
M421	-	1.16	-	-	-	-	-	-
M435	-	17.96	-	-	-	-	-	-
M435	-	-	-	-	-	12.72	-	-
M471	-	-	-	-	-	4.49	-	-
M547_31.1min*	0.65	-	0.88	-	-	-	-	-
M547_37.5min*	-	-	0.99	-	-	-	1.07	-
M547_38.1min*	-	-	-	-	-	-		-
M550F006E	-	4.13	-	5.8	-	3.78	-	4.44
M550F006Z	-	1.08	-	1.35	-	-	-	1.06
M550F007E	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F007Z	-	3.49	-	3.75	-	3.31	-	3.77
M550F008	-	1.08	-	-	-	-	-	-
M550F009_38.0min	-	17.96	0.99	17.25	-	13.7	1.07	13.7
M550F009_40.6min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F011_41.0min	-	3.85	-	1.41	-	3.43	-	2.55
M550F011_43.4min	-	5.91	1.66	9.52	-	-	1.83	9.57
M550F012_44.1min	-	4.13	-	5.8	-	-	-	-
M550F012_47.2min	-	1.08	-	1.35	-	-	-	-
M550F013_31.4min	0.7	-	2.37	-	0.32	-	1.3	-
M550F015_32.9min	0.74	-	0.98	-	0.56	-	-	-
M550F015_34.5min	-	-	-	-	-	2.86	-	-
M550F016_37.8min	-	17.96	0.99	17.25	-	13.7	1.07	13.7
M550F016_41.1min	-	3.85	-	1.41	-	-	-	-
M550F018_44.6min	-	4.13	-	5.8	-	-	-	-
M550F018_45.0min	-	3.55	-	3.33	-	-	-	-
M550F018_45.6min	-	3.49	-		-	3.04	-	3.53
M550F021 (Morpholine)	-	-	-	-	0.95	-	0.59	-
M550F022_36.2min	-	1.16	-	-	-	2.15	-	-
M550F022_39.3min	-	-	-	1.19	-	4.49	-	-
M550F023_33.5min	-	3.39	-	-	-	2.72	-	1.94

Table 5.1.1-17: Identified metabolites in urine and faeces of rats

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F023_36.2min	-	1.16	-	-	-	-	-	-
M550F024_28.6min	-	-	-	-	-	1.27	-	1.22
M550F024_32.9min	-	-	-	-	-	-	-	1.27
M550F025_36.1min	-	-	-	-	-	2.15	-	-
M550F025_37.3min	-	1.6	-	17.25	-	-	-	0.98
M550F025_38.3min	-	-	0.99		-	4.49	-	3.29
M550F025_40.3min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F027_34.5min	-	-	-	1.53	0.15	2.86	-	-
M550F027_38.4min	-	17.96	0.99	17.25	-	4.49	1.07	3.29
M550F027_41.4min	-	3.85	-	-	-	-	-	-
M550F028_40.5min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F028_41.7min	-	3.85	-	-		-	-	-
M550F028_43.1min	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F029_30.4min	0.65	-	0.88	-	-	-	-	-
M550F029_31.3min	0.7	-	2.37	-	-	-	1.3	-
M550F030_41.2min	-	3.85	-	1.41	-	-	-	-
M550F030_43.4min	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F031_45.1min	-	3.55	-	9.13	-	3.78	-	4.44
M550F033_34.6min	-	1.66	-	1.53	-	2.86	-	-
M550F033_38.6min	-	17.96	0.99	17.25	-	4.49	1.07	3.29
M550F034_37.9min	-	17.96	0.99	-	-	-	-	-
M550F034_41.0min	-	3.85	-	1.41	0.26	3.43	-	2.55
M550F035_36.5min	-	1.16	-	1.82	-	2.15	-	1.97
M550F035_38.8min	-	-	-	-	-	4.49	1.07	3.29
M550F036	-	1.6	-	-	-	-	-	-
M550F037	-	3.39	-	-	-	-	-	-
M550F038	-	3.85	-	1.2	-	-	-	-
M550F039_31.1min	-	-	0.88	-	-	-	-	-
M550F039_40.5min	-	3.55	-	-	-	-	-	-
M550F040_38.6min	-	-	0.99	-	-	-	-	-
M550F040_41.3min	-	3.85	-	-	-	-	-	-
M550F041_30.1min	-	0.98	-	0.72	-	-	-	-
M550F041_34.4min	-	1.66	-	1.53	-	2.86	-	-
M550F042	0.74	-	-	-	-	-	-	-
M550F043	-	1.16	-	-	-	-	-	-
M550F044	-	1.6	-	-	-	-	-	-
M550F045	-	3.85	-	-	-	-	-	-
M550F046	-	-	-	1.82	-	2.15	-	1.97
M550F047_30.7min	-	4.16	-	2.61	0.34	4.08	-	3.03
M550F047_35.1min	-	1.66	-	1.53	-	-	-	-
M550F049_35.4min	-	-	-	-	-	2.86	-	-
M550F049_39.0min	-	17.96	0.99	17.25	-	-	-	-
M550F050_37.1min	-	1.6	-	17.25	-	-	-	0.98
M550F050_39.5min	-	-	-	1.19	-	4.49	-	-
M550F051_49.8min	-	1.96	-	-	-	-	-	-

Table 5.1.1-17: Identified metabolites in urine and faeces of rats

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F051_51.2min	-	16.87	-	26.35	-	18.14	-	15.5
M550F052	-	4.13	-	5.8	-	-	-	-
M550F053_43.3min	-	5.91	1.66	-	-	12.72	-	-
M550F053_51.4min	-	16.87	-	-	-	18.14	-	-
M550F055_30.8min	0.65	4.16	0.88	-	0.34	-	-	-
M550F055_32.0min	0.7	-	-	-	-	1.12	-	0.28
M550F056_30.5min	-	4.16	-	0.72	-	-	-	-
M550F056_34.5min	-	1.66	-	-	-	-	-	-
M550F057_38.3min	-	-	-	-	-	4.49	-	3.29
M550F057_41.3min	-	3.85	-	1.41	-	-	-	-
M550F058_34.5min	-	1.66	-	-	-	2.86	-	-
M550F058_38.0min	-	17.96	-	-	-	13.7	-	-
M550F060	-	3.49	-	-	-	-	-	-
M550F061	-	3.55	-	-	-	3.52	-	-
M550F062_31.4min	0.7	-	2.37	-	-	-	-	-
M550F062_34.6min	-	1.66	-	-	-	-	-	-
M550F063_31.8min	0.7	-	2.37	-	-	-	-	-
M550F063_35.1min	-	1.66	-	-	-	-	-	-
M550F064_30.9min	0.65	-	-	-	-	-	-	-
M550F065	0.7	-	-	-	-	-	-	-
M550F066	-	16.87	-	26.35	-	18.14	-	-
M550F067_25.5min	-	-	0.96	-	-	-	-	-
M550F068_36.1min	-	1.16	-	-	-	-	-	-
M550F069_30.3min	0.65	-	0.88	-	0.34	-	-	-
M550F069_35.5min	-	-	-	-	-	2.86	-	-
M550F070_25.5min	0.64	-	-	-	-	-	-	-
M550F070_32.2min	0.74	-	-	-	-	-	-	-
M550F071	-	-	0.99	-	-	-	-	-
M550F072_26.5min	0.64	-	-	-	-	-	-	-
M550F073_32.1min	0.7	-	-	-	-	-	-	-
M550F074_25.4min	0.64	-	0.96	-	-	-	-	-
M550F074_27.7min	-	-	-	-	0.16	-	-	-
M550F074_31.0min	-	-	-	-	0.34	-	-	-
M550F075_32.1min	-	-	-	-	-	-	1.3	-
M550F076_30.8min	0.65	-	-	-	0.34	4.08	-	-
M550F076_31.3min		-	0.88	-		-	-	-
M550F078_30.5min	0.65	-	0.88	-	-	-	-	-
M550F079_30.4min	-	-	-	-	0.34	-	-	-
M550F081_32.1min	0.7	-	-	-	-	-	1.3	-
M550F083_37.4min	-	-	-	17.25	-	-	-	13.7
M550F083_37.9min	-	-	0.99	-	-	13.7	1.07	
M550F084	0.64	-	-	-	-	-	-	-
M550F087	0.74	-	0.98	-	-	-	-	-
M550F088	-	-	-	-	0.16	-	-	-
M550F090_31.9min	0.7	-	-	-	0.32	1.12	1.3	0.28

Table 5.1.1-17: Identified metabolites in urine and faeces of rats

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F090_34.6min	-	-	-	1.53	-	2.86	-	-
M563	0.07	-	-	-	-	-	-	-
M565	0.64	-	-	-	-	-	-	-
M567	0.64	-	-	-	-	-	-	-
M567_25.4min	-	-	0.96	-	-	-	-	-
M567_25.4min	-	-	0.42	-	-	-	-	-
M581	-	-	-	-	0.16	-	-	-
M595	-	-	-	1.53	-	-	-	-

* This metabolite was not assigned a metabolite code name (designation contains the M550F prefix) as it is considered to be a degradation product of an original metabolite

Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response

Bile (Phase 2)

The metabolite patterns in bile were again complex and were largely comparable for both sexes and both dose levels. BAS 550 F was not observed in bile. The most abundant metabolite in bile was M550F013, in amounts from 22-40% dose (sum of isomers at 31.4min and 34.7min). The following metabolites were also present in bile in significant amounts: M550F069_30.3min (up to 12% of dose), M550F076_30.8min (up to 6% of dose), M547_31.1min (up to 6% of dose), M550F075_32.1min (up to 8% of dose), M547_37.5min (up to 9% of dose), M550F083_37.9min (up to 9% of the dose), M550F027_38.4min (up to 9% of dose) and M550F007E (up to 7% of dose).

Although the morpholine label bile samples generally contained an additional polar radioactive region which contained the metabolite M550F021 (morpholine), this metabolite was not present in significant amounts in bile.

Table 5.1.1-18: Identified metabolites in bile from rats

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F021 (Morpholine)	-	-	-	-	0.34	-	-	-
M550F069_23.5min / M550F086_24.0min / M550F062_24.3min	1.03	-	-	-	-	-	-	-
M550F080_25.4min / M550F074_25.4min / M550F070_25.5min / M550F082_25.9min / M550F084	4.00	1.36	2.29	1.11	3.21	1.79	2.00	1.44
M550F089_27.6min / M550F074_27.7min / M550F064_27.7min / M550F088	2.20	1.43	2.84	1.13	3.38	1.91	2.46	1.08
M550F076_28.6 / M550F077 M550F067_29.8min M550F068_29.7min / M550F086_30.0min / M550F069_30.3min / M550F029_30.4min M550F055_30.8min M550F072_29.9min M550F079_30.2min ;M623 M550F086_30.0min M550F079_30.4min	-	-	-	-	-	-	3.10	0.99
M550F085 / M550F078_28.7	-	1.19	-	-	-	1.47	-	0.41
M550F077 / M550F068_29.7min / M550F086_30.0min	2.79	-	-	-	2.27	-	-	-

Table 5.1.1-18: Identified metabolites in bile from rats

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F069_30.3min / M550F079_30.4min / M550F029_30.4/ M550F078_30.5 / **M550F076_30.8min /** M550F055_30.8min / M550F064_30.9min / M550F074_31.0min / M579 **M547_31.1min /** M550F076_31.3min / M480 M547_31.5min	11.53	5.46	5.97	2.65	8.65	6.42	3.94	2.27
M550F029_31.3 M550F062_31.4min / M550F081_31.4min / M550F082_31.5min / **M550F013_31.4min** / M550F063_31.8min	32.52	24.97	20.77	17.90	25.13	27.54	26.40	23.90
M550F073_32.1min M550F081_32.1min / **M550F075_32.1min** / M550F070_32.2min M550F089_32.6	7.86	5.33		1.45	3.84	5.37		
M550F090_31.9min / M550F055_32.0min / M550F081_32.1min / M550F073_32.1min / M550F075_32.1min / M550F070_32.2min / M623_32.2min / M550F086_32.6min / M550F089_32.6min	-	-	-	-	-	-	-	1.43
M550F015_32.9min	1.52	2.41	3.60	2.61	2.41	2.17	4.02	3.43
M550F074_33.7min	1.98	-	-	-	-	-	-	-
M550F027_34.5min / M550F015_34.5min / M550F073_34.6min/ M550F090_34.6min / **M550F013_34.7min /** M550F033_34.6min	7.55	7.35	5.55	4.37	8.02	7.77	5.41	8.15
M547_37.5min / **M550F083_37.9min /** M550F009_38.0min/ **M550F027_38.4min** M550F059/ M547_38.1min / M550F071 M550F040_38.6min M550F033_38.6min	9.17	6.11	6.32	6.01	6.97	7.64	7.18	7.48

Table 5.1.1-18: Identified metabolites in bile from rats

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F025_40.3min / M550F028_40.5min / M550F009_40.6min / M577_41.6min	-	-	-	-	-	-	-	0.64
M577_42.6 / M550F018_42.6min M550F028_43.1min / M550F030_43.4min M550F053_43.3 M550F011_43.4min / **M550F007E**	4.31	3.51	2.62	2.44	5.54	6.82	3.19	4.25
M550F006E / M417 / M550F031_45.1min	-	-	-	-	-	-	-	0.48

#: Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response. Not all metabolites were identified during every sample period.

Plasma and tissues (Phase 3)
The metabolite patterns in plasma were complex despite containing relatively small amounts of radioactivity and were similar for both sexes and both dose levels. BAS 550 F was a major metabolite in plasma and was observed in the both isomeric forms (BAS 550 F (E): up to 0.01% of the dose, BAS 550 F (Z): up to 0.15% of the dose). The following metabolites were also present in the major radioactive regions in plasma: M550F013_31.4min (up to 0.02% of dose), M550F022_39.3min (up to 0.02% of dose), M550F018_45.0min (up to 0.10% of dose, sum of isomers at 45.0 and 45.6 min).

The metabolite patterns in kidney were complex and largely comparable for both sexes and both dose levels. BAS 550 F was a major metabolite in kidney and was observed in the both isomeric forms (BAS 550 F (E): up to 0.01% of the dose, BAS 550 F (Z): up to 0.06% of the dose). The following metabolites were also present in the major radioactive regions in kidney: M550F027_38.4min (up to 0.04% of dose), M550F011_41.0min (up to 0.01% of dose), M550F028_43.1min (up to 0.07% of dose), M550F007E (up to 0.02% of dose), M550F031_45.1min (up to 0.03% of dose), M550F011_43.4min (up to 0.01% of dose) and M550F018_45.6min (up to 0.04% of dose).

The metabolite patterns in liver were complex and largely comparable for both sexes and both dose levels. BAS 550 F (Z) was present in the major radioactive regions in liver (up to 0.43% of dose). The following metabolites were also present in the major radioactive regions in liver: M550F076_28.6min (up to 0.22% of dose), M550F009_38.0min (up to 0.10% of dose), M550F027_38.4min (up to 0.41% of dose), M550F028_40.5min (up to 0.06% of dose), M550F028_43.1min (up to 0.63% of dose), M550F007E (up to 0.63% of dose), M550F011_43.4min (up to 0.08% of dose), M550F031_45.1min (up to 0.21% of dose) and M550F018_45.6min (up to 0.41% of dose).

The morpholine label samples of plasma, kidney and liver generally contained an additional polar radioactive region (up to 0.03%, 0.05% and 0.74% of dose in plasma, kidney and liver, respectively). In matrices which contained sufficient radioactivity to allow additional analysis (urine and bile), M550F021 (morpholine) was identified as one of the radioactive components present in that region, thus it is very likely that the polar regions in plasma, kidney and liver partly contain this metabolite as well.

Table 5.1.1-19: Identified metabolites in plasma, kidney, and liver (p-chlorophenyl label)

Metabolite / Component	Plasma male low	Plasma female low	Plasma male high	Plasma female high	Kidney male low	Kidney female low	Kidney male high	Kidney female high	Liver male low	Liver female low	Liver male high	Liver female high
	Composition of radioactive residues in % of the dose											
BAS 550 F (Z)	0.03	0.15	0.02	0.05	-	0.04	0.01	0.02	-	0.43	-	0.10
BAS 550 F (E)	-	-	0.01	0.01	-	-		0.01	-	-	-	-
M550F009_38.0	-	-	-	-	-	-	-	-	-	-	0.10	-
M550F009_40.6											0.06	
M550F007E					0.03				0.28		0.05	0.08
M550F011_41.0	-	-	-	-			0.01	-	-	-	0.06	-
M550F011_43.4	-	-	-	-	0.03	0.07	-	-	0.28	0.42	0.05	0.08
M550F018_45.0	0.02	0.04	-	-	0.03	-	-	-	-	-	-	0.08
M550F018_45.6	0.04	0.06	0.01	0.01		-	0.01	0.01		0.41	-	0.07
M550F027_38.4	-	-	-	-	0.04	0.04	-	-	0.38	-	-	-
M550F028_40.5	-	-	-	-	-	-	0.01	-	-	-	0.06	-
M550F028_43.1	-	-	-	-	0.03	0.07	-	-	0.28	0.42	-	0.08
M550F030_41.2							0.01					
M550F030_43.4						0.07				0.42		0.08
M550F031_45.1	-	-	-	-	0.03	-	0.01	-	-	-	-	0.07
M550F031_43.4												0.08
M550F034_41							0.01					
M550F076_28.6	-	-	-	-	-	-	-	-	0.22	-	-	-
M550F083_37.9											0.10	

Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response.

Table 5.1.1-20: Identified metabolites in plasma, kidney, and liver (morpholine label)

Metabolite / Component	Plasma male low	Plasma female low	Plasma male high	Plasma female high	Kidney male low	Kidney female low	Kidney male high	Kidney female high	Liver male low	Liver female low	Liver male high	Liver female high
	Composition of radioactive residues in % of the dose											
BAS 550 F (Z)	0.02	0.09	0.01	0.03	-	0.06	0.01	0.01	-	0.22	-	0.04
BAS 550 F (E)	-	-	-	-	-	-	0.00	-	-	-	-	-
M550F007E	-	-	-	-	0.02	-	0.00	-	0.53	0.63	0.04	0.12
M550F009_38.0	-	-	-	-	-	-	-	-	-	-	0.05	0.04
M550F009_40.6											0.05	
M550F011_43.4	-	-	-	-	0.02	0.04	0.00	0.01	0.53	0.63	0.04	0.12
M550F013_31.4	0.02	0.01	0.01	0.00	-	-	-	-	-	-	-	-
M550F018_45.0	-	0.03	-	-	0.02	0.04	-	-	0.21	-	-	0.12
M550F018_45.6	0.02	0.03	0.01	0.01			-	-		-	-	-
M550F022_39.3	0.02	-	-	-	-	-	-	-	-	-	-	-
M550F027_38.4	-	-	-	-	0.03	-	-	0.00	0.41	0.29	0.05	0.04
M550F028_40.5	-	-	-	-	-	-	-	-	-	-	0.05	-
M550F028_43.1	-	-	-	-	0.02	0.04	0.00	0.01	0.53	0.63	0.04	0.12
M550F029_31.3			0.01	0.01								
M550F030_43.4						0.04	0.00	0.01		0.63	0.04	0.12
M550F031_45.1	-	-	-	0.01	0.02	-	0.00	-	0.21	-	0.04	0.12
M550F083_37.9											0.05	0.04

Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response.

E/Z isomer ratio of dimethomorph

The E/Z isomer ratio for BAS 550 F in the application formulations were approx. 44:52.

The E and Z isomers of BAS 550 F were identified in most faeces samples and some plasma/tissue samples.

In faeces, the E/Z isomer ratio was relatively stable at ca. 13:87 in the first sampling period (0-24 hours), regardless of sex and label. In the later sampling periods (24-48 and 48-72 hours), only smaller amounts of the Z isomer were observed.

In plasma, kidney and liver, the E/Z isomer ratio (where calculable) ranged between 57:43 and 7:93 with most samples containing more Z isomer.

Storage stability

Preliminary HPLC analysis (method LC02) was performed on selected samples of urine (male and female, both labels) and bile (male and female, both labels, 10 mg/kg) ca. 3 months after sampling. Additional preliminary HPLC analysis (method LC02) was performed on selected samples of urine (male, p-chlorophenyl label), bile (male, p-chlorophenyl label, 10 mg/kg) and faeces (male, p-chlorophenyl label) ca. 6 months after sampling (within 1 month of extraction for faeces).

All quantitative HPLC runs (method LC02) of urine, faeces and bile were accomplished within ca. 9 months after sampling. Comparison of preliminary HPLC analysis and actual analysis showed that the metabolic patterns obtained were similar, demonstrating stability during storage.

The quantitative HPLC runs (method LC02) of plasma/kidney/liver extracts were accomplished with the LC-MS analysis ca. 6 months after extraction. The extraction of the plasma/kidney/liver samples was generally accomplished within ca. 6 months after sampling.

Metabolic pathway

The proposed metabolic pathway in rats is depicted in Figure 5.1.1-1.

The observed transformation steps in the metabolism of dimethomorph are

- hydroxylation of either the dimethoxy or chlorophenyl ring and subsequent glucuronidation (Metabolic steps 1 and 2)
- demethylation of the dimethoxy ring and subsequent hydroxylation and/or glucuronidation (Metabolic step 3)
- hydroxylation of the morpholine ring and subsequent modification (further hydroxylation, ring opening, degradation, conjugation) (Metabolic step 5)
- cleavage and release of the intact morpholine ring (Metabolic step 4)

Generally, many observed metabolites combine different metabolic steps with their sequence not being able to be exactly stated.

The metabolic step 3 leads to a considerable number of different metabolites, combining single or double demethylation, hydroxylation/opening of the morpholine ring and glucuronide conjugation.

Metabolic step 5 comprises many metabolic structures arising from several subsequent metabolic steps following the hydroxylation of the morpholine ring.
Substep 5a includes double hydroxylation of the morpholine ring, its opening, demethylation and glucuronidation.

Substep 5b includes metabolites with a reduction at the morpholine ring
Substep 5c includes metabolites with an additional hydroxylation of the dimethoxy ring.
Substep 5d summarizes all structures with an opening of the morpholine ring at the oxygen atom and subsequent degradation as well as glucuronidation/acetylation.
Substep 5e summarizes all structures with an opening of the morpholine ring at the nitrogen atom and subsequent degradation as well as glucuronidation/acetylation

Although difficult to say due to the complexity of the metabolic patterns, it seems that metabolic steps 3 and 5 are the predominant ones also in quantitative terms. Step 4 (Cleavage and free morpholine) was clearly found to be a minor pathway in the rat.

The metabolic pathway observed in the new study is in good agreement with the information coming from the already peer reviewed studies (DK-440-001 and DK-440-002). Metabolite CUR 7586 (=M550F010), depicted in the pathway based on previous data, was found in its other isomeric form as M550F051. Metabolite Z43 (=M550F005) from the previous pathway was observed in its single or double demethylated form as M550F039, M550F040 and M550F037, respectively. This confirms that all metabolic steps observed in the previous studies are observed in the new study as well.

III. CONCLUSION

After a single oral dose application of [^{14}C]-BAS 550 F (250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female rats, the excretion of radioactivity during the 168 hours post-dose was similar for both sexes and both labels (>95% of the dose). Excretion of radioactivity was rapid (most was excreted within the first 48 hours after administration), with the majority excreted via faeces (ca. 84-92% of the dose) and smaller amounts via urine (ca. 6-12 % of the dose).

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 250 mg/kg bw, labelled in two separate locations: p-chlorophenyl or morpholine ring) to male and female bile duct cannulated rats, the excretion of radioactivity during the 72 hours post-dose (p-chlorophenyl label) or 48 hours post dose (morpholine label) was generally similar for both sexes, both dose levels and both labels (>83 % dose in the low dose group and >70 % of the dose in the high dose group). The majority of the radioactivity was excreted via bile (ca. 61-88% of the dose for the low dose group and 46-60% of the dose for the high dose group), with smaller amounts via urine (ca. 3-5 % dose in males and 10-23 % of the dose in females) and faeces (ca. 5-7 % dose in the low dose group and 13-39% of the dose in the high dose group). The slightly lower recoveries in the bile from the high dose group in comparison to the low dose suggest a possible saturation of absorption at the high dose level (250 mg/kg).

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 500 mg/kg bw, labelled in two separate locations: p-chlorophenyl or morpholine ring) to male and female rats, radioactivity in the investigated tissues (kidney, liver, pancreas, whole blood and plasma) at the sampling times used (1 hour at the low dose level, 12 hours at the high dose level) were in the same range for both sexes and both labels. Highest proportions of administered radioactivity were observed in liver (3-5 % dose in the low dose group and 0.5-0.9 % dose in the high dose group). The radioactivity residues in kidney were considerably lower (ca. 0.2-0.3 % dose in the low dose group and 0.05-0.07 % dose in the high dose group) and even less in the pancreas. Residue levels in whole blood ranged from 0.3-0.5 % dose at the low dose level or 0.1-0.2 % dose at the high dose level, plasma residue levels were similar to whole blood.

BAS 550 F was extensively metabolised, all analysed matrices (urine, faeces, bile, plasma, kidney and liver) showed HPLC patterns with a multitude of peaks, each often containing multiple metabolites (due to the possibility of E/Z isomerism and also because of very similar polarity of many metabolites due to their structural similarity).

As a conclusion, the metabolism of Dimethomorph in rat was shown to be very extensive. The main transformation steps observed in this study confirm and complement the metabolic pathway known from the previous studies. The main metabolic steps were identified as:

- hydroxylation of either the dimethoxy or chlorophenyl ring and subsequent glucuronidation
- demethylation of the dimethoxy ring and subsequent glucuronidation
- hydroxylation and oxidative opening of the morpholine ring and subsequent conjugation
- cleavage and release of the intact morpholine ring

Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (representing already peer reviewed and new data)

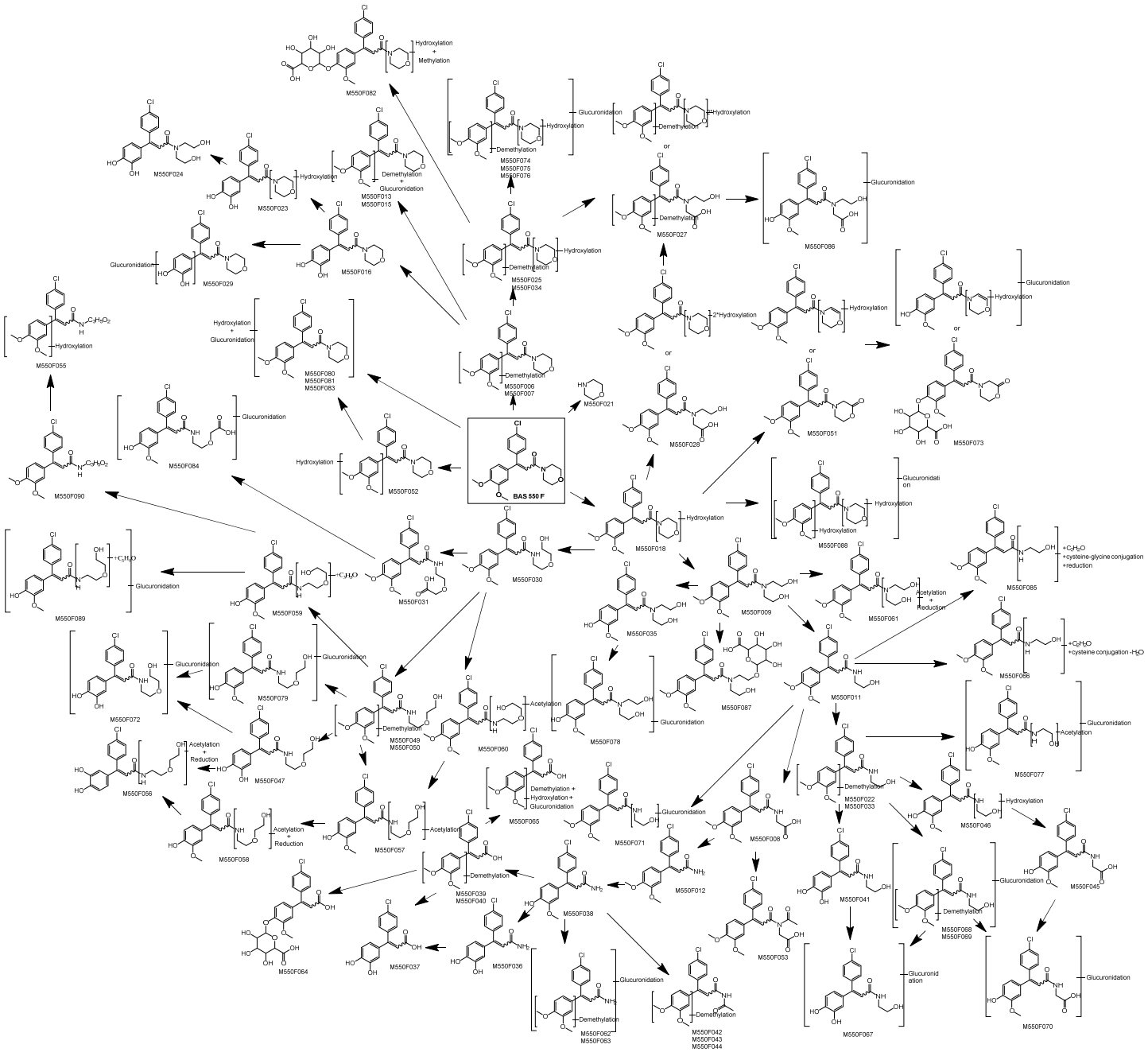
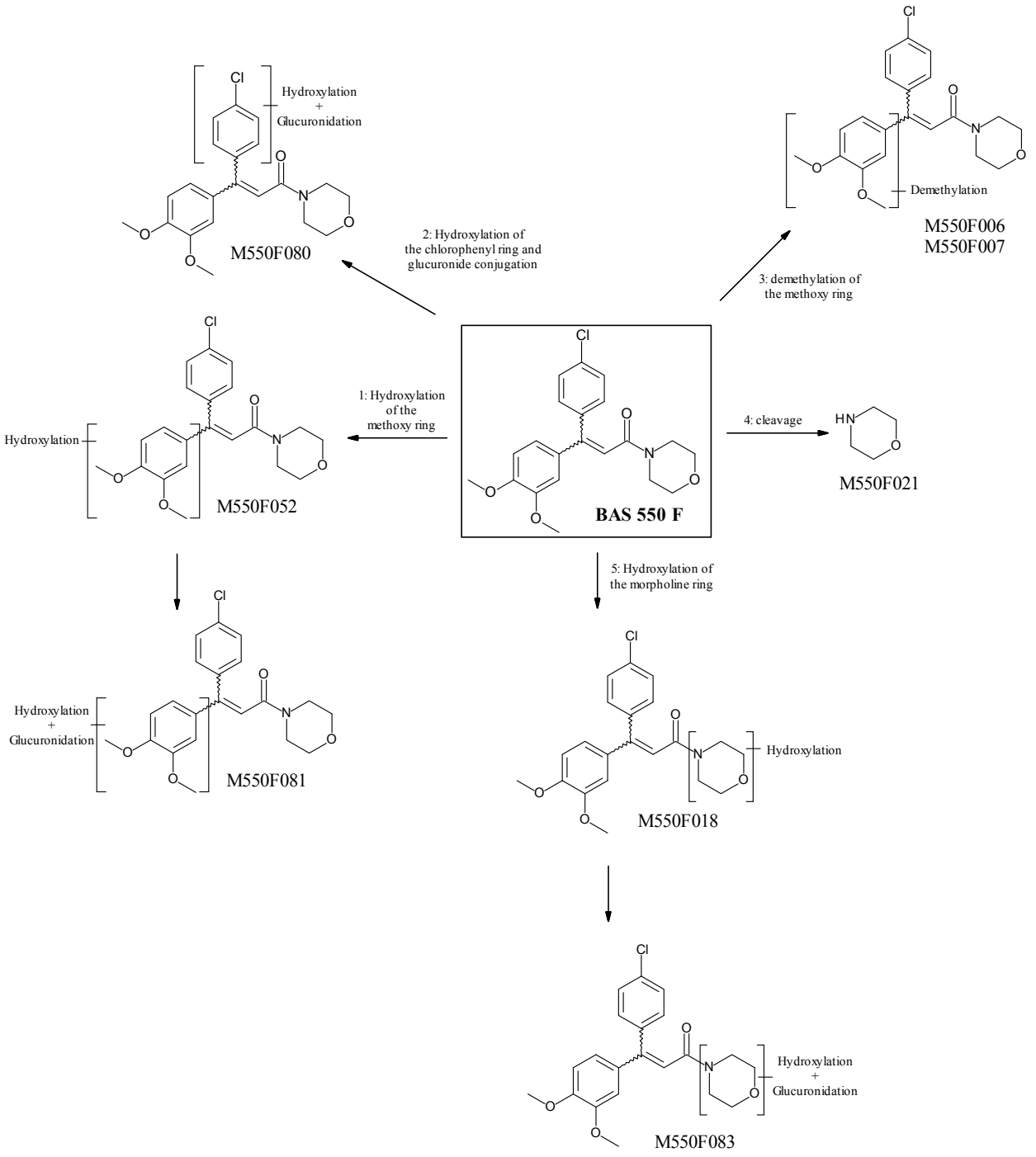


Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial 5 metabolic steps



Steps 3 and 5 have further metabolic steps, as shown in the following figures.

Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 3: Demethylation of the dimethoxy ring

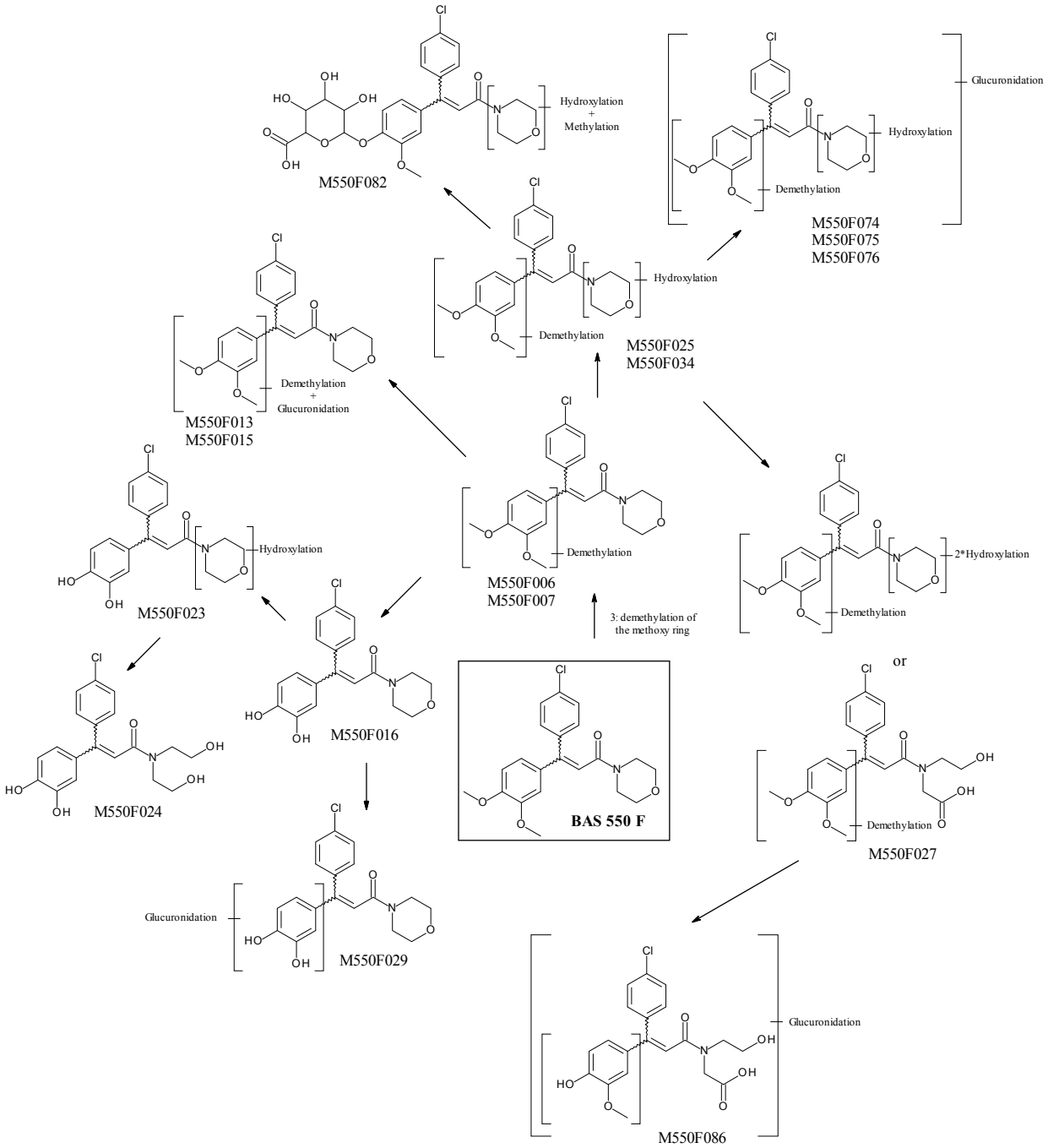


Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and further modification of the morpholine ring (opening, degradation, conjugation)

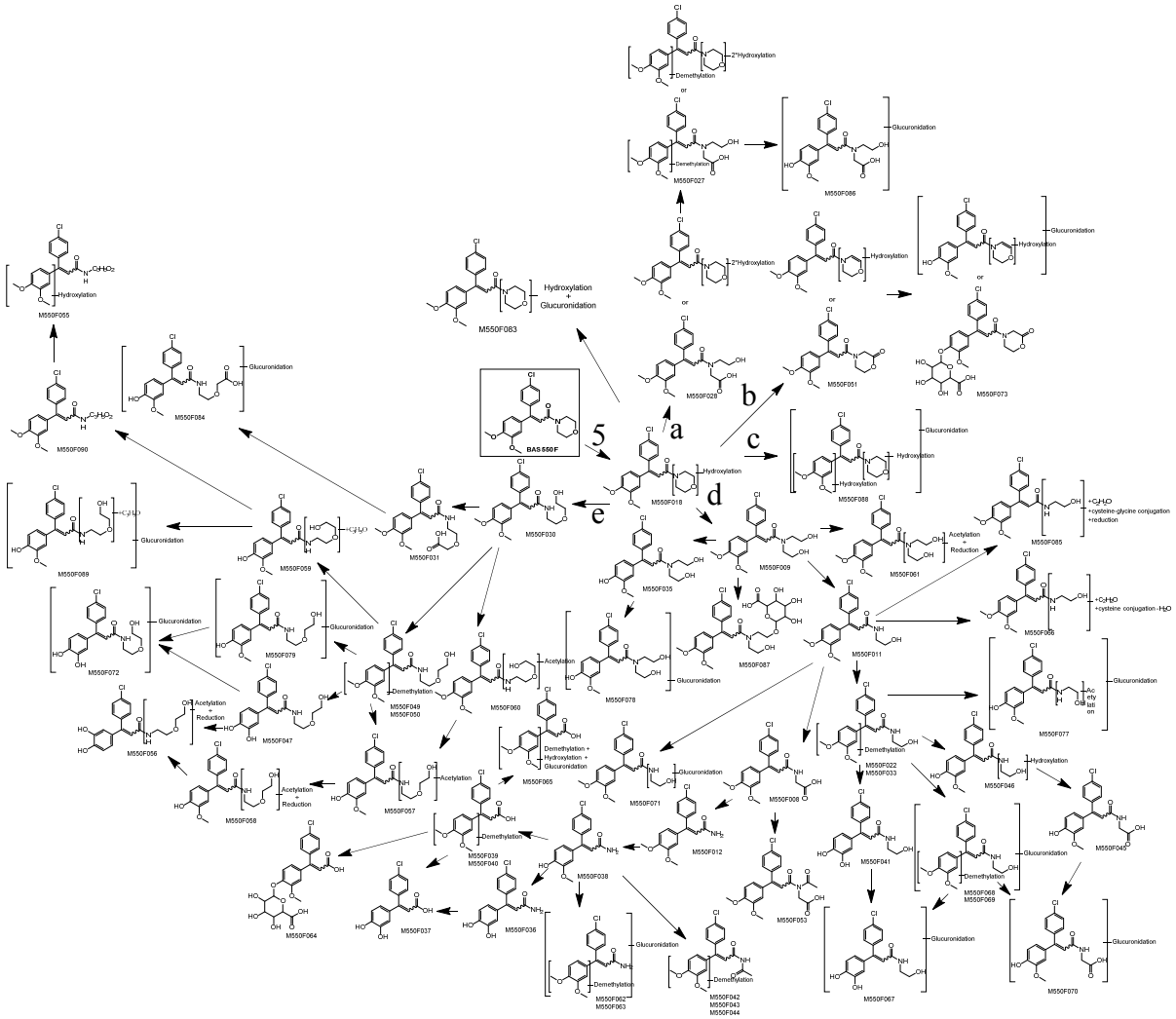


Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and further modification of the morpholine ring, sub-steps a, b and c:

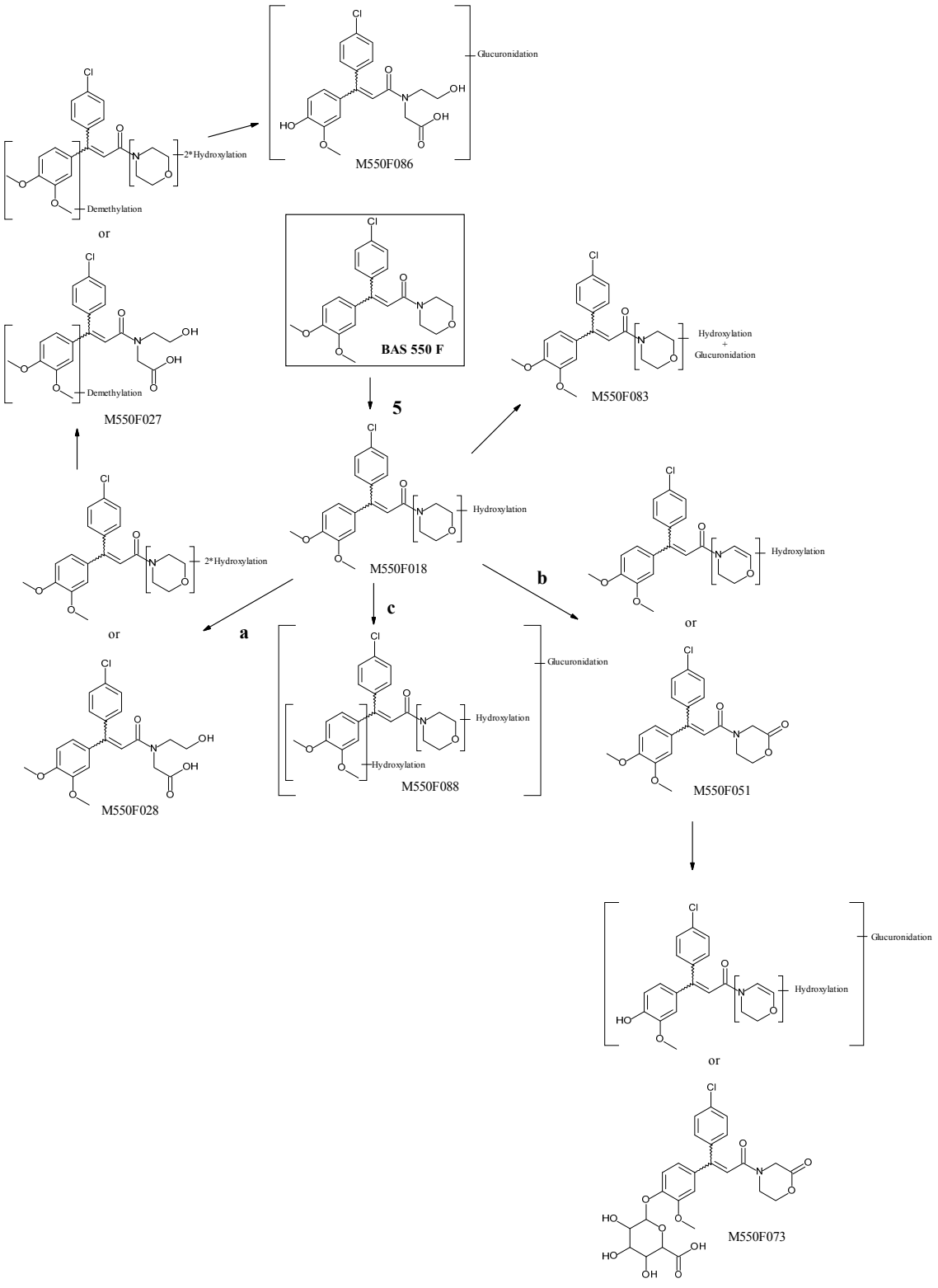
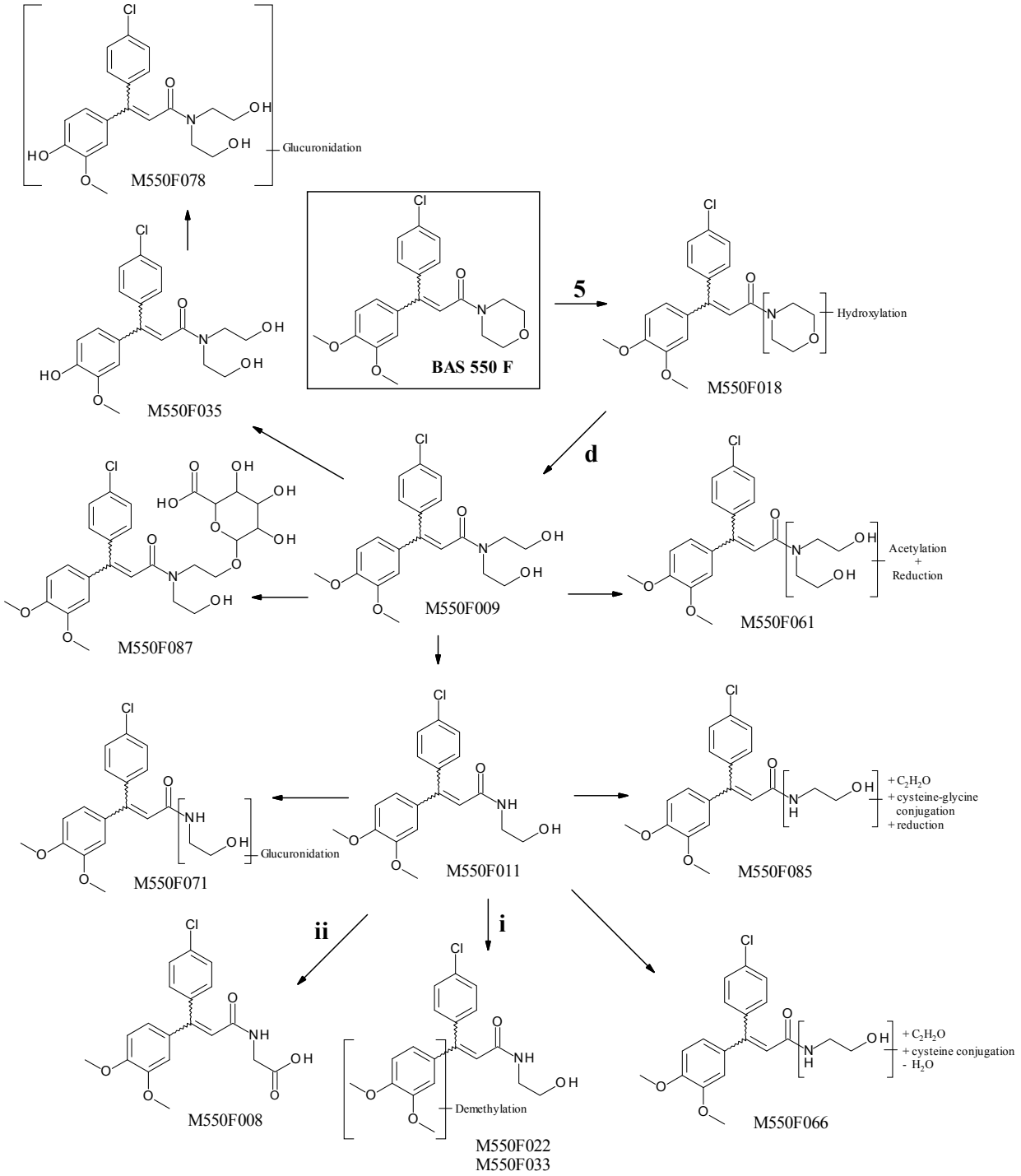


Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d (ring opening at the oxygen and degradation)



Steps 5 d i and 5 d ii have further metabolic steps, as shown in the following figures.

Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d i

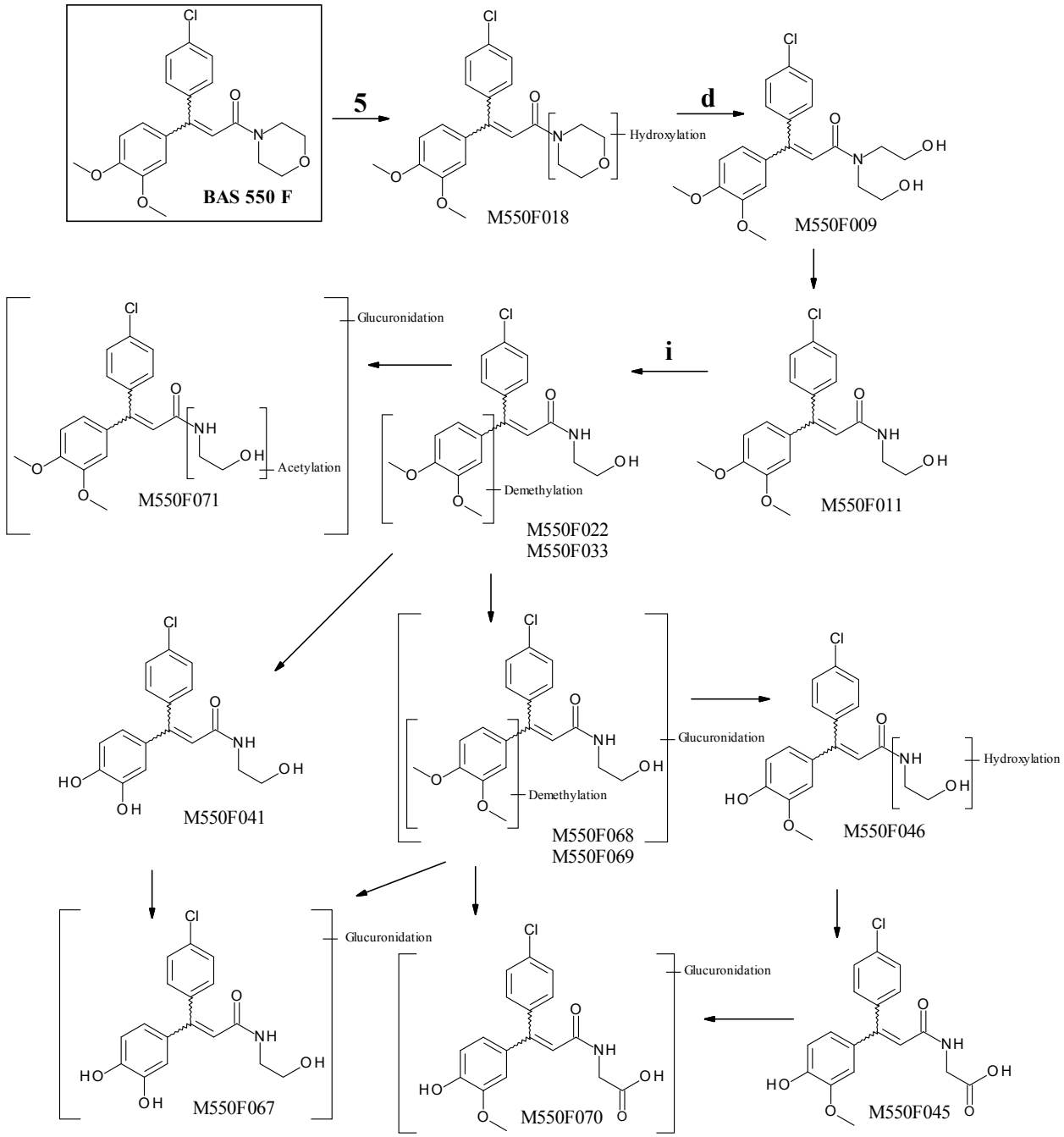
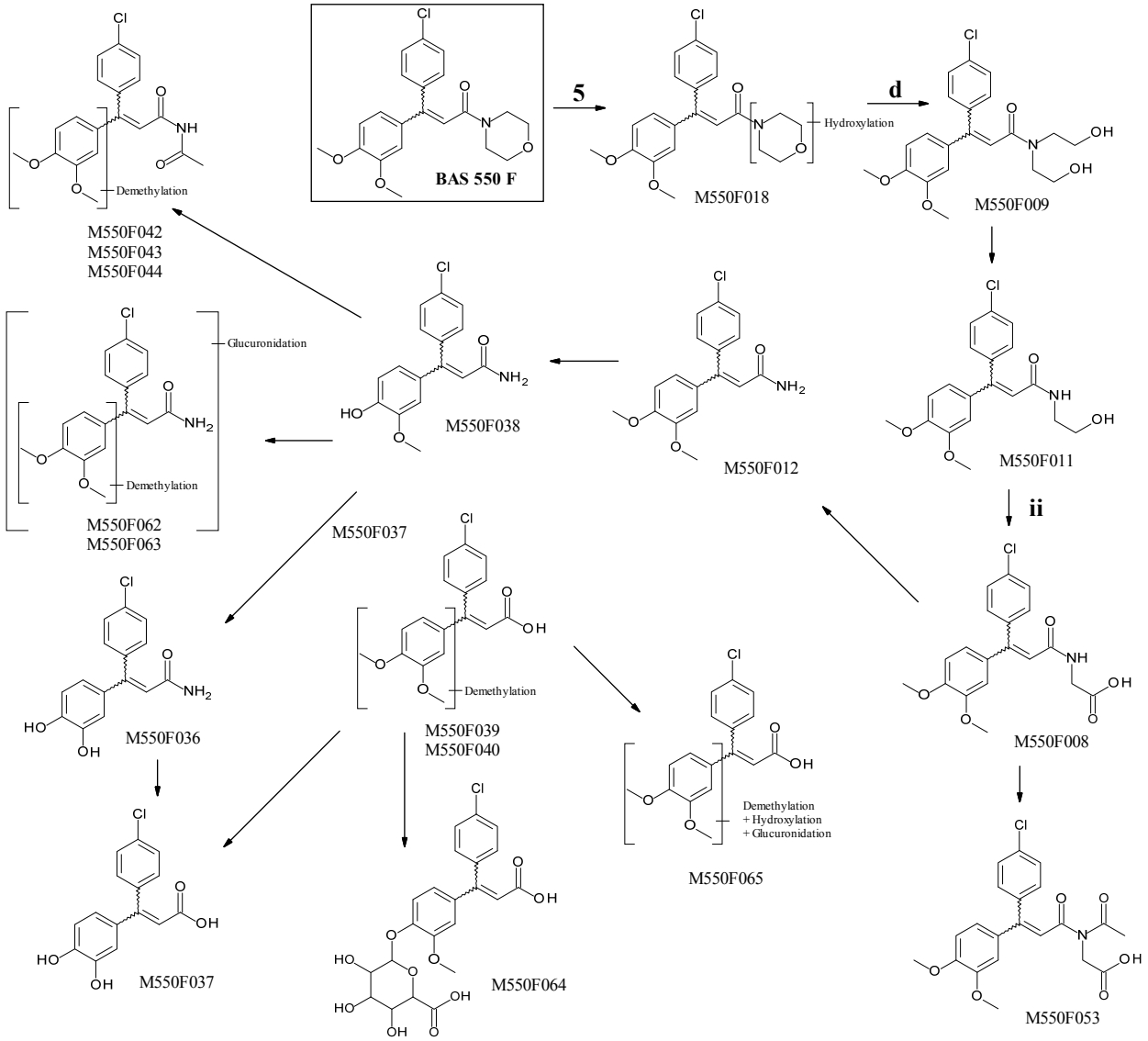


Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d ii[1]



[1] Please note the metabolites M550F039, M550F040, M550F037, M550F064 and M550F065 could also occur as products of the Cleavage pathway (Step 4)

Figure 5.1.1-1: Proposed metabolic pathway of BAS 550 F in rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step e (ring opening at the nitrogen and degradation)

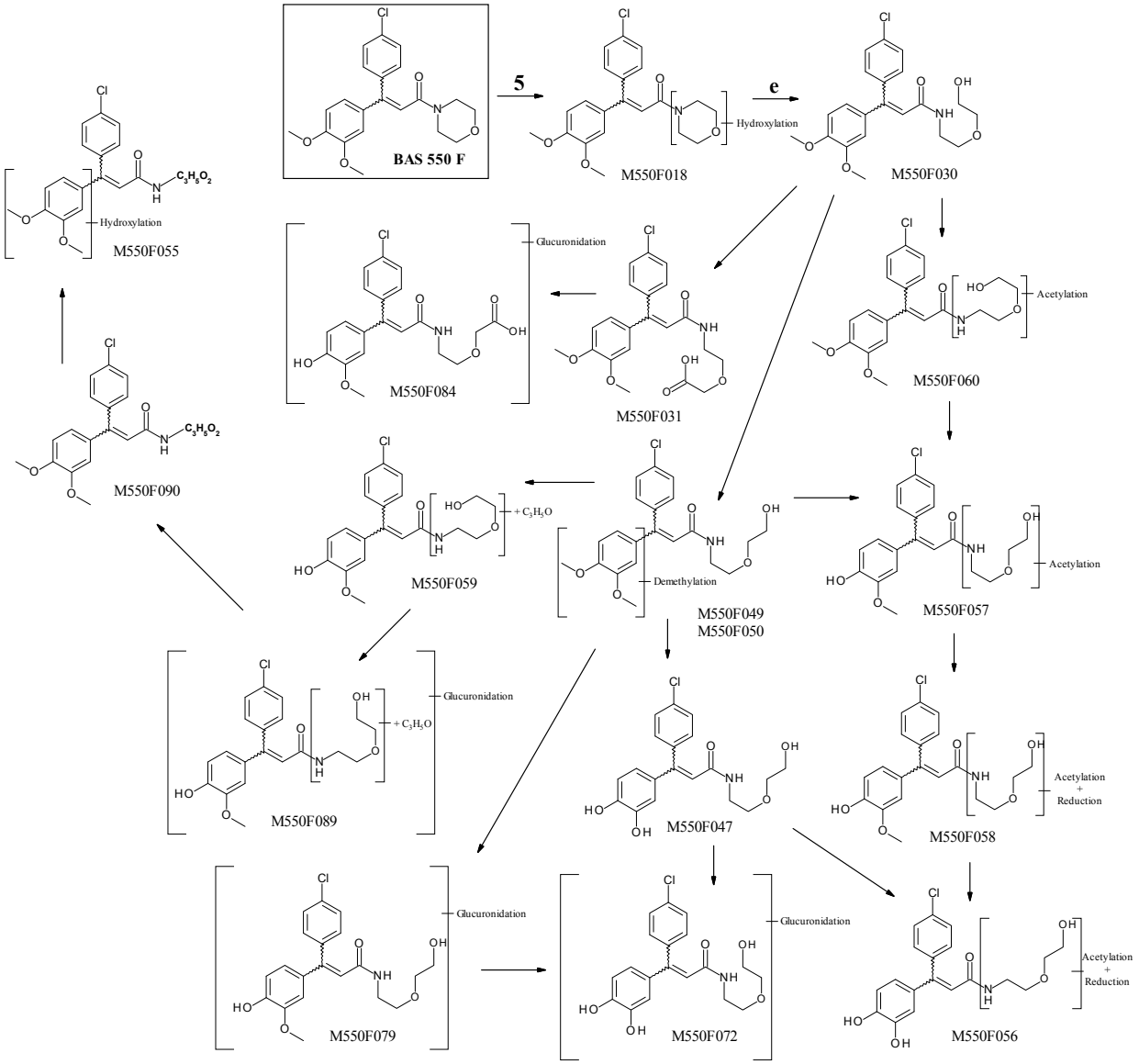


Table 5.1.1-21 List of identified metabolites in rats

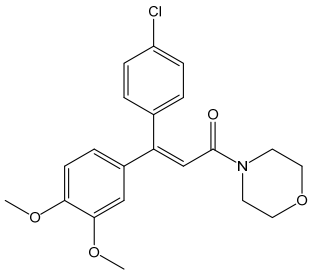
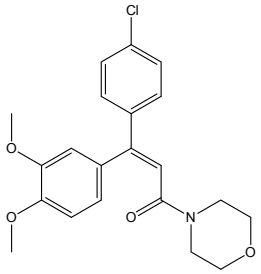
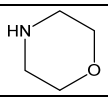
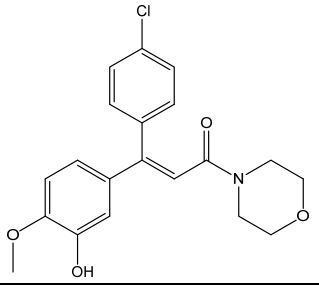
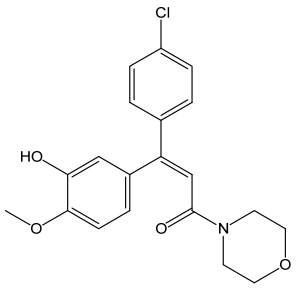
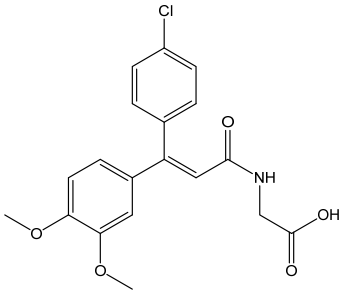
Metabolite code	MW	Proposed structure
BAS550F(E)	387	
BAS550F(Z)	387	
M550F021	87	
M550F006E	373	
M550F006Z	373	
M550F008	375	

Table 5.1.1-21 List of identified metabolites in rats

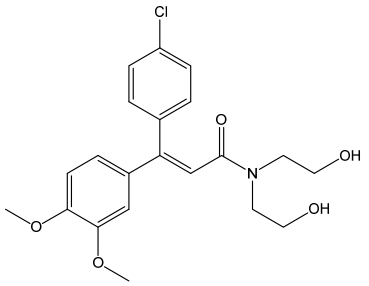
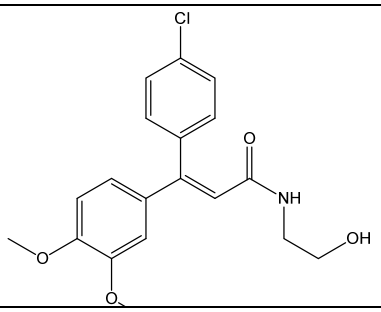
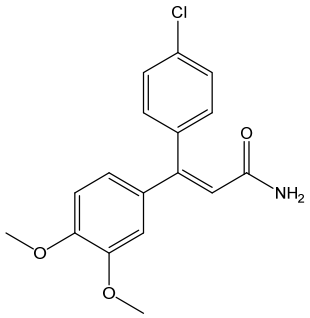
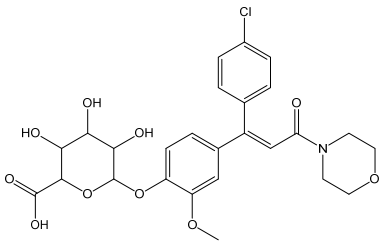
Metabolite code	MW	Proposed structure
M550F009	405	
M550F011	361	
M550F012	317	
M550F013	549	

Table 5.1.1-21 List of identified metabolites in rats

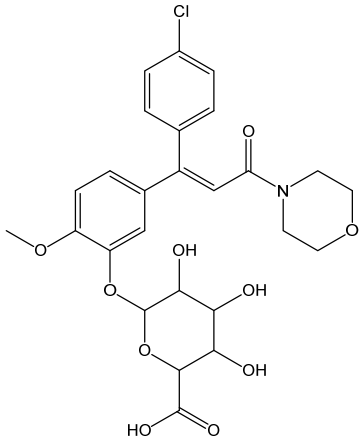
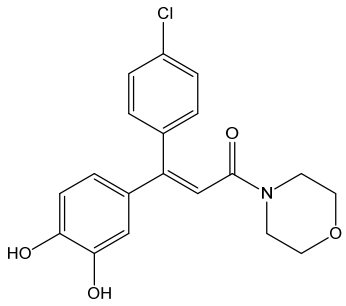
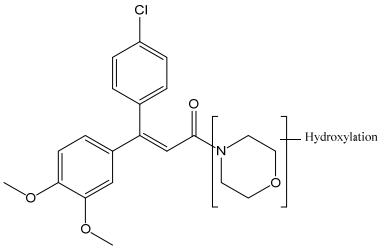
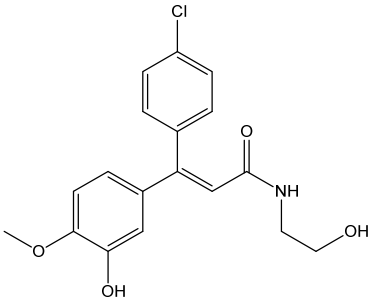
Metabolite code	MW	Proposed structure
M550F015	549	
M550F016	359	
M550F018	403	
M550F022	347	

Table 5.1.1-21 List of identified metabolites in rats

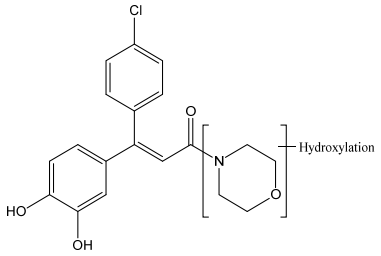
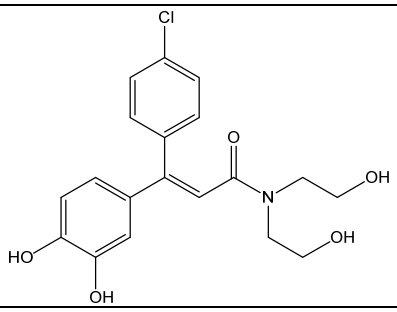
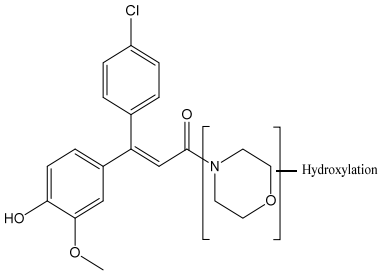
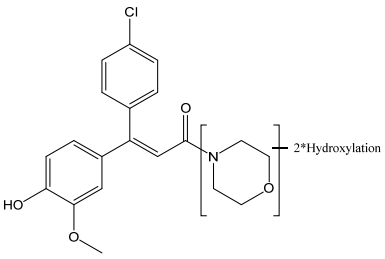
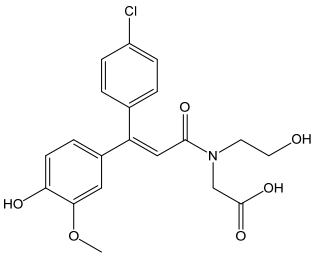
Metabolite code	MW	Proposed structure
M550F023	375	
M550F024	377	
M550F025	389	
M550F027	405	 or 

Table 5.1.1-21 List of identified metabolites in rats

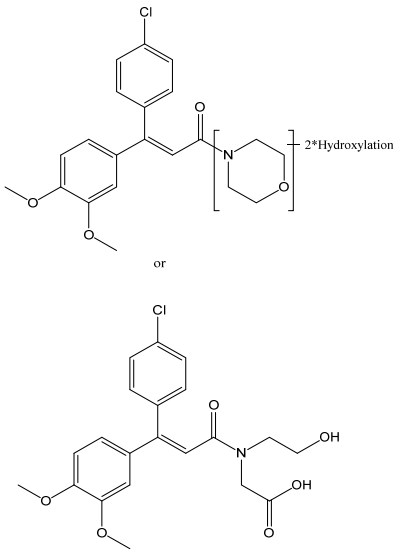
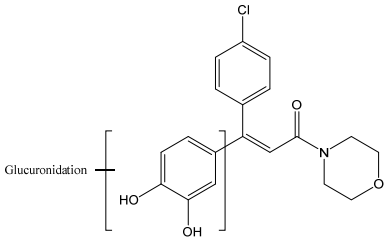
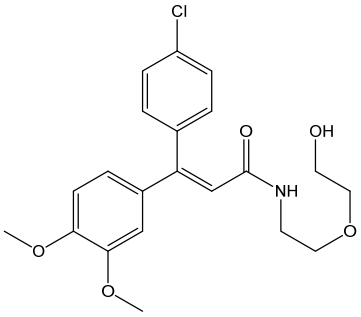
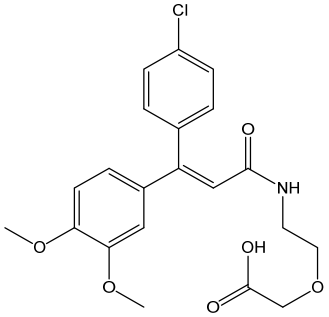
Metabolite code	MW	Proposed structure
M550F028	419	 or
M550F029	535	
M550F030	405	
M550F031	419	

Table 5.1.1-21 List of identified metabolites in rats

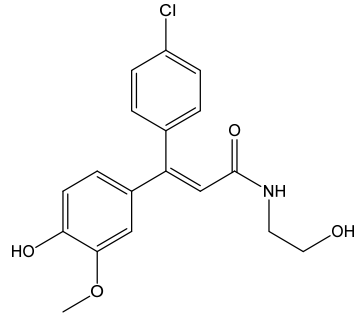
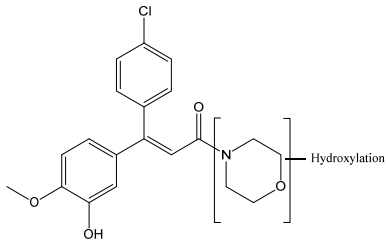
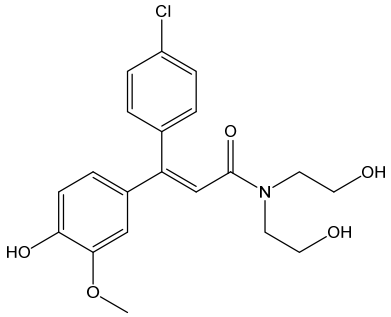
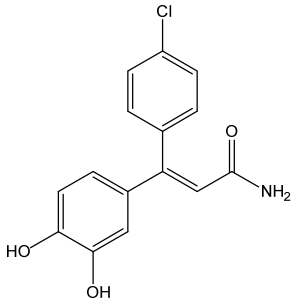
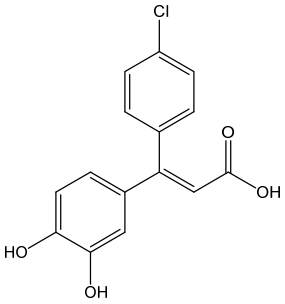
Metabolite code	MW	Proposed structure
M550F033	347	
M550F034	389	
M550F035	391	
M550F036	289	
M550F037	290	

Table 5.1.1-21 List of identified metabolites in rats

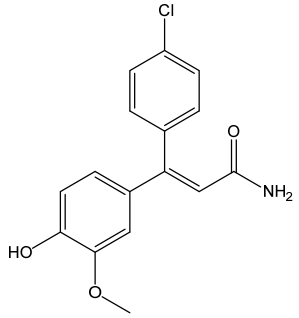
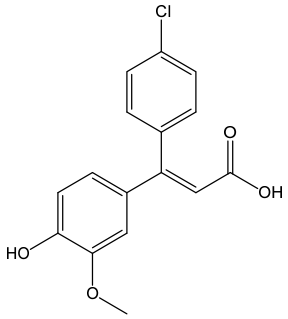
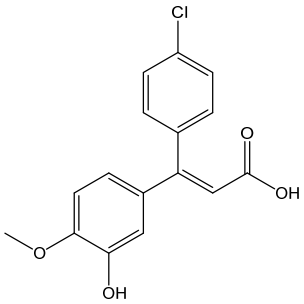
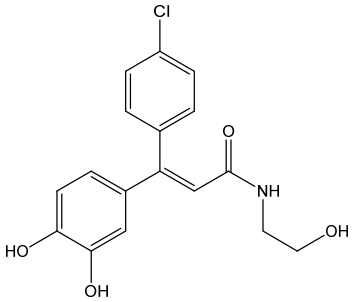
Metabolite code	MW	Proposed structure
M550F038	303	
M550F039	304	
M550F040	304	
M550F041	333	

Table 5.1.1-21 List of identified metabolites in rats

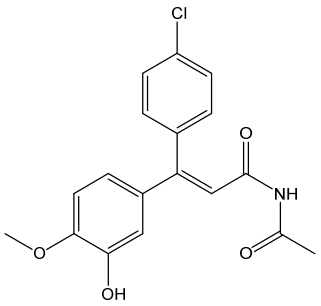
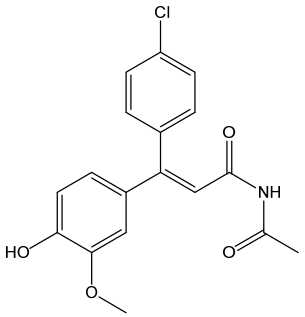
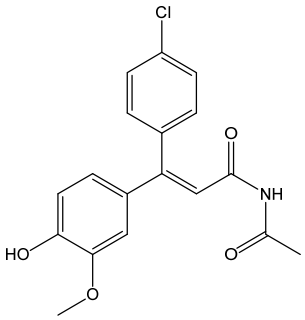
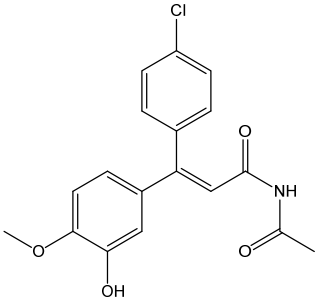
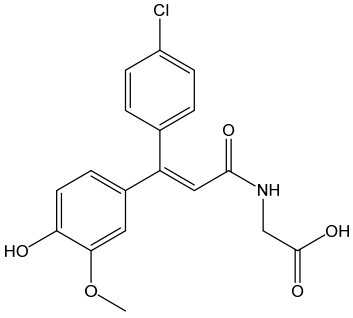
Metabolite code	MW	Proposed structure
M550F042	345	 or 
M550F043	345	
M550F044	345	
M550F045	361	

Table 5.1.1-21 List of identified metabolites in rats

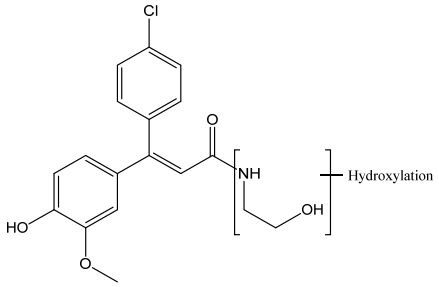
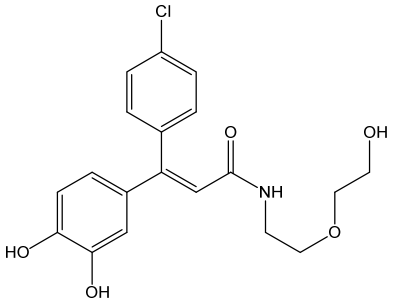
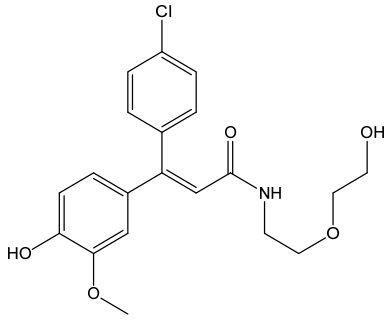
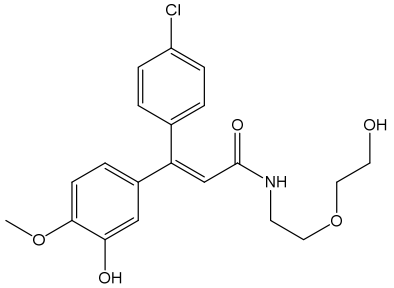
Metabolite code	MW	Proposed structure
M550F046	363	
M550F047	377	
M550F049	391	
M550F050	391	

Table 5.1.1-21 List of identified metabolites in rats

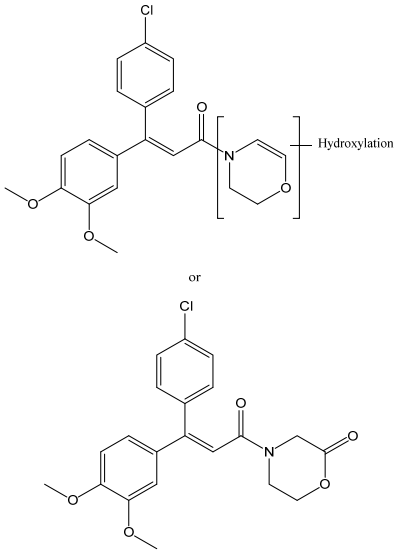
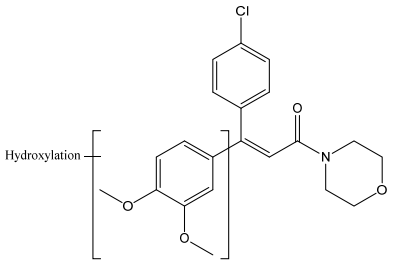
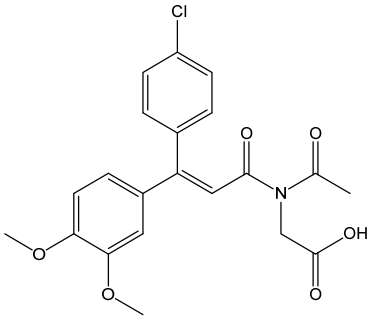
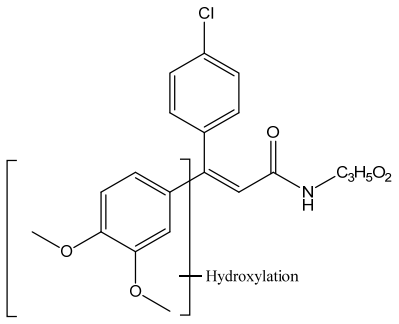
Metabolite code	MW	Proposed structure
M550F051	401	 or
M550F052	403	
M550F053	417	
M550F055	405	

Table 5.1.1-21 List of identified metabolites in rats

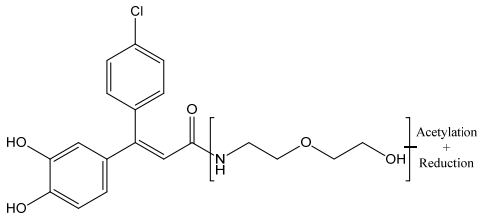
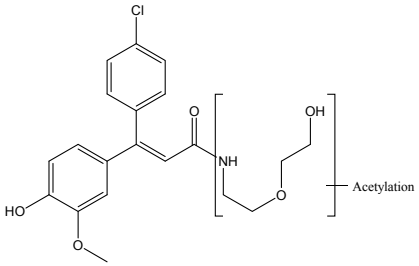
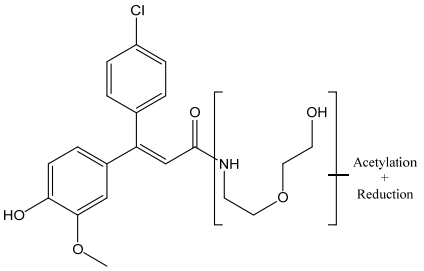
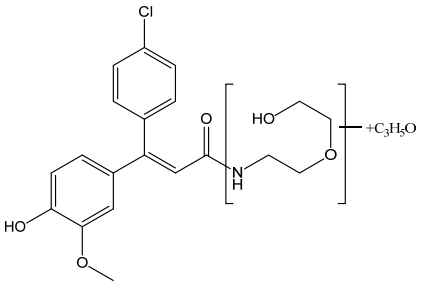
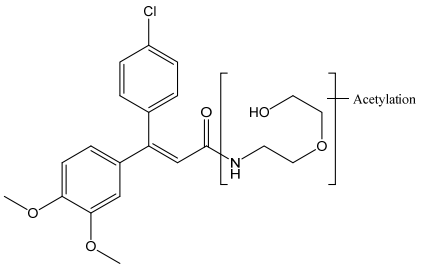
Metabolite code	MW	Proposed structure
M550F056	421	
M550F057	433	
M550F058	435	
M550F059	447	
M550F060	447	

Table 5.1.1-21 List of identified metabolites in rats

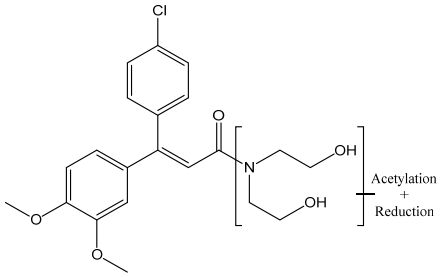
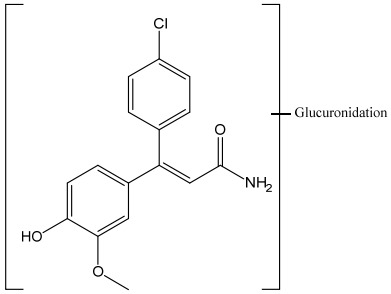
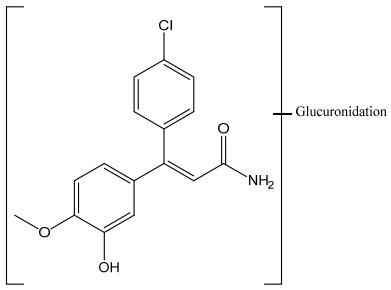
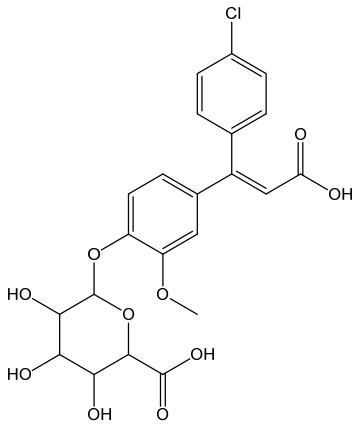
Metabolite code	MW	Proposed structure
M550F061	449	
M550F062	479	
M550F063	479	
M550F064	480	

Table 5.1.1-21 List of identified metabolites in rats

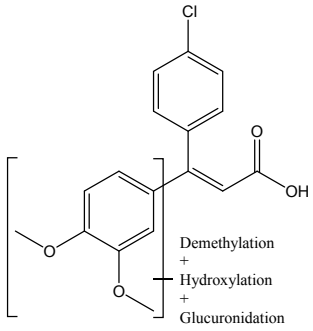
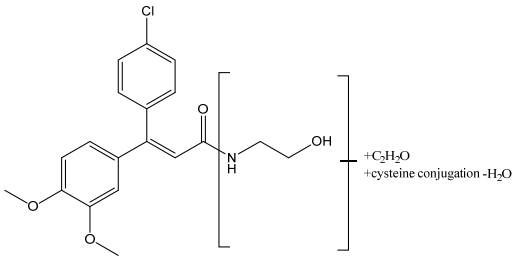
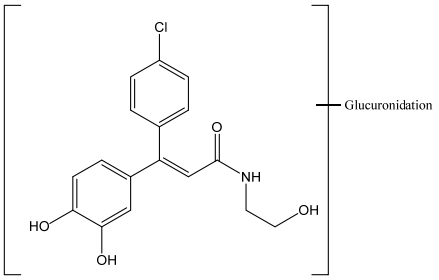
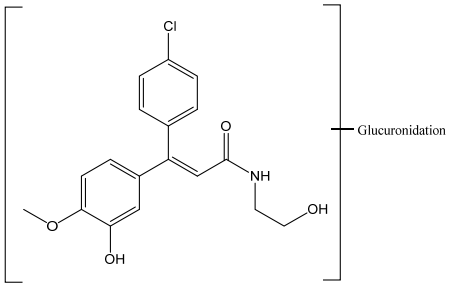
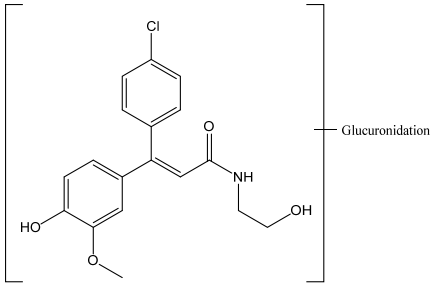
Metabolite code	MW	Proposed structure
M550F065	496	
M550F066	504	
M550F067_25.5min	509	
M550F068	523	
M550F069	523	

Table 5.1.1-21 List of identified metabolites in rats

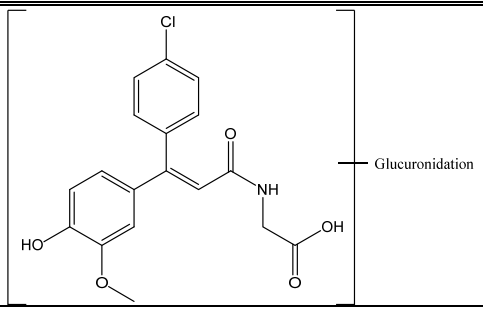
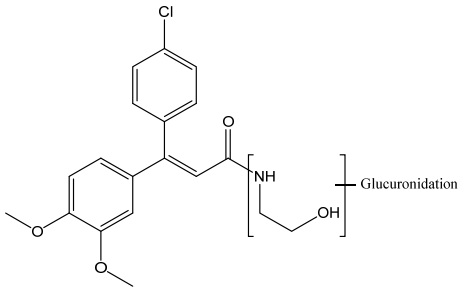
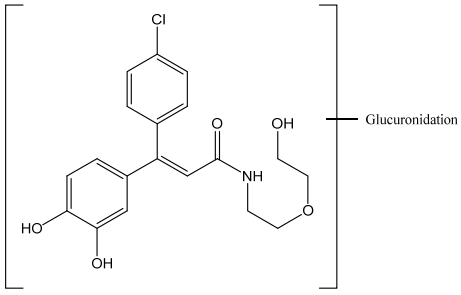
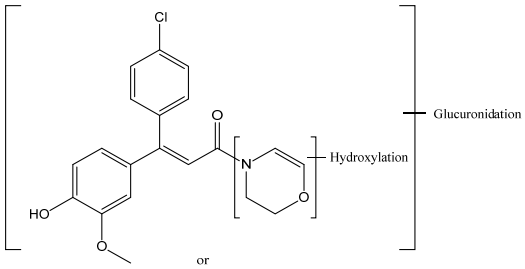
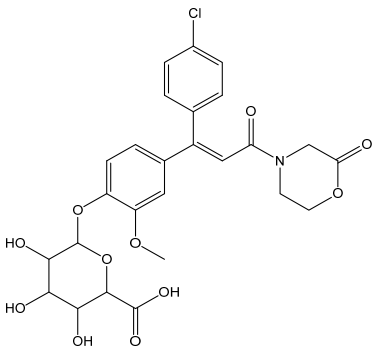
Metabolite code	MW	Proposed structure
M550F070	537	
M550F071	537	
M550F072	553	
M550F073	563	 or 

Table 5.1.1-21 List of identified metabolites in rats

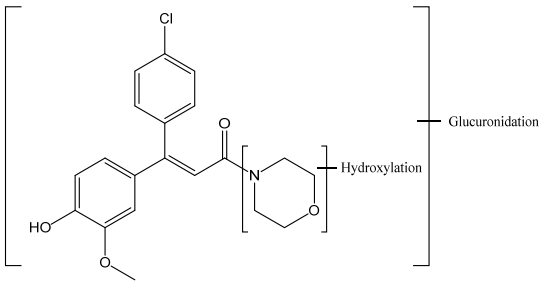
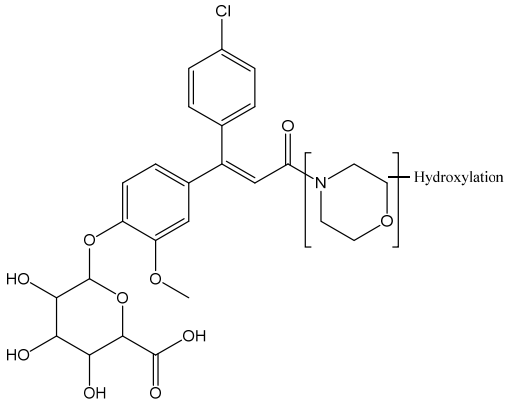
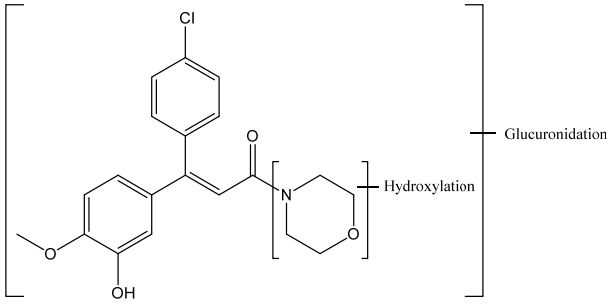
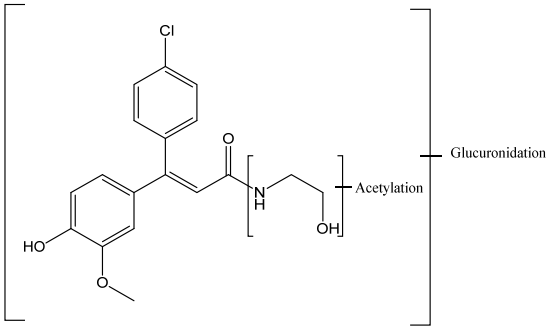
Metabolite code	MW	Proposed structure
M550F074	565	
M550F075	565	
M550F076	565	
M550F077	565	

Table 5.1.1-21 List of identified metabolites in rats

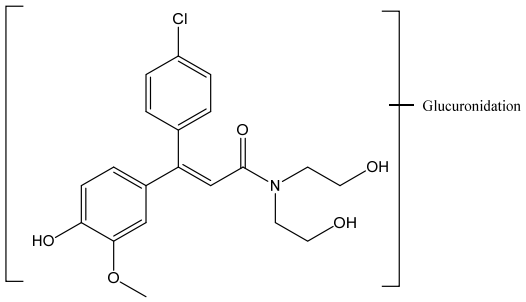
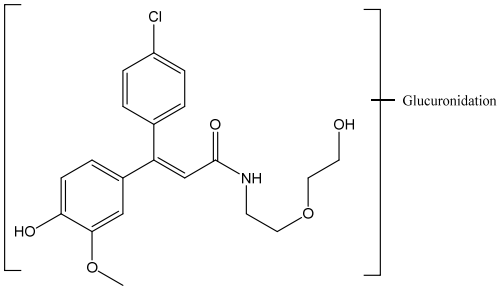
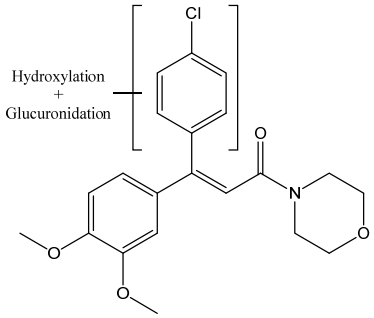
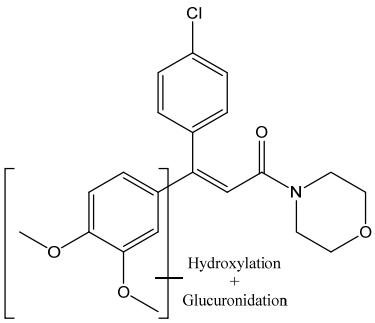
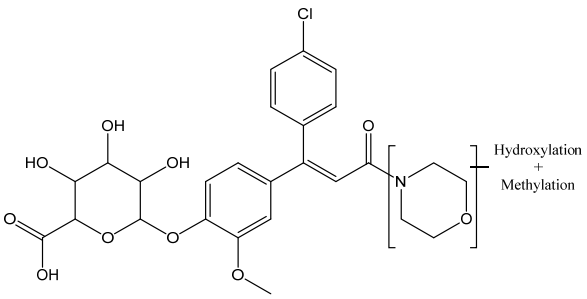
Metabolite code	MW	Proposed structure
M550F078	567	
M550F079	567	
M550F080	579	
M550F081	579	
M550F082	579	

Table 5.1.1-21 List of identified metabolites in rats

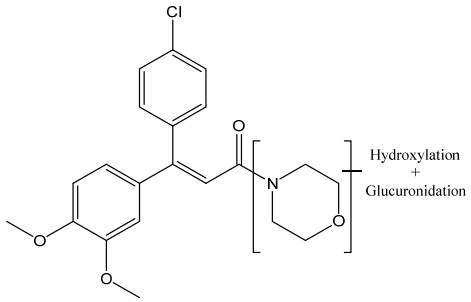
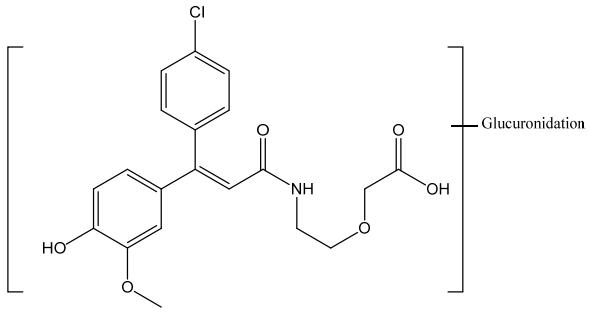
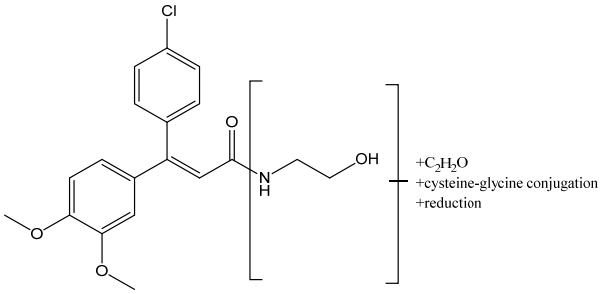
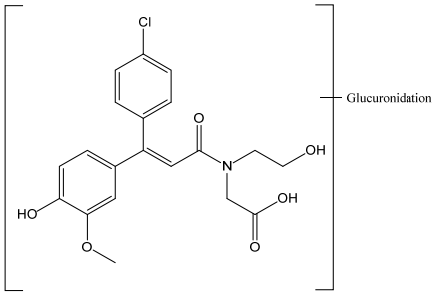
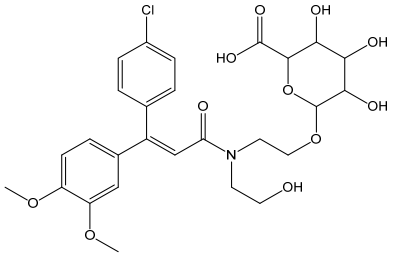
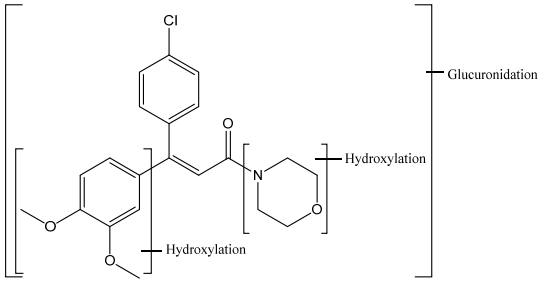
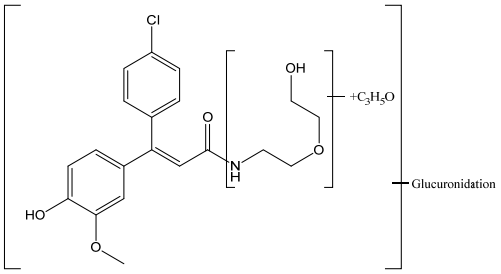
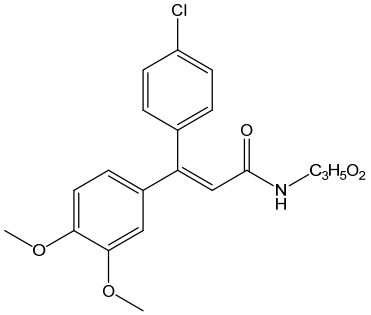
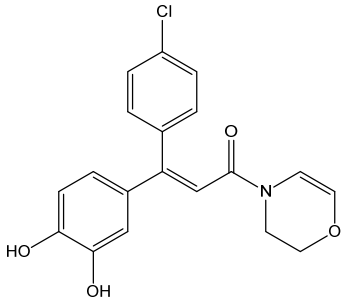
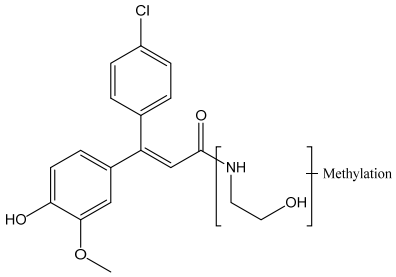
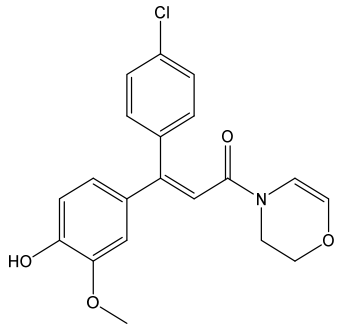
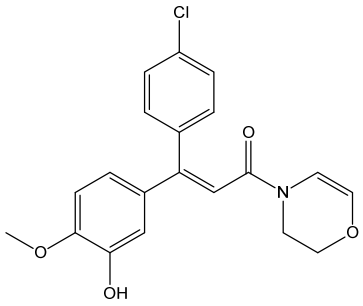
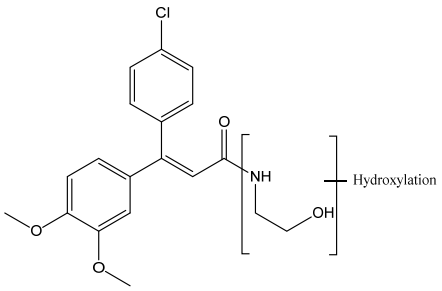
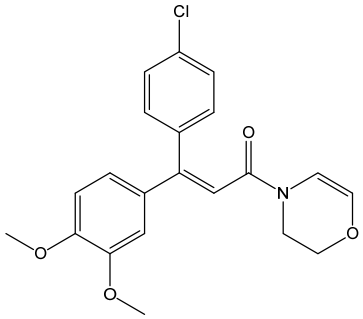
Metabolite code	MW	Proposed structure
M550F083	579	
M550F084	581	
M550F085	581	
M550F086	581	
M550F087	581	

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M550F088	595	
M550F089	623	
M550F090	389	
M357*	357	
M361	361	

* This metabolite is considered to be a degradation product of an original metabolite.
Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M371*	371	
M371*	371	
M377	377	
M385*	385	

* This metabolite is considered to be a degradation product of an original metabolite.
Metabolites with designation "Mxxx" (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Table 5.1.1-21 List of identified metabolites in rats

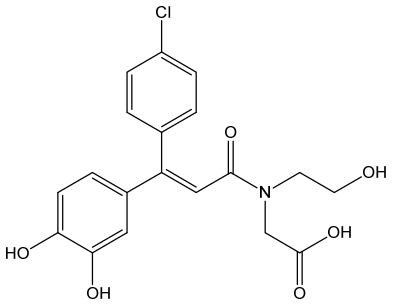
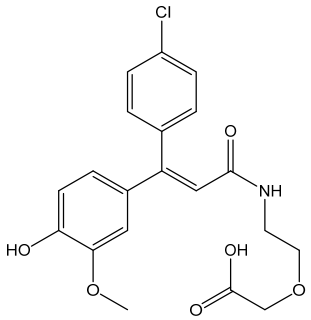
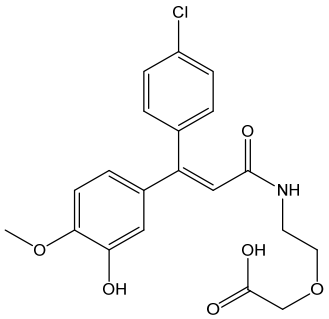
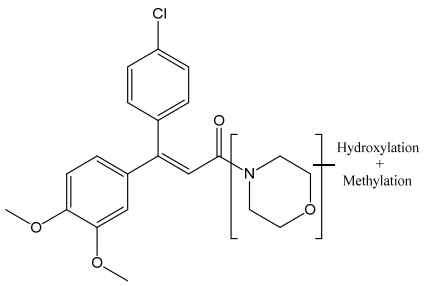
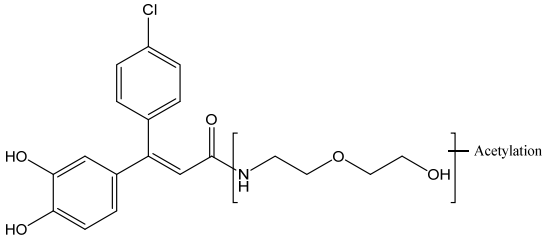
Metabolite code	MW	Proposed structure
M391	391	
M405	405	
M405	405	
M417	417	
M419	419	

Table 5.1.1-21 List of identified metabolites in rats

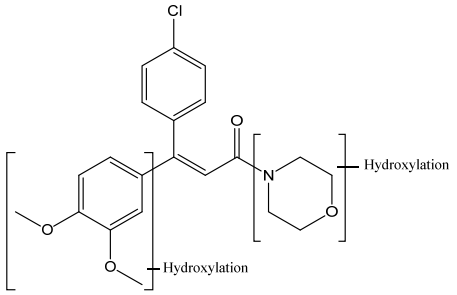
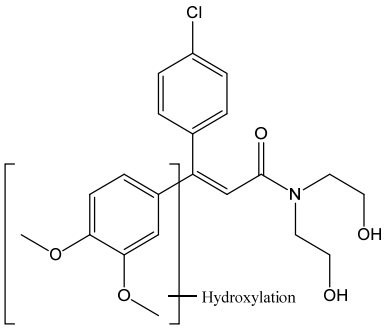
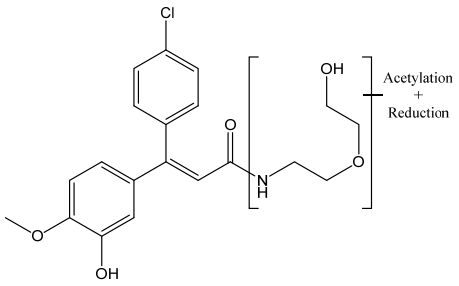
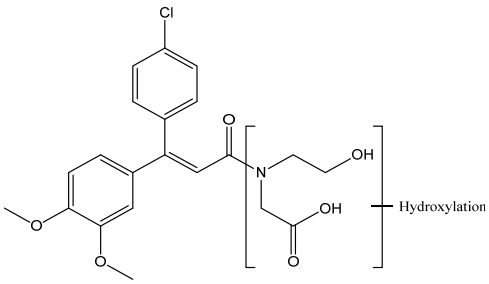
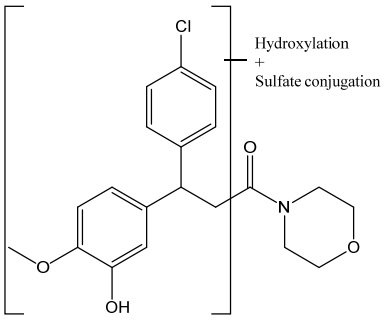
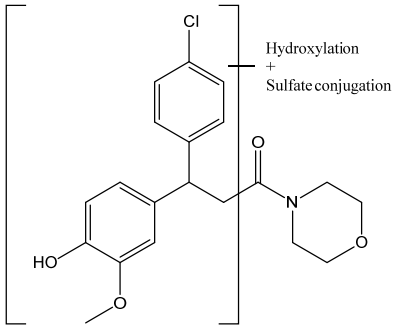
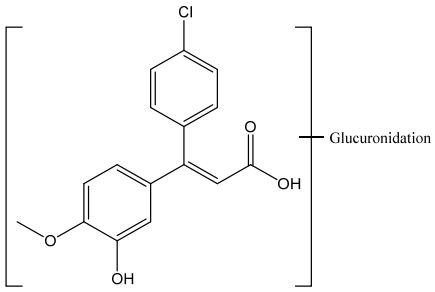
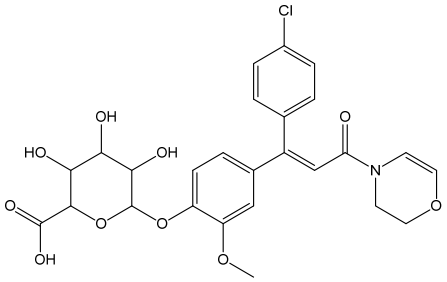
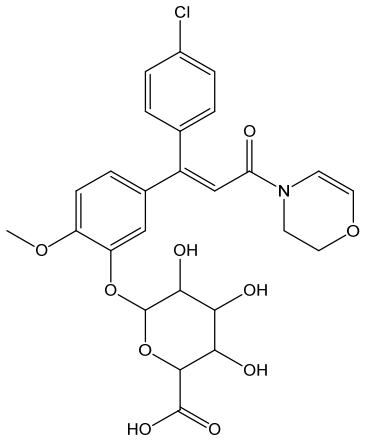
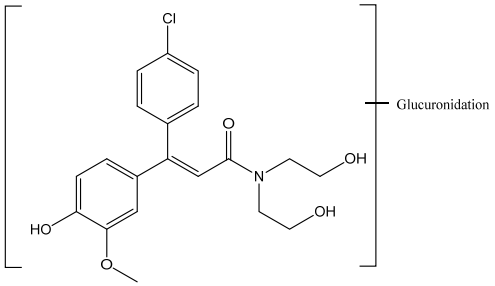
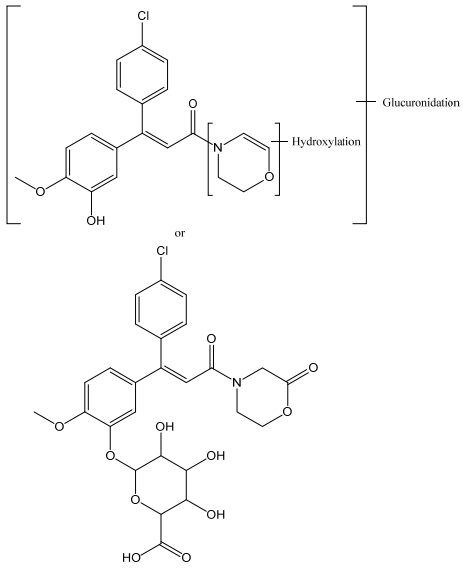
Metabolite code	MW	Proposed structure
M419	419	
M421	421	
M435	435	
M435	435	

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M471	471	 or 
M480	480	
M547*	547	

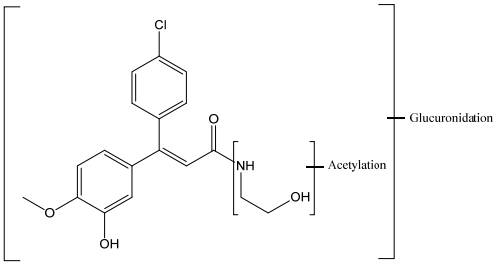
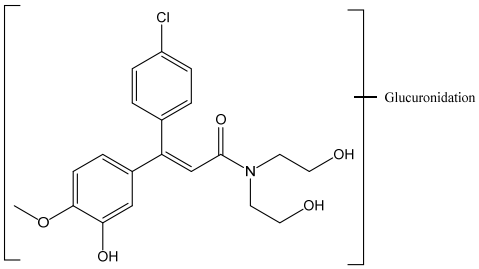
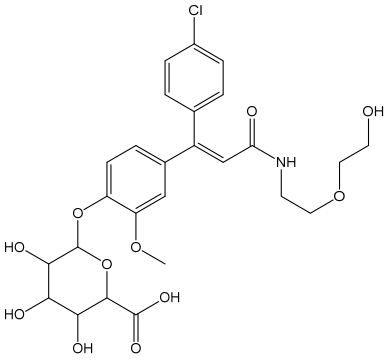
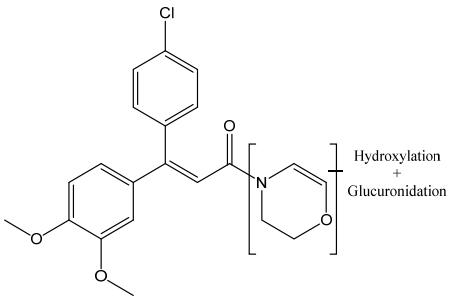
* This metabolite is considered to be a degradation product of an original metabolite.
Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M547*	547	
M553	553	
M563*	563	

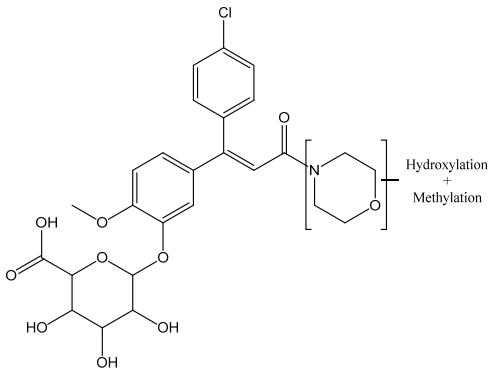
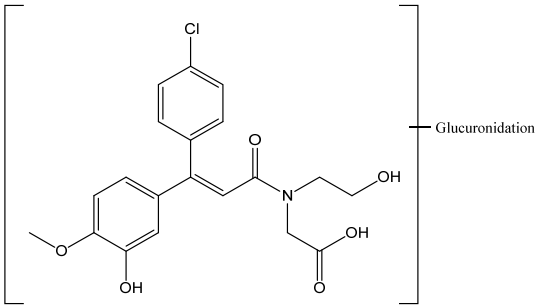
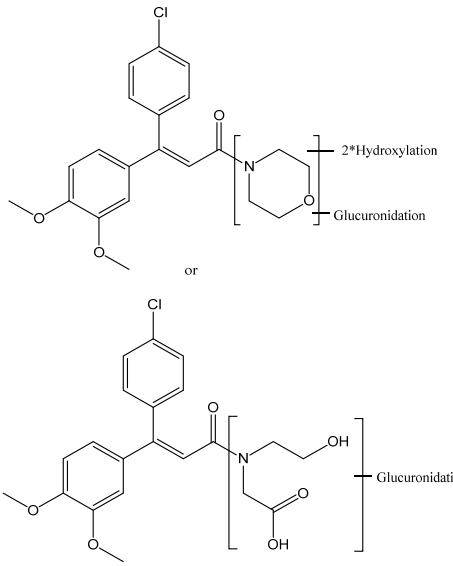
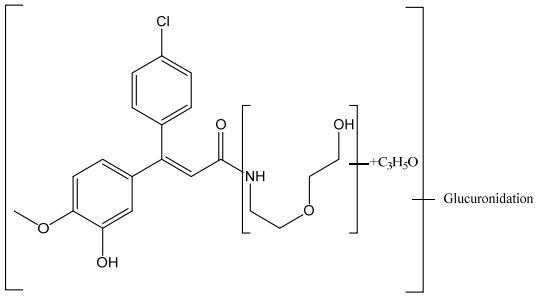
* This metabolite is considered to be a degradation product of an original metabolite.
Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M565	565	
M567	567	
M567	567	
M577*	577	

* This metabolite is considered to be a degradation product of an original metabolite.
Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Table 5.1.1-21 List of identified metabolites in rats

Metabolite code	MW	Proposed structure
M579	579	
M581	581	
M595	595	
M623	623	

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Report: CA 5.1.2/1
Birks V., 2015 a
14C-BAS 550 F: Comparative in-vitro metabolism studies with rat, dog and human cryopreserved hepatocytes
2015/1245078
Guidelines: 2004/10/EC of 11 February 2004
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: [morpholine-2,3-C14]-BAS 550 F (morpholine label)
[p-chlorophenyl-U-C14]-BAS 550 F (chlorophenyl label)
[acrolyl-2-C13]-BAS 550 F (^{13}C- label)
[morpholine-N15]-BAS 550 F (^{15}N label)
Unlabeled BAS 550 F

Lot/Batch #: 858-1101 (morpholine label)
1068-1101 (chlorophenyl label)
1070-0101 (^{13}C label)
1071-0105 (^{15}N label)
AC9978-68A (unlabeled)

Purity: 97.6% (unlabeled)
91.3% (^{13}C label)
97.3% (^{15}N label)
Radiochemical purity: 95.4% (morpholine label)
99.1% (chlorophenyl label)
Specific activity: 4.39 MBq/mg (morpholine label)
7.4 MBq/mg (chlorophenyl label)

CAS#: 110488-70-5

Stability of test compound:
The test item was stable over the test period. Stability controls without cells showed nearly identical HPLC profiles that contained only peaks corresponding to the unchanged active substance BAS 550 F.

2. Vehicle and/or positive control:
Vehicle: hepatocytes
Positive control: testosterone or 7-ethoxycoumarin instead of the test substances were incubated with hepatocytes from the three different species to indicate the metabolic activity of the different hepatocytes.

3. Test animals: Mammals
Species: Human, rat, dog
Strain: -, Wistar, Beagle

B. STUDY DESIGN AND METHODS

1. Dates of work: 28-Apr-2015 - 16-June-2015

This study was carried out at Quotient Bioresearch (Rushden) Ltd., United Kingdom.

Test procedure
The objective of this study was to compare the *in vitro* metabolism in hepatocytes of animal species used in toxicological testing of dimethomorph (BAS 550 F, Reg. No. 247723) to the metabolism in human hepatocyte samples and to determine whether metabolic profiles are similar and whether unique human metabolites occur.

To address this question, the radiolabeled test item was incubated with hepatocytes from human, rat and dog (all mixed gender) at a final concentration of 10 µM. The concentration was chosen after a cell viability pre-test. Two differently ^{14}C-labeled test items (p-chlorophenyl-U-^{14}C-BAS 550 F and morpholine-2,3-^{14}C-BAS 550 F) were used. Hepatocytes were incubated with 10 µM BAS 550 F of both labels, respectively. The viability of the hepatocytes was determined after 180 min of incubation using the AQ$_{ueous}$ One Solution cell viability assay by Promega.

After incubation for 0, 10, 30, 60 or 180 min, the reaction was terminated by addition of acetonitrile (25% of incubation sample volume) and the resulting supernatant was analysed by HPLC. Selected samples were additionally investigated by LC-HR-MS.

All the supernatants contained at least >88% of the applied radioactivity (% AR), therefore pellet extraction was not required.

Negative and positive controls were run in parallel to demonstrate the absence of non metabolic degradation and the metabolic activity of the hepatocytes (Phase I and Phase II metabolic reactions), respectively. The control experiments yielded the expected results.

Test design and analytical procedures

Test solutions
Stock solutions of BAS 550 F were prepared in DMSO. The radioactive concentration of the solutions was confirmed by LSC. Radiochemical purity was confirmed on each experimental day by HPLC.
For the unlabeled test item a 2 mM solution of ^{12}C-BAS 550 F (unlabeled) was prepared by dissolving an aliquot of ^{12}C-BAS 550 F in DMSO and subsequent dilutions.

For the preparation of the application solutions (either morpholine or chlorophenyl label), specific amounts of labeled test items were reduced to dryness under N_2 gas and reconstituted in DMSO. Then, 2 mM solutions of ^{14}C/^{15}N/^{12}C-BAS 550 F (morpholine label) and ^{14}C/^{13}C/^{12}C-BAS 550 F (chlorophenyl label) were prepared by combining suitable amounts of the prepared solutions to give the final stocks. The final stock solutions were analyzed by LC-HR-MS in order to confirm the identity of BAS 550 F and the initial isotope pattern.

Positive control incubations were performed with ^{14}C-testosterone and ^{14}C-7-ethoxycoumarin, respectively. The final concentration of ^{14}C-testosterone in incubations was 150 µM at a target of 1% (v/v). Therefore, a stock solution of 15 mM ^{14}C-testosterone was prepared. Initially, unlabeled testosterone was weighed and dissolved in methanol to give a 15 mM solution. An aliquot of ^{14}C-testosterone was reduced to dryness under N_2 gas and reconstituted in the unlabeled testosterone. The final concentration of ^{14}C-7-ethoxycoumarin in incubations was 25 µM at a target of 1% (v/v). Therefore, a stock solution of 2.5 mM ^{14}C-7-ethoxycoumarin was prepared. An aliquot of ^{14}C-7-ethoxycoumarin in toluene was reduced to dryness under N_2 gas and reconstituted in methanol.

Negative controls:
In the negative controls no metabolism should occur. For the “stability control”, the application solution was mixed with incubation medium instead of the cell suspension. For the “zero incubation control” (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.

Preparation of hepatocytes
Mixed gender rat and dog hepatocytes were purchased from Bioreclamation IVT. Mixed gender Hepatosure™ Pooled Cryopreserved Human Hepatocytes (100 donors) were sourced from Xenotech. Hepatocytes were resuscitated as per the instruction provided by the supplier.

Viability tests and non-specific binding tests
The viability of human, rat and dog hepatocytes after incubation with 1, 5 and 10 µM BAS 550 F (non-radiolabeled) was tested using the cell proliferation assay AQ_{ueous} One Solution (Promega) in order to select the appropriate concentration of the test item in the *in vitro* assays.
Prior to the species comparison of ^{14}C-BAS 550 F metabolism, an assessment of the non-specific binding to the incubation plate was performed at 10 µM ^{14}C-BAS 550 F (2 radiolabeled forms, separately).

In vitro assays
On each incubation day, the application solutions in DMSO were diluted with hepatocyte incubation medium (WME) by a factor of 200 to prepare the respective application medium. Aliquots of the application media were analyzed by LSC to calculate the amounts of applied radioactivity per well (representing 100% AR). The application media were incubated at a final concentration of 10 µM BAS 550 F (both labels) with human, rat or dog hepatocytes. In the case of testosterone and 7-ethoxycoumarin, the incubations were performed at 150 µM and 25 µM, respectively and were diluted with hepatocyte incubation medium (WME) by a factor of 100 to prepare the respective application medium.

Each sample (4.02 mL total incubation volume) comprised 0.02 mL of application medium and 4 mL of hepatocyte cell suspension in one of the wells of a 24-well plate. The final cell concentration was approximately 1 x 10^6 cells/mL. The reactions were performed for 0, 10, 30, 60 or 180 min at 37 °C and at 5% CO_2 in an incubator.
Two negative controls (stability and zero incubation control), two positive controls (testosterone and 7-ethoxycoumarin) and a blank control (application medium with DMSO instead of the test item) were performed for each analyzed species and label.

In each experimental setup, the incubation of BAS 550 F as well as the test compound control assays were performed in triplicates. Incubations with testosterone or 7-ethoxycoumarin were performed in singlicate. In parallel, the viability of the human, rat and dog hepatocytes after 180 minutes incubation with 10 µM BAS 550 F (chlorophenyl label) was tested using a cell proliferation assay.

Sampling and sample storage
The incubation was terminated by pipetting the incubation mixture into a tube containing cold acetonitrile to adjust the sample to an acetonitrile concentration of approximately 20% (v/v). All incubations were sonicated for a few seconds, vortex mixed and centrifuged for 5 min at 16,000 x g at 4°C prior to analysis. The radioactive residues in the supernatants were determined by LSC analysis of aliquots pre and post centrifugation. The remaining pellet was then redissolved in water and the percentage of radioactivity remaining determined by LSC analysis. All samples were stored in a freezer at -20 °C.

Work-up of the residual pellet
In the supernatant the radioactive residues were quantified and ranged from 88.0% to 112%. Therefore, pellet extraction was not required. The remaining pellet was redissolved in water and aliquots were measured via LSC.

Evaluation of the data by HPLC and MS

Each *in vitro* assay was analyzed in triplicates by HPLC and one sample per triplicate was further investigated by LC-HR-MS for the qualitative evaluation of the masses of peaks representing more than 5% AR in human hepatocytes.

Samples were removed from the freezer and allowed to thaw in the refrigerator. Samples were sonicated for 10 minutes and centrifuged at 13,000 rpm at 10°C for 5 minutes in order to sediment out any particulate matter. An aliquot was transferred to a polypropylene vial, capped and transferred to the autosampler for LC-MS analysis. The mass spectra of those ^{14}C-peaks were evaluated to obtain the m/z values of prominent ions corresponding to the test item or its conversion products.

The m/z values were assigned to prominent peaks in the supernatant and are listed together with the retention times and the % AR values.

II. RESULTS AND DISCUSSION

The radio-HPLC analyses of human, rat and dog hepatocyte samples were compared in order to determine whether a unique human metabolite occurred or not. Selected human, rat and dog supernatant samples were also analysed by LC-HR-MS to assign m/z values to prominent peaks representing more than 5% AR in human samples.

For the triplicates of each negative control (stability controls without cells and zero incubation controls), all replicates showed an HPLC profile that contained only peaks corresponding to the unchanged active substance BAS 550 F. Therefore no significant metabolism or degradation of BAS 550 F occurred without the influence of hepatocytes.
The positive controls with testosterone showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. Testosterone was metabolized (>40%) after incubation with human, rat and dog hepatocytes.
HPLC analyses of the positive controls with 7-ethoxycoumarin revealed mean portions of the metabolised 7-ethoxycoumarin reaching values of 29.9% AR after incubation with human hepatocytes and 27.9% and 83.3% AR after incubation with rat and dog hepatocytes respectively. Hydroxylated, sulphated and glucuronidated metabolites of 7-ethoxycoumarin were detected in human, rat and dog with similar levels of 7-hydroxycoumarin (7HC) sulphate and 7HC glucuronide formed in dog (30-34% AR). In rat, 7HC sulphate was the major metabolite (12% AR). However, in human both 7HC sulphate and 7HC glucuronide were formed at lower levels (4.4-7.4% AR).

After the incubation of BAS 550 F with human hepatocytes, four ^{14}C peaks were detected that represented more than 5% AR on at least one time point in one label. Two of these signals represented the *E*- and *Z*-isomers of unchanged active substance BAS 550F. The other peaks, named Region 9 and Region 12, corresponded to metabolites of BAS 550 F.

The peak at 42.0 min (Region 9) was detected after 60 min in the chlorophenyl and morpholine label with 1.58% AR and 1.45% AR, respectively. After 180 min, the mean % AR for Region 9 increased to a maximum of 4.97% AR (chlorophenyl label) and 4.85% AR (morpholine label).
The peak at 44.0 min (Region 12) was detected after 10 min in the chlorophenyl and morpholine label with 2.46% AR and 1.76% AR, respectively. After 60 min, the mean % AR for Region 12 increased to 13.76% AR (chlorophenyl label) and 11.64% AR (morpholine label) and further to 16.78% AR (chlorophenyl label) and 16.13% AR (morpholine label) after 180 min of incubation.

Both regions were found to be consisting of multiple components, following LC-MS/MS analysis.
Region 9 comprised components with retention times of 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420) and 41.4 min (m/z 420).
Region 12 comprised components retention times of 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374).

The identified components were similar for both labels, with the exception of an additional component detected in the region 9 of the morpholine label, with a retention time of 41.4 min (m/z 404). The results are shown in Table 5.1.2-1 and Table 5.1.2-2.

Table 5.1.2-1: Comparison of the relevant metabolites of BAS 550 F (morpholine label) after incubation with human, rat and dog hepatocytes

Relevant Peak		Region 9 [a]	Region 12 [a]	BAS 550 F *E*-isomer	BAS 550 F *Z*-isomer
		[% AR] [b]			
0 min	Human	n.d.	n.d.	41.94	55.47
	Rat	n.d.	n.d.	42.19	55.72
	Dog	n.d.	n.d.	42.33	55.37
10 min	Human	n.d.	1.76	41.05	53.25
	Rat	5.27	1.36	35.92	53.28
	Dog	n.d.	2.60	41.35	51.26
30 min	Human	1.02	6.35	36.49	45.52
	Rat	8.84	5.22	25.61	46.76
	Dog	0.95	7.74	38.76	45.22
60 min	Human	1.45	11.64	31.28	38.20
	Rat	7.63	9.81	15.99	36.93
	Dog	1.89	11.28	35.09	36.30
180 min	Human	4.85	16.13	21.75	24.85
	Rat	5.24	15.41	5.70	17.66
	Dog	3.75	13.11	31.48	25.88

[a] Found to be multicomponent following LC-MS/MS analysis;
Region 9: 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420), 41.4 min (m/z 420) and 41.4 min (m/z 404, morpholine label only)
Region 12: 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374)

[b] % AR of supernatant

n.d. not detected

Table 5.1.2-2: Comparison of the relevant metabolites of BAS 550 F (chlorophenyl label) after incubation with human, rat and dog hepatocytes

Relevant Peak		Region 9 [a]	Region 12 [a]	BAS 550 F *E*-isomer	BAS 550 F *Z*-isomer
		[% AR] [b]			
0 min	Human	n.d.	n.d.	39.10	60.90
	Rat	n.d.	n.d.	38.77	61.00
	Dog	n.d.	n.d.	38.40	59.67
10 min	Human	n.d.	2.46	37.20	56.58
	Rat	5.80	1.98	32.60	58.57
	Dog	n.d.	3.14	38.33	57.34
30 min	Human	n.d.	8.05	33.94	50.55
	Rat	8.52	6.97	21.73	51.17
	Dog	n.d.	9.29	35.54	48.05
60 min	Human	1.58	13.76	28.61	40.91
	Rat	6.99	12.41	12.77	38.74
	Dog	1.13	13.19	32.91	39.73
180 min	Human	4.97	16.78	18.29	24.81
	Rat	5.91	18.13	4.12	17.87
	Dog	2.64	14.69	28.84	27.86

[a] Found to be multicomponent following LC-MS/MS analysis;
Region 9: 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420) 41.4 min (m/z 420) and 41.4 min (m/z 404, morpholine label only)
Region 12: 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374)
[b] % AR of supernatant
n.d. not detected

III. CONCLUSION

In this comparative *in vitro* metabolism study with dimethomorph (BAS 550 F, Reg. No. 247723) no significant differences were noted between the two different radiolabels (p-chlorophenyl-U-^{14}C and morpholine-2,3-^{14}C).

After the incubation of human hepatocytes with the active substance, four ^{14}C peaks were detected that represented more than 5% AR on at least one time point in one label. These were the *E*- and *Z*-isomers of the unchanged active substance BAS 550 F plus two regions (Region 9 and Region 12) that comprised several metabolites. The components identified were generally similar for both labels and all tested species.

All the components identified in human hepatocytes were also detected in rat and dog hepatocyte samples. Therefore it can be concluded that no human-specific metabolites were found and that the metabolic degradation in the tested species was similar.

CA 5.2 Acute Toxicity

Studies submitted in the original Annex I Dossier (2004):
Dimethomorph (BAS 550 F) has been tested in various species and via different routes of administration. All studies are scientifically valid. The studies listed in Table 5.2-1 have been evaluated and peer reviewed during the previous Annex I inclusion process.

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with dimethomorph

Route/species/sex	Dose range	Vehicle	Result	Reference (BASF DocID)
Oral Rat, Sprague-Dawley, m/f	3200, 4000, 5000 mg/kg bw	0.1% aqueous Tween 80	LD_{50} (female) = 3500 mg/kg bw LD_{50} (male) = 4300 mg/kg bw	DK-411-004
Oral (Z-isomer) Rat, Wistar, m/f	5000 mg/kg bw	0.1% aqueous Tween 80	LD_{50} > 5000 mg/kg bw	DK-411-008
Oral (E-isomer) Rat, Wistar, m/f	4000, 5000 mg/kg bw	0.1% aqueous Tween 80	LD_{50} (female) = 4754 mg/kg bw LD_{50} (male) = 4715 mg/kg bw	DK-411-009
Dermal Rat, Wistar, m/f	5000 mg/kg bw	None	LD_{50} > 5000 mg/kg bw	DK-412-001
Dermal Rat, Fisher 344, m/f	2000 mg/kg bw	None	LD_{50} > 2000 mg/kg bw	DK-412-002
Inhalation Rat, Wistar, m/f	4.24 mg/L	None	LC_{50} > 4.24 mg/L	DK-413-001
Skin irritation Rabbit, (NZW), m/f	0.5 g/animal	None	Non irritant	DK-412-002
Eye irritation Rabbit, (NZW), m/f	50 mg/animal	None	Non irritant	DK-412-002
Skin sensitisation, Maximisation Test Guinea pig, Crl:(HA)BR, m	Intradermal: 5% in mineral oil or Freund's adjuvant /water Epidermal: 25% in petrolatum	Petrolatum	Not sensitising	DK-416-003

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
In vivo studies characterizing the acute oral, dermal and inhalation toxicity profile and the skin and eye irritation profile as well as the skin sensitizing profile were performed with dimethomorph, which were not submitted during the previous Annex I inclusion process. Furthermore, an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells has been performed and is described in detail in chapter M-CA 5.2.7. The respective studies are listed in Table 5.2-2.

Table 5.2-2: Summary of not yet peer-reviewed acute toxicity studies with dimethomorph

Type of study	Test substance	Result Classification	Reference (BASF DocID)
Oral route - rat	Dimethomorph (solvent: 0.5% CMC)	LC_{50} > 2000 mg/kg bw	2010/1091144, 2010/1091145 2010/1140958
Oral route - rat	Dimethomorph (solvent: 0.5% CMC)	LC_{50} > 2000 mg/kg bw	2010/1210734, 2011/1071936
Dermal route - rat	Dimethomorph (solvent: 0.5% CMC)	LC_{50} > 2000 mg/kg bw	2010/1091146, 2010/1091147, 2010/1140957
Dermal route - rat	Dimethomorph (solvent: 0.5% CMC)	LC_{50} > 2000 mg/kg bw	2010/1210735, 2011/1071937
Inhalation route - rat	Dimethomorph (solvent: none)	LC_{50} > 5.2 mg/L	2010/1141914
Inhalation route - rat	Dimethomorph (solvent: none)	LC_{50} > 5.2 mg/L	2011/1121982
Skin irritation - rabbit	Dimethomorph (solvent: none)	Not irritant	2011/1148561
Eye irritation - rabbit	Dimethomorph (solvent: none)	Not irritant	2011/1148562
Skin sensitization (LLNA) – mouse	Dimethomorph (solvent: DMF)	Not sensitizing	2011/1148560, 2012/1135754
In vitro NRU Phototoxicity Test in Balb/c 3T3 cells	Dimethomorph	Not phototoxic	2013/1110115

Dimethomorph has low acute toxicity by the oral, dermal and inhalation route of administration.

Dimethomorph is not irritant to the skin or eye.

In a LLNA test dimethomorph is not sensitizing.

No indications for a phototoxic potential is observed in an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells.

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the Review Report of dimethomorph (EFSA Scientific Report 82, 1+-69, 2006:

Rat LD_{50} oral:	3900 mg/kg bw (racemat) > 5000 mg/kg bw (Z-isomer) 4472 mg/kg bw (E-isomer)
Rat LD_{50} dermal:	> 2000 mg/kg bw
Rat LC_{50} inhalation:	> 4.24 mg/L
Skin irritation:	Not irritant
Eye irritation:	Not irritant
Skin sensitization (test method used and result):	Not sensitising (M & K maximisation test)

The proposed endpoints based on all available studies are shown below in Table 5.2-3.

Table 5.2-3: Proposed acute toxicity endpoints of dimethomorph

Study type/species	Results
Acute oral toxicity, rat	LD_{50} > 2000 mg/kg bw
Acute dermal toxicity, rat	LD_{50} > 2000 mg/kg bw
Acute inhalation toxicity, rat	LC_{50} > 5.2 mg/L
Dermal irritation, rabbit	Not Irritating
Eye irritation, rabbit	Not irritating
Skin sensitization, LLNA	Not sensitizing
In vitro NRU Phototoxicity Test, Balb/c 3T3 cells	Not phototoxic

CA 5.2.1 Oral

Report:	CA 5.2.1/1 [redacted], 2010a BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats 2010/1091144
Guidelines:	OECD 423 (Acute Toxic Class Method), EPA 870.1100, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)
Report:	CA 5.2.1/2 [redacted]., 2010b Amendment No. 1 to the report - BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats 2010/1091145
Guidelines:	OECD 423, EPA 870.1100, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)
Report:	CA 5.2.1/3 Class T., 2010 b Concentration and homogeneity analysis of BAS 550 F in 0.5% CMC in water 2010/1140958
Guidelines:	none
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Note: The report amendment (CA 5.2.1/2) corrects organizational issue concerning the test facility for substance analysis, which does not affect the outcome or interpretation of the study.

Executive Summary

The study was performed to assess the acute toxicity following oral administration of BAS 550 F in Wistar rats.
Single doses of 2000 mg/kg bw (female) of BAS 550 F (batch: COD-001244, purity: 99.8%) in 0.5% CMC were given to two groups of three fasted animals, by gavage in a sequential manner. Animals were observed for 14 days.

After administration of 2000 mg/kg bw of BAS 550 F all animals survived until the termination of the study. Accordingly, the oral LD_{50} was found to be greater than 2000 mg/kg bw.

Oral LD_{50} > 2000 mg/kg bw

Clinical observation revealed impaired general state, dyspnea, piloerection, exsiccosis, diarrhea and reduced feces.

No apparent abnormalities were observed in any animal at necropsy at the termination of the study.

According to the EU and GHS classification criteria, no classification is warranted as to acute oral toxicity for BAS 550 F.

(DocID 2010/1091144)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	solid / beige
Lot/Batch #:	COD-001244
Purity	99.8% (tolerance ± 1.0%)
Stability of test compound:	The stability under storage conditions over the study period was guaranteed by the sponsor.

2. Vehicle: 0.5% carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	Wistar / Crl:WI (Han)
Sex:	female
Age:	female: approx. 8 -12 weeks
Weight at dosing (mean):	group 1: 175 ± 6 g; group 2: 181 ± 0 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	VRF1(P); SDS Special Diets Services, Germany, ad libitum
Water:	Tap water ad libitum
Housing:	Animals were housed individually in Makrolon cages, type III enriched with wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria

Environmental conditions:

Temperature:	22 ± 3°C
Humidity:	30 to 70%
Air changes:	Fully air-conditioned rooms
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: (in life phase: 26-Jan-2010 (first treatment) to 23-Feb-2010 (necropsy of the last animal))

2. Animal assignment and treatment:

A starting dose of 2000 mg/kg bw by gavage was used. The animals were deprived of food overnight approximately 16 hours before dosing, but had free access to water. Food was offered again approximately 4 hours after administration. Test substance was given to the animals at a volume of 10 mL/kg bw.

As none of the animals died, 2000 mg/kg bw. (limit test) were administered to 3 further female animals in a second step.

Because no mortality occurred either, the study fulfilled the criteria for a limit test and was terminated.

Observations were performed frequently during the hours following administration for detection of possible treatment-related clinical signs. Thereafter, the animals were observed at least once a day for at least 14 days.

Body weights were recorded at day 0 (prior to dosing), and weekly thereafter and on the last day of administration.

Necropsy with gross-pathology examination on the last day of the observation period after sacrificed by CO_2-inhalation in a chamber with increasing concentrations over time. No histological examinations were performed.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred after sequential administration of 2000 mg/kg bw of BAS 550 F to two groups of females rats [see Table 5.2.1-1].

Table 5.2.1-1: Mortality of rats administered BAS 550 F by the oral route (gavage)

Step No.	Sex	Dose (mg/kg bw)	Mortality / animals treated
1	Females	2000	0/3
2	Females	2000	0/3

B. CLINICAL OBSERVATIONS

In the first test group impaired general state, dyspnea, piloerection, exsiccosis and reduced feces in two out of three animals at study day 3 after administration was observed. Clinical observation in the second test group revealed diarrhea from hour 4 to hour 5 in two animals and reduced feces in all three animals on study day 1 after administration.

C. BODY WEIGHT

The mean body weight of the test groups increased throughout the study period within the normal range.

D. NECROPSY

Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

III. CONCLUSION

Under the experimental conditions of this study the oral LD_{50} of BAS 550 F in rats was determined to be higher than 2000 mg/kg bw. In absence of any mortality and substantial signs of systemic toxicity a classification of BAS 550 F as to acute oral toxicity is not warranted according to EU (Council Regulation 1272/2008/EC) and OECD Globally Harmonized System (GHS, revision 2, 2007) classification criteria.

Report:	CA 5.2.1/4 [redacted] 2010 a BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats 2010/1210734
Guidelines:	OECD 423 (2001), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.1100, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Report:	CA 5.2.1/5 Keller C.,Kamp H., 2011 a BAS 550 F (Dimethomorph) - Homogeneity and concentration control analysis in 0.5% Carboxymethylcellulose in deionized water 2011/1071936
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The study was performed to assess the acute toxicity following oral administration of BAS 550 F in Wistar rats.

Single doses of 2000 mg/kg bw (female) of BAS 550 F (batch: DMM20100610, purity: 98.5%) in 0.5% CMC were given to two groups of three fasted animals, by gavage in a sequential manner. Animals were observed for 14 days.

After administration of 2000 mg/kg bw of BAS 550 F all animals survived until the termination of the study. Accordingly, the oral LD_{50} was found to be greater than 2000 mg/kg bw.

Oral LD_{50} > 2000 mg/kg bw

Clinical observation in the first group revealed impaired general state, dyspnea and piloerection. No clinical signs were observed in the second administration group.

No apparent abnormalities were observed in any animal at necropsy at the termination of the study.

According to the EU and GHS classification criteria, no classification is warranted as to acute oral toxicity for BAS 550 F.

(DocID 2010/1210734)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	solid / beige
Lot/Batch #:	DMM20100610
Purity	98.5%
Stability of test compound:	The stability under storage conditions over the study period was guaranteed by the sponsor.

2. Vehicle: 0.5% carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	Wistar / Crl:WI (Han)
Sex:	female
Age:	female: approx. 10 weeks
Weight at dosing (mean):	group 1: 184 - 190 g; group 2: 170 - 175 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	VRF1(P); SDS Special Diets Services, Germany, ad libitum
Water:	Tap water ad libitum
Housing:	Animals were housed individually in Makrolon cages, type III enriched with wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria

Environmental conditions:

Temperature:	22 ± 3°C
Humidity:	30 to 70%
Air changes:	Fully air-conditioned rooms
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: (in life phase: 15-Nov-2010 (first treatment) to 07-Dec-2010 (necropsy of the last animal))

2. Animal assignment and treatment:

A starting dose of 2000 mg/kg bw by gavage was used. The animals were deprived of food overnight approximately 16 hours before dosing, but had free access to water. Test substance was given to the animals at a volume of 10 mL/kg bw.
As none of the animals died, 2000 mg/kg bw. (limit test) were administered to 3 further female animals in a second step.
Because no mortality occurred either, the study fulfilled the criteria for a limit test and was terminated.
Observations were performed frequently during the hours following administration for detection of possible treatment-related clinical signs. Thereafter, the animals were observed at least once a day for at least 14 days.
Body weights were recorded at day 0 (prior to dosing), and weekly thereafter and on the last day of administration.
Necropsy with gross-pathology examination on the last day of the observation period after sacrificed by CO_2-inhalation in a chamber with increasing concentrations over time. No histological examinations were performed.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred after sequential administration of 2000 mg/kg bw of BAS 550 F to two groups of females rats [see Table 5.2.1-2].

Table 5.2.1-2: Mortality of rats administered BAS 550 F by the oral route (gavage)

Step No.	Sex	Dose (mg/kg bw)	Mortality / animals treated
1	Females	2000	0/3
2	Females	2000	0/3

B. CLINICAL OBSERVATIONS

In the first test group impaired general state and dyspnea in all three animals from hour 0 until hour 5 after administration was observed. Piloerection was noted in two out of three animals from hour 1 or 2 until hour 5. No clinical signs were observed in the second administration group.

C. BODY WEIGHT

The mean body weight of the test groups increased throughout the study period within the normal range.

D. NECROPSY

Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

III. CONCLUSION

Under the experimental conditions of this study the oral LD_{50} of BAS 550 F in rats was determined to be higher than 2000 mg/kg bw. In absence of any mortality and substantial signs of systemic toxicity a classification of BAS 550 F as to acute oral toxicity is not warranted according to EU (Council Regulation 1272/2008/EC) and OECD Globally Harmonized System (GHS, revision 2, 2007) classification criteria.

CA 5.2.2 Dermal

Report: CA 5.2.2/1
[redacted], 2010c
BAS 550 F (Dimethomorph) - Acute dermal toxicity study in rats
2010/1091146
Guidelines: OECD 402, EPA 870.1200, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Report: CA 5.2.2/2
[redacted], 2010a
Amendment No. 1 to the report - BAS 550 F (Dimethomorph) - Acute dermal toxicity study in rats
2010/1091147
Guidelines: OECD 402, EPA 870.1200, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Report: CA 5.2.2/3
Class T., 2010a
Concentration and homogeneity analysis of BAS 550 F in 0.5 % CMC in water
2010/1140957
Guidelines: none
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Note: The report amendment (CA 5.2.2/3) corrects organizational issue concerning the test facility for substance analysis, which does not affect the outcome or interpretation of the study.

Executive Summary

In an acute dermal toxicity study (Limit Test), Wistar rats (5 males and 5 females) were exposed to a single dermal dose of 2000 mg/kg of BAS 550 F (Batch COD-001244; Purity: 99.8%) to the clipped skin on the dorsal area of the trunk under semi-occlusive conditions for 24 hours. The test item was applied as a suspension in 0.5% CMC-solution. The animals were observed for 14 days after administration. Based on the absence of mortality in this study the acute dermal LD_{50} was determined to be

Rat dermal LD_{50} > 2000 mg/kg bw

No signs of systemic toxicity or local skin effects were observed in the animals. The mean body weight of the animals increased within the normal range throughout the study period. No abnormal findings were observed in the animals at terminal necropsy.

According to the EU and GHS classification criteria, no classification is warranted as to acute dermal toxicity for BAS 550 F.

(DocID2010/1091146)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	Solid / beige
Lot/Batch #:	COD-001244
Purity/content:	99.8% (tolerance ± 1.0%)
Stability of test compound:	The stability under storage conditions over the study period was guaranteed by the sponsor.

2. Vehicle: 0.5% carboxymethylcellulose (CMC)-solution

3. Test animals:

Species:	Rat
Strain:	Wistar (Crl:WI (Han) SPF)
Sex:	male and female
Age:	males: approximately 8 weeks females: approximately 11 weeks
Weight at dosing (mean):	males: 220 ± 10 g; females: 206 ± 8 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	VRF1 (P); SDS Special Diets Services, Altrip, Germany, ad libitum
Water:	Tap water, ad libitum.
Housing:	Single housing in Makrolon cages type III enriched with wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	22 ± 3°C
Humidity:	30 to 70%
Air changes:	Fully air-conditioned rooms
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 21-Jan-2010 - 22-Feb-2010
(in life phase: 26-Jan-2010 (treatment) to 09-Feb-2010 (necropsy of the animals))

2. Animal assignment and treatment:

BAS 550 F was administered to the clipped skin of five male and five female rats at a dose of 2000 mg/kg under semiocclusive conditions for 24 hours. For this the test substance suspended in 0.5% CMC-solution and was applied to the clipped epidermis on the dorsal area corresponding to at least 10% of the estimated body surface (i.e. about 40 cm^2). The hair of the dorsal trunk was clipped about 24 hours before treatment.
The test item is covered with an air-permeable dressing (4 layers of absorbent gauze) and stretch bandage. After removal of the semi occlusive dressing, the application site was rinsed with warm water.

The animals were observed for possible treatment-related clinical signs frequently during the hours following administration of the test substance and then at least once a day for a total of 2 weeks. Individual readings of local skin reactions were done 30 – 60 minutes after removal of the semi-occlusive dressing (day 1), as a rule weekly thereafter and on the last day of observation.

Individual body weights were determined shortly before application (day 0), and weekly thereafter.

Necropsy with gross-pathology examination was performed on the last day of the observation period after sacrifice with CO_2 in a chamber with increasing concentrations over time.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in this study.

B. CLINICAL OBSERVATIONS

No systemic clinical signs were observed during clinical examination. No local effects were observed

C. BODY WEIGHT

The mean body weight of the animals increased throughout the study period within the normal range.

D. NECROPSY

Macroscopic examination of the main organs revealed no abnormal findings in any of the animals at the termination of the study.

III. CONCLUSION

Under the experimental conditions of this study the dermal LD_{50} of BAS 550 F in rats was determined to be higher than 2000 mg/kg bw. In absence of any mortality and signs of systemic toxicity, a classification of BAS 550 F as to acute dermal toxicity is not warranted according to EU (Council Regulation 1272/2008/EC) and OECD Globally Harmonized System (GHS, revision 2, 2007) classification criteria.

Report:	CA 5.2.2/4 [redacted]., 2010a BAS 550 F (Dimethomorph) - Acute dermal toxicity study in rats 2010/1210735
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, JMAFF No 12 Nosan No 8147, OECD 402 (1987), EPA 870.1200
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Report:	CA 5.2.2/5 Keller C.,Kamp H., 2011b BAS 550 F (Dimethomorph) - Homogeneity and concentration control analysis in 0.5% Carboxymethylcellulose in deionized water 2011/1071937
Guidelines:	None
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an acute dermal toxicity study (limit test) Wistar rats (5 males and 5 females) were exposed to a single dermal dose of 2000 mg/kg of BAS 550 F (batch DMM20100610; purity: 98.5%) to the clipped skin on the dorsal area of the trunk under semi-occlusive conditions for 24 hours. The test item was applied as a suspension in 0.5% CMC-solution. The animals were observed for 14 days after administration. Based on the absence of mortality in this study the acute dermal LD_{50} was determined to be

Rat dermal LD_{50}, > 2000 mg/kg bw

No signs of systemic toxicity or local skin effects were observed in the animals. The mean body weight of the animals increased within the normal range throughout the study period. No abnormal findings were observed in the animals at terminal necropsy.

According to the EU and GHS classification criteria, no classification is warranted as to acute dermal toxicity for BAS 550 F.

(DocID2010/1210735)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	Solid / beige
Lot/Batch #:	DMM20100610
Purity/content:	98.5%
Stability of test compound:	The stability under storage conditions over the study period was guaranteed by the sponsor.

2. Vehicle: 0.5% carboxymethylcellulose (CMC)-solution

3. Test animals:

Species:	Rat
Strain:	Wistar (Crl:WI (Han) SPF)
Sex:	male and female
Age:	males: approximately 8 weeks females: approximately 12 weeks
Weight at dosing (mean):	males: 220 ± 10 g; females: 206 ± 8 g
Source:	[redacted]
Acclimation period:	At least 5 days
Diet:	VRF1 (P); SDS Special Diets Services, Altrip, Germany, ad libitum
Water:	Tap water, ad libitum.
Housing:	Single housing in Makrolon cages type III enriched with wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	22 ± 3°C
Humidity:	30 to 70%
Air changes:	Fully air-conditioned rooms
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 12-Nov-2010 - 03-Dec-2010
(in life phase: 17-Nov-2010 (treatment) to 30-Nov-2010 (necropsy of the animals))

2. Animal assignment and treatment:

BAS 550 F was administered to the clipped skin of five male and five female rats at a dose of 2000 mg/kg under semi occlusive conditions for 24 hours. For this the test substance suspended in 0.5% CMC-solution and was applied to the clipped epidermis on the dorsal area corresponding to at least 10% of the estimated body surface (i.e. about 40 cm^2). The hair of the dorsal trunk was clipped about 24 hours before treatment.
The test item is covered with an air-permeable dressing (4 layers of absorbent gauze) and stretch bandage. After removal of the semi occlusive dressing, the application site was rinsed with warm water.

The animals were observed for possible treatment-related clinical signs frequently during the hours following administration of the test substance and then at least once a day for a total of 2 weeks. Individual readings of local skin reactions were done 30 – 60 minutes after removal of the semi-occlusive dressing (day 1), as a rule weekly thereafter and on the last day of observation.

Individual body weights were determined shortly before application (day 0), and weekly thereafter.

Necropsy with gross-pathology examination was performed on the last day of the observation period after sacrifice with CO_2 in a chamber with increasing concentrations over time.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in this study.

B. CLINICAL OBSERVATIONS

No systemic clinical signs were observed during clinical examination. No local effects were observed

C. BODY WEIGHT

The mean body weight of the male animals increased throughout the study period within the normal range. The mean body weight of the female animals slightly decreased during the first post-exposure observation week, probably due to the bandage procedure, but increased during the second week.

D. NECROPSY

Macroscopic examination of the main organs revealed no abnormal findings in any of the animals at the termination of the study.

III. CONCLUSION

Under the experimental conditions of this study the dermal LD_{50} of BAS 550 F in rats was determined to be higher than 2000 mg/kg bw. In absence of any mortality and signs of systemic toxicity, a classification of BAS 550 F as to acute dermal toxicity is not warranted according to EU (Council Regulation 1272/2008/EC) and OECD Globally Harmonized System (GHS, revision 2, 2007) classification criteria.

CA 5.2.3 Inhalation

Report:	CA 5.2.3/1 [redacted] 2010a BAS 550 F (Dimethomorph) - Acute inhalation toxicity study in Wistar rats - 4-hour dust exposure (head-nose only) 2010/1141914
Guidelines:	OECD 403 (2009), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.2, EPA 870.1300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an acute inhalation toxicity study, groups of 5 males and 5 females Wistar rats were exposed to BAS 550 F as a dust (batch COD-001244; Purity: 99.8%) at a concentration of 5.2 mg/L for 4 hours. The animals were observed for 14 days after exposure.

No mortality occurred at the tested concentration. Accordingly, the acute inhalation LC_{50} for after dust inhalation exposure of BAS 550 F was determined to be

LC_{50} (male and female rats) > 5.2 mg/L

Clinical signs of toxicity consisted of accelerated respiration, abdominal respiration, respiration sounds, salivation, red encrusted eyes, piloerection and substance contaminated fur. Findings were observed from hour 2 of exposure through to study day 3. No abnormalities were detected in the animals from study day 4 onwards. The mean body weights of the animals decreased during the first few post exposure observations days and increased from study day 7 onward. No gross pathological abnormalities were detected during necropsy in the animals at the termination of the post exposure observation period.

Cascade impactor measurements resulted in particle size distributions with mass median aerodynamic diameters (MMADs) of 6.0 and 6.9 µm. Based on the two cascade impactor measurements 26% of the particles had a diameter of MMAD < 3 µm.

According to the EU and GHS classification criteria, no classification is warranted as to acute inhalation toxicity for BAS 550 F.

(DocID 2010/1141914)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	Solid / beige
Lot/Batch #:	COD-001244
Purity/content:	99.8% (tolerance ± 1.0%)
Stability of test compound:	The stability was guaranteed for the duration of the study.

2. Vehicle: none, the test substance was dosed unchanged

3. Test animals:

Species:	Rat
Strain:	Wistar / RccHan:WIST(SPF)
Sex:	male and female
Age:	males: approx. 9 weeks, female animals: approx. 11 weeks
Weight at dosing (mean):	males: 268.8 ± 3.8 g; females: 211.3 ± 7.0 g
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	Kliba-Labordiaet (Maus / Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages type M III, floor area about 800 cm^2 or caged in groups up to 5 animals, if animals were free of clinical signs and findings in Polysulfon cages (H-Temp [PSU]), floor area about 2065 cm^2 enriched with Wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70%
Air changes:	15 air changes per hour
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 01-July-2010 - 22-July-2010
(in life phase: 05-July-2010 (day of exposure) - 19-July-2010 (last day of observation))

2. Animal assignment and treatment:

For determination of the acute inhalation toxicity (single head-nose inhalation, 4-hour-exposure) groups of five male and five female rats were exposed to 5.2 mg/L of the test substance BAS 550 F, that was dosed unchanged as a dust aerosol. The animals were randomly selected from a pool of animals. After exposure, animals were observed for at least 14 days.
Individual body weights were recorded once during acclimatization period, shortly before exposure (day 0), and at least on days 1, 3 and 7, and before the sacrifice of the animals at the end of the observation period.
Detailed clinical observations were recorded for each animal separately several times during exposure and at least once daily on the pre-exposure day and during the observation period.
A check for any dead or moribund animal was made twice each workday and once on Saturdays, Sundays, and on public holidays. At the end of the observation period the surviving animals were sacrificed with CO_2-inhalation in a chamber with increasing concentration over time, and were subjected to gross-pathological examination.

3. Statistics/calculations

For results of the type "LC_{50} greater than", "LC_{50} approx.", or "LC_{50} smaller than", the binomial test was used for statistical evaluation. [Steel R.G.D., Torrie J.H. (1984): Principles and procedures of statistics a biometrical approach. McGraw - Hill]
The calculation of the particle size distribution was carried out in the inhalation laboratory on the basis of mathematical methods for evaluating particle measurements. [DIN 66141: Darstellung von Korngrößenverteilungen, DIN 66161: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, Germany)]

4. Generation of the test atmosphere and exposure:

The dust aerosol was generated inside the inhalation system with a dosing-wheel dust generator and compressed air. The test substance was stirred in its container before a sample for dust generation was taken. The test substance was desagglomerated in a mixer (mixing for 2x10 seconds) under addition 1% Aerosil E200 and 1% Aerosil R972 before introduction into the dust generator, in order to improve dust formation. The concentration was adjusted by varying the aperture width and the rotation of the dosing wheel.
A head-nose inhalation system INA (glass-steel construction, BASF SE, volume V $\approx$ 34 L) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. Supply airflow (compressed air) of 0.8 m³/h was used for the exposure. The exhaust airflow was set at 0.6 m³/h. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals. An air change of about 24 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

5. Analytical investigation:
The nominal concentration was calculated from the amount of substance dosed and the supply air flow.

The concentration of the test substance in the inhalation atmosphere was determined via gravimetrical measurement. Preweighed filters were placed into the filtration equipment. By means of a vacuum pump (Millipore) metered volumes of the dust were drawn through the filter. For each sample the dust concentration in mg/L was calculated from the difference between the preweight of the filter and the weight of the filter after sampling with reference to the sample volume of the inhalation atmospheres. Mean and standard deviation for the concentration were calculated based on the results from individual measurements.

- Sampling devices: Filtration equipment with probe, internal diameter: 7 mm, (Millipore)
- Filter: MN 85/90 BF (d = 47 mm)
- Equipment: balance Mettler AT 250
- Sampling position: immediately adjacent to the animals' noses at a separate spare port
- Sampling flow: 3 L/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: 6 L

6. Particle Size Analysis:
The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter of 6.9 mm.
Before sampling, the impactor were assembled with preweighed glass-fiber and equipped with a backup particle filter. The impactor was connected to the vacuum pump and three samples were taken from the breathing zone of the animals starting approx. 30 minutes after the beginning of the exposure. The sample volume for each sample was 6 L.
After sampling the impactor was taken apart. The collecting discs and the backup particle filter were re-weighed. The results from the particle size analysis were corrected for the additive.

II. RESULTS AND DISCUSSION

A. MORTALITY

No lethality occurred at the tested concentration of 5.2 mg/L during the study period of 14 days. Therefore, the study satisfies the criteria of a limit test.
Based on the absence of mortality the following LC_{50} value was determined:

LC_{50} (both sexes combined):	> 5.2 mg/L
LC_{50} (male rats)	> 5.2 mg/L
LC_{50} (female rats)	> 5.2 mg/L

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity comprised accelerated respiration, abdominal respiration, respiration sounds, salivation, red encrusted eyes, piloerection and substance contaminated fur. Findings were observed from hour 2 of exposure through to study day 3. No abnormalities were detected in the animals from study day 4 onwards. The nature and duration of the observations are indicated in Table 5.2.3-1.

Table 5.2.3-1: Nature and duration of clinical signs observed in rats exposed for 4 hours to BAS 550 F as a dust aerosol

Test group 1 (5.2 mg/L)	Males	Females
Fur, substance contaminated	d0 – d1	d0 – d2
Eye, red encrusted	-	d1 – d3
Respiration, abdominal	-	d0 – d1
Respiration, accelerated	h2 – h4	h2 – h4
Respiratory sounds	-	d0
Piloerection	d0	d0 – d2
Salivation	-	d0

hn: hour n of exposure; d0: post-exposure on the day of exposure; dn: day n after exposure

C. BODY WEIGHT

The mean body weights of the animals decreased during the first few post exposure observation days and increased throughout from study day 7 onward.

D. NECROPSY

No gross pathological abnormalities were detected in the animals that underwent necropsy at termination of the study.

E. ANALYTICAL MEASUREMENTS

The exposure conditions are summarized in Table 5.2.3-2.

Table 5.2.3-2: Exposure conditions

Supply air (compressed) (m^3/h)	Exhaust air (m^3/h)	Substance flow (g/h)	Temperature (°C)	Relative humidity (%)
0.8	0.6	54.7	19.0 ± 0.7	35.4 ± 1.9

The result of the analytical concentration measurements are presented in Table 5.2.3-3.

Table 5.2.3-3: Measurement of the analytical concentration

Mean concentration (mg/L)	Standard deviation	Nominal concentration (mg/L)
5.2	0.6	68.4

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) in the range of 6.0 and 6.9 µm with geometric standard deviations of 3.0 and 5.3, respectively [see Table 5.2.3-4]. In spite of several technical measures (mixing and addition of 1% Aerosil E200 and 1% Aerosil R972 to reduce hygroscopic tendency and improve the flow ability) no smaller particle size could be reached. Other technical measures for the reduction of particle size (e.g. use of a cyclonic separator) were not taken because of the tremendous consumption of test substance for reaching suitable concentrations.

Table 5.2.3-4: Particle size measurements

Sample	MMAD (µm)	Mass < 3 µm (%)	Geometric standard deviation
1	6.0	26.2	3.0
2	6.9	30.9	5.3

III. CONCLUSION

Under the conditions of this study the 4 hour inhalation LC_{50} of BAS 550 F for male and female rats was estimated to be > 5.2 mg/L. Based on the results of this study, BAS 550 F does not warrant classification as to acute inhalation toxicity according to EU criteria (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

Report:	CA 5.2.3/2 [redacted], 2011a BAS 550 F (Dimethomorph) - Acute inhalation toxicity study in Wistar rats - 4-hour dust exposure (head-nose only) 2011/1121982
Guidelines:	OECD 403 (2009), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.2, EPA 870.1300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an acute inhalation toxicity study, groups of 5 male and 5 female Wistar rats were exposed to BAS 550 F as a dust (batch DMM20100610; Purity: 98.5%) at a concentration of 5.2 mg/L for 4 hours. The animals were observed for 14 days after exposure.

No mortality occurred at the tested concentration. Accordingly, the acute inhalation LC_{50} for after dust inhalation exposure of BAS 550 F was determined to be

LC_{50} (male and female rats) > 5.2 mg/L

Clinical signs of toxicity consisted of accelerated and depressed respiration, red crusts of the eye and the nose, piloerection and substance contaminated fur. Findings were observed from hour 1 of exposure through to study day 2. No abnormalities were detected in the animals from study day 3 onwards. The mean body weights of the animals decreased during the first few post exposure observations days and increased from study day 3 onward. No gross pathological abnormalities were detected during necropsy in the animals at the termination of the post exposure observation period.
Cascade impactor measurements resulted in particle size distributions with mass median aerodynamic diameters (MMADs) of 4.7 and 5.5 µm. Based on the two cascade impactor measurements at least 28% of the particles had a diameter of MMAD < 3 µm.

According to the EU and GHS classification criteria, no classification is warranted as to acute inhalation toxicity for BAS 550 F.

(DocID 2011/1121982)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 550 F
Description:	Solid / beige
Lot/Batch #:	DMM20100610
Purity/content:	98.5%
Stability of test compound:	The stability was guaranteed for the duration of the study.
2. Vehicle:	none, the test substance was dosed unchanged
3. Test animals:	
Species:	Rat
Strain:	Wistar / RccHan:WIST
Sex:	male and female
Age:	males: approx. 8 weeks, female animals: approx. 10 weeks
Weight at dosing (mean):	males: 236.0 ± 3.8 g; females: 194.8 ± 2.4 g
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	Kliba-Labordiaet (Maus / Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages type M III, floor area about 800 cm^2 or caged in groups up to 5 animals, if animals were free of clinical signs and findings in Polysulfon cages (H-Temp [PSU]), floor area about 2065 cm^2 enriched with wooden gnawing blocks (Typ NGM E-022); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70%
Air changes:	15 air changes per hour
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 06-Dec-2010 - 19-Jan-2011
(in life phase: 07-July-2010 (day of exposure) - 21-Dec-2010 (last day of observation))

2. Animal assignment and treatment:

For determination of the acute inhalation toxicity (single head-nose inhalation, 4-hour-exposure) groups of five male and five female rats were exposed to 5.2 mg/L of the test substance BAS 550 F, that was dosed unchanged as a dust aerosol. The animals were randomly selected from a pool of animals. After exposure, animals were observed for at least 14 days.
Individual body weights were recorded once during acclimatization period, shortly before exposure (day 0), and at least on days 1, 3 and 7, and before the sacrifice of the animals at the end of the observation period.
Detailed clinical observations were recorded for each animal separately several times during exposure and at least once daily on the pre-exposure day and during the observation period.
A check for any dead or moribund animal was made twice each workday and once on Saturdays, Sundays, and on public holidays. At the end of the observation period the surviving animals were sacrificed with CO_2-inhalation in a chamber with increasing concentration over time, and were subjected to gross-pathological examination.

3. Statistics/calculations:

For results of the type "LC_{50} greater than", "LC_{50} approx.", or "LC_{50} smaller than", the binomial test was used for statistical evaluation. [Steel R.G.D., Torrie J.H. (1984): Principles and procedures of statistics a biometrical approach. McGraw - Hill]
The calculation of the particle size distribution was carried out in the inhalation laboratory on the basis of mathematical methods for evaluating particle measurements. [DIN 66141: Darstellung von Korngrößenverteilungen, DIN 66161: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, Germany)]

4. Generation of the test atmosphere and exposure:

The dust aerosol was generated inside the inhalation system with a dosing-wheel dust generator and compressed air. The test substance was stirred in its container before a sample for dust generation was taken. The test substance was desagglomerated in a mixer (mixing for 3x10 seconds) under addition 1% Aerosil® 200 and 1% Aerosil® R972 before introduction into the dust generator, in order to improve dust formation. The concentration was adjusted by varying the aperture width and the rotation of the dosing wheel.
A head-nose inhalation system INA (glass-steel construction, BASF SE, volume V ≈ 34 L) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.
The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. Supply airflow (compressed air) of 0.8 m^3/h was used for the exposure. The exhaust airflow was set at 0.6 m^3/h. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals. An air change of about 27 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

5. Analytical investigation:

The nominal concentration was calculated from the amount of substance dosed and the supply air flow.

The concentration of the test substance in the inhalation atmosphere was determined via gravimetrical measurement. Preweighed filters were placed into the filtration equipment. By means of a vacuum pump (Millipore) metered volumes of the dust were drawn through the filter. For each sample the dust concentration in mg/L was calculated from the difference between the preweight of the filter and the weight of the filter after sampling with reference to the sample volume of the inhalation atmospheres. Mean and standard deviation for the concentration were calculated based on the results from individual measurements.

- Sampling devices: Filtration equipment with probe, internal diameter: 7 mm, (Millipore)
- Filter: MN 85/90 BF (d = 47 mm)
- Equipment: balance Mettler AT 250
- Sampling position: immediately adjacent to the animals' noses at a separate spare port
- Sampling flow: 3 L/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: 6 L

6. Particle Size Analysis:
The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter of 6.9 mm.
Before sampling, the impactor were assembled with preweighed glass-fiber and equipped with a backup particle filter. The impactor was connected to the vacuum pump and three samples were taken from the breathing zone of the animals starting approx. 30 minutes after the beginning of the exposure. The sample volume for each sample was 6 L.
After sampling the impactor was taken apart. The collecting discs and the backup particle filter were re-weighed. The results from the particle size analysis were corrected for the additive.

II. RESULTS AND DISCUSSION

A. MORTALITY

No lethality occurred at the tested concentration of 5.2 mg/L during the study period of 14 days. Therefore, the study satisfies the criteria of a limit test.
Based on the absence of mortality the following LC_{50} value was determined:

LC_{50} (both sexes combined):	> 5.2 mg/L
LC_{50} (male rats)	> 5.2 mg/L
LC_{50} (female rats)	> 5.2 mg/L

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity consisted of accelerated and depressed respiration, red crusts of the eye and the nose, piloerection and substance contaminated fur. Findings were observed from hour 1 of exposure through to study day 2. No abnormalities were detected in the animals from study day 3 onwards. The nature and duration of the observations are indicated in Table 5.2.3-5.

Table 5.2.3-5: Nature and duration of clinical signs observed in rats exposed for 4 hours to BAS 550 F as a dust aerosol

Test group 1 (5.2 mg/L)	Males	Females
Eye, encrusted	d0 – d1	d0 – d1
Fur, substance contaminated	d0 – d2	d0 – d2
Nose, red encrusted	d0 – d2	d0 – d2
Respiration, accelerated	d0 – d1	d0 – d2
Respiration, depressed	h1 – h4	h1 – h4
Piloerection	d0 – d2	d0 – d2

hn: hour n of exposure; d0: post-exposure on the day of exposure; dn: day n after exposure

C. BODY WEIGHT

The mean body weights of the animals decreased during the first few post exposure observation days and increased throughout from study day 3 onward.

D. NECROPSY

No gross pathological abnormalities were detected in the animals that underwent necropsy at termination of the study.

E. ANALYTICAL MEASUREMENTS

The exposure conditions are summarized in Table 5.2.3-6.

Table 5.2.3-6: Exposure conditions

Supply air (compressed) (m^3/h)	Exhaust air (m^3/h)	Substance flow (g/h)	Temperature (°C)	Relative humidity (%)
0.8	0.6	44.8	21.6 ± 0.7	33.3 ± 2.4

The result of the analytical concentration measurements are presented in Table 5.2.3-7

Table 5.2.3-7: Measurement of the analytical concentration

Mean concentration (mg/L)	Standard deviation	Nominal concentration (mg/L)
5.2	0.4	56.0

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) in the range of 4.7 and 5.5 µm with geometric standard deviations of 2.6 and 2.9, respectively [see Table 5.2.3-8]. In spite of several technical measures (mixing and addition of 1% Aerosil® 200 and 1% Aerosil® R972 to reduce hygroscopic tendency and improve the flow ability) no smaller particle size could be reached. Other technical measures for the reduction of particle size (e.g. use of a cyclonic separator) were not taken because of the tremendous consumption of test substance for reaching suitable concentrations.

Table 5.2.3-8: Particle size measurements

Sample	MMAD (µm)	Mass < 3 µm (%)	Geometric standard deviation
1	4.7	32.0	2.6
2	5.5	28.6	2.9

III. CONCLUSION

Under the conditions of this study the 4 hour inhalation LC_{50} of BAS 550 F for male and female rats was estimated to be > 5.2 mg/L. Based on the results of this study, BAS 550 F does not warrant classification as to acute inhalation toxicity according to EU criteria (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

CA 5.2.4 Skin irritation

Report: CA 5.2.4/1
[redacted], 2011a
BAS 550 F (Dimethomorph) - Acute dermal irritation / corrosion in rabbits
2011/1148561

Guidelines: OECD 404 (2002), EPA 870.2500, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In a primary dermal irritation study the skin irritation/corrosion potential of BAS 550 F (batch: DMM20100610, Purity: 98.5%) was tested. The intact clipped skin of 3 White New Zealand rabbits was exposed to 0.5 g of the test-substance using a patch of 2.5 cm x 2.5 cm for 4 hours. The application of the test substance was performed in a stepwise procedure starting with one animal and supplementing two additional animals. The application area was covered with a semi-occlusive dressing. The cutaneous reactions were assessed immediately after removal of the patch and approximately 1, 24, 48 and 72 hours after removal of the patch.

No test item related clinical observations were recorded during the course of the study. The average score (24 to 72 hours) for irritation was calculated to be 0.0 for erythema and 0.0 for edema. Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for erythema and 3x 0.0 for edema. Consequently, the overall 24 to 72 hour skin irritation scores were 0.0 for erythema and 0.0 for edema.

Based on the findings of this study BAS 550 F does not show a skin irritation potential and thus has not to be classified for skin irritation potential according to EU criteria (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

(DocID 2011/1148561)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	solid / beige
Lot/Batch #:	DMM20100610
Purity/content:	98.5%
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle: The test substance was applied unchanged**.** Minimally moistened with highly deionized water.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White Crl:KBL (NZW)
Sex:	male and female
Age:	6 - 7 months
Weight at dosing (mean):	3.57 - 4.28 kg
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	STANRAB (P) SQC; SDS Special Diets Services, 67122 Altrip, Germany
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating with shallow cage body, floor area of 4225 cm² enriched with wooden gnawing blocks (Typ KNH E-041); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	17 - 23 °C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 29-Jun-2011 to 18-Jul-2011
(in life phase: 04-Jul-2011 (treatment) to 15-Jul-2011 (last day of observation))

2. In-vitro pre-test:

An in vitro study using the EpiDerm™ human skin model was not performed.

3. Animal assignment and treatment:

The potential of BAS 550 F to cause acute dermal irritation or corrosion was assessed by a single topical application of 0.5 g of the unchanged test substance for 4 hours to the intact untreated skin of three New Zealand White rabbits using a patch of 2.5 cm x 2.5 cm. The test patch was secured in position by means of a semi-occlusive dressing (Idealbinde, Pfaelzische Verbandstoff-Fabrik, Kaiserslautern) and Fixomull® stretch (adhesive fleece, Beiersdorf AG, Germany).
After removal of the patch the residuals of the test substance were washed off with Lutrol® E400 and Lutrol®/water (1:1).
At least 24 hours before treatment, the dorsolateral part of the trunk of the animals was clipped. The cutaneous reactions were assessed immediately after removal of the patch, approximately 1, 24, 48 and 72 hours, and then in weekly intervals up to day 14.
Body weights were determined just before application and after the last reading. A check for dead or moribund animals was performed at least once each workday.

II. RESULTS AND DISCUSSION

No cutaneous reactions at all were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for erythema. Consequently, the overall 24 to 72 hour skin irritation scores were 0.0 for erythema and 0.0 for edema. Individual and mean irritation scores after 4 hour dermal application of BAS 550 F are presented in Table 5.2.4-1.

Table 5.2.4-1: Individual and mean skin irritation scores after 4 hour dermal application of BAS 550 F

Readings	Animal	Erythema	Edema	Additional findings
0 h	01	0	0	
	02	0	0	
	03	0	0	
1 h	01	0	0	
	02	0	0	
	03	0	0	
24 h	01	0	0	
	02	0	0	
	03	0	0	
48 h	01	0	0	
	02	0	0	
	03	0	0	
72 h	01	0	0	SD
	02	0	0	SD
	03	0	0	SD
Individual Means 24 - 72 h	01	0.0	0.0	
	02	0.0	0.0	
	03	0.0	0.0	
Overall Means		0.0	0.0	

SD: study discontinued because the animal was free of findings

III. CONCLUSION

Based on the findings of this study BAS 550 F does not show a skin irritation potential under the test conditions chosen. No classification of BAS 550 F for skin irritation potential according to EU criteria (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007) is needed.

CA 5.2.5 Eye irritation

Report:	CA 5.2.5/1 [redacted] 2011b BAS 550 F (Dimethomorph) - Acute eye irritation in rabbits 2011/1148562
Guidelines:	OECD 405 (2002), EPA 870.2400, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In a primary eye irritation study, the eye irritation/corrosion potential of BAS 550 F (batch: DMM20100610, purity: 98.5%) was determined by instillation of 0.1 ml bulk volume (about 52 mg) of the test substance into the conjunctival sac of the right eye of three New Zealand White rabbits. The application of the test substance was performed in a stepwise procedure starting with one animal and supplementing two additional animals. About 24 hours after application the eye was rinsed with tap water.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after administration of the test substance.

After 1 hour, slight conjunctival redness and chemosis (grade 1), moderate reaction at the iris (grade 1) and slight or obvious discharge (grade 1 to grade 2) were observed. Conjunctival redness and chemosis (grade 1) persist in one animal after 24h of administration. Additional findings like injected scleral vessels in a circumscribed or circular area were found in one animal from hour 1 to hour 48. The ocular reactions were reversible in all animals within 72 hours after application. Accordingly, the individual mean eye irritation scores (24 to 72 hours) were 0.0, 0.0 and 0.0 for corneal opacity, 0.0, 0.0 and 0.0 for iris lesions, 0.0, 0.0 and 0.3 for conjunctival redness and 0.0, 0.0 and 0.3 for chemosis.

Based on the results of the study the test substance BAS 550 F does not show any eye irritation potential and no classification as to eye irritation is warranted according to the EU (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

(DocID 2011/1148562)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	solid / beige
Lot/Batch #:	DMM20100610
Purity/content:	98.5%
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle: The test substance was applied unchanged**.**

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White Crl:KBL (NZW)
Sex:	male and female
Age:	ca. 3 months
Weight at dosing (mean):	2.23 - 2.64 kg
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	STANRAB (P) SQC; SDS Special Diets Services, 67122 Altrip, Germany
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating with shallow cage body, floor area of 4225 cm² enriched with wooden gnawing blocks (Typ KNH E-041); Abedd ® Lab. and Vet. Service GmbH Vienna, Austria
Environmental conditions:	
Temperature:	17 - 23 °C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 29-Jun-2011 to 18-Jul-2011
(in life phase: 04-Jul-2011 (first application) to 14-Jul-2011 (last day of observation))

2. In-vitro pre-test:

No in-vitro study for the determination of the potential for serious damage to the eyes was performed.

3. Animal assignment and treatment:

The potential of BAS 550 F to cause acute eye irritation was assessed by instillation of 0.1 mL bulk volume (about 51.8 mg of the comminuted test item) of the undiluted test substance into the conjunctival sac of the right eye. The left eye, which remained untreated, served as the negative control. About 24 hours after application of the test substance the treated eye was rinsed with 3 to 6 mL of hand warm tap water for 1 to 2 minutes using a syringe.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after application of the test substance. Body weights were measured just before application and after the last reading. The animals were checked for mortality and morbidity at least once each workday.

II. RESULTS AND DISCUSSION

Slight conjunctival redness (grade 1) was observed in all animals 1 hour after application and persisted in only one animal up to 24 hours. Moderate reaction at the iris (grade 1) was noted in one animal 1 hour after application only. Furthermore slight conjunctival chemosis (grade 1) was observed in one animal 1 hour after application up to 24 hours. Slight or obvious discharge (grade 1 or 2) was noted solely in two animals 1 hour after application. Additional findings like injected scleral vessels in a circumscribed or circular area were noted only in one animal from hour 1 to hour 48 [see Table 5.2.5-1]. The ocular reactions were reversible in all animals within 72 hours after application. Accordingly, the mean individual scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity, 0.0, 0.0 and 0.0 for iris lesions, 0.0, 0.0 and 0.3 for redness of the conjunctiva, and 0.0, 0.0 and 0.3 for chemosis.

Table 5.2.5-1: Individual and mean eye irritation scores after ocular application of BAS 550 F

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Opacity	Area involved		Redness	Chemosis	Discharge	
1 hour	01 (♂)	0	0	0	1	0	1	
	02 (♀)	0	0	0	1	0	0	
	03 (♀)	0	0	1	1	1	2	49
24 hours	01	0	0	0	0	0	0	FL negative
	02	0	0	0	0	0	0	FL negative
	03	0	0	0	1	1	0	48, FL negative
48 hours	01	0	0	0	0	0	0	
	02	0	0	0	0	0	0	
	03	0	0	0	0	0	0	48
72 hours	01	0	0	0	0	0	0	SD
	02	0	0	0	0	0	0	SD
	03	0	0	0	0	0	0	SD
Individual 24 to 72 h means	**01**	**0.0**		**0.0**	**0.0**	**0.0**		
	02	**0.0**		**0.0**	**0.0**	**0.0**		
	03	**0.0**		**0.0**	**0.3**	**0.3**		
Overall 24 – 72 h mean	**all**	**0.0**		**0.0**	**0.1**	**0.1**		

48: scleral vessels injected, circumscribed area, central ingrown
49: scleral vessels injected, circular, ingrown
FL: fluorescein
SD: study discontinued because the animal was free of findings

III. CONCLUSION

Under the test conditions chosen BAS 550 F showed a very slight eye irritation potential. Consequently, no classification as to eye irritation is required according to the EU (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

CA 5.2.6 Skin sensitisation

Report:	CA 5.2.6/1 [redacted], 2012a BAS 550 F (Dimethomorph) - Local lymph node assay (LLNA) in mice (CBA/CaCrl) 2011/1148560
Guidelines:	OECD 429 (2010), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.42
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Report:	CA 5.2.6/2 Becker M.,Kamp H., 2012a Analytical report - BAS 550 F (Dimethomorph) - Homogeneity and concentration control analyses in dimethylformamide 2012/1135745
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

For the determination of potential sensitizing properties of the test item BAS 550 F a Murine Local Lymph Node Assay (LLNA) was conducted.

Groups of 5 female mice were treated with three different concentrations of the test substance (10, 25 and 50% (w/w) in dimethylformamide (DMF)) or with the vehicle alone for three consecutive days.

A skin sensitizing effect for BAS 550 F is not considered since Stimulation Indices (S.I.) of 1.06, 0.93 and 0.91 were determined with BAS 550 F at concentrations of 10, 25 and 50% (w/w) in DMF, respectively.

A statistically significant increase in lymph node cell count (1.51) was obtained in the low dose groups in comparison to the vehicle control group. The cut-off value for a positive response regarding the lymph node cell count index (1.55) was not exceeded. Therefore, this was not considered as biological relevant as also the S.I.s determined for all concentrations did not exceed the threshold value of 3. Lymph node and ear weights indices did not result in a biologically significant increase.

No signs of systemic and local toxicity and no mortalities were observed.

Positive control studies performed twice a year with the sensitizer alpha-hexylcinnamaldehyde proved the sensitivity of the method used.

In conclusion, based on the results of this study BAS 550 F does not display skin sensitizing properties under the conditions of the test. Thus, classification of BAS 550 F as a skin sensitizer is not required according to EU criteria (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC /Council Regulation 1272/2008/EC) as well as according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

(DocID 2011/1148560)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F

Description:	beige solid
Lot/Batch #:	DMM20100610
Purity:	98.5%
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor. Stable until 14 July 2013 Stability in DMF was assessed in report CA 5.2.6/2.

2. Vehicle and/or positive control: dimethylformamide (vehicle),
□-hexylcinnamaldehyde (positive control)

3. Test animals:

Species:	Mouse
Strain:	CBA/CaCrl
Sex:	females
Age:	8 to 9 weeks
Weight at dosing:	17.6 ± 1.6 g
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	pelleted standard diet (Harlan Laboratories B.V., 5960 AD Horst, Netherlands), ad libitum
Water:	tap water ad libitum
Housing:	group housing in Makrolon Type III cages with wire mesh top (EHRET GmbH, Germany)
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	40 - 65%
Air changes:	not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00/18:00 – 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 20-Jul-2011 - 28-Sep-2011

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 550 F was assessed using the radioactive Murine Local Lymph Node Assay. For this, female mice were allocated to groups of 5 animals at random. The groups were treated either with

- the control group with dimethylformamide,
- a 10% (w/w) dilution of the test article in dimethylformamide,
- a 25% (w/w) dilution of the test article in dimethylformamide, or
- a 50% (w/w) dilution of the test article in dimethylformamide.

3. Analysis of treatment solutions:
The highest test item concentration, which could be technically used was a 50% (w/w) suspension in dimethylformamide. From a concentration of 10% and below, test item solutions could be prepared. At higher concentrations, an applicable formulation of the test item was not achieved, neither by the use of other vehicles nor by using additional methods to formulate the test item (e.g. warming to 37°C).

A homogeneity analysis was performed in a separate study. Considering the low standard deviation in the homogeneity analysis, it can be concluded that BAS 550 F (Dimethomorph) was distributed homogeneously in dimethylformamide.
The single and mean values of BAS 550 F (Dimethomorph) in dimethylformamide were found to be in the range between 90 % and 110 % of the nominal concentrations. These results demonstrated the correctness of the concentration of BAS 550 F (Dimethomorph) in dimethylformamide.

4. Statistics:
The mean values and standard deviations were calculated in the body weight tables, for the ear weights, the lymph node weights and lymph node cell count, and for the DPM values (group mean DPM ± standard deviation).
A statistical analysis was conducted on the DPM values, the ear weights, the lymph node weights and the lymph node cell count to assess whether the difference was statistically significant between test item groups and negative control group. For all statistical calculations SigmaStat for Windows (Version 2.0) was used. A One-Way-Analysis-of-Variance was used as statistical method. In case of significant results of the One-Way-ANOVA, multiple comparisons were performed with the Dunnett test or the Student Newman Keuls test. Statistical significance was set at the five per cent level ($p < 0.05$). The Dean-Dixon-Test was used for identification of possible outliers (performed with Microsoft Excel 2003).
However, both biological and statistical significance were considered together.

5. Clinical observation:
Mortality was checked at least once daily from experimental start to necropsy. Clinical signs (local irritation at the application site or systemic toxicity) were recorded at least once daily. Especially the treatment sites were observed carefully.

6. Body weights:
Individual body weights were determined on day 0 prior to the first application and on day 5 prior to the sacrifice of the animals.

7. Treatment of animals:
The dosing solutions were applied daily to the dorsal part of the ears at a volume of 25 µl per ear per day for 3 consecutive days. A further group of mice (control animals) was treated with an equivalent volume of the relevant vehicle alone. Five days after the first topical application (day 6), 20.2 µCi of ^{3}H-thymidine in 250 µl phosphate-buffered saline was injected into the tail vein of each mouse.

8. Terminal procedures:
Approximately 5 hours after ^{3}H-thymidine injection the animals were euthanized by intraperitoneal injection of Pentobarbital-Natrium.
Immediately after the death of each animal a circular piece of tissue (diameter 0.8 cm) was punched out of the apical part of each ear of all animals. The weight of the pooled punches was determined for each test group. These measurements served for the detection of a potential inflammatory ear swelling.
Immediately after removal of the ear punches the left and right auricular lymph nodes were dissected. The weight of the pooled lymph nodes from both sides was determined for each animal.
After weight determination, a single cell suspension was prepared per test group from the pooled lymph nodes by carefully passing all lymph nodes through an iron mesh (mesh size 200 µm) into phosphate-buffered physiological saline. Subsequently the cell counts were determined with an aliquot of each suspension using a Casy®- Counter.
The remaining cell suspensions were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloro-acetic acid. Each precipitate was transferred to scintillation fluid and incorporation of ^{3}H-thymidine into the cells was measured in a □-scintillation counter.

9. Data evaluation and interpretation

The stimulation indices (SI) of cell count, ^{3}H-thymidine incorporation, lymph node weight and ear weight were calculated as the ratio of the test group values for these parameters divided by those of the vehicle control group.

The lymph node cell count and the ^{3}H-thymidine incorporation into the lymph node cells as well as to a certain extent lymph node weight are used to determine the potential sensitizing properties of a test article. Because not only sensitization induction but also irritation of the ear skin by the test substance may induce lymph node responses, the weight of ear punches taken from the area of test-substance application is determined as a parameter for inflammatory ear swelling as an indicator for the irritant action of the test substance.

Stimulation indices of >1.5 for cell count and/or of ≥ 3 for ^{3}H-thymidine incorporation are generally considered as indicative for a sensitizing potential of a test substance. If applicable, the EC (estimated concentration) leading to the respective SI values were calculated by linear or semi-logarithmical regression.

If the increase in cell count, ^{3}H-thymidine incorporation and/or lymph node weight is accompanied by a biologically relevant increase in ear weights it cannot be ruled out that the lymph node response was caused by irritation and not by skin sensitization. Depending on the magnitude of lymph node response, based on expert judgment, the evaluation of the sensitizing potential may be modified or additional studies might be necessary.

If a test article – despite of concentration related increase - does not elicit a biological relevant increase in cell count and/or ^{3}H-thymidine incorporation, further investigation of the sensitization potential at higher concentrations may be considered.

10. Positive controls

A concurrent positive control (reliability check) with a known sensitizer was not included into this study. Studies using the positive control substance alpha-hexylcinnamaldehyde are performed twice a year in the laboratory in order to show that the test system is able to detect sensitizing compounds under the test conditions chosen.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

No deaths occurred during the study period. No symptoms of local toxicity at the ears of the animals and no systemic findings were observed during the study period.

B. BODY WEIGHTS

There were no effects on body weight development. The increase of body weights during the study was within the expected range.

C. STIMULATION INDICES

The stimulation indices (SI) for lymph node cell counts, ^{3}H-thymidine incorporation and lymph node and ear weights are given in Table 5.2.6-1.

Table 5.2.6-1: Stimulation indices for cell counts, ^{3}H-thymidine incorporation, lymph node and ear weight in mice after treatment with BAS 550 F

Test Group	Treatment	Parameter evaluated	Stimulation index[1]
		Cell count [counts/lymph node pair]	
1	vehicle 1% DMF	8.54 ± 2.31	1.00
2	10% in DMF	12.93 ± 2.08*	1.51
3	25% in DMF	10.45 ± 1.91	1.22
4	50% in DMF	9.36 ± 0.98	1.10
		^{3}H-Thymidine incorporation [DPM/lymph node pair]	
1	vehicle 1% DMF	748.8 ± 179.7	1.00
2	10% in DMF	790.4 ± 306.4	1.06
3	25% in DMF	696.4 ± 238.7	0.93
4	50% in DMF	682.4 ± 84.3	0.91
		Lymph node weight [mg/lymph node pair]	
1	vehicle 1% DMF	5.62 ± 0.56	1.00
2	10% in DMF	5.53 ± 0.44	0.98
3	25% in DMF	5.60 ± 0.32	1.00
4	50% in DMF	5.40 ± 0.54	0.96
		Ear weight [mg/animal]	
1	vehicle 1% DMF	25.7 ± 1.1	1.00
2	10% in DMF	24.2 ± 0.8	0.90
3	25% in DMF	26.4 ± 1.9	1.03
4	50% in DMF	26.0 ± 0.3	1.01

[1] test group x / test group 1 (vehicle control)
* statistically significant increase vs. control group ($p < 0.05$)
DMF: dimethylformamide

At any concentration tested, topical treatment of mouse ears with BAS 550 F did not result in a biologically significant increase of the stimulation index for ^{3}H-thymidine incorporation, lymph node weight and ear weight

A statistically significant increase in lymph node cell count was observed in the lowest dose group in comparison to the vehicle control group ($p<0.05$); however, as the Stimulation Index obtained for this group was well below the threshold value of 3, this was not regarded as biologically relevant. The cutoff-value for a positive response regarding the lymph node cell count index of 1.55 reported for BALB/c mice (Ehling G. et al. (2005): An European inter-laboratory validation of alternative endpoints of the murine local lymph node assay 2nd round. Toxicology, 212, 69–79) was not exceeded in any test item treated group.

D. POSITIVE CONTROL

The sensitivity of mice (CBA) and the reliability of experimental techniques is assessed regularly using a known sensitizer. Positive results were consistently obtained over the years using several variations of the methods and different vehicles. The results of 6 control studies are presented in Table 5.2.6-2.

Table 5.2.6-2: Positive control LLNA studies performed

Date of performance	Aug 2011	Oct 2010	Sep 2010	May 2010	Mar 2010	Feb 2010
Name of test substance	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-exylcinnam aldehyde
Concentrations tested	5%, 10%, 25%	25%	25%	25%	25%	25%
Vehicle	DMF	DMF	DMF	DMF	DMF	DMF
Stimulation index 3H-thymidine incorporation[b]	1.35, 2.18, 8.08	10.03	6.45	6.14	7.48	6.49
Evaluation of study results	Positive	Positive	Positive	Positive	Positive	Positive

[b] = Ratio of test group values to control group values (Stimulation index) greater than 3.0 indicates a positive result
[#] = Individual lymph nodes

III. CONCLUSION

Based on the results of this study it is concluded that BAS 550 F has no sensitizing properties under the test conditions chosen. Consequently, no classification as a skin sensitizer is required according to the EU (28th ATP of Council Directive 67/548/EEC by Commission Directive 2001/59/EC / Council Regulation 1272/2008/EC) and according to the OECD Globally Harmonized System (GHS, revision 2, 2007).

CA 5.2.7 Phototoxicity

Report:	CA 5.2.7/1 Cetto V.,Landsiedel R., 2013a BAS 550 F (Dimethomorph) - *In vitro* 3T3 NRU phototoxicity test 2013/1110115
Guidelines:	OECD 432 (2004) *In vitro* 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Dimethomorph (batch COD-001244; purity 99.8%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells *in vitro*. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. A single experiment was carried out with and without irradiation with solar simulator. Vehicle and positive controls were included and clearly fulfilled the acceptance criteria.

Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in this study with and without irradiation: 0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 and 464.2 µg/mL.

Precipitation was seen at 215.4 µg/mL and above with and without irradiation. In the absence and the presence of irradiation in the highest concentrations applied cytotoxicity was noted and EC_{50} values were calculated. Based on the results of the present study, the test substance was predicted to have no phototoxic potential as indicated by Photo-Irritancy-Factor (PIF) values of 1.2. The threshold for a negative response is ≤ 2. The positive control chlorpromazine led to the expected increased cytotoxicity with irradiation as indicated by PIF values of 29.8.
Thus, under the experimental conditions of this study, dimethomorph is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

(DocID 2013/1110115)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 550 F (dimethomorph)

Description:	Solid, beige
Lot/Batch #:	COD-001244
Purity:	99.8% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01 May 2015
Solvent used:	Dimethylsulfoxide (DMSO) Even though the report indicated that the stability in DMSO was not determined analytically in the course of this study, the stability of dimethomorph in DMSO was demonstrated in earlier studies (In vitro mammalian cell gene mutation test. DocID DK-435-014).

2. Control Materials:

Vehicle control:	DMSO 1% (v/v) in PBS
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO; 8 concentrations tested - 1.9 to 180 µg/mL without irradiation, 0.03 to 3.2 µg/mL with irradiation

3. Test organisms: BaIb/c 3T3, clone A31: fibroblast cell line isolated from the muscle tissue of a mouse embryo. The Balb/c 3T3 cell line which was used in this experiment was obtained from the "European Collection of Cell Cultures" Salisbury, Wiltshire SP4 OJG, UK on 09 Aug 2006 and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral Red solution:	- 0.4 g Neutral Red powder (NR; Sigma N4638) - 100 mL deionized water
Neutral Red medium:	- 1 mL Neutral Red solution - 79 mL culture medium (DMEM incl. supplements) (incubated overnight at 37°C with 5% CO_2 and filtered with a 0.22 µm filter prior to use)
Other solutions and reagents:	- phosphate buffered saline (PBS) without Ca/Mg - trypsin/EDTA solution (0.05%; 0.02%) - Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source: The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1 produced a light spectrum corresponding to the conditions recommended in OECD 432, i.e. the mainly UVA but also sufficient amounts of UVB to to excite chemicals typically absorbing in the UVB range.. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest:	Up to 1000 µg/mL with and without irradiation. The EC_{50} values determined were 174.1 µg/mL without and 159.8 µg/mL with irradiation (no detailed data provided in the report).
Main NRU test:	Based on the results of the pretest the following concentrations were used in the main study: Without irradiation: 0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 and 464.2 µg/mL With irradiation: 0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 and 464.2 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 11-Mar-2013 to 13-May-2013

2. Treatment and NRU Phototoxicity Test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control. After pre-incubation for 1 hour in the dark (5% (v/v) CO_2, ≥90% humidity; 37°C) one 96 well-plate per substance was irradiated for 50 minutes with artificial sunlight (UVA intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm^2) whereas the respective reference plate was kept in the dark for the same period. Irradiation was performed according to OECD 432. Details on the irradiation spectrum can be found in the guideline in annex 3 figure 1. Thereafter the test- respectively control-substance was removed and the cells washed at least once with 100 µL PBS. After replenishing the wells with culture medium the cells were incubated overnight under the conditions indicated above. The medium was removed 24 hours after the start of treatment and after washing with 100 µL PBS the wells were filled with 100 µL medium containing 50 µg/mL Neutral Red. Subsequently the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Finally, the cells were washed again with 100 µL and the dye was extracted by 100 µL Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake by means of a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

3. Evaluation/Assessment

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100%.

$$Viability^{\S}\ [\%] = \frac{\text{Absorbance}_{\text{mean of the test group}}}{\text{Absorbance}_{\text{mean of the vehicle control}}} \times 100$$

§ The authors of the study denominate the above quotient as 'cytotoxicity', which is strictly speaking not correct. Thus, in this summary the appropriate term 'viability' is used. This applies also to Table 5.2.7-1 to Table 5.2.7-2.

In case of cytotoxicity, an EC_{50} value (concentration at which the viability is reduced by 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve). Therefore two viability values were needed: one between 100% and 50% and one between 50% and 0%. From these two points the concentration that inhibits the Neutral Red uptake by 50% of the respective control was calculated.

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor (PIF) prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes two special cases: Case 1 accounts for situations in which an EC_{50} can only be calculated in the presence of irradiation with artificial sunlight. Case 2 accounts for situations where an EC_{50} cannot be calculated in absence and presence of irradiation. These special cases do not apply to this study. Even though described in the report these cases are not described in this summary.
- The Mean Photo Effect prediction model which is used if no EC_{50} was obtained in the absence and presence of artificial sunlight. This is not the case in this study. Even though described in the report this prediction model is not described in this summary.

3.2 Photo-Irritancy-Factor (PIF)

For substances which induce a 50% cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of irradiation with artificial sunlight.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥5	phototoxic potential predicted
2 < PIF < 5:	probable phototoxic potential predicted
PIF ≤ 2:	no phototoxic potential predicted

3.3 Other parameters

pH:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Osmolarity:
Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Solubility:
Test substance precipitation was checked immediately after treatment and at the end of treatment.

Cell morphology:
Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.

4. Statistics:

No special statistical tests were performed.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD550 value (with and without irradiation) should be > 0.3.
  - Cell viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC50 value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of irradiation precipitation in culture medium was observed at test substance concentrations of ≥ 215.4 µg/mL.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

After treatment with the test substance, clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the main experiments in the absence and the presence of irradiation in at least the highest concentration (see Table 5.2.7-1).
Without irradiation, there was a decrease in the cell number at concentrations ≥ 215.4 µg/mL (EC50: 170.3 µg/mL). With UVA irradiation, there was a decrease in the cell number at concentrations ≥ 215.4 µg/mL (EC50: 147.0 µg/mL). Cell morphology changes were observed from 215.4 µg/mL onward with and without irradiation.

Based on the EC50 values a PIF of 1.2 was calculated, indicating no phototoxic potential for dimethomorph.

Table 5.2.7-1: Mean relative viability of dimethomorph with (+) and without (-) irradiation with artificial sunlight in Balb 3T3 cells

Test group	Irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-		0.350	-	16.8
Vehicle control 2	-		0.388	-	11.0
Vehicle mean	-		0.369	100.0	14.3
Dimethomorph					
2.2 µg/mL	-	-	0.425	115.3	5.9
4.6 µg/mL	-	-	0.441	119.7	6.2
10.0 µg/mL	-	-	0.475	128.8	5.7
21.5 µg/mL	-	-	0.462	125.2	4.4
46.4 µg/mL	-	-	0.481	130.5	5.2
100.0 µg/mL	-	-	0.454	123.1	10.4
215.4 µg/mL	-	+	0.011	3.0	2.2
464.2 µg/mL	-	+	0.002	0.5	0.9
Vehicle control 1	+		0.404	-	1.3
Vehicle control 2	+		0.478	-	4.3
Vehicle mean	+		0.441	100.0	9.3
Dimethomorph					
2.2 µg/mL	+	-	0.449	101.7	3.7
4.6 µg/mL	+	-	0.480	108.9	4.2
10.0 µg/mL	+	-	0.486	110.3	4.6
21.5 µg/mL	+	-	0.484	109.8	2.1
46.4 µg/mL	+	-	0.470	106.5	4.9
100.0 µg/mL	+	-	0.368	83.4	27.8
215.4 µg/mL	+	+	0.006	1.3	2.1
464.2 µg/mL	+	+	0.004	1.0	0.8

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

C. CYTOTOXICITY OF THE POSITIVE CONTROL

After treatment with the positive control chlorpromazine clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the absence and the presence of artificial sunlight (see Table 5.2.7-2).
In the experiment without irradiation, there was a decrease in viability at ≥ 30.0 µg/mL (EC50: 21.2 µg/mL). With irradiation, there was a decrease in viability at ≥ 0.8 µg/mL (EC50: 0.7 µg/mL). Cell morphology was distinctively changed in both experiments at ≥ 30 µg/mL and ≥ 0.8 µg/mL without and with irradiation.

Based on the EC50 values PIF's of 29.8 was obtained, indicating a strong phototoxic potential, thus confirming the sensitivity of the test system.

Table 5.2.7-2: Mean relative viability of Chlorpromazine with (+) and without (-) irradiation with artificial sunlight in Balb/c 3T3 cells

Test group	Irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative viability [% of control]	
				Mean	SD
Blank	-	0.038	-	-	-
Vehicle control 1	-	0.429	0.392	-	9.6
Vehicle control 2	-	0.524	0.486	-	3.5
Vehicle mean	-	0.476	0.439	100.0	12.9
Chlorpromazine					
1.9 µg/mL	-	0.476	0.438	99.8	3.1
3.8 µg/mL	-	0.490	0.452	103.1	2.9
7.5 µg/mL	-	0.466	0.428	97.6	3.9
15.0 µg/mL	-	0.367	0.330	75.1	4.4
30.0 µg/mL	-	0.101	0.064	14.5	3.1
60.0 µg/mL	-	0.062	0.025	5.7	14.1
90.0 µg/mL	-	0.040	0.002	0.5	1.1
180.0 µg/mL	-	0.038	0.000	0.1	0.1
Blank	+	0.038	-	-	-
Vehicle control 1	+	0.501	0.463	-	3.4
Vehicle control 2	+	0.567	0.529	-	6.4
Vehicle mean	+	0.534	0.496	100.0	8.6
Chlorpromazine					
0.03 µg/mL	+	0.504	0.466	94.1	2.5
0.05 µg/mL	+	0.509	0.471	95.1	3.1
0.10 µg/mL	+	0.509	0.472	95.1	4.7
0.20 µg/mL	+	0.502	0.464	93.6	5.5
0.40 µg/mL	+	0.495	0.458	92.3	6.2
0.80 µg/mL	+	0.226	0.188	38.0	12.9
1.60 µg/mL	+	0.042	0.004	0.8	0.6
3.20 µg/mL	+	0.040	0.002	0.3	0.3

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

III. CONCLUSION

According to the results of the present study, dimethomorph is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test.

CA 5.3 Short-Term Toxicity

The short-term toxicity of dimethomorph technical (E/Z racemat) was investigated in 2 dietary 28-day studies in Sprague-Dawley rats. In addition 2 dietary 28-day studies in Fisher 344 rats with E isomer and with Z isomer respectively were performed. Furthermore, dimethomorph technical (E/Z racemat) was investigated in a dietary 90-day study in Sprague-Dawley rats and Beagle dogs each, a 6 week dietary dose range finding study in CD-1 mice and a one-year dietary study in Beagle dogs.

Short-term (28-day) exposure of Sprague-Dawley rats to dimethomorph at dietary concentrations of 200, 1000, and 5000 ppm resulted in a NOAEL of 1000 ppm (equal to 80 mg/kg bw/day based on food consumption data). The highest dietary concentration tested (5000 ppm, equal to 280-300 mg/kg bw) induced increased morbidity and clinical signs of toxicity, reductions in body weight gains and in food consumption, increased liver to body weight ratios and increased blood urea nitrogen for both sexes. No microscopic examinations were performed. In a second short-term (28-day) toxicity study in Sprague-Dawley rats, using dietary concentrations of 0, 2000, 3000, and 4000 ppm, the NOAEL was less than 2000 ppm based on decreased body weight gains for both sexes at all dietary concentrations, and dose-related increases in liver-to-body weight ratios and hepatocellular hypertrophy for both sexes at 3000 and 4000 ppm and for females at 2000 ppm. Based on food consumption data, the 2000 ppm concentration is equal to 150 mg/kg bw/day.

A 28-day exposure of Fisher 344 rats to E isomer dimethomorph at dietary concentrations of 10, 100 or 750 mg/kg bw/day resulted in a NOAEL of 10 mg/kg bw/day, based on a dark discoloration and enlargement of the liver, an increase in adjusted liver weight in the males and a midzonal hepatocellular cytoplasmic lipid vacuolation in male and female rats at 100 mg/kg bw/day. A 28-day exposure of Fisher 344 rats to Z isomer dimethomorph at dietary concentrations of 0, 10, 100 and 750 mg/kg bw/day resulted in a NOAEL of 10 mg/kg bw/day, based on a slight midzonal lipid vacuolation at 100 or 750 mg/kg bw/day in both sexes.

In the subchronic (90-day) feeding study in Sprague-Dawley rats with dimethomorph, the NOAEL of 200 ppm was based on increased liver weights for females at 1000 ppm. Based on food consumption data, the NOAEL of 200 ppm is equal to approximately 16 mg/kg bw/day. In a 6 week dietary dose range finding study in mice a liver weight increase in males (5000 ppm and above) and females (4000 ppm and above) was observed.

In the subchronic (90-day) feeding study in beagle dogs with dimethomorph, increases in alkaline phosphatase activity (both sexes), increases in relative liver weight in females, reductions in absolute and relative prostate weights, and an increased incidence of prostatic interstitial fibrosis in males were observed at 1350 ppm (highest concentration tested). The NOAEL for this study is 450 ppm, equal to approximately 15 mg/kg bw/day as based on food consumption data.

In the 1-year feeding study in beagle dogs with dimethomorph technical, increased relative liver weights in females and increased relative testes weights in males occurred at 450 ppm. The NOAEL for this study is 150 ppm, equal to an approximate daily intake of 5 mg/kg bw/day as based on food consumption data.

Table 5.3-1: Summary of reviewed dimethomorph short-term toxicity studies

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
28-day dietary; Sprague-Dawley rats; 0, 200, 1000, 5000 ppm	Males: 15.8, 80.9, 305.9 Females: 17.5, 81.1, 283.3	80	280	↑morbidity; ↓body weight gain (or weight losses) and food consumption; ↑liver weights, ↑ BUN (males & females)	DK-420-005
28-day dietary; Sprague-Dawley rats; 0, 2000, 3000, 4000 ppm	Males: 150, 300, 400 Females: 200, 300, 400	-	150	↓body weight gain (males & females); ↑liver weights (females); hepatocellular hypertrophy (females)	DK-420-002; DK-420-004
28-day dietary; E isomer; Fischer 344 rat;	0, 10, 100, 750	10	100	Liver: dark discoloration, enlargement, adjusted liver weight ↑ (males), midzonal hepatocellular cytoplasmic lipid vacuolation (males & females)	DK-470-015
28-day dietary; Z isomer; Fischer 344 rat;	0, 10, 100, 750	10	100	Liver and caecal enlargement, midzonal hepatocellular cytoplasmic lipid vacuolation (males & females)	DK-470-016
90-day dietary; Sprague-Dawley rat; 0, 40, 200, 1000 ppm	Males: 2.9, 14.2, 73 Females: 3.2, 15.8, 82	16	73	↑ liver weights (females)	DK-425-001
6-week dietary; CD-1 mouse; Males: 0, 300/10000, 800, 2000/5000 ppm Females: 0, 300/8000, 800, 2000/4000 ppm	-		4000/5000 ppm	Absolute and adjusted liver weight ↑ (males & females) low dose was increased to 10000 ppm in males and 8000 ppm in females; high dose was increased to 5000 in males and 4000 in females	DK-420-003; DK-420-004
7-day dietary range finding; Beagle dog; 750, 900, 1000, 1200 ppm	-		750 ppm	≥750 ppm: few clinical signs (male), 900 ppm: ↓body weight 1000 ppm: ↓food consumption maximum tolerated dose: >1200 ppm	DK-420-001;

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
14-day dietary range finding; Beagle dog; 1200 ppm	-		1200 ppm	Few clinical signs (male), maximum tolerated dose: >1200 ppm	DK-420-001;
90-day dietary; Beagle dog; 0, 150, 450, 1350 ppm	Males: 5, 15, 43 Females: 6, 15, 44	15	43	↑ alkaline phosphatase activity (males); ↓ prostrate weights (males); prostatic interstitial fibrosis (males); ↑ liver weights (absolute and relative) (females)	DK-425-002
Chronic (1-year) dietary; Beagle dog; 0, 150, 450, 1350 ppm	Males: 4.9, 14.7, 44.6 Females: 5.0, 15.7, 47.0	5	15	↑ liver weights (females) ↑ testes weights (males)	DK-427-003

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the DAR/EFSA conclusion of dimethomorph (EFSA scientific report, 82, 1-69, 2006):

Short term toxicity	
Target / critical effect:	Liver; testes and prostate (dog only)
Lowest relevant oral NOAEL / NOEL:	1yr dog: (5 mg/kg bw/d) 90d dog: (15 mg/kg bw/d) 90d rat: (16 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL:	No data - not required
Lowest relevant inhalation NOAEL/NOEL	No data - not required

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

There is one 28-day dermal toxicity study in Wistar rats performed in 2010 that was not submitted in the former registration process ([see KCA 5.3.3/1 2010/1151903]).

Study	**Dosages (mg/kg bw/ day)**	**NOAEL (mg/kg bw/day)**	**Main adverse effect**	**Reference (DocID)**
28-day dermal study Wistar rat	100, 300, 1000	1000	No systemic and local effects were observed	2010/1151903

Thus, the conclusion for relevant endpoints for the current re-registration is as follows:

Short term toxicity	
Target / critical effect:	Liver; testes and prostate (dog only)
Lowest relevant oral NOAEL / NOEL:	1yr dog: (5 mg/kg bw/d) 90d dog: (15 mg/kg bw/d) 90d rat: (16 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL:	>1000 mg/kg bw/d
Lowest relevant inhalation NOAEL/NOEL	No data - not required

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of are provided under the respective chapters.

CA 5.3.1 Oral 28-day study

Rat:

[redacted] 1985, ZTH 236 Z50 Preliminary assessment of toxicity to rats by dietary admixture for four weeks, [redacted], unpublished, BASF DocID DK-420-005

Material and Methods:
Test material: Dimethomorph (ZTH 236 Z50); Batch No. L 5000; purity 99 %
Test animals: Male and female Sprague-Dawley rats
Dimethomorph technical was fed to male and female Sprague-Dawley rats (5/sex/group) for 28 days at dietary concentrations of 0, 200, 1000 and 5000 ppm. At treatment initiation, animals were approximately 7-8 weeks of age, males weighed approximately 250 g and females weighed approximately 160 g.

Food consumption and body weight were determined weekly. The state of health was checked each day. During the weekly weighing the animals were subjected to an additional comprehensive clinical examination. Clinical chemistry, haematological examinations and urinalysis were carried out towards the end of the administration period. The animals were subjected to gross-pathological assessment.

Findings:
Average test substance intake values for these concentrations were approximately 16, 80 and 300 mg/kg bw/day, respectively, for males, and 17, 80 and 280 mg/kg bw/day, respectively, for females based on food consumption data. Clinical signs of toxicity were observed only in the 5000 ppm group and included soft feces, swollen abdomen, hunched posture, piloerection, emaciation, lethargy and unsteady gait. Due to the severity of these findings, two females and one male in the 5000 ppm group were sacrificed in a moribund condition and it was necessary to sacrifice the remaining animals in the 5000 ppm group following the completion of the clinical chemistry evaluations performed during week 4. Statistically significant reductions in body weight gains were observed for males and females in the 5000 ppm group when compared to controls, with three males and three females exhibiting a net weight loss over the 28-day treatment period. These reductions in body weight gains correlated with reductions in food consumption noted for both sexes at 5000 ppm. No treatment-related effects on body weight or body weight gains were observed at 200 or 1000 ppm.

Changes in hematology were observed for both sexes in the 5000 ppm group and included statistically significant increases in platelet and neutrophil counts. Total leukocyte counts were also increased for both sexes in the 5000 ppm group when compared to controls (reflecting the increases in neutrophil counts), but the differences were not statistically significant. Quantitative changes, relative to control values, in the concentration of plasma proteins were noted for males and females at the 5000 ppm level as indicated by a reduction in total albumin levels and a concomitant increase in globulin levels. Other clinical chemistry changes observed included a reduction in total plasma proteins for females at 5000 ppm, statistically significant increases in blood urea nitrogen (BUN) for both sexes at 5000 ppm and a slight but statistically significant increase in BUN for females at 1000 ppm. The increase in BUN observed for females at 1000 ppm was not considered adverse because no treatment-related renal effects, including BUN, were observed at dietary concentrations of 1000 ppm in the 13-week dietary toxicity study in rats or at dietary concentrations of 2000 ppm in two 24-month chronic feeding studies in rats. Urinalyses data indicated that both males and females in the 5000 ppm group voided an increased volume of urine, when compared to control data, but the differences were only statistically significant for females.

Absolute liver weights for both sexes in all treatment groups were comparable to controls, but increased liver to body weight ratios were observed for males (non-statistically significant) and females (statistically significant) in the 5000 ppm group when compared to controls. No other organ weight changes were observed. No microscopic examinations were performed in this study.

Conclusion:
The NOAEL for this study was 1000 ppm, based on increased moribundity and clinical signs of toxicity, reductions in body weight gains (or weight losses) and in food consumption, increased liver to body weight ratios and increased blood urea nitrogen for both sexes at 5000 ppm. Based on food consumption data, this dietary concentration representing the NOAEL is equal to an approximate daily intake of 80 mg/kg bw/day.

[redacted], 1986, CME 151: 4 week dietary dose range finding study in rats, [redacted] unpublished, BASF DocID DK-420-002 and DK-420-004

Material and Methods:
Test material: Dimethomorph (CME 151); Batch No. DW 11/86; purity 96.6 ± 0.8 % reported in DK-380-002
Test animals: Male and female Sprague-Dawley rats
Dimethomorph technical was fed to four groups of male and female Sprague-Dawley rats (10/sex/group) for 28 days at dietary concentrations of 0, 2000, 3000 and 4000 ppm. At treatment initiation, animals were approximately 6-7 weeks of age, males weighed approximately 195 g and females weighed approximately 135 g.

Findings:
The concentrations of the diet were analytically confirmed. The stability and homogeneous distribution of the test substance in the diet have been confirmed in the above study.
Test substance intake values for the 2000, 3000 and 4000 ppm groups were approximately 150, 300 and 400 mg/kg bw/day, respectively, for males, and approximately 200, 300 and 400 mg/kg bw/day, respectively, for females based on food consumption data. The calculated test substance intake values for this study are higher than in the previous study because the rats in the second study were smaller in size and one week younger than in the first study. All animals in this study survived to study termination. Clinical signs of toxicity were observed only in the 4000 ppm group, which included piloerection, swollen abdomen and emaciation.
Dose-related reductions in body weights (statistically significant) and body weight gains (non-statistically significant) were noted for females at all dietary concentrations as compared to controls. Body weight gains for males at all dietary levels were reduced when compared to controls, but the differences were only statistically significant at 4000 ppm.

Reductions in food consumption were noted for males and females at all dietary levels but the differences from the controls were not statistically significant at any level. Hematology, clinical chemistry and urinalysis studies were not performed in this study. Macroscopic findings noted at terminal necropsy included distended intestines, often fluid filled or with gelatinous contents, for both sexes in the 4000 ppm group and for females in the 3000 ppm group. Dose-related increases in liver-to-body weight ratios were observed for both sexes at 3000 and 4000 ppm and for females at 2000 ppm. Absolute organ weights for both sexes in all treatment groups were comparable to controls.

Histological examinations were only performed on livers from animals in all groups. Microscopic liver changes noted in this study included hepatocellular hypertrophy for both sexes at 3000 and 4000 ppm groups and for females at 2000 ppm.

When comparing the food consumption data for both studies, animals in the 5000 ppm group (first study) ate substantially less food during the 4-week treatment period than animals in the 4000 ppm group (second study). The food consumption patterns for the control animals were comparable in both studies.

Conclusion:
The NOAEL is less than 2000 ppm, based on decreased body weight gains for both sexes at all dietary concentrations, and dose-related increases in liver-to-body weight ratios and hepatocellular hypertrophy for both sexes at 3000 and 4000 ppm and for females at 2000 ppm. Based on food consumption data, the 2000 ppm concentration is equal to an average daily intake of approximately 150 mg/kg bw/day in males and 200 mg/kg bw/day in females.

[redacted] 1990, SAG 151 E isomer: A 28 day oral toxicity study in rats, [redacted], unpublished, Shell Agrar DocID 151AA-432-005, BASF DocID DK-470-015.

Material and Methods:
Test material: Dimethomorph (SAG 151 - E isomer); Batch No. L4785; purity: E isomer 99.5 - 99.7 %, Z-isomer 0.3 - < 0.5 %
Test animals: male and female Fisher 344 rats (SPF) supplied by [redacted].
Dimethomorph technical E isomer was fed by oral gavage to four groups of male and female Fisher 344 rats (7/sex/group) for 28 days at dietary concentrations of 0, 10, 100 or 750 mg/kg bw/day. At treatment initiation, animals were approximately 6-8 weeks of age, males weighed approximately 105 g and females weighed approximately 93 g.

Rats were checked for clinical signs twice daily. Bodyweight and food intake were measured weekly. At day 29 the rats were necropsied and blood samples were taken by cardiac puncture for hematology and clinical chemistry. All organs were subjected to macroscopic examination and brain, heart, liver, kidney, spleen, adrenals and testes were weighed. Histopathology was performed on the adrenals, heart, kidney, intestines, liver, lymph nodes, pituitary, spleen, caecum, stomach and gross lesions from the control and top dose groups. Additionally livers were examined from the intermediate dose levels. Bone marrow smears were prepared from the femur of all rats.

Findings:
The concentrations of the diet were analytically confirmed. The stability and homogeneous distribution of the test substance in the diet have been confirmed in the above study.
There was no treatment-related effect on clinical signs. A statistical significant increase in food intake occurred in the top dose males during week 3 and 4. This coincided with a slight, but not statistical significant increase in body weight, which was seen in 750 mg/kg bw/day dosed males during week 3 and 4.

Rats of both sexes treated with 750 mg/kg bw/day showed slight decreases in total blood hemoglobin indicating a mild normocytic normochromic anemia. Additionally, rats at the top dose group had increased platelet counts, males also had a small increase in platelet volume.

A range of small but statistically significant differences were seen in the clinical chemistry parameters. The observed increases in protein, bilirubin, gamma glutamyl transpeptidase, cholesterol, calcium and triglyceride were attributed to treatment related hepatic changes at the 750 mg/kg bw/day treatment level. Increases in serum urea and creatinine concentrations suggested renal toxicity but no related histopathological observations were made.

A slight dose increase in adjusted liver weight was seen in the males at 100 and 750 mg/kg bw/day and in females at the 750 mg/kg bw/day treatment level. The liver weight effect was dose related and supported by clinical chemical and histopathological changes indicating a treatment related response. Other organ weight differences included a decreased splenic weight in top dose males and an increase in unadjusted and adjusted adrenal weight in the 100 and 750 mg/kg bw/day dose group males. These differences were small and only seen in one sex and were considered to be chance effects.

Slight to moderate hepatic enlargement was seen macroscopically in one male at 100 mg/kg bw/day and in all male and female rats at 750 mg/kg bw/day. Dark discoloration of the liver was observed in most males and one female at 750 mg/kg bw/day and occasionally in males at 100 mg/kg bw/day. Slight to moderate caecal enlargement and fluid caecal contents were seen at 750 mg/kg bw/day but no related changes were observed histologically. Patchy mid zonal hepatocellular cytoplasmic lipid vacuolation was observed at 750 mg/kg bw/day. The overall severity was slight and the frequency dose related, being apparent in all males and females at the top dose and some 100 mg/kg bw/day treated rats.

Conclusion:
Significant treatment-related changes were observed in the liver. The NOAEL is 10 mg/kg bw/day, based on a dark discoloration and enlargement of the liver, an increase in adjusted liver weight in the males and a mid zonal hepatocellular cytoplasmic lipid vacuolation in male and female rats at 100 mg/kg bw/day.

[redacted] 1991, SAG 151 Z isomer: A 28 day oral toxicity study in rats, [redacted] unpublished, Report-No. SBGR.90.107; Project-No. 151AA-432-004; (Study-No. SRC58690); BASF DocID DK-470-016.

Material and Methods:
Test material: SAG 151 Z isomer; Batch No. Th H296, ST90/106; purity 95.6 %
Test animals: Male and female SPF Fischer 344 rats
Dimethomorph was fed to groups of 7 male and 7 female rats for 4 weeks at dietary concentrations of 0, 10, 100 and 750 mg/kg bw/day. All animals were examined for clinical signs. Haematological and clinical chemistry examination was performed. Post-mortem examination included gross pathology, organ weights and histopathological evaluation.

Findings:
In both sexes increases in serum bilirubin and protein, as well as decreased albumin to globulin ration were seen at 750 mg/kg bw/day. A dose related, statistical significant increase in liver weight was seen in rats treated with 100 or 750 mg/kg bw/day. Slight caecal enlargement was observed in 6 males and 2 females at 750 mg/kg bw/day. Slightly fluid caecal contents were recorded in 3 males at 750 mg/kg bw/day. Histopathological examination of the liver revealed a patchy midzonal cytoplasmic lipid vacuolation at 100 or 750 mg/kg bw/day in both sexes.

Conclusion:
Some clinical chemistry variates showed changes at 750 mg/kg bw/day in both sexes, reflecting functional hepatic changes. Histopathological examination of the liver revealed a slight midzonal lipid vacuolation at 100 or 750 mg/kg bw/day in both sexes. No treatment related effects were seen at 10 mg/kg bw/day.

CA 5.3.2 Oral 90-day study

Rat:

[redacted] **1987, CME 151: Toxicity to rats by dietary admixture for 13 weeks with a 4-week withdrawal period,** [redacted] **Report No. CMK 7/8624, unpublished, BASF Document No. DK-425-001.**

Material and Methods:
Test material: Dimethomorph (CME 151); Batch No. T2/85; purity 98.7 ± 1.5 % reported in DK-380-003 (see Document J.)
Test animals: Male and female Sprague-Dawley rats
Dimethomorph technical was fed to four groups of male and female Sprague-Dawley rats for 13 weeks at dietary concentrations of 0, 40, 200 and 1000 ppm. Twenty male and twenty female rats were assigned to the control and 1000 ppm groups while 10 animals/sex were assigned to the 40 and 200 ppm groups. Following 13 weeks of treatment, 10 animals/sex/group were sacrificed and 10 animals/sex in the control and 1000 ppm groups received control diet for a 4-week recovery period.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The concentrations of the diets were analytically confirmed.

All animals survived to study termination and no overt signs of toxicity were observed that could be attributed to administration of the test material. Food consumption values for males and females in all treated groups were comparable to controls during the treatment and recovery periods. Average test substance intake values for the 40, 200 and 1000 ppm groups were 2.9, 14.2, and 73 mg/kg bw/day, respectively for males, and 3.2, 15.8 and 82 mg/kg bw/day, respectively for females, based on food consumption data.

No treatment-related changes in body weights or body weight gains were observed for either male or female rats at any concentration. No treatment-related ophthalmological findings were observed at study termination and microscopic examination of vaginal smears obtained during weeks 9 - 12 of the study did not indicate any treatment-related effects on the estrous cycle of female rats.
Hematological investigations performed in week 13 indicated that total white blood cell counts for males receiving 1000 ppm were lower than those of controls. This was associated with lower lymphocyte counts for these animals. Total white blood cell counts and lymphocyte counts for males of the high dose group were noted to be similar to those of controls at the investigation performed during week 4 of the withdrawal period. Marginal group differences noted among erythrocyte parameters in week 13 and week 4 of the withdrawal period were considered to be of no toxicological significance. Values were generally within background data ranges and in week 4 of withdrawal males and females generally showed opposing trends.

No adverse effects of treatment were evident from the clinical chemistry data. A slight increase in the acidity of the urine of treated rats was noted in comparison with controls at the investigation performed in week 13, but this was not dose-related in degree. Urinary pH investigated during week 4 of the withdrawal period for rats which had received 1000 ppm showed regression of this effect for these animals. There were no other toxicological significant differences in urinalysis parameters.

There were no treatment-related organ weight changes observed for males at termination of the 13-week treatment period. For females, absolute heart and liver weights, heart-to-body weight ratios and liver-to-body weight ratios were statistically significantly increased, relative to controls, at 1000 ppm following 13 weeks of treatment. Absolute and relative liver weight were also slightly increased (statistically significant) for females at 1000 ppm following the 4-week recovery period. Kidney weights for treated females were marginally higher than those of controls, after adjustment for final bodyweight as covariate, but not to a dose-related degree. After 4 weeks of withdrawal there was no significant difference. There were no macroscopic or microscopic changes, which were attributable to treatment with dimethomorph technical in any of the tissues evaluated.

Conclusion:
Based on increased liver weights for females at 1000 ppm, the NOAEL for this study is 200 ppm. Based on food consumption data, this concentration is equal to an approximate average daily intake of 16 mg/kg bw/day.

Mouse:

[redacted] 1986, CME 151 6 week dietary dose range finding study in mice, [redacted], Celamerck Document Report No. 151AE-431-003, unpublished. BASF Document No. DK-420-003

Material and Methods:
Test material: Dimethomorph (CME 151); Batch No. DW 11/86; purity not specified in the report
Test animals: Male and female CD-1 mice
Dimethomorph was administered to mice (10/sex/group) at dietary concentrations of 0, 300, 800 and 2000 ppm. Due to the lack of any toxic effects the dose levels were increased from day 23 of dosing. The changes in dose level were as follows: 300 ppm was increased to 10000 ppm (males) and 8000 ppm (females); 2000 ppm was increased to 5000 ppm (males) and 4000 ppm (females). It was decided to increase the duration of dosing to a total of 6 weeks. After 6 weeks of dosing all animals were killed and autopsied with selected organs. Livers from selected animals were examined histopathologically.

Findings:
Males in the groups receiving 300/10000 ppm or 2000/5000 ppm and females receiving 300/8000 or 2000/4000 ppm showed significant increases in absolute liver weight and when adjusted for terminal body weight.

Conclusion:
There was a liver weight increase in males (5000 ppm and above) and females (4000 ppm and above) which could not be attributed to a specific histopathological lesion.

Additional study in rats and mice:

[redacted] **Histopathological examination of ileum sections taken from the rat [redacted] project No. 435025) and mouse [redacted] project No. 435067) dose range finding studies, [redacted] Report No. 3770, unpublished. BASF Document No. DK-425-004.**

Material and Methods:
Test material: Dimethomorph (CME 151); Batch No. DW 11/86; purity 96.6 ± 0.8 %
Details about test material, animals and methods were reported in [redacted] 1986, CME 151: 4 week dietary dose range finding study in rats, [redacted] BASF Document No. DK-420-002 and [redacted], 1986, CME 151 6 week dietary dose range finding study in mice, [redacted] Celamerck Document Report No. 151AE-431-003, unpublished.BASF Document No. DK-420-003. Ileum haematoxylin and eosin sections were prepared from all the rats and mice and histopathologically evaluated.

Findings:
Based upon the histopathological findings on the ileum, oral administration of dimethomorph to rats at up to 4000 ppm was associated with ileal dilatation and inflammation which appeared to originate in the serosa and become chronic. Female rats appeared to be more susceptible to dimethomorph than male rats. In mice given at up to 10000 ppm had some effect on ileal dilatation and also increased the incidence of villous atrophy in both sexes.

Conclusion:
Overall the toxic effects in mice were less than those seen in rats.

Dog:

[redacted] **1986, CME 151: 13 week dietary toxicity study in dogs, [redacted] Report No. 3722, unpublished.BASF Document No. DK-425-002.**

Material and Methods:
Test material: Dimethomorph (CME 151); Batch No. DW 11/86; purity 96.6 ± 0.8 % reported in DK-380-002 (see Document J).
Test animals: Male and female purebred Beagle dogs
Dimethomorph technical was administered to purebred Beagle dogs (4/sex/group) at dietary concentrations of 0, 150, 450 and 1350 ppm. All animals were provided 400 grams of food per day for 90 days.
The animals were observed daily for any signs of ill health or reaction to treatment. Food consumption was monitored daily and body weight was monitored weekly throughout the dosing period. Laboratory investigations of hematology, clinical chemistry and urinalysis were undertaken pretrial and during weeks 6 and 13 of treatment. Opthalmoscopic examination was undertaken pretrial and during week 13 of treatment. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Findings:
The concentrations of the diets were analytically confirmed.

All animals survived to study termination. Treatment-related clinical signs were noted at 1350 ppm and consisted of lip-licking, occasional subdued behaviour and a few incidences of body tremors. Lip-licking was frequently observed over the dosing period and was considered to be due to the fact that 1350 ppm was approximately the emetic threshold demonstrated in a previous maximum tolerated dose study in dogs. Salivation was observed in all groups, including controls, during week 1 of the study and was observed intermittently in all treatment groups at various times during treatment. Because no dose-related increase in the incidence of salivation was evident, this finding was not considered treatment-related. No treatment-related ophthalmoscopic abnormalities were observed at study termination.

Food consumption patterns for males and females in all treated groups were comparable to controls during the 13-week treatment period. Average test substance intake values during the 13-week treatment period for the 150, 450 and 1350 ppm groups were 5, 15 and 43 mg/kg bw/day, respectively for males, and 6, 15 and 44 mg/kg bw/day, respectively for females, based on food consumption data.

No treatment-related changes in body weights or body weight gains were observed at any dietary concentration. No changes in hematology parameters were observed for any treatment group during the study. Statistically significant increases in serum alkaline phosphatase activity were observed at weeks 6 and 13 for males at 1350 ppm when compared to controls. Although increases in serum alkaline phosphatase activity were not observed for females at any dietary concentration at weeks 6 and 13 during the 13-week study, statistically significant increases for this enzyme were observed at weeks 13, 26, and 51 for male and female dogs at 1350 ppm in the 1-year dietary toxicity study. No other clinical chemistry changes were observed during the study. No adverse effects of treatment were evident from the urinalysis data.

Anatomic pathology findings showed no treatment-related macroscopic changes during necropsy. Some changes in absolute and relative organ weight were observed (see Table 5.3.2-1, Table 5.3.2-2, Table 5.3.2-3). Absolute thymus weights and thymus-to-body weight ratios were statistically significantly increased in males at 1350 ppm when compared to controls, but these organ weight changes were not correlated with any microscopic lesions, and therefore, were not considered to be treatment-related. Increases in mean absolute liver weight (non-statistically significant) and mean liver-to-body weight ratios (statistically significant) were observed for females at 1350 ppm when compared to controls. Absolute and relative liver weights for males at 1350 ppm were comparable to controls. Absolute prostate weights and prostate-to-body weight ratios were significantly reduced, relative to controls, for males at 1350 ppm. The changes in prostate weights noted for males in the 1350 ppm group were consistent with an apparent increase in the incidence of prostatic interstitial fibrosis for all animals of this group, as compared to the control group. No other treatment-related organ weight changes or microscopic findings were observed.

Table 5.3.2-1: Absolute organ weight (g) in males (statistically significant changes).

Group/Dose level (ppm)		Body weight (kg)	Thymus	Prostate
1 (0)	Number	4	4	4
	Mean	12.1	8.34	5.68
	S.D.	1	1.35	3.67
2 (150)	Number	4	4	4
	Mean	11.1	5.63	6.63
	S.D.	0.6	3.10	1.18
3 (450)	Number	4	4	4
	Mean	11.7	9.86	6.49
	S.D.	1.2	3.42	2.08
4 (1350)	Number	4	4	4
	Mean	12.1	14.95*	3.27**
	S.D.	0.9	6.84	1.31

*Significantly different from controls $p<0.05$
** Significantly different from controls $p<0.01$

Table 5.3.2-2: Relative organ weight (as percentage of body weight) in males (statistically significant changes).

Group/Dose level (ppm)		Thymus x 100	Prostate x 100
1 (0)	Number	4	4
	Mean	6.92	7.01
	S.D.	1.25	2.64
2 (150)	Number	4	4
	Mean	5.11	5.98
	S.D.	2.86	0.79
3 (450)	Number	4	4
	Mean	8.30	5.70
	S.D.	2.23	2.29
4 (1350)	Number	4	4
	Mean	12.15*	2.71**
	S.D.	4.85	1.04

*Significantly different from controls $p<0.05$
** Significantly different from controls $p<0.01$

Table 5.3.2-3: Relative organ weight (as percentage of body weight) in females (statistically significant changes).

Group/Dose level (ppm)		Liver x 10
1 (0)	Number	4
	Mean	28.21
	S.D.	2.09
2 (150)	Number	4
	Mean	32.97
	S.D.	0.91
3 (450)	Number	4
	Mean	32.08
	S.D.	2.47
4 (1350)	Number	4
	Mean	35.85**
	S.D.	5.10

** Significantly different from controls $p<0.01$

Conclusion:
Based on increases in alkaline phosphatase activity (males), increases in mean relative liver weight in females, reductions in absolute and relative prostate weights, and an increased incidence of prostatic interstitial fibrosis in males at 1350 ppm, the NOAEL for this study is 450 ppm. Based on food consumption data, this concentration is equal to an approximately average daily intake of 15 mg/kg bw/day.

Additional studies in dogs:

[redacted]., 1986, CME 151 Dietary maximum tolerated dose study in dogs, [redacted], unpublished, Celamerck Document 151AE-431-001; unpublished.BASF Document No. DK-420-001.

Material and Methods:
Test material: Dimethomorph CME 151; Batch No. DW 11/86, purity not specified
Test animals: Male and female purebred Beagle dogs
The objective of the study was to determine the dietary maximum tolerated dose. The study was conducted in 2 parts. Dimethomorph was administered to groups of 1 male and 1 female dog at dietary concentrations of 0, 1000, 750, 900 and 1200 ppm for 7 days in part A of the study. In part B of the study 1 male and 1 female dog were dosed with 1200 ppm dimethomorph for 14 consecutive days. The animals were observed daily for clinical signs and viability. Food consumption of the animals was determined daily and their body weight twice a week. Clinical chemistry and hematological examinations were carried out following overnight starvation pretrial and prior to necropsy. Urine samples were collected over the final period of water deprivation, pretrial and one day prior to necropsy. Faecal samples were collected at the time of urine collection. All animals were subjected to gross pathological evaluation and organ weight analysis.

Findings:
Emesis, subdued behaviour and increased micturition on very few occasions was observed for the male dog were absent in the last week of treatment in part A of the study. Body weight losses were demonstrated by the male dog when dosed with 900 ppm or above. There was a reduction in food intake for both animals when dosed with 1000 ppm. In case of the female dog, food intake was also noted to be below maximal pretrial. In part B of the study slightly body tremors were only observed on a few occasions for the male dog.

Conclusion:
It was concluded that the maximum tolerated dose of dimethomorph is > 1200 ppm.

1-year study in dog:

[redacted] 1990, SAG 151: 52 week dietary toxicity study in dogs, [redacted], unpublished, BASF Document No. DK-427-003.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. DW 11/86; purity 96.6 %
Test animals: Male and female purebred Beagle dogs
Dimethomorph was administered to groups of 4 male and 4 female purebred Beagle dogs at dietary concentrations of 0, 150, 450 and 1350 ppm for about 12 months. All dogs were offered limited diet (400 g/dog/day) for 52 weeks. The animals were observed daily for any signs of ill health or reaction to treatment. Food consumption of the animals was determined daily and their body weight once a week. Clinical chemistry and hematological examinations as well as urinalyses were carried out once pretrial and during weeks 13, 26, and 51 of the administration period. Ophthalmological examinations were carried out pretrial and during weeks 26 and 51 of treatment. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Findings:
The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis.

All dogs survived to study termination and no treatment-related clinical signs of toxicity or ophthalmological abnormalities were detected during the 52-week study period. Sporadic convulsive seizures (lasting for 30-60 seconds) were observed in one male at the 1350 ppm treatment level on two days during week 44 and two days during week 47. Since the incidence of spontaneous convulsive episodes may occur up to 12 % in a closed colony of Beagle dogs, the sporadic convulsions observed in the male dog in the 1350 ppm group were not considered treatment-related. In addition, a slight convulsive seizure was observed for one male in the 150 ppm group prior to weighing during weeks 32 and 34, and was attributed to stress.
Food consumption patterns for males and females in all treated groups were comparable to controls during the 52-week treatment period. Average test substance intake values during the 52-week treatment period for the 150, 450 and 1350 ppm groups were 5, 15 and 45 mg/kg bw/day, respectively, for males and 5, 16 and 47 mg/kg bw/day, respectively, for females based on food consumption data. Slightly reduced body weight gains were observed for dimethomorph treated groups with the exception of the 150 mg/kg bw/d dose group females which showed an increase of body weight gain (See Table 5.3.2-4, Table 5.3.2-5).

Table 5.3.2-4: Group mean body weight (kg) – Males

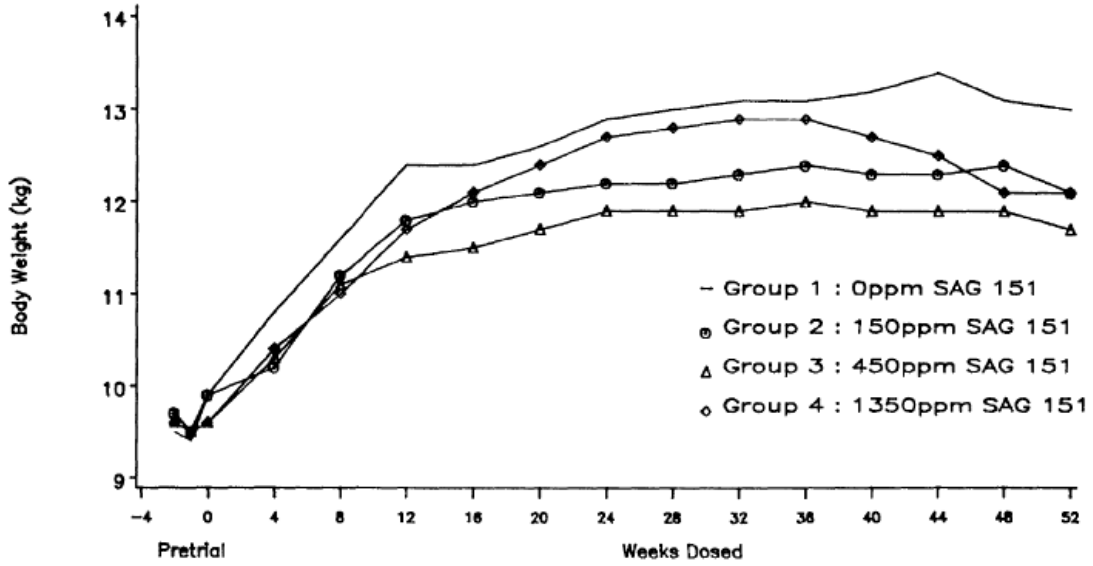
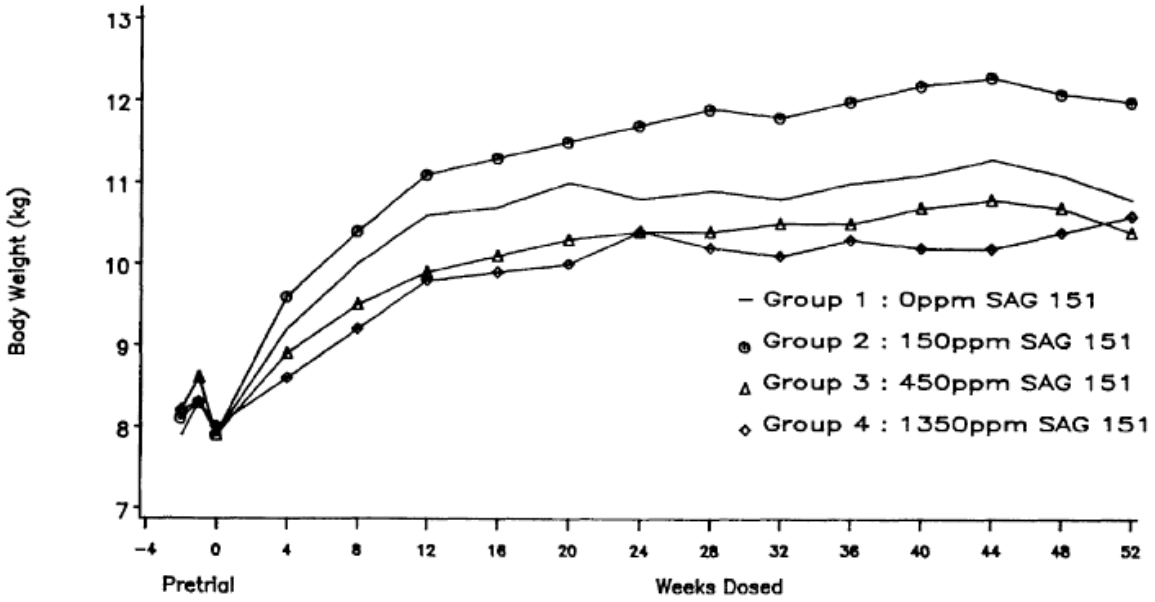


Table 5.3.2-5: Group mean body weight (kg) – Females



Hematology and urinalysis parameters were unaffected by treatment with dimethomorph technical. Statistically significant increases in serum alkaline phosphatase were observed at weeks 13, 26 and 51 for males and females at 1350 ppm when compared to controls. No other treatment-related clinical chemistry changes were observed for either sex at any dietary level.
There were no treatment-related macroscopic changes observed at necropsy. Absolute liver weights and liver-to-body weight ratios were significantly increased for males at 1350 ppm when compared to controls. Liver-to-body weight ratios were increased, relative to controls, for females at 450 ppm (not statistically significant) and at 1350 ppm (statistically significant). Statistically significant increases in testes-to-body weight ratios were observed for males at 450 and 1350 ppm without any histopathological findings. Decreases in absolute prostate weights (statistically significant) and prostate-to-body weight ratios (non-statistically significant) were observed for males at 1350 ppm when compared to controls (See Table 5.3.2-6, Table 5.3.2-7, Table 5.3.2-8).

Table 5.3.2-6: Absolute organ weight (g) – Males (statistically significant changes)

Group/Dose level (ppm)		Body weight (kg)	Liver	Prostate
1 (0)	Number	4	4	4
	Mean	13.1	371.66	8.25
	S.D.	1	33.52	2.40
2 (150)	Number	4	4	4
	Mean	12.1	327.71	8.97
	S.D.	1	31.93	2.52
3 (450)	Number	4	4	4
	Mean	11.4	346.36	5.55
	S.D.	1.5	23.96	1.35
4 (1350)	Number	4	4	4
	Mean	12.1	433.44*	4.27*
	S.D.	01.1	30.87	1.86

*Significantly different from controls $p<0.05$

Table 5.3.2-7: Relative organ weight – Males

Group/Dose level (ppm)		Liver	Testes	Prostate
1 (0)	Number	4	4	4
	Mean	368.14	25.44	7.40
	S.E.	15.65	1.57	0.94
2 (150)	Number	4	4	4
	Mean	328.01	27.56	9.04
	S.E.	15.65	1.57	0.94
3 (450)	Number	4	4	4
	Mean	349.20	31.49*	6.23
	S.E.	15.65	1.57	0.94
4 (1350)	Number	4	4	4
	Mean	433.82*	32.73*	4.36
	S.E.	15.65	1.57	0.94

*Significantly different from controls p<0.05

Table 5.3.2-8: Relative organ weight – Females (statistically significant changes)

Group/Dose level (ppm)		Liver
1 (0)	Number	4
	Mean	280.25
	S.E.	25.95
2 (150)	Number	4
	Mean	264.79
	S.E.	25.95
3 (450)	Number	4
	Mean	350.02
	S.E.	25.95
4 (1350)	Number	4
	Mean	412.26**
	S.E.	25.95

** Significantly different from controls p<0.01

The changes in prostate weights noted for males in the 1350 ppm group were consistent with a slight increase in the incidence and severity of prostatic interstitial fibrosis, as compared to controls. Thus, the tissue shrinkage resulting from interstitial fibrosis was reflected by a reduction in prostate weights only in the 1350 ppm group (Table 5.3.2-9). The only other microscopic finding was increased hepatic lipid content which was observed in 3 of 4 males at 1350 ppm as compared to none in control males. This small increase in hepatic lipid was more equivocal in females where the incidences for the control, 150, 450 and 1350 ppm groups were 1 of 4, 0 of 4, 2 of 4 and 2 of 4, respectively.

Table 5.3.2-9: Histopathology findings in the prostate - males

Group/dose (ppm)	Control	150	450	1350
Number of animals	4	4	4	4
Incidence of Prostatitis	0	1	0	2
Incidence of increased fibrosis	0	0	0	4*

*p<0.05 in pairwise Fishers´s test

Conclusion:
Based on increased relative liver weights in females and increased relative testes weights in males at 450 ppm, the NOAEL for this study is 150 ppm. Based on food consumption data, this concentration is equal to an approximate daily intake of 4.9 mg/kg bw/day.

CA 5.3.3 Other routes

Report: CA 5.3.3/1
[redacted] 2010 a
BAS 550 F (Dimethomorph) - Repeated-dose 28-day dermal toxicity study in Wistar rats
2010/1151903
Guidelines: OECD 410, EPA 870.3200
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dermal administration of dimethomorph (BAS 550 F) to male and female Wistar rats over a period of 28 days did not reveal test substance related adverse signs of systemic toxicity at dose levels of 100, 300, or 1000 mg/kg bw/d. Moreover, during functional observational battery as well as measurement of motor activity, no signs of neurotoxicity were obtained. Regarding clinical pathology no treatment-related, adverse effects were observed during dermal application of the compound corresponding to a dose of 1000 mg/kg bw/d. Regarding pathology significant weight changes noted in adrenal glands and liver of male and females occurred without dose-relationship and, therefore, were regarded as incidental and not related to treatment. Histopathology did not reveal treatment-related findings.
The no observed adverse effect level (NOAEL) for systemic toxicity and local effects under the conditions of the present study was 1000 mg/kg bw/day in male and female Wistar rats.
(DocID 2010/1151903)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F (Dimethomorph)

Description:	solid / beige
Lot/Batch #:	COD-001244
Purity:	99.8%
Stability of test compound:	The test substance was stable over the study period (until 01 Mar 2015)

2. Vehicle and/or positive control: CMC (1%) in drinking water

3. Test animals:

Species:	Rat
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	61-63 days
Weight at dosing:	235-279 g (males), 158-178 g (females)
Source:	[redacted]
Acclimation period:	7 days
Diet:	Kliba maintenance diet mouse/rat “GLP”, ad libitum
Water:	Drinking water, ad libitum
Housing:	Single housing in type M III polycarbonate cages (Becker&Co., Castrop-Rauxel, Germany)

Environmental conditions:

Temperature:	20-24°C
Humidity:	30-70%
Air changes:	15/h
Photo period:	12 h / 12 h (12 hours light from 06:00-18:00 h, 12 hours dark from 18:00-06:00 h)

B. STUDY DESIGN

1. Dates of experimental work: 27-Jan-2010 - 05-Mar-2010 (in-life phase)

2. Animal assignment and treatment:
BAS 550 F was administered dermally to groups of 10 male and 10 female Wistar rats at doses of 0, 100, 300, and 1000 mg/kg bw/day for 28 days. The animals were randomized and assigned to the treatment groups. The test substance was administered uniformly to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk; at least 10% of the body surface) using 3 mL syringes (Becton Dickinson & Co. USA) for about 28 days (5 days per week). The administration volume was 4 mL/kg bw, based upon the latest individual body weight determination. The skin was covered for 6 hours after administration using a semiocclusive dressing, consisting of 4 layers of porous gauze dressing ("Verbandmull Ph. Eur.", Lohmann GmbH & Co KG, Neuwied, Germany) and an elastic dressing (Fixomull Stretch, Beiersdorf AG, Hamburg, Germany). After removal of the dressing, the treated skin was washed with lukewarm water. Control animals received the vehicle, only. At the end of the administration period all surviving animals were sacrificed after a fasting period (withdrawal of food) for about 16-20 hours.

3. Test substance preparation and analysis:
The test substance was applied as a suspension in 1% aqueous carboxymethylcellulose (CMC). To prepare the suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then the vehicle was filled up to the desired volume, subsequently mixed using a magnetic stirrer. During application the test substance preparations were kept homogeneous using a magnetic stirrer. The test substance preparations were prepared twice a week and kept cold in a refrigerator during this time.
The stability of the test substance in the vehicle over a period of 7 days was proven prior to the study (study code PCP06240). For homogeneity and concentration control analyses, each 6 samples of all concentrations were drawn before the start of the administration period (will be used as a concentration control at the same time). The sampling was done under administration conditions out of the beaker (each 2 samples from bottom, mid, and top of the beaker). Each one sample was analyzed in the analytical laboratory; the other one was frozen until finalization of this report.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics applied

Parameter	Statistical test
Body weight, feed consumption, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians
Clinical pathology parameters, urine volume, urine specific gravity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians
Urinalysis, except color, turbidity, volume and specific gravity	Pairwise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions
Weight parameters (pathology)	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.
All animals were checked daily for any clinically abnormal signs. Abnormalities and changes were documented for each animal. Moreover, the findings on the treated skin were obtained once each workday (as a rule, immediately before application).
Detailed clinical observations (DCO) were performed in all animals prior to the administration period and thereafter at weekly intervals. The findings were ranked according to the degree of severity, if applicable. The animals were transferred to a standard arena (50×37.5 cm with sides of 25 cm high). The following parameters were examined:

1. abnormal behavior during “handling”	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on study day 0 (start of the administration period) and twice weekly thereafter. The difference between the body weight on the respective day of weighing and the body weight on study day 0 was calculated as body weight change.

3. Food and water consumption:

Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and per day. Drinking water consumption was observed by daily visual inspection of the water bottles for any overt changes in volume.

4. Functional observational battery:

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 h. The FOB started with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. The observations were performed at random.

Home cage observations:

The animals were observed in their closed home cages; any disturbing activities (touching the cage or rack, noise) were avoided during these examinations in order not to influence the behavior of the animals. Attention was paid to:

1. posture
2. tremor
3. convulsions
4. abnormal movements
5. impairment of gait
6. other findings

Open field observations:

The animals were transferred to a standard arena (50×50 cm with sides of 25 cm high) and observed for at least 2 minutes. Following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

Sensorimotor Tests/Reflexes:

The animals were removed from the open field and subjected to following sensorimotor or reflex tests:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hindlimbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

5. Motor activity assessment:

Motor activity assessment (MA) was carried out in all animals at the end of the administration period. Motor activity was measured on the same day as FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the animals were placed in new clean polycarbonate cages for the time of measurement. Eighteen beams were allocated per cage. The numbers of beam interrupts were counted over 12 intervals of 5 minutes. The sequence at which the animals were placed in the polycarbonate cages was selected at random. Motor activity measurements were carried out starting at 13:00 h. On account of the measuring variant "staggered", the starting time varied according to the time needed to place the animals in the cages. For each animal, measurement started individually when the 1st beam was interrupted and was finished exactly 1 hour later. The animals did not receive any food or water during the measurements. After the transfer of the last animal the room of measurement was darkened.

5. Ophthalmoscopy:

The eyes of all animals were examined prior to the start of the administration period. At the end of the administration period, i.e. study day 28, the eyes of animals in test groups 0 (control) and 3 (1000 mg/kg bw/d) were examined for any changes using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after administration of a mydriatic (Mydrum, Chauvin ankerpharm GmbH, Rudolstadt, Germany).

6. Hematology and clinical chemistry:

In the morning, blood was taken from the retroorbital venous plexus from fasted animals. The animals were anaesthetized using isoflurane (Isoba®, Essex GmbH, Munich, Germany). The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. For urinalysis the individual animals were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight. The urine samples were evaluated in a randomized sequence. With the exception of volume, color, turbidity, sediment examination and the specific gravity, all the urine constituents were determined semiquantitatively using test strips (Combur-9-test M, Roche, Mannheim, Germany) and a reflection photometer (Miditron M; Roche, Mannheim, Germany).The assays of blood and serum parameters were performed under internal laboratory quality control conditions with commercial reference controls to assure reliable test results. The results of the clinical pathology examinations are expressed in units of the International System (SI). The following examinations were carried out in 10 animals per test group and sex.

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Differential blood count	✓	Prothrombin time
✓	Hematocrit (Hct)				
✓	Mean corpuscular volume (MCV)				
✓	Mean corpuscular hemoglobin (MCH)				
✓	Mean corpuscular hemoglobin concentration (MCHC)				
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Potassium	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Sodium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Chloride	✓	Globulin (by calculation)	✓	Lactic dehydrogenase
✓	Phosphate (inorganic)	✓	Glucose (fasted)	✓	γ-GT
✓	Magnesium	✓	Protein (total)		
		✓	Urea		
		✓	Creatinine		
		✓	Triglycerides		
		✓	Bile acids		
	Urinalysis				
			Semiquantitative parameters		
✓	Specific gravity	✓	Bilirubin	✓	Protein
✓	Colour	✓	Glucose	✓	pH-value
✓	Turbidity	✓	Ketones	✓	Urobilinogen
✓	Volume		Blood	✓	Sediment (microscopical exam.)

7. Sacrifice and pathology:

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Weight assessment was carried out on all animals sacrificed at scheduled dates. The organs were sampled, weighed, and examined histopathologically as indicated in the table below.

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	aorta	✓		#	mammary gland (♀)	✓	✓	#	thymus
✓		#	bone marrow[§]	✓			muscle, skeletal	✓	✓	#	thyroid glands
✓	✓	#	brain	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	caecum	✓		#	nose	✓		#	urinary bladder
✓		#	colon	✓	✓	#	ovaries	✓	✓	#	uterus, oviducts, vagina
✓		#	duodenum	✓		#	pancreas	✓	✓		body (anesthetized animals)
✓		#	esophagus	✓	✓	#	parathyroid glands				
✓	✓	#	epididymides	✓		#	pharynx				
✓		#	eyes (with optic nerve)	✓		#	pituitary				
✓		✓	gross lesions	✓		#	prostate				
			Harderian gland	✓		#	rectum				
✓	✓	#	heart	✓		#	salivary glands				
✓		#	ileum	✓		#	seminal vesicle				
✓		#	jejunum (w. Payer's plaque)	✓		#	skin (treated and untreated)				
✓	✓	#	kidneys	✓		#	spinal cord (3 levels)				
✓	✓	✓	liver	✓	✓	#	spleen				
✓		#	larynx	✓			sternum w. marrow				
✓		#	lung	✓		#	stomach (cardia, fundus and pylorus)				

[§] from femur; [#] axillary and mesenteric

The organs or tissues were fixed in 4% formalin solution. The hematoxylin-eosin (HE) stained slides were examined and assessed by light microscopy.

II. RESULTS AND DISCUSSION

A. ANALYSIS

The stability of the test substance BAS 550 F (Dimethomorph) in M4-, OECD- and tap water over a period of 7 days at room temperature was verified analytically before the start of the study. Considering the low standard deviation in the homogeneity analysis, it can be concluded that BAS 550 F (Dimethomorph) was distributed homogeneously in drinking water containing 1% carboxymethylcellulose. The concentration control analyses of all concentrations revealed that the values were in the expected range of the target concentrations, i.e. were always in a range of 97.9-102.5% of the nominal concentration.

B. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related effects were observed**.**

2. Mortality

No animal died prematurely in the present study.

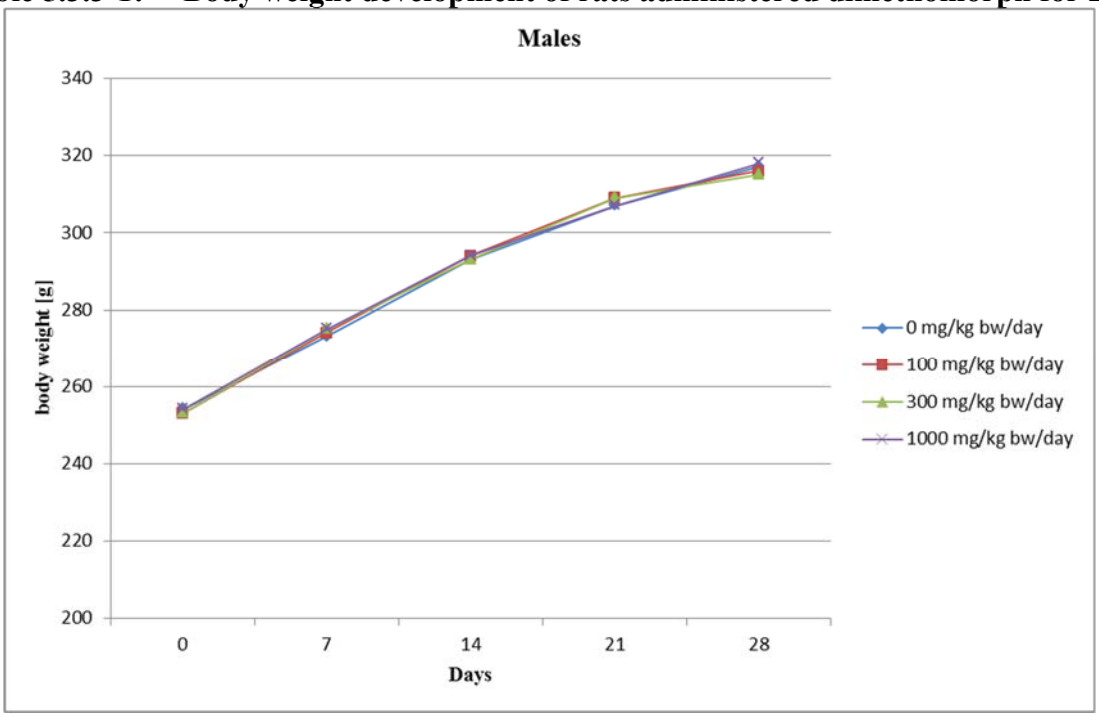
3. Ophthalmoscopy

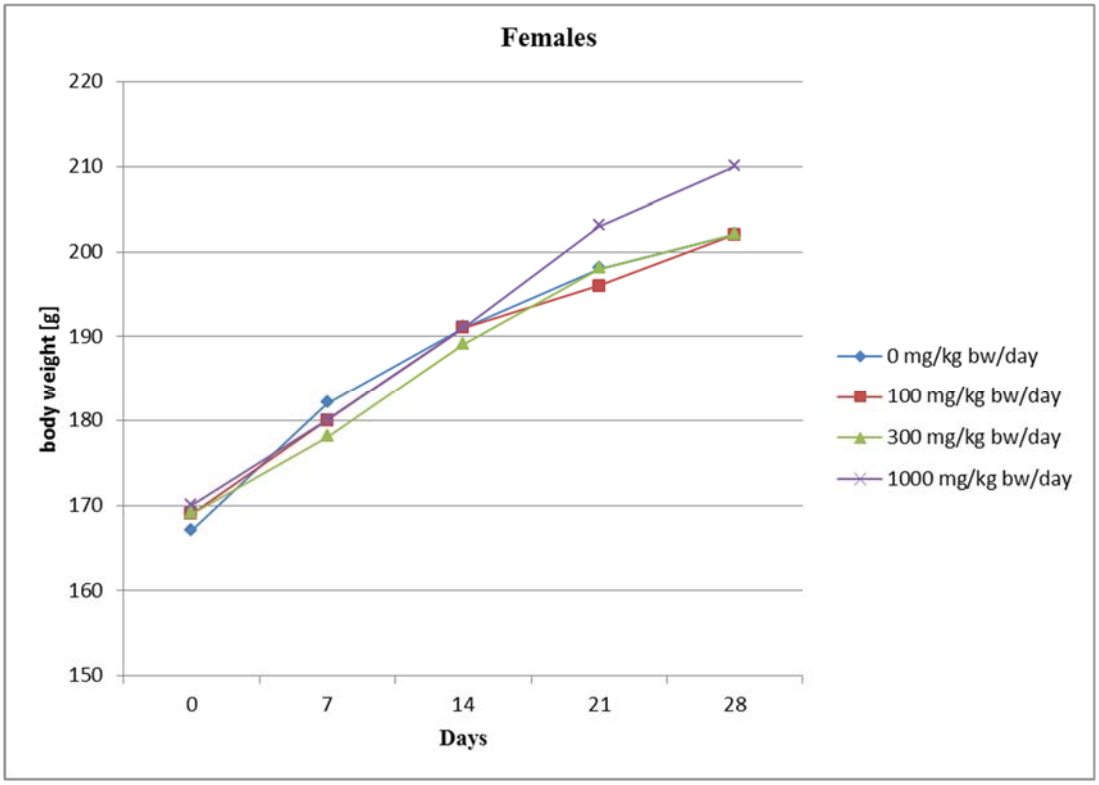
Because findings occurred in single animals only and a dose-response relationship was not observed all findings were assessed as being incidental in nature.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No test substance-related effects on body weights in both sexes were observed. Body weight change in females was decreased in all dosed animals, with the exception of animals of test group 3 (1000 mg/kg bw/day) on days 21 and 28 (+15.7%) and a statistically significant maximum of -35.4% in females of test group 2 on day 7 of the application period. Due to the fact that no correlation to food consumption and body weight was observed this variability could be assessed as not related to treatment.

Table 5.3.3-1: Body weight development of rats administered dimethomorph for 28 days





D. FOOD AND WATER CONSUMPTION

No test substance-related changes on food and water consumption were observed.

E. FUNCTIONAL OBSERVATIONAL BATTERY

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed between test substance-treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered to have been incidental.
Furthermore no test substance-related findings were observed at “Home cage observations”, “Open field observations”, “Sensorimotor tests/reflexes”, and “Quantitative parameters”.

F. MOTOR ACTIVITY MEASUREMENT

Regarding the overall motor activity, a significantly increased value was detected only in female animals of test group 2 (300 mg/kg bw/d). As there was no comparable effect at higher doses and, thus, no dose-response relationship observable this finding was assessed as being not related to treatment. Comparing the single intervals of test substance-treated groups with the control groups, the isolated significant increase of interrupts in females of test group 2 (300 mg/kg bw/d) at interval 6 and of test group 3 (1000 mg/kg bw/d) at interval 3 and was considered to be spontaneous in nature and not test substance-related.

F. CLINICAL PATHOLOGY

1. Hematological findings

No treatment-related changes among hematological parameters were detected. In males of test group 1 (100 mg/kg bw/d) red blood cell (RBC) counts and hematocrit values were lower compared to controls, but this parameter was not dose-dependently changed. Therefore, this alteration was regarded as incidental and not treatment-related. In females of test group 3 (1000 mg/kg bw/d) relative reticulocyte counts were higher compared to controls, but this increase was still within the historical control range (1.0-4.9%) and, therefore, it was regarded as incidental and not treatment-related.

Table 5.3.3-2: Selected hematology findings in rats administered dimethomorph for 28 days (group means)

	RBC [tera/L]	HGB [mmol/L]	HCT [L/L]	RETI [%]
		Males		
Control	8.65±0.28	9.7±0.2	0.434±0.009	1.5±0.3
100 mg/kg bw/day	8.18±0.30**	9.4±0.2	0.419±0.008**	1.6±0.2
3000 mg/kg bw/day	8.49±0.24	9.6±0.2	0.426±0.008	1.4±0.3
1000 mg/kg bw/day	8.57±0.44	9.5±0.3	0.430±0.012	1.6±0.3
		Females		
Control	8.07±0.22	9.3±0.3	0.411±0.016	2.3±1.1
100 mg/kg bw/day	8.04±0.34	9.2±0.3	0.407±0.013	1.9±0.5
300 mg/kg bw/day	8.22±0.40	9.5±0.5	0.421±0.017	1.9±0.6
1000 mg/kg bw/day	7.69±0.61	9.2±0.5	0.397±0.033	2.8±0.7*

* = p≤0.05; ** = p≤0.01

2. Clinical chemistry findings

No treatment-related, adverse changes among clinical chemistry parameters were measured. In males of test group 3 (1000 mg/kg bw/d) inorganic phosphate levels were higher compared to controls but these values were within the historical control range (1.90-2.63 mmol/L) and, therefore, were regarded as incidental and not treatment-related. In females of test group 2 (300 mg/kg bw/d) the potassium levels were higher and the total bilirubin levels were lower compared to controls and, additionally, in females of test group 1 (100 mg/kg bw/d) the total bilirubin levels were decreased. These values were not changed dose-dependently and, therefore, the alterations were considered as incidental and not treatment-related. In females of test group 3 (1000 mg/kg bw/d) the cholesterol levels were increased, but the values were still within the historical control range (0.95-1.96 mmol/L). Therefore, this effect was regarded as incidental and not treatment-related.

Table 5.3.3-3: Selected clinical chemistry findings in rats administered dimethomorph for 28 days (group means)

	Males				Females			
Dose [mg/kg bw/d]	0	100	300	1000	0	100	300	1000
Phosphate (inorganic) [mmol/L]	2.21±0.11	2.31±0.18	2.22±0.13	2.38±0.11**	2.08±0.23	2.12±0.23	2.14±0.24	2.04±0.24
Potassium [mmol/L]	4.75±0.20	4.70±0.29	4.62±0.29	4.71±0.27	4.23±0.23	4.35±0.18	4.66±0.21**	4.22±0.44
Cholesterol [mmol/L]	1.92±0.21	1.87±0.26	1.94±0.33	1.89±0.33	1.37±0.44	1.20±0.23	1.22±0.31	1.71±0.40*
Bili, total [µmol/L]	2.29±0.42	2.31±0.51	2.36±0.23	2.60±0.33	3.15±0.70	2.32±0.37**	2.48±0.48*	2.60±0.59

* = p≤0.05; ** = p≤0.01

3. Urinalysis

No treatment-related changes among urinalyses parameters were detected.

E. NECROPSY

1. Organ weight

Absolute weights

The significant weight increase of the adrenal glands in males of test groups 1 (100 mg/kg bw/d) and 3 (1000 mg/kg bw/d) occurred without dose-relationship and was therefore regarded as incidental and not related to treatment. All other mean absolute weight parameters did not show relevant differences when compared to the control group and were considered to be within the normal range.

Relative weights

The significant weight increase observed in adrenal glands in males of test groups 1 (100 mg/kg bw/d) and 3 (1000 mg/kg bw/d), as well as the significant liver weight decrease in male animals of test groups 1-3 (100, 300 and 1000 mg/kg bw/d) occurred without a dose-relationship. In females, a weak significant liver weight increase without dose-relationship was observed at all dose levels. All these changes in males and females were without a histopathological correlate and considered to be incidental and not treatment-related. All other mean relative weight parameters of treated animals did not show relevant differences when compared to the control groups.

Table 5.3.3-4: Mean absolute organ weights in rats administered dimethomorph for 28 days (group means)

	Terminal bw [g]	Liver [g]	Adrenals [mg]
	Males		
Control	288±18	7.59±0.70	58.7±7.6
100 mg/kg bw/day	288±18	7.20±0.63	66.7±4.4**
300 mg/kg bw/day	285±22	6.96±0.90	63.4±8.0
1000 mg/kg bw/day	288±21	7.25±0.78	68.0±8.7*
	Females		
Control	183±6	4.60±0.28	77.9±9.3
100 mg/kg bw/day	181±10	4.75±0.36	74.5±6.6
300 mg/kg bw/day	182±9	4.93±0.49	77.8±10.3
1000 mg/kg bw/day	188±11	5.11±0.49	79.5±5.74

* $p \leq 0.05$; ** $p \leq 0.01$

Table 5.3.3-5: Mean relative organ weights in rats administered dimethomorph for 28 days (group means)

	Terminal bw [%]	Liver [%]	Adrenals [%]
	Males		
Control	100	2.63±0.1	0.02±0.003
100 mg/kg bw/day	100	2.49±0.05	0.023±0.002*
300 mg/kg bw/day	100	2.43±0.18	0.022±0.003
1000 mg/kg bw/day	100	2.50±0.13	0.024±0.003**
	Females		
Control	100	2.51±0.12	0.043±0.006
100 mg/kg bw/day	100	2.62±0.08*	0.041±0.004
300 mg/kg bw/day	100	2.70±0.23*	0.043±0.005
1000 mg/kg bw/day	100	2.70±0.18*	0.042±0.005

* $p \leq 0.05$; ** $p \leq 0.01$

2. Gross and histopathology

Gross lesions:
All macroscopic findings occurred either individually and were considered to be incidental or spontaneous in origin and without any relation to treatment.

Histopathology
All findings were either single observations, or were biologically equally distributed between control and treated rats. All of them were considered to be incidental and/or spontaneous in origin.

III. CONCLUSIONS

The dermal administration of dimethomorph (BAS 550 F) to male and female Wistar rats over a period of 28 days did not reveal test substance related adverse signs of systemic toxicity at dose levels of 100, 300, or 1000 mg/kg bw/d. Increased liver and adrenal weights were observed, but were considered incidental, lacking a dose-response relationship. Histopathology revealed no findings. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) for systemic and local effects was 1000 mg/kg bw/d in male and female animals.

CA 5.4 Genotoxicity Testing

The potential genotoxicity of dimethomorph was investigated in a series of both *in vitro* and *in vivo* studies. All regular end points for genetic damage (point mutations, chromosome damage and DNA-damage and repair) were assessed. Dimethomorph was evaluated for its potential genotoxicity *in vitro* using bacterial and mammalian cell mutagenicity tests and an unscheduled DNA synthesis test, and *in vivo* clastogenicity was evaluated by two micronucleus tests. No point mutation was detected in bacterial cells (Ames test) and mammalian cells (V79 Chinese hamster cells). Whereas in *in vitro* cytogenectic assays (V79 Chinese hamster cells and human lymphocytes) an increase in chromosome aberrations was detected only at high concentrations, which were strongly cytotoxic and partly caused precipitation, no clastogenicity was observed in two assays at higher concentrations in vitro in CHL cells and *in vivo* in two mouse micronucleus tests. No genotoxicity was found in an *in vitro* UDS assay. Dimethomorph did not induce cell transformation in the Syrian hamster embryo (SHE) cell cultures. Considering all these findings, it can be concluded that dimethomorph is not mutagenic nor genotoxic.

Table 5.4-1: Summary of reviewed dimethomorph mutagenicity studies

Study/strain/species	Test conditions	Results	Reference (BASF DocID)
Ames mutagenicity test; S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538 E. coli WP2 uvrA$^-$	All six strains received 5000, 2000, 1000, 500, 250, 125, 62.5, and 31.25 µg/mL with and without S9	Negative; Negative	DK-435-018; DK-435-011 DK-435-010
V79/HGPRT mutagenicity test	133, 180, 237 and 333 µg/mL with S9; 33, 100, 133, and 180 µg/mL without S9	Negative; Negative	DK-435-014
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells (Prestudy)	13, 60, and 170 µg/mL with S9; 12, 60 and 160 µg/mL without S9 (200 metaphases scored per dose group	Slight increase in aberrations at 160 µg/mL without S9 and 170 µg/mL with S9 (marked cytotoxicity) at 7 hours and at 160 µg/mL without S9 at 18 hours	DK-435-004
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells (Main study)	170 µg/mL with S9; 160 µg/mL without S9 (400 metaphases scored per dose group)	Slight increase in aberrations at 7 h fixation time at 170 µg/mL with S9 (marked cytotoxicity); Negative without S9 (including 18 hours)	DK-435-006
In vitro cytogenetics: chromosome aberration in cultured human peripheral lymphocytes	Up to 422 µg/mL with S9; Up to 333 µg/mL without S9	Negative, except for positive results at a high, precipitating and strongly cytotoxic concentration (422 µg/mL with S9)	DK-435-013
In vitro UDS, rat hepatocytes	0, 2.5, 10, 25, 100, and 250 µg/mL	Negative	DK-435-002
In vitro cell transformation assay with Syrian hamster embryo cells	Up to 50 µg/mL without S9, 6 and 48 h Up to 265 µg/mL with S9, 6 h	Negative	DK-435-005
In vivo chromosome aberration: Mouse micronucleus test	0, 20, 100, and 200 mg/kg bw (intraperitoneal)	Negative	DK-435-012

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of dimethomorph and are provided under the respective chapters.

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the DAR/EFSA conclusion of dimethomorph (EFSA scientific report, 82, 1-69, 2006):

Genotoxicity	No genotoxic potential

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

An additional set of genotoxicity studies is available that was not yet submitted during the previous Annex I inclusion process. An AMES test, a HGPRT test on V79 cells, three chromosomal aberration tests, one on V79 cells and two in CHL cells, a DNA repair test and an *in vivo* micronucleus test via the oral route. The respective studies are listed in Table 5.4-2. All studies were negative both *in vivo* an *in vitro* and thus clearly confirmed that dimethomorph does not have a genotoxic potential.

Table 5.4-2: Summary of not peer-reviewed dimethomorph genotoxicity studies.

Study/strain/species	Test conditions	Results	Reference (BASF DocID)
Ames mutagenicity test; S. typhimurium TA 98, TA 100, TA 1535, TA 1537, E. coli WP2 uvrA$^-$	All six strains received 5000, 1580, 1000, 500, 158, 50 and 15, µg/mL with and without S9. Additional dose of 5 µg/mL with S9.	Negative; Negative	DK-435-001
V79/HGPRT mutagenicity test	15.4, 30, 100, 250 and 300 µg/mL with S9; 23, 100, 200, and 230 µg/mL without S9	Negative; Negative	DK-435-003
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells	120, 170 and 250 µg/mL with S9; (200-1000 metaphases scored per dose group)	Negative	DK-435-007
In vitro cytogenetics: chromosome aberration in CHL	93.8 to 1500 µg/mL with S9; 11.7 to 187 µg/mL without S9	Negative; Negative	DK-435-015
In vitro cytogenetics: chromosome aberration in CHL	93.8, 187.5, 375, 750 and 1500 µg/mL with and without S9;	Negative; Negative	DK-435-016
DNA repair	20, 50, 100, 200, 500, 1000 µg/plate with and without S9.	Negative	DK-435-017
In vivo chromosome aberration: Mouse micronucleus test oral route	5000 mg/kg bw	Negative	DK-435-009

Full OECD summaries of the respective studies are provided under the respective chapters.

Based on the results of studies which were not yet peer-reviewed, no changes are needed in the agreed end-points.

Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)

In vitro studies	Ames test: Negative HPRT: Negative Chromosome aberration assay in human lymphocytes: weakly positive only w S9 at cytotoxic, precipitating concentrations Chromosome aberration assay in V79 cells: weakly positive only at cytotoxic, precipitating concentrations Chromosome aberration assays in CHL cells: negative	
In vivo studies	Mouse micronucleus assay (i.p. route): Negative Mouse micronucleus assay (oral route): Negative	
Photomutagenicity	Not required based on a negative phototoxicity test in vitro	
Potential for genotoxicity	No evidence for genotoxic potential	

Comparison with CLP criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), a mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

In a series of *in vitro* and *in vivo* tests with dimethomorph several endpoints of potential genotoxicity were measured such as gene mutation in bacterial and mammalian cells, chromosomal aberration and DNA damage/repair. Dimethomorph has been tested negative for point mutations in four independent Ames-tests and two mammalian cell gene mutation assays (V79). In a series of *in vitro* clastogenicity assays dimethomorph was weakly positive only at doses that induced strong cytotoxicity and/or precipitation in V79 cells or human lymphocytes but were negative in 2 assays in CHL cells at higher concentrations. The absence of any clastogenic or aneugenic potential *in vivo* was confirmed in two mouse micronucleus assays via oral and i.p. route.

Conclusion on classification and labelling

Overall, based on the available studies dimethomorph was evaluated to have no genotoxic potential. In conclusion, in comparison with the criteria on classification and labelling for mutagenicity, dimethomorph is not subject to classification for "mutagenicity" according to Regulation 1272/2008/EC.

CA 5.4.1 In vitro studies

Report: CA 5.4.1/1
Glatt H.R., 1985a
Bacterial mutagenicity test on ZTH 236Z40
DK-435-001
Guidelines: OECD
GLP: no

Executive Summary
S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2 uvrA were exposed to Dimethomorph (batch: X84, purity: 97.8%) using DMSO as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the Ames standard plate test (SPT) dimethomorph was tested in concentrations of 5 to 5000 µg/plate in the presence of metabolic activation and from 15 to 5000 µg/plate in the absence of metabolic activation (Aroclor-induced rat liver S9 mix).
Precipitation of the test substance was found from about 500 µg/plate onward. No cytotoxicity was observed up to the highest concentration tested. An increase in the number of his^+ or trp^+ revertants was not observed both in the standard plate test either without S-9 mix or after the addition of a metabolizing system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study, dimethomorph is not mutagenic in the Ames standard plate under the experimental conditions of the study.
(BASF DocID DK-435-001)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Dimethomorph

Test material name:	ZTH 236Z40
Description:	Solid (powder), white
Lot/Batch #:	X84
Purity:	97.8%
Stability of test compound:	Expiry date May 1986.
Solvent used:	DMSO

2. Control Materials:

Vehicle control:	The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds for all tester strains tested without addition of metabolic activation system were N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 10 µg/plate), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 15 µg/plate) and benzo[a]pyrene-4-5-oxide (1 µg/plate).

Positive control compounds for all tester strains tested with addition of rat metabolic activation system were benzo[a]pyrene (10 µg/plate), 3-methylcholanthrene (90 µg/plate) and 2-aminoanthracene (10 µg/plate).

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Six days after administration the animals were sacrificed and the livers are prepared. The S9 fraction and the S9-mix was prepared freshly prior to each experiment. One volume of S9-fraction is mixed with one volume of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	75 mM
Glucose 6-phosphate	7.5 mM
NADP	6 mM
KCl	50 mM
$MgCl_2$	12 mM
S9	50%

4. Test organisms: S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli WP2 uvrA

5. Test concentrations:

Plate incorporation assay: In the first and second experiment triplicate plates were prepared for each concentration (neg. control; 5, 15, 50, 150, 500, 1500, 5000 µg/plate and positive controls at the concentrations indicated above) with rat liver S9 mix) for all tester strains indicated above. Without S9 mix the concentration of 5 µg/plate was not used.

B. TEST PERFORMANCE:

1. Dates of experimental work: 29-Aug-1985 to 30-Sep-1985

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 50 µM histidine, 50 µM biotin and 50 µM tryptophan), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL KCl (150 mM) (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (2% glucose). After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ and trp^+ revertants) are counted.

3. Toxicity experiments:

To estimate toxicity, his^+ bacteria (about 600 cfu) were added as internal standard to otherwise normal mutagenicity plates (spontaneous TA 1537 revertants). The difference in the number of colonies on plates with and without added his^+ bacteria, in the presence of test compound, is compared to the value obtained with solvent controls. The ratio of these two values gives the surviving fraction.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if comparable "clear effects" or "weak increases" are observed in both experiments conducted. The result of the test is positive if at least one test unit shows a positive result.

The result of a test unit is considered negative if "no noticeable effects" are observed in both experiments or if "no noticeable effect" is seen in one experiment and a "weak increase" is seen in the other experiment.
Detailed definitions of evaluation criteria are described in the following table:

Mean number of colonies on solvent control (X_0)	Mean maximal increase in presence of test compound over solvent control		
	"No noticeable effect"	"Clear effect"	"Possible weak effect"
≤ 10	≤ 9 and ≤ X_0	≥ 30	All other results
≤ 30	≤ 19 and ≤ X_0	≥ 40	
≤ 80	≤ 29	≥ 80	
≤ 200	≤ 39	≥ 120	

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO and in water for at least 2 h was guaranteed.

B. CYTOTOXICITY

No cytotoxicity was observed up to the highest dose applied.

C. MUTATION ASSAYS

Neither in the experiment with metabolic activation nor without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Test item precipitation was observed from about 500 µg/plate onward.

III. CONCLUSION

According to the results of the present study, Dimethomorph is not mutagenic in the Ames standard plate test in the presence or absence of metabolic activation.

Report:	CA 5.4.1/2 Heidemann A.,Miltenburger H.G., 1987a Detection of gene mutations in somatic mammalian cells in culture: HGPRT-test with V79 cells DK-435-003
Guidelines:	OECD recommendations of 1983, EPA guidelines of 1982
GLP:	no

Executive Summary

Dimethomorph (Batch: Dw 11/86) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in V79 cells. Two independent evaluable experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 300 µg/mL were used in the main experiment. As heavy cytotoxicity was observed in the main experiment (I/II) further experiments were performed with additional concentrations in the range of 150 µg/mL to 210 µg/mL with lower concentration intervals. The treatment intervals for both experiments in the presence and absence of metabolic activation were 4 hours. EMS and DMBA served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for one week for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.
The test substance showed high toxicity from 200 µg/ml onwards, yielding relative cloning efficiencies <20% in the presence and absence of metabolic activation. The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in the independent experiments. Solvent and positive controls were valid. Based on the results of the study it is concluded that under the conditions of this test dimethomorph does not induce forward mutations in mammalian cells in vitro.

(BASF DocID DK-435-003)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Dimethomorph

Name of test material: CME 151-Z50
Description: N.A.
Lot/Batch #: Dw 11/86
Purity: N.A.
Stability of test compound: The stability of the test substance at room temperature in the vehicle DMSO over a period of 2 hours was verified analytically.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: Incubations without test substance and vehicle
Vehicle control: 1% (v/v) DMSO in culture medium
Positive control -S9: Ethylmethanesulfonate (EMS) 1 mg/mL (= 8 mM)
Positive control +S9: 9,10-Dimethyl-1,2-benzanthracene (DMBA) 15.4 µg/mL (= 60.1 µM)

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and mixed with S9-supplement (cofactors) yielding a final protein concentration of 20-45 mg/mL in the S9 mix.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	5 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organism:

Chinese hamster fibroblasts (V79) cells. V79 cells have a high proliferation rate (doubling time about 12-16 h), high plating efficiency (about 70-90%) and karyotype with a modal number of 22 chromosomes.

5. Culture media:

Culture medium:	MEM medium supplemented with 10% (v/v) fetal calf serum (FCS).
Selection medium:	Culture medium with 6-thioguanine at a final concentration of 11 µg/mL

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

Preliminary toxicity assay:	No details were provided
Mutation assay:	
Experiment I/II:	23, 100, 200, 230 µg/mL without metabolic activation
	30, 100, 250, 300 µg/mL with metabolic activation
Experiment III/IV and V/VI:	150, 180, 200 µg/mL without metabolic activation
	150, 210, 250 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-June-1986 to 11-Nov-1986

2. Preliminary cytotoxicity assay:

The toxicity was determined by establishing a concentration related plating efficiency. No details were provided. Based on the results of the preliminary cytotoxicity assay experiments I and II were performed with the test substance at concentrations from 23 – 300 µg/mL.

3. Mutation Assay:

Cell treatment:	For each test group, about $5x10^5$ cells per flask were seeded into flasks containing about 15 mL MEM medium supplemented with 10% FCS and incubated for with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. Two flasks were used for each test group per experiment. After 24 h the medium was replaced with serum-free medium containing the test substance without S9 mix or with S9 mix (20 µL/mL). Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.
Plating efficiency:	About 500 cells were seeded in 5 mL medium (in duplicate per experimental point), subcultured and stained after 1 week with methylene blue (10% in 0.01 n KOH solution).

Mutagenicity:	For the mutant selection, one 175-cm^2 flask each was seeded with $8x10^5$ cells in 30 mL and treated one day later. After one week cells were cultured in five 80 cm^2 flasks containing selective medium ($6x10^5$ cells/flask) and incubated for 7 days. At the end of the selection period, colonies were stained with methylene blue and counted.

5. Statistics:

Due to the clearly negative findings, a statistical evaluation was not carried out.

6. Evaluation criteria:

The test chemical is considered positive in this assay if one of the following criteria are met:

- Reproducible induction of mutation frequency (MF) that is 3 times higher than the spontaneous MF with one of the test substance concentration
- Concentration related induction of MF

Nevertheless, in a case by case evaluation bot decisions depend on the corresponding negative control data. In case of very low spontaneous mutation rates the criteria may not apply.

II. RESULTS AND DISCUSSION

B. CYTOTOXICITY

Based on the preliminary toxicity test, the concentration of 300 µg/mL was chosen as the top dose.

In contrast to the preliminary experiment on toxicity the test groups 200 – 300 µg/mL in experiment I and II produced unexpected heavy toxicity.

In experiment III and IV toxicity (CE < 20%) was observed at 200 µg/mL onward. A similar toxicity was observed in experiments V and VI.

A. MUTANT FREQUENCY

No increase in the number of mutant colonies was observed either with or without S9 mix. The mutant frequencies at any dose were close to the range of that of the concurrent negative control values.

Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

The result tables below contain all relevant data. These include the results from experiment I and II up to 100 µg/mL and from experiments V and VI. The cytotoxic concentrations of experiments I and II (> 100 µg/mL) were not included. Furthermore, experiments III and IV were not included in the tables as no positive controls were performed within these experiments and furthermore they are redundant to experiments V and VI as the same conditions and concentrations were used.

Table 5.4.1-1: Cytotoxicity and mutagenicity data from experiments I and II

Test group	Concentration (µg/mL)	S9 mix	Mutant frequency (per 10^6 cells)	Cloning efficiency (%)	
				Absolute	Relative
Negative control	0	-	14.1 / 15.6	72.7 / 81.4	100
Solvent control	0	-	33.8 / 50.3	68.1 / 80.6	100
Test substance	23	-	31.2 / 58.2	82.1 / 80.0	120.5 / 99.2
Test substance	100	-	21.2 / 14.8	74.3 / 78.9	109.2 / 97.9
EMS	1000	-	366 / 1113	14.2 / 10.2	19.5 / 12.5
Negative control	0	+	19.7 / 46	88.9 / 81.6	100
Solvent control	0	+	10.2 / 46.5	79.2 / 81.7	100
Test substance	30	+	25.8 / 25.7	86.9 / 84.9	109.8 / 104
Test substance	100	+	18.7 / 55.2	77.6 / 78.5	98.0 / 96
DMBA	15.4	+	176 / 247	40.7 / 14.4	51.5 / 17.7

Table 5.4.1-2: Cytotoxicity and mutagenicity data from experiments V and VI

Test group	Concentration (µg/mL)	S9 mix	Mutant frequency (per 10^6 cells)	Cloning efficiency (%)	
				Absolute	Relative
Negative control	0	-	28.2 / 7.3	47 / 56.8	100
Solvent control	0	-	2.3 / 3.7	48 / 63.7	100
Test substance	180	-	12.2 / 5.8	37.9 / 34.2	78.9 / 53.7
Test substance	200	-	21.9 / 2.1	14.5 / 33.3	30.3 / 52.3
EMS	1000	-	310 / 535	2.6 / 20	5.5 / 35.2
Negative control	0	+	17.5 / 22.9	57.2 / 73.2	100
Solvent control	0	+	6.9 / 8.4	52 / 63.1	100
Test substance	150	+	3.1 / 3.2	48.7 / 47	93.6 / 74.4
Test substance	210	+	0 / 18.8	25.9 / 14.3	49.7 / 22.7
DMBA	15.4	+	344 / 312	35.7 / 54.1	68.6 / 85.8

III. CONCLUSION

In conclusion, under the conditions of the test Dimethomorph does not induce forward mutations in the HPRT locus in V79 cells in vitro.

Report: CA 5.4.1/3
Kramer P.J., 1987a
In vitro chromosomal aberration assay - Chinese hamster V79 cells
DK-435-007
Guidelines: none
GLP: no

Executive Summary
Dimethomorph (BAS 550 F, Batch: Dw 11/86, Purity: not specified) was tested in vitro for the ability to induce chromosome aberrations in Chinese Hamster V79 cells in the presence of metabolic activation system consisted of liver S9-mix of Aroclor 1254 pretreated rats. Test substance in concentration of 120, 170 and 250 µg/mL: Negative (culture medium), vehicle (DMSO) and positive controls (DMBA) were included to demonstrate the sensitivity of the test system. At least 200 well spread metaphases per treatment were analysed for chromosomal aberrations.
Test substance induced cytotoxicity at 250 µg/mL, and thus it was not possible to evaluate the highest dose tested. After metabolic activation, dimethomorph did not induce chromosome-damaging activity up to 170 µg/mL, as the number of noticed metaphases was comparable with negative and solvent controls. The positive control DMBA demonstrated the ability of the test system to recognize known mutagens that requires metabolic activation.
Based on the results of this study dimethomorph is considered to have no clastogenic potential in vitro in Chinese hamster V79 cells in the presence of metabolic activation.

(BASF DocID DK-435-007)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimethomorph; BAS 550 F (CME 151)

Description:	not specified
Lot/Batch #:	Dw 11/86
Purity:	not specified
Stability of test compound:	not specified

2. Control Materials:

Negative:	culture medium
Solvent control:	Dimethylsulfoxide (DMSO)
Positive, +S9:	10 and 15 µg/mL 9,19-Dimethyl-1,2-benzanthrancene (DMBA) in culture medium

3. Activation: S9 derived from male rats pretreated with Aroclor 1254

4. Test organisms: Chinese hamster V79 cells (Klone 108/7)

5. Culture medium: DMEM with 10% FCS

6. Test concentrations:

Test substance concentrations of 120, 170 and 250 µg/mL were tested after metabolic activation.

B. TEST PERFORMANCE:

1. Dates of experimental work: finalization date: 26-May-1987

2. Cytogenicity assay:

Cell treatment: Cultures containing 8 x 10^4 cells were exposed to the test article for 4 hours at 3 concentrations (120, 170 and 250 µg/mL) in presence of metabolic activation system. The treatment medium was replaced by normal medium and cultures incubated for another 3 hours.

Spindle inhibition: not specified

Cell harvest: The sampling time was 7 hours after start of the treatment.

3. Statistics The proportion of metaphases with aberrations was calculated for each group.

4. Evaluation criteria: not specified

II. RESULTS AND DISCUSSION

Cytotoxicity at 250 µg/mL test substance was noticed, however the methodology was not specified in the study report. Thus it was not possible to evaluate the highest dose tested. After metabolic activation, dimethomorph did not induce chromosome-damaging activity up to 170 µg/mL, as the number of noticed metaphases was comparable with negative and solvent controls [see Table 5.4.1-3]. The positive control DMBA demonstrated the ability of the test system to recognize known mutagens that requires metabolic activation.

III. CONCLUSION

Based on the results of the study it is concluded that dimethomorph has a no clastogenic potential in vitro in the presence of metabolic activation system.

Table 5.4.1-3: Chromosome aberration test with dimethomorph after metabolic activation (4 hours treatment, harvest after 7 hours)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Gaps (g)		Breaks (br)		Exchanges (ex)		Multiple aberrations (mul)		Special aberrations (spec)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Culture medium	1	100	n.d.	5		4		1		4		0		0		0	
	2	100	n.d.	9		6		3		6		0		0		0	
	3	100	n.d.	1		1		0		1		0		0		0	
	4	100	n.d.	9		9		0		8		1		0		0	
	5	100	n.d.	3		2		1		2		0		0		0	
	6	100	n.d.	5		3		2		3		0		0		0	
	7	100	n.d.	4		4		0		4		0		0		0	
	8	100	n.d.	2		2		0		2		0		0		0	
	9	100	n.d.	3		3		0		3		0		0		0	
	10	100	n.d.	3		2		1		2		0		0		0	
	sum	**1000**	n.d.	**44**	**4.0**	**36**	**3.5**	**8**		**35**		**1**		**0**		**0**	
Vehicle DMSO	1	100	n.d.	5		4		1		4		0		0		0	
	2	100	n.d.	6		4		2		4		0		0		0	
	3	100	n.d.	5		4		1		4		0		0		0	
	4	100	n.d.	3		3		0		3		0		0		0	
	5	100	n.d.	5		5		0		5		0		0		0	
	6	100	n.d.	4		3		1		3		0		0		0	
	7	100	n.d.	1		0		1		0		0		0		0	
	8	100	n.d.	2		2		0		1		0		0		1	
	9	100	n.d.	0		0		0		0		0		0		0	
	10	100	n.d.	5		4		1		3		0		1		0	
	sum	**1000**	n.d.	**36**	**3.4**	**29**	**2.8**	**7**		**27**		**0**		**1**		**1**	

Table 5.4.1-3: Chromosome aberration test with dimethomorph after metabolic activation (4 hours treatment, harvest after 7 hours)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Gaps (g)		Breaks (br)		Exchanges (ex)		Multiple aberrations (mul)		Special aberrations (spec)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
120 µg/mL	1	100	n.d.	10		10		0		10		0		0		0	
	2	100	n.d.	3		1		2		1		0		0		0	
	3	100	n.d.	6		4		2		4		0		0		0	
	4	100	n.d.	5		2		3		2		0		0		0	
	5	100	n.d.	4		2		2		2		0		0		0	
	6	100	n.d.	6		2		4		2		0		0		0	
	7	100	n.d.	1		1		0		0		0		0		1	
	8	100	n.d.	4		3		1		3		0		0		0	
	sum	**800**	n.d.	**38**	**4.25**	**25**	**3.5**	**14**		**24**		**0**		**0**		**1**	**0.13**
170 µg/mL	1	100	n.d.	6		5		1		3		0		0		2	
	2	100	n.d.	5		3		2		3		0		0		0	
	3	100	n.d.	10		8		2		8		0		0		0	
	4	100	n.d.	6		3		3		3		0		0		0	
	5	100	n.d.	6		5		1		5		0		0		0	
	6	100	n.d.	6		4		2		4		0		0		0	
	7	100	n.d.	2		1		1		1		0		0		0	
	8	100	n.d.	3		2		1		2		0		0		0	
	9	100	n.d.	1		1		0		1		0		0		0	
	10	100	n.d.	6		6		0		0		5		0		1	
	sum	**1000**	n.d.	**52**	**4.7**	**39**	**3.7**	**13**		**30**		**5**		**0**		**3**	
250 µg/mL	1	100	n.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	100	n.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	sum	**200**	n.d.	toxic effect													
10 µg/mL	1	100	n.d.	16		13		3		12		0		0		1	
	2	100	n.d.	8		5		3		5		0		0		0	
	sum	**200**	n.d.	**24**	**9.5**	**18**	**7.5**	**6**		**17**		**0**		**0**		**1**	
15 µg/mL	1	100	n.d.	25		21		4		21		0		0		0	
	2	100	n.d.	14		11		3		11		0		0		0	
	sum	**200**	n.d.	**39**	**16.0**	**32**	**14.5**	**7**		**32**		**0**		**0**		**0**	

Report: CA 5.4.1/4
Matsumura H., 1993a
Dimethomorph: In vitro cytogenetics test
DK-435-015
Guidelines: OECD 473, JMAFF 59 NohSan No 4200
GLP: no

Executive Summary

Dimethomorph (BAS 550 F, Batch: 92226, Purity: 96.2%) was tested in vitro for the ability to induce chromosome aberrations in Chinese Hamster lung cells (CHL) in the absence and presence of metabolic activation system consisting of liver S9-mix of phenobarbital/benzoflavone pretreated rats. Without metabolic activation CHL cells were incubated with dimethomorph at concentrations of 23.4, 46.9, 93.8 and 187.5 µg/mL and 11.7, 23.4, 46.9, and 93.8 µg/mL for 24 and 48 hours, respectively. With metabolic activation CHL cells were incubated with dimethomorph at concentrations of 93.8, 187.5, 375, 750 and 1500 µg/mL for 6 hours and cells were harvested after 24 hour. Negative (culture medium), vehicle (DMSO) and positive controls (-S9: MMC; +S9: BaP) were included to demonstrate the sensitivity of the test system. Metaphases were arrested by addition of 0.5 µg/mL colchicine 2 hours prior harvest, visualized by Giemsa staining. At least 200 well spread metaphases per treatment were analysed for chromosomal aberrations.
In experiments with or without metabolic activation, no increased number of cells with aberration was observed. The frequency of metaphases with structural aberrations (excluding gap) was in the range of 0 - 2.0%, and of polyploidy in the range of 0 - 0.5%. The frequencies of aberrant metaphases in negative and solvent controls were within the historical data range, for any treatment. The positive controls induced marked increases of the incidence of aberrant metaphases, thus demonstrating the ability of the test system to detect known clastogens that do or do not require metabolic activation.
Based on the results of this study dimethomorph is considered to have no clastogenic or aneugenic potential in vitro in Chinese hamster lung cells (CHL) in the presence and absence of metabolic activation system.

(BASF DocID DK-435-015)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimethomorph; BAS 550 F

Description: white powder (crystalline)

Lot/Batch #: 92226

Purity: 96.2%

Stability of test compound: stable for acid, alkali, light and heat

2. Control Materials:

Negative: untreated

Solvent control: DMSO (0.5% final concentration)

Positive, -S9: 0.1 µg/mL mitomycin C (MMC) in Hanks' BSS

Positive, +S9: 40 µg/mL benzo[a]pyrene (BaP) in DMSO

3. Activation: S9 derived from male Sprague-Dawley rats. The rats were induced by an i.p. injections of 30 mg/kg phenobarbital on day 1 and 60 mg/kg phenobarbital on days 2-4. Additionally, 80 mg/kg 5,6-benzoflavone was injected i.p. on day 3.

The S9-mix had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADH	4 mM
NADPH	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	30 %

4. Test organisms: Chinese hamster lung cells (CHL)

5. Culture medium: Eagle's MEM medium with glutamine supplemented with
- 10% (v/v) newborn calf serum
- 0.01% (v/v) amphotericine B (2.5 µg/mL)

6. Test concentrations:

a) Prelim. toxicity assay: Nine concentrations ranging from 11.7 to 3000 µg/mL

b) Mutation assay

-S9/24 h: 23.4, 46.9, 93.8 and 187.5 µg/mL

-S9/48 h: 11.7, 23.4, 46.9, and 93.8 µg/mL

+S9/6h: 93.8, 187.5, 375, 750 and 1500 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 01-Feb-1993 to 10-Mar-1993

2. Preliminary cytotox. assay: Cultures containing 2.5 x 10^5 cells in 5 mL medium and 0.025 mL test item solutions, were exposed for 24 or 48 hours without metabolic activation with 9 concentrations up to solubility limit at 3000 µg/mL. Same concentrations were incubated for 6 hours in the presence of S9 mix and for another 18 hours with fresh culture medium without additives. Cells were fixed for 5 min with ethanol and stained with 2% Giemsa solution (pH 6.8) for 20 min. Test item concentration inducing 50% or more reduction of the relative cell growth was selected as the top-dose for the main experiment.

3. Cytogenicity Assay:

Cell treatment: Cultures containing 1 x 10^6 cells in 10 mL medium and 0.05 mL test item solutions, were exposed for 24 or 48 hours without metabolic activation. In the presence of S9 mix, CHL cells were incubated in 5 mL medium with 0.025 mL test item preparation for 6 hours and for another 18 hours with fresh culture medium without additives. Cells were fixed for 5 min with ethanol and stained with 2% Giemsa solution (pH 6.8) for 20 min.

Duplicate cultures were used.

Spindle inhibition: 0.5 µg/mL of colchicine was added to the cultures 2 hours prior to harvesting.

Metaphase analysis: Slides were coded prior to analysis. At least 100 well-spread metaphases of each culture (200 in total) were counted for all test groups, and if cells had 25 ± 1 chromosomes (typical karyotype of CHL cells), they were analyzed for structural chromosome aberrations. Polyploidy and endreduplication were also recorded. A metaphase with one or more chromosome aberrations was counted as one aberrant metaphase.

A mitotic index based on 1000 cells/culture was determined for all evaluated test groups.

4. Statistics The number of aberrant metaphases excluding gaps in each dose was statistically compared with that of corresponding solvent control using chi-square test.

5. Evaluation criteria:

The test chemical is considered positive in this assay if a dose-related and reproducible significant increase in the number of structural chromosomal aberrations was observed.

The results of structural and numerical chromosome aberrations were evaluated separately.

II. RESULTS AND DISCUSSION

A. Preliminary cytotoxicity assay

Without metabolic activation, the concentrations reducing the relative growth by about 50% were 187.5 and 93.8 µg/mL after 24- and 48-hour treatment, respectively. With addition of S9 mix, no growth inhibition effect was observed at any concentration. Due to test substance floating at the solubility limit of 3000 µg/mL, the top-dose for the main experiment with S9 was set to 1500 µg/mL.

In both methods test item precipitation was observed at ≥ 187.5 µg/mL immediately after addition of the test item preparation to the culture medium, but not after 6 hours incubation.

B. Cytogenicity assays

In experiments with or without metabolic activation, no increased number of cells with aberration was observed [see Table 5.4.1-4, Table 5.4.1-5 and Table 5.4.1-6]. The frequency of metaphases with structural aberrations (excluding gap) was in the range of 0 - 2.0%, and of polyploidy in the range of 0 - 0.5%. No statistically significant difference to the concurrent negative and solvent controls was obtained by the chi-square method in any treatment mode.

The frequencies of aberrant metaphases in negative and solvent controls were within the historical data range, for any treatment.

The positive controls induced marked increases of the incidence of aberrant metaphases, thus demonstrating the ability of the test system to detect known clastogens that do or do not require metabolic activation.

III. CONCLUSION

Based on the results of the study it is concluded that dimethomorph has no clastogenic or aneugenic potential in vitro in the presence and absence of metabolic activation.

Table 5.4.1-4: Chromosome aberration test on dimethomorph without metabolic activation (24 hours treatment)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps	Aberrant cells excluding gaps	Chromatide type		Chromosome type		Gap	Fragmen-tation	Polyploidy	Other
		n	%	n	n	br	ex	br	ex	n	n	%	n
Negative control	A	100	7.4	3	2	1	0	0	1	1	0	1	0
	B	100	6.5	3	1	1	0	0	0	2	0	0	0
	Total (mean)	**200**	(7.0)	6 (3.0)	1 (0.5)	2 (1)	0	0	1 (0.5)	2 (1)	0	1 (0.5)	0
Vehicle DMSO	A	100	6.4	2	1	1	0	0	0	1	0	0	0
	B	100	5.4	2	0	0	0	0	0	2	0	1	0
	Total (mean)	**200**	(5.9)	4 (2.0)	1 (0.5)	1 (0.5)	0	0	0	3 (1.5)	0	1 (0.5)	0
Dimethomorph													
23.4 µg/mL	A	100	5.2	2	1	1	0	0	0	1	0	1	0
	B	100	5.7	3	1	0	0	1	0	2	0	1	0
	Total (mean)	**200**	(4.7)	5 (2.5)	2 (1)	1 (0.5)	0	1 (0.5)	0	3 (1.5)	0	2 (1)	0
46.9 µg/mL	A	100	3.3	3	0	0	0	0	0	3	0	1	0
	B	100	5.2	2	2	0	0	0	2	0	0	0	0
	Total (mean)	**200**	(4.3)	5 (2.5)	2 (1)	0	0	0	2 (1)	3 (1.5)	0	1 (0.5)	0
93.8 µg/mL	A	100	3.7	0	0	0	0	0	0	0	0	1	0
	B	100	3.6	2	1	1	0	0	0	1	0	0	0
	Total (mean)	**200**	(3.7)	2 (1)	1 (0.5)	1 (0.5)	0	0	0	1 (0.5)	0	1 (0.5)	0
187.5 µg/mL	A	100	2.2	2	1	0	1	0	0	1	0	0	0
	B	100	2.4	2	0	0	0	0	0	2	0	0	0
	Total (mean)	**200**	(2.3)	4 (2.0)	1 (0.5)	0	1 (0.5)	0	0	3 (1.5)	0	0 (0)	0
Positive control MMC													
0.1 µg/mL	A	100	3.8	26	26	21	13	0	0	0	0	0	0
	B	100	3.3	23	22	12	17	1	0	5	0	0	0
	Total (mean)	**200**	(3.6)	49 (24.5)	48 (24.0)	33 (16.5)	30 (15.0)	1 (0.5)	0	5 (2.5)	0	0 (0)	0

br = break, ex = exchange

Table 5.4.1-5: Chromosome aberration test on dimethomorph without metabolic activation (48 hours treatment)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps	Aberrant cells excluding gaps	Chromatide type		Chromosome type		Gap	Fragmen tation	Polyploidy	Other
		n	%	n	n	br	ex	n	ex	n	n	%	n
Negative control	A	100	3.5	4	2	2	0	0	0	2	0	2	0
	B	100	3.1	5	2	2	0	0	0	4	0	1	0
	Total (mean)	**200**	(3.3)	9 (4.5)	4 (2.0)	4 (2)	0	0	0	6 (3)	0	3 (1.5)	0
Vehicle DMSO	A	100	3.2	2	1	1	0	0	0	1	0	0	0
	B	100	2.9	2	2	1	1	0	0	0	0	0	0
	Total (mean)	**200**	(3.1)	4 (2)	3 (1.5)	2 (1)	1 (0.5)	0	0	1 (0.5)	0	0	0
Dimethomorph													
11.7 µg/mL	A	100	2.6	1	0	0	0	0	0	0	0	0	0
	B	100	2.7	1	1	0	1	0	0	0	0	0	0
	Total (mean)	**200**	(2.7)	2(1)	1 (0.5)	0	1 (0.5)	0	0	1 (0.5)	0	0	0
23.4 µg/mL	A	100	2.9	3	3	1	0	1	1	0	0	1	0
	B	100	2.0	1	1	1	0	0	0	0	0	0	0
	Total (mean)	**200**	(2.5)	4 (2)	4 (2)	2 (1)	0	1 (0.5)	1 (0.5)	0	0	1 (0.5)	0
46.9 µg/mL	A	100	2.5	0	0	0	0	0	0	0	0	0	0
	B	100	2.2	1	0	0	0	0	0	1	0	0	0
	Total (mean)	**200**	(2.4)	1 (0.5)	0	0	0	0	0	1 (0.5)	0	0	0
93.8 µg/mL	A	100	1.4	3	2	0	0	2	0	1	0	0	0
	B	100	1.6	1	0	0	0	0	0	1	0	0	0
	Total (mean)	**200**	(1.5)	4 (2)	2 (1)	0	0	2 (1)	0	2 (1)	0	0	0
Positive control MMC													
0.1 µg/mL	A	100	3.6	34	34	18	28	1	0	1	0	0	0
	B	100	3.3	31	29	13	23	1	1	7	0	1	0
	Total (mean)	**200**	(3.5)	65 (32.5)	63 (31.5)	31 (15.5)	51 (25.5)	2 (1)	1 (0.5)	8 (4)	0	1 (0.5)	0

br = break, ex = exchange

Table 5.4.1-6: Chromosome aberration test on dimethomorph with metabolic activation (6 hours treatment, harvest after 24 hours)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps	Aberrant cells excluding gaps	Chromatide type		Chromosome type		Gap	Fragmen-tation	Polyploidy	Other
		n	%	n	n	br	ex	n	ex		n	%	n
Negative control	A	100	6.7	1	0	0	0	0	0	1		0	0
	B	100	5.5	2	0	0	0	0	0	2	0	0	0
	Total (mean)	**200**	(6.1)	3 (1.5)	0	0	0	0	0	3 (1.5)	0	0	0
Vehicle DMSO	A	100	5.8	2	1	1	0	0	0	1	0	0	0
	B	100	5.0	3	1	1	0	0	0	3	0	0	0
	Total (mean)	**200**	(5.4)	5 (2.5)	2 (1)	1 (0.5)	0	0	0	4 (2)	0	0	0
Dimethomorph													
93.8 µg/mL	A	100	6.7	1	0	0	0	0	0	1	0	0	0
	B	100	5.3	3	0	0	0	0	0	3	0	0	0
	Total (mean)	**200**	(6.0)	4 (2)	0	0	0	0	0	4 (2)	0	0	0
187.5 µg/mL	A	100	5.8	1	0	0	0	0	0	1	0	0	0
	B	100	5.3	0	0	0	0	0	0	0	0	0	0
	Total (mean)	**200**	(5.6)	1 (0.5)	0	0	0	0	0	1 (0.5)	0	0	0
375 µg/mL	A	100	4.9	0	0	0	0	0	0	0	0	1	0
	B	100	4.9	1	0	0	0	0	0	1	0	0	0
	Total (mean)	**200**	(4.9)	1 (0.5)	0	0	0	0	0	1 (0.5)	0	1 (0.5)	0
750 µg/mL	A	100	4.4	4	1	0	0	1	0	4	0	0	0
	B	100	4.9	1	0	0	0	0	0	1	0	0	0
	Total (mean)	**200**	(4.7)	5 (2.5)	1 (0.5)	0	0	1 (0.5)	0	5 (2.5)	0	0	0
1500 µg/mL	A	100	6.9	3	1	1	1	0	0	2	0	1	0
	B	100	5.2	2	1	0	1	0	0	1	0	0	0
	Total (mean)	**200**	(6.1)	5 (2.5)	2 (1)	1 (0.5)	2 (1)	0	0	3 (1.5)	0	1 (0.5)	0
Positive control BaP													
0.1 µg/mL	A	100	5.4	45	45	13	40	3	3	3	0	0	0
	B	100	5.9	40	38	11	34	5	3	3	0	0	0
	Total (mean)	**200**	(5.7)	85 (42.5)	83 (41.5)	24 (12)	74 (37)	8 (4)	6 (3)	6 (3)	0	0	0

br = break, ex = exchange

Report: CA 5.4.1/5
Kurita T., 1995a
Dimethomorph: In vitro cytogenetics test in the presence of metabolic activation
DK-435-016
Guidelines: JMAFF 59 NohSan No 4200, OECD 473
GLP: no

Executive Summary
Dimethomorph (BAS 550 F, Batch: 92226, Purity: 96.2%) was tested in vitro for the ability to induce chromosome aberrations in Chinese Hamster lung cells (CHL) in the absence and presence of metabolic activation system consisted of liver S9-mix of phenobarbital/benzoflavone pretreated rats. With and without metabolic activation CHL cells were incubated for 6 h with the test substance at concentrations of 93.8, 187.5, 375, 750, and 1500 µg/mL and were fixed after 18 h post-exposure without test substance. Negative (culture medium), vehicle (DMSO) and positive controls (-S9: MMC; +S9: BaP) were included to demonstrate the sensitivity of the test system. Metaphases were arrested by addition of 0.5 µg/mL colchicine 2 hours prior harvest, visualized by Giemsa staining. At least 200 well spread metaphases per treatment were analysed for chromosomal aberrations.
In experiments with or without metabolic activation, no increased number of cells with aberration was observed. The frequency of metaphases with structural aberrations (excluding gap) was in the range of 0 - 3.0%, and of polyploidy in the range of 0 - 0.5%. The frequencies of aberrant metaphases in negative and solvent controls were within the historical data range for any treatment. The positive controls induced marked increases of the incidence of aberrant metaphases, thus demonstrating the ability of the test system to detect known clastogens that do or do not require metabolic activation.
Based on the results of this study dimethomorph is considered to have no clastogenic or aneugenic potential in vitro in Chinese hamster lung cells (CHL) in the presence and absence of metabolic activation system.

(BASF DocID DK-435-016)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimethomorph; BAS 550 F

Description: white powder (crystalline)

Lot/Batch #: 92226

Purity: 96.2%

Stability of test compound: stable for acid, alkali, light and heat

2. Control Materials:

Negative: untreated

Solvent control: DMSO (0.5% final concentration)

Positive, -S9: 0.2 µg/mL mitomycin C (MMC) in Hanks' BSS

Positive, +S9: 40 µg/mL benzo[a]pyrene (BaP) in DMSO

3. Activation: S9 derived from male Sprague-Dawley rats. The rats were induced by an i.p. injections of 30 mg/kg phenobarbital on day 1 and 60 mg/kg phenobarbital on days 2-4. Additionally, 80 mg/kg 5,6-benzoflavone was injected i.p. on day 3.

The S9-mix had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADH	4 mM
NADPH	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	30 %

4. Test organisms: Chinese hamster lung cells (CHL)

5. Culture medium: Eagle's MEM medium with glutamine supplemented with
- 10% (v/v) newborn calf serum
- 0.01% (v/v) amphotericine B (2.5 µg/mL)

6. Test concentrations:

a) Prelim. toxicity assay: Nine concentrations ranging from 11.7 to 3000 µg/mL

b) Mutation assay

±S9/6 h treatment: 93.8, 187.5, 375, 750, 1500 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-July-1995 to 22-Aug-1995

2. Preliminary cytotox. assay: Cultures containing 1 x 10^5 cells in 5 mL medium were cultivated for 48 h, and thereafter medium was changed and 0.015 mL test item solutions was added. The cultures were exposed to the test substance in the presence of S9 mix for 6 h. Medium was changed and cells were allowed to grow for another 18 h without test substance. Cells were fixed for 5 min with ethanol and stained with 3% Giemsa solution (pH 6.8) for 20 min. Test item concentration inducing 50% or more reduction of the relative cell growth was selected as the top-dose for the main experiment.

3. Cytogenicity Assay:

Cell treatment: Cultures containing 1 x 10^6 cells in 10 mL medium and 0.05 mL test item solutions, were exposed for 24 or 48 hours without metabolic activation. In the presence of S9 mix, CHL cells were incubated in 5 mL medium with 0.025 mL test item preparation for 6 hours and for another 18 hours with fresh culture medium without additives. Cells were fixed for 5 min with ethanol and stained with 2% Giemsa solution (pH 6.8) for 20 min.

Duplicate cultures were used.

Spindle inhibition: 0.5 µg/mL of colchicine was added to the cultures 2 hours prior to harvesting.

Metaphase analysis: Slides were coded prior to analysis. At least 100 well-spread metaphases of each culture (200 in total) were counted for all test groups, and if cells had 25 ± 1 chromosomes (typical karyotype of CHL cells), they were analyzed for structural and numerical chromosome aberrations.

A mitotic index based on 1000 cells/culture was determined for all evaluated test groups.

4. Statistics The number of aberrant metaphases excluding gaps in each dose was statistically compared with that of corresponding solvent control using chi-square test.

5. Evaluation criteria:

The test chemical is considered positive in this assay if a dose-related and reproducible significant increase in the number of structural chromosomal aberrations was observed.

The results of structural and numerical chromosome aberrations were evaluated separately.

II. RESULTS AND DISCUSSION

A. Preliminary cytotoxicity assay

Up to 3000 µg/mL no reduction of the mitotic index of 50% or more was observed in the presence of metabolic activation. Test item precipitation was observed at ≥ 187.5 µg/mL immediately after addition of the test item preparation to the culture medium. Precipitation of the test substance was observed at 3000 µg/mL with lumps floating at the surface. Therefore, 1500 µg/mL was determined as the highest dose used in the main test.

B. Cytogenicity assays

In experiments with or without metabolic activation, no increased number of cells with aberration was observed [see **Table 5.4.1-7** and **Table 5.4.1-8**]. The frequency of metaphases with structural aberrations (excluding gap) was in the range of 0 - 3.0%, and of polyploidy in the range of 0 – 0.5%. No statistically significant difference to the concurrent negative and solvent controls was obtained by the chi-square method in any treatment mode.

The frequencies of aberrant metaphases in negative and solvent controls were within the historical data range, for any treatment.

The positive controls induced marked increases of the incidence of aberrant metaphases, thus demonstrating the ability of the test system to detect known clastogens that do or do not require metabolic activation.

Table 5.4.1-7: Chromosome aberration test on dimethomorph without metabolic activation (6 hours treatment)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps	Aberrant cells excluding gaps	Chromatide type		Chromosome type		Gap	Fragmen-tation	Polyploidy	Other
		n	%	n	n	ctb	cte	csb	cse	n	n	%	n
Negative control	A	100	77	1	0	0	0	0	0	1	0	0	0
	B	100	61	2	1	0	0	1	0	1	0	0	0
	Total (mean)	**200**	69	3 (1.5)	1 (0.5)	0	0	1 (0.5)	0	2 (1)	0	0	0
Vehicle DMSO 0.5%	A	100	73	2	1	1	0	0	0	1	0	0	0
	B	100	64	0	0	0	0	0	0	0	0	1	0
	Total (mean)	**200**	69	2 (1.0)	1 (0.5)	1 (0.5)	0	0	0	1 (0.5)	0	1 (0.5)	0
Dimethomorph													
93.8 µg/mL	A	100	61	0	0	0	0	0	0	0	0	0	0
	B	100	44	0	0	0	0	0	0	0	0	0	0
	Total (mean)	**200**	53	0	0	0	0	0	0	0	0	0	0
187.5 µg/mL	A	100	42	0	0	0	0	0	0	0	0	0	0
	B	100	56	1	1	0	0	1	0	0	0	0	0
	Total (mean)	**200**	49	1 (0.5)	1 (0.5)	0	0	1 (0.5)	0	0	0	0	0
375 µg/mL	A	100	30	3	2	1	2	0	0	1	0	0	0
	B	100	29	3	2	1	2	0	0	1	0	0	0
	Total (mean)	**200**	30	6 (3.0)	4 (2)	2 (1)	4 (2)	0	0	2 (1)	0	0	0
750 µg/mL	A	100	27	4	3	1	3	0	0	1	0	0	0
	B	100	39	4	3	1	1	0	0	3	0	0	0
	Total (mean)	**200**	33	8 (4)	6 (3)	2 (1)	4 (2)	0	0	4 (2)	0	0	0
1500 µg/mL	A	100	51	0	0	0	0	0	0	0	0	0	0
	B	100	55	0	0	0	0	0	0	0	0	0	0
	Total (mean)	**200**	53	0	0	0	0	0	0	0	0	0	0
Positive control MMC													
0.1 µg/mL	A	100	47	30	30	13	25	0	0	1	0	0	0
	B	100	58	37	37	19	27	1	0	4	0	0	0
	Total (mean)	**200**	53	67 (33.5)	67 (33.5)	32 (16)	52 (26)	1 (0.5)	0	5 (2-5)	0	0	0

Ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; - g: excluding gaps

Table 5.4.1-8: Chromosome aberration test on dimethomorph with metabolic activation (6 hours treatment)

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps	Aberrant cells excluding gaps	Chromatide type		Chromosome type		Gap	Fragmen-tation	Polyploidy	Other
		n	%	n	n	ctb	cte	csb	cse	n	n	%	n
Negative control	A	100	70	1	0	0	0	0	0	0	0	0	0
	B	100	56	0	0	0	0	0	0	1	0	0	0
	Total (mean)	**200**	63	1 (0.5)	0	0	0	0	0	1 (0.5)	0	0	0
Vehicle DMSO 0.5%	A	100	63	3	3	2	0	0	0	0	0	0	0
	B	100	64	0	0	0	0	0	1	0	0	0	0
	Total (mean)	**200**	64	3 (1.5)	3 (1.5)	2(1)	0	0	1 (0.5)	0	0	0	0
Dimethomorph													
93.8 µg/mL	A	100	77	0	0	0	0	0	0	0	0	0	0
	B	100	56	1	1	0	0	1	0	0	0	0	0
	Total (mean)	**200**	67	1 (0.5)	1 (0.5)	0	0	1 (0.5)	0	0	0	0	0
187.5 µg/mL	A	100	58	2	0	0	0	0	0	2	0	0	0
	B	100	41	1	1	1	0	0	0	0	0	1	0
	Total (mean)	**200**	50	3 (1.5)	1 (0.5)	1 (0.5)	0	0	0	2 (1)	0	1 (0.5)	0
375 µg/mL	A	100	34	2	2	0	0	0	0	1	0	0	0
	B	100	41	2	1	1	1	0	1	1	0	0	0
	Total (mean)	**200**	38	4 (2)	3 (1.5)	1 (0.5)	1 (0.5)	0	1 (0.5)	2 (1)	0	0	0
750 µg/mL	A	100	51	0	0	0	0	0	0	0	0	0	0
	B	100	45	1	1	0	0	1	0	0	0	0	0
	Total (mean)	**200**	48	1 (0.5)	1 (0.5)	0	0	1 (0.5)	0	0	0	0	0
1500 µg/mL	A	100	65	1	1	0	0	0	0	0	0	0	0
	B	100	43	1	1	0	0	1	1	0	0	0	0
	Total (mean)	**200**	54	2 (1)	2 (1)	0	0	1 (0.5)	1 (0.5)	0	0	0	0
Positive control B(a)P													
40 µg/mL	A	100	34	45	43	16	36	2	1	4	0	0	0
	B	100	29	43	41	7	40	1	1	3	0	0	0
	Total (mean)	**200**	32	88 (44)	84 (42)	23 (11.5)	76 (38)	3 (1.5)	2 (1)	7 (3.5)	0	0	0

Ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; - g: excluding gaps

III. CONCLUSION

Based on the results of the study it is concluded that dimethomorph has no clastogenic or aneugenic potential in vitro in the presence and absence of metabolic activation.

Report: CA 5.4.1/6
Akanuma M., 1993a
Dimethomorph: DNA repair test (REC-assay)
DK-435-017
Guidelines: JMAFF 59 NohSan No 4200
GLP: no

Executive Summary

Dimethomorph was tested in the DNA repair test (Rec-assay) with Bacillus subtilis H17 and M45. The strains were incubated in 90 mm Petri dishes with dimethomorph at concentrations of 20, 50, 100, 200, 500, and 1000 µg/disk in duplicate cultures. A negative control (Kanamycin, 0.2 µg/disk) and positive controls with S9 (Trp-P-1, 5 µg/disk) or without S9 (mitomycin C, 0.01 µg/disk) were used in the study. As endpoint of the study the growth inhibitory zones determined in strain H17 and M45 were measured and the difference between strains was calculated.
Dimethomorph induced growth inhibitory zones in both the recombination-deficient strain M45 and the recombination-proficient strain H17 with and without metabolic activation. The differences of growth inhibitory zones between both strains were 1 mm or less and were thus below the trigger of 4 - 5 mm for a positive result as indicated in the evaluation criteria. Growth inhibitory zones induced by the negative control kanamycin were 1 mm. The positive controls mitomycin C (-S9 mix) and Trp-P-1 (+S9 mix) induced larger inhibitory zones in the strain M45 compared with those observed in the strain H17. The differences of growth inhibitory zones 19 – 20 mm and 11 – 12 mm for mitomycin C and Trp-P-1, respectively. In the solvent control no growth inhibitory zone was observed in both strains M45 and H17.
According to the results of the present study, Dimethomorph was negative in the DNA repair test under the conditions used in this experiment.

(BASF DocID DK-435-017)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Dimethomorph

Description:	Solid (crystalline powder), white
Lot/Batch #:	92226
Purity:	96.2%
Stability of test compound:	Stable for acid, alkali, light and heat
Solvent used:	DMSO

2. Control Materials:

Negative control:	Kanamycin (0.2 µg/disk)
Solvent control:	DMSO (20 µL/disk)
Positive control	Mitomycin C (0.01 µg/disk) (-S9)
	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (5 g/disk) (+S9)

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received four intraperitoneal injection of phenobarbital (day 1-4) and one intraperitoneal injection of 5,6-benzoflavone (day 3). The S9-fraction is mixed with the S9-supplement (cofactors) yielding the so-called S9-mix.

The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	20 mg/mL
NADPH + NADH	40 mg/mL
KCl	33 mM
$MgCl_2$	8 mM

The S9 fraction was checked in advance in Salmonella typhimurium strains TA 100 and TA 98 with 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene.

4. Test organisms: Recombination-wild (rec+) strain H17 and recombination-deficient (recE-) strain M45 of Bacillus subtilis. At the time of spore preparation UV sensitivity (recE) and response to control chemicals were checked.

5. Test concentrations:

Rec-assay (+/- S9): 20, 50, 100, 200, 500, 1000 µg/disk

B. TEST PERFORMANCE:

1. Dates of experimental work: 01-Feb-1992 to 01-Feb-1992

2. Spore preparation:

Overnight cultures were transferred into modified Schaeffer´s medium (1.6% nutrient broth, 0.2% KCl, 0.1% glucose, 0.05% MgSO4*7H2O, 1 µM FeSO4, 1 mM Ca(NO3)2, and 0.1 mM MnCl2). Cells were incubated for 3 (strain H17) or 5 (strain M45) days. Sporulated cells were washed with 1/15 M phosphate buffer (pH 7.4) and then treated with lysozyme (100000 units/mg) at the final concentration of 2 mg/mL in Tris-buffer. Sodium dodecyl sulfate was added at the final concentration of 1% and incubated at 37°C for 30 min. The obtained spores were washed and stored in pure water (4°C).

3. Rec-assay:

Without metabolic activation:

Molten B2 top agar medium (B2 medium supplemented with 0.8% agar) was prepared and kept at 45°C. One hundred µL of spore suspension (3 x 10^7/mL) of tester strain was placed in an empty 90-mm Petri dish and mixed with the agar (5 mL). A paper filter soaked with 20 µL test substance solution was placed on the plate. Duplicate plates were made for each experimental point. Diameter of a growth inhibitory zone of each strain was measured after incubation at 37°C for 24 h.

With metabolic activation:

Molten B2 top agar medium (B2 medium supplemented with 0.8% agar) was prepared and kept at 45°C. One hundred µL of spore suspension (3 x 10^7/mL) of tester strain and 0.05 mL of S9 fraction was placed in an empty 90-mm Petri dish and mixed with the agar (5 mL). A paper filter soaked with 20 µL test substance and 20 µL of a co-factor solution was placed on the plate. Duplicate plates were made for each experimental point. Diameter of a growth inhibitory zone of each strain was measured after incubation at 37°C for 24 h.

4. Evaluation and acceptance criteria:

Evaluation criteria

- Results are judged negative when the test substance causes no growth inhibition in either strain
- In the case that the test substance causes growth inhibition at least in one strain, results are judged positive when growth inhibitory zone of M45 is larger than that of H17, and the difference in diameter is 5 mm or more at one or more dose levels that causes growth inhibitory zones of 4 mm or less in diameter in the H17 (rec+) strain. In this case, a retest is conducted to confirm reproducibility of the positive result.

Acceptance criteria

An assay is considered acceptable if all of the following criteria are satisfied:

- No growth inhibition is observed in the solvent control
- In a positive control, growth inhibitory zone of M45 is larger than that of H17, and the difference in diameter is 5 mm or more
- In a negative control, the difference in diameter of growth inhibitory zone between the strains is 4 mm or less

II. RESULTS AND DISCUSSION

In the absence of metabolic activation, Dimethomorph induced growth inhibitory zones of 1 – 2 mm in diameter in recombination-proficient strain H17 at dose levels of 200 µg/disk or more, and 1 – 3 mm in diameter in the recombination-deficient strain M45 at dose levels of 100 µg/disk or more. The differences of growth inhibitory zones between the strains H17 and M45 were 0 - 1 mm. In the presence of metabolic activation system, growth inhibitory zones 1 – 3 mm and 1 – 4 mm in diameter were observed in the strains H17 and M45, respectively, at dose levels of 100 µg/disk or more. At these doses, the differences of growth inhibitory zones between the strains H17 and M45 were 0 – 1 mm in diameter.

Growth inhibitory zones induced by the negative control kanamycin were 1 mm. The positive controls mitomycin C (-S9 mix) and Trp-P-1 (+S9 mix) induced larger inhibitory zones in the strain M45 compared with those observed in the strain H17. The differences of growth inhibitory zones 19 – 20 mm and 11 – 12 mm for mitomycin C and Trp-P-1, respectively. In the solvent control no growth inhibitory zone was observed in both strains M45 and H17.

Table 5.4.1-7: Results of the DNA repair test

Compound	Dose	S9 (-)			S9 (+)		
	µg/disk	Inhibitory zone#		Difference	Inhibitory zone#		Difference
		M45	H17	(mm)	M45	H17	(mm)
Solvent control		0	0	0	0	0	0
		0	0	0	0	0	0
Dimethomorph	20	0	0	0	0	0	0
		0	0	0	0	0	0
	50	0	0	0	0	0	0
		0	0	0	0	0	0
	100	1	0	1	1	1	0
		1	0	1	0	0	0
	200	2	2	0	2	2	0
		3	2	1	2	2	0
	500	2	1	1	3	3	0
		2	1	1	3	3	0
	1000	2	2	0	4	3	1
		3	2	1	3	3	0
Negative control Kanamycin	0.2	10	9	1	-	-	-
		9	8	1	-	-	-
Positive control Mitomycin C	0.01	22	3	19	-	-	-
		23	3	20	-	-	-
Positive control Trp-P-1	5	-	-	-	11	0	11
		-	-	-	12	0	12

diameter of growth inhibitory zone subtracted the diameter of disk (8 mm)

III. CONCLUSION

According to the results of the present study, Dimethomorph was negative in the DNA repair test under the conditions used in this experiment.

Peer-reviewed studies:

Brooks and Wiggins, 1989, Bacterial mutagenicity study with CME-151 (DocID DK-435-018, DK-435-011)

Dimethomorph was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation test. Testing employed a standard plate incorporation assay using six bacterial tester strains TA98, TA100, TA1535, TA1537 and TA1538 of *S. typhimurium* and WP2 uvrA of E. coli. All six tester strains received either the test material in dimethylsulfoxide (DMSO) at dose levels of 5000, 2000, 1000, 500, 250, 125, 62.5 and 31.25 µg/plate, or DMSO as the negative control, with and without S9 metabolic activation for three replicates per dose. The assay was conducted, in full, twice to confirm results.

Findings:
The formulations of the test and control substances were considered to be stable for the duration of the study. Precipitate was noted at dose levels of 1000 µg/plate and above. Dimethomorph did not induce toxicity (except in strain TA1537 where evidence of cytotoxicity was noted at the 5000 µg/plate dose level upon microscopic evaluation of the background). Thus, the results obtained from both trials showed no positive responses or dose-related increases in revertant frequencies in the presence or absence of metabolic activation. The positive control materials elicited positive responses indicating that the test system was capable of detecting base-pair and frameshift mutations and that the metabolic activation system was functioning properly.

Conclusion:
According to the results of the study, dimethomorph was judged to be negative for inducing base pair or frame shift mutations in these strains of bacteria.

Van de Waart, 1991, Evaluation of the mutagenic activity of dimethomorph in an *in vitro* mammalian cell gene mutation test with V79 Chinese hamster cells (with independent repeat) (DocID DK-435-014)

Dimethomorph was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79 Chinese hamster cells *in vitro*. The dose levels used in the definitive study were based on results from a dose-range finding test performed with dimethomorph at 3, 10, 33, 100, 333 and 1000 µg/mL with and without S9 metabolic activation. Toxicity was observed in the range-finding study at 100 µg/mL and above without metabolic activation and at 333 µg/ml and above with metabolic activation. Dimethomorph was tested in the definitive trials at 33, 100, 133 and 180 µg/mL without metabolic activation and at 133, 180, 237 and 333 µg/mL with metabolic activation.

Findings:

Dimethomorph did not induce a significant dose-related increase in the mutant frequency at the HGPRT-locus either with or without metabolic activation in two independent trials. The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the V79 Chinese hamster cell line. Both of the positive control chemicals, i.e. EMS (ethylmethanesulfonate) and DMN (dimethylnitrosamine), led to the expected increase in the frequencies of forward mutations.

Conclusion:

Under the experimental conditions of this assay, dimethomorph does not induce forward mutations *in vitro* in the V79/ HGPRT mutation assay.

Heidemann and Miltenburger, 1986, Chromosome aberrations in cells of Chinese hamster cell line V79 (DocID DK-435-004)
Dimethomorph was assessed for its potential to induce chromosome aberrations in V79 cells both in the presence and absence of a metabolic activation system (S9 from Aroclor-treated rats). Cultured cells were harvested for chromosome preparation at 7 hours (high dose); 18 hours (low, medium, and high dose) and 28 hours (high dose) after the start of the treatment with the test substance. The treatment time was 4 hours both with and without metabolic activation. Using the criterion of > 50 % inhibition of mitotic index as an indicator of toxicity, and colony forming ability of treated cells in a pre-experiment, the doses evaluated for chromosomal aberrations in the main assay were 12, 60, and 160 µg/mL without metabolic activation, and 13, 60, and 170 µg/mL with activation. Dimethylsulfoxide (DMSO) was used as the vehicle; the test solutions were prepared on day of experiment and were handled under reduced light conditions. In each experimental group 200 metaphases were scored for chromosomal aberrations.

Findings:
The validity of the test system was demonstrated by significant increases in aberration rates after treatment with the positive controls, i.e., ethylmethanesulfonate (EMS) (without S9) and cyclophosphamide (CPA) (with S9). Cultures treated with the highest concentrations of the test substance showed a slight increase in chromosome aberrations compared with the vehicle controls with and without activation at 7 hour harvest, but also demonstrated cytotoxicity (inhibition of mitotic index without and without metabolic activation, respectively). Another weak response was observed at 18 hours in the absence of activation at the high dose.

Discussion: The results are difficult to interpret because the enhancement is weak and a statistical analysis was not performed because of the small sample size of only 200 metaphases per test group.

Conclusion:
Because of the one weak response at the highest dose tested, with and without metabolic activation, another study was performed using a larger sample size of 400 metaphases per dose group.

Heidemann and Miltenburger, 1987, Chromosome aberrations in cells of Chinese hamster cell line V79 (DocID DK-435-006)

In order to assess the results of the preceding study, dimethomorph was tested for its mutagenic potential to induce chromosome aberrations in V79 cells. Cultured cells were harvested for chromosome preparations 7 hours (high dose) and 18 hours (low, medium and high dose); after the start of the treatment with the test substance. The treatment time was 4 hours both with and without S9 metabolic activation. The doses were selected from the preceding study (5.4.1.3/1) to assess the positive responses, i.e., 160 µg/mL without S9 mix and 170 µg/mL with S9 mix at preparation interval 7 hours, and 12, 60, and 160 µg/mL without S9 mix at preparation interval 18 hours. Dimethylsulfoxide (DMSO) was used as the vehicle; the test solutions were prepared on day of experiment. In this study, 400 metaphases were scored per dose group of the test article. For the positive control groups, ethylmethanesulfonate (EMS) and cyclophosphamide (CPA), 200 metaphases were scored per group.

Findings:

Results showed a slight but statistically significant increase in the number of cells with aberrations in the cultures treated with 170 µg/mL of the test substance with activation at 7 hours as compared with the negative control. The magnitude of the response was, however, very small at this high dose level. As seen in the previous study, this dose produced marked cytotoxicity (73.8% inhibition of mitotic index). In contrast to the previous study, which sampled only 200 metaphases per group, the results of testing 160 ug/mL without activation at the 18-hour interval demonstrated no positive response when using a larger sample size of 400 metaphases scored per group. The positive controls (EMS and CPA) showed significant increases in chromosome aberrations, validating the test system.

Conclusion:

It was concluded from both studies that dimethomorph induced only a weak response in increasing chromosome aberrations at 7 hours in V79 cells in the presence of S9 mix at a dose level which induced marked cytotoxicity in a previous study.

Van de Waart, 1991, Evaluation of the ability of dimethomorph to induce chromosome aberrations in cultured peripheral human lymphocytes, (DocID DK-435-013)
Dimethomorph was tested for chromosome aberrations in cultured peripheral human lymphocytes in the presence and absence of a metabolic activation system (Aroclor – 1254 induced rat liver S9 mix). Dimethomorph was tested up to 333 µg/mL in the absence and up to 422 µg/mL in the presence of S9 mix for a 24-hour fixation period and up to 422 µg/mL in the absence and in the presence of S9 mix for a 48-hour fixation period. The doses were selected based on the inhibition of mitotic indices of 50 % or greater at the indicated high doses. Dimethylsulfoxide (DMSO) was used as the vehicle; the test substance concentrations were prepared directly prior to use.

Findings:
No increase in the number of cells with chromosome aberrations was observed at concentration levels up to 333 µg/mL in the absence as well as in the presence of S9 mix. At 422 µg/mL, a statistically significant increase in the number of cells with chromosome aberrations was found in the presence of S9-mix at 24 hour harvest only. However, this concentration was strongly cytotoxic (68 % inhibition of mitotic index). Furthermore, at the concentration of 422 µg/mL, the test substance precipitated in the culture medium. Positive control chemicals, mitomycin C (without S9) and cyclophosphamide (with S9), both produced a statistically significant increase in the incidence of cells with chromosome aberrations indicating the validity of the system.

Conclusion:
It was concluded that the test substance is positive in human lymphocytes only at a very high (precipitate present) and strongly cytotoxic concentration of 422 µg/mL with metabolic activation and negative up to 333 µg/mL with and without metabolic activation.

Timm and Miltenburger, 1986, Unscheduled DNA synthesis in hepatocytes of male rats in vitro (UDS test) (DocID DK-435-002)

Dimethomorph was tested for its ability to induce DNA repair synthesis (unscheduled DNA synthesis; UDS) in primary hepatocytes from male Wistar rats. DMSO was used as the solvent and negative control. Cytotoxicity was determined after 3 hours exposure to dimethomorph by Trypan blue exclusion in a preliminary experiment at 0, 1.00, 3.33, 10, 33.33, 100, and 250 µg/mL. Based on the results of survival in the prestudy, concentrations selected for the definitive study were 0, 2.5, 10, 25, 100, and 250 µg/mL. Dimethylsulfoxide (DMSO) was used as the vehicle; the test substance concentrations were prepared directly prior to use. The test material, negative (DMSO; dimethylsulfoxide) and positive control (DMBA; 7, 12-dimethylbenz(a)anthracene), along with tritiated thymidine were added to six replicate cultures per dose and incubated for three hours. After washing, cells were incubated for a further four hours before liquid scintillation counting. A test substance is regarded as positive when an increase occurs in the ^{3}H-thymidine incorporation of treated cells by two-fold over the negative control in more than one concentration of the test substance.

Findings:

In the preliminary experiment, survival was reduced to 63 % of the control value at the highest concentration tested (250 µg/mL). The tritiated thymidine incorporation, given as disintegrations per minute (dpm) per µg DNA was not increased in the treated groups; the values for dpm/µg DNA were comparable to that of the negative control. In addition, no indication of a dose response was observed. The positive control substance (DMBA at 25.64 µg/mL) induced a three-fold increase of dpm/µg DNA as compared to the negative control thus demonstrating the sensitivity of the test system.

Conclusion:

It was concluded that dimethomorph did not damage the DNA of rat hepatocytes expressed by unscheduled DNA synthesis (UDS).

Miltenburger, 1986, CME 151-Z50: Cell transformation assay in Syrian hamster embryo (SHE) cells (DocID DK-435-005)
Dimethomorph was assessed for its potential to transform mammalian cells in vitro. Embryos from healthy Syrian golden hamster were used for the preparation of the primary cells. Secondary cultures of SHE cells were treated for 6 hours in the presence of S9 mix with concentrations of dimethomorph of 25, 250, 260, 265 µg/mL. In addition SHE cells were treated for 6 and 48 hours in the absence of S9 mix with concentrations of dimethomorph of 5, 10, 25, 50 µg/mL. The concentrations and the treatment times were determined in a pre-experiment.

Findings:
In two groups treated with dimethomorph a single transformation colony was found in a sample of 1000 randomly selected colonies. This finding was not regarded as an indication for the property of the test substance to transform cells. A single transformed colony was also found occasionally in the negative control group. In the historical negative controls a spontaneous rate of 0.06 % is found. The development of transformed colonies in the positive control groups treated with Benzo(a)pyrene (BaP) (with metabolic activation) and N-methyl-N`-nitro-N-nitrosoguanidine (MNNG) (without metabolic activation) demonstrated the sensitivity of the test system.

Conclusion:
Dimethomorph did not induce cell transformation in the SHE cell cultures.

CA 5.4.2 In vivo studies in somatic cells

Report: CA 5.4.2/1
[redacted] 1989a
Mouse micronucleus test on CME 151 technical material
DK-435-009
Guidelines: OECD 474
GLP: no

EXECUTIVE SUMMARY

Dimethomorph (Batch: 3 SRC Ref ST89/116; Purity: 98.5%) was tested for chromosomal damage (clastogenicity) in CD1 mice using the micronucleus test method. For this purpose, the test substance, suspended in 1% methylcellulose, was administered once orally to groups of 15 male and female mice at a dose level of 5000 mg/kg body weight in a volume of 20 mL/kg body weight. The vehicle served as negative and mitomycin C as positive control. Each 5 animals of the test substance treated group were sacrificed 24 h, 48 h, or 72 h after the administration. Positive control groups (5 aniamls per sex) were sacrificed at 24 h. After the sacrifice the bone marrow of the two femora was prepared from each animal. After staining of the preparations, 1000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes occurring per 1000 polychromatic erythrocytes were also recorded.

The oral administration of Dimethomorph did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was close to the concurrent negative control and was within the range of the historical control data. No inhibition of erythropoiesis induced by the treatment of mice with the test substance was observed. In contrast, a significant inhibition of erythropoiesis was observed in the animals treated with mitomycin C.

Clinical signs observed included piloerection and hunched posture within 2 hours after administration. No signs of systemic toxicity were observed in any of the animals treated with the positive control or the vehicle. The positive control chemical mitomycin C led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system. Thus, under the experimental conditions of this study, the test substance Dimethomorph does not induce cytogenetic damage in bone marrow cells of CD1 mice in vivo.

(BASF DocID DK-435-009)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Dimethomorph

Test material name:	CME 151 Technical Material
Description:	Solid, beige coloured
Lot/Batch #:	3 SRC Ref ST89/116
Purity:	98.5%
Stability of test compound:	Expiry date of test material: April 1990. Stability in formulation was not determined.
Vehicle used:	aqueous methylcellulose (1% MC)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	DMSO/corn oil (2:3, v/v)
Positive control:	Mitomycin C (MMC) 12 mg/kg bw (dissolved in 0.9% saline)

3. Test animals:

Species:	Mouse
Strain:	CD1
Sex:	Male and female
Age:	35 days
Body weight:	22-24 g
Source:	[redacted]
Number of animals per dose:	
Range finding study:	2 per sex and dose
Micronucleus assay:	15 per sex and dose (vehicle control/tast substance), 5 per sex and dose in the positive control group
Acclimation period:	At least 5 days
Diet:	Pelleted Biosure LAD 1 rodent diet, ad libitum
Water:	Tap water, ad libitum
Housing:	Group housing (5 per cage) in plastic disposable cages

4. Environmental conditions:

Temperature:	22 °C
Humidity:	NA
Air changes:	30 per hour
Photo period:	12-hour light-dark cycle

5. Test compound doses:

Range finding test:	375, 750, 1500, 3000, 6000 mg/kg bw
Micronucleus assay:	5000 mg/kg bw
	The test substance was administered once by oral gavage using an application volume of 20 mL/kg bw.

B. TEST PERFORMANCE:

1. Dates of experimental work: 02-May-1989 to 21-June-1989

2. Preliminary range finding test

In a pretest for the determination of the acute oral toxicity, 2 male and 2 female animals were treated once by oral gavage at doses of 375, 750, 1500, 3000, 6000 mg/kg bw. The animals were observed regularly during the working day for a period of 72 h and any mortalities or clinical signs during the experiment were recorded.

3. Micronucleus test

Treatment and sampling:
Groups of 15 male mice were treated once with either the vehicle or 5000 mg/kg bw test substance by oral gavage. Each five animals per sex and dose group were sacrificed after 24 h, 48 h and 72 h, respectively. The application volume was 20 mL/kg bw and the animals were deprived of diet overnight prior to and for 2 h after dosing. The positive control substance MMC was administered once by oral gavage and the mice were sacrificed after 24 h. The animals were observed for evident clinical signs of toxicity throughout the study.

Slide preparation
At the end of the respective treatment period the mice killed by cervical dislocation. From each animal, both femora were prepared free of all soft tissue. After cutting the epiphyses a direct smear was made onto a slide containing a drop of calf serum. One smear was prepared per femur.

The smear was air-dried and stained for 10 min with Giemsa solution. Cover slips were mounted with DPX.

Slide evaluation

In general, 1000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.

The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.

The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoiesis and thus, that the test substance actually reached the target organ.

4. Statistics

For a comparison of an individual treated group with a concurrent control group, Wilcoxon´s sum of ranks test is used.

II. RESULTS AND DISCUSSION

A. PRELIMINARY RANGE FINDING TEST

In the pretest no mortality up to 6000 mg/kg bw was observed. Therefore, 5000 mg/kg bw was chosen as the limit dose in the main experiment according to the limit dose recommended for acute oral toxicity testing at that time.

B. MICRONUCLEUS ASSAY

Clinical examinations

No mortalities were observed after treatment with the test substance. No adverse clinical signs were observed in the vehicle or positive control treated animals. Piloerection and hunched posture were observed in the test substance treated animals of both sexes within the first two hours after administration.

Micronucleus test results

The test substance did not cause any statistically significant increase in the number of micronucleated polychromatic or normochromatic erythrocytes at any of the three kill times. The incidences were within the historical control values (mean: 0.84; range from 0.1 to 2.5).

The positive control substance Mitomycin C led to a statistically significant increase (28.6‰) in the number of polychromatic erythrocytes.

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the vehicle control group or in the various dose groups at any of the sacrifice intervals.

No inhibition of erythropoiesis induced by the test substance was observed. In contrast, an inhibition of erythropoiesis induced by the treatment of mice with Mitomycin C was observed.

Table 5.4.2-1: Summary of micronucleus test results at the 24 h, 48 h, and 72 h sacrifice time point

Sacrifice	Compound	Ratio PCE/NCE	MN-PCE [‰]	MN-NCE [‰]
24 h	Vehicle control	0.802	0.3	0
	Test substance (5000 mg/kg bw)	0.788	0.3	0
	Mitomycin C (12 mg/kg bw)	**0.296*** **	**28.6*** **	0
48 h	Vehicle control	0.752	0.3	0.2
	Test substance (5000 mg/kg bw)	0.928	0.3	0
72 h	Vehicle control	1.132	0.7	0.2
	Test substance (5000 mg/kg bw)	1.023	0.6	0

***: $p < 0.001$

III. CONCLUSION

Based on the result of this study Dimethomorph did not induce micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic activity in vivo.

Peer-reviewed study:

[redacted], 1991, Micronucleus test in bone marrow cells of the mouse with dimethomorph (DocID DK-435-012)

Dimethomorph was tested in the micronucleus test in mice to evaluate the clastogenic potential *in vivo.* Dose levels of the test substance for the definitive test were selected based on results from a range-finding test conducted at 500, 400, 300, and 100 mg/kg bw using corn oil as the vehicle, and at 500, 400, 300, and 200 mg/kg bw using dimethylsulfoxide (DMSO) as the vehicle. Three mice/sex/dose were dosed intraperitoneally and were observed for signs of toxicity for 5 days (120 hours). Based on the results of the preliminary study, groups of 15 Swiss DF-1 mice/sex received a single intraperitoneal dose of dimethomorph at doses of 200, 100, or 20 mg/kg bw in the definitive mouse micronucleus assay. Dimethylsulfoxide (DMSO) was used as the vehicle and negative control, cyclophosphamide (CPA) at 50 mg/kg body weight served as the positive control. The test substance concentrations were prepared directly prior to use. The vehicle and test article-treated animals (5/sex/group) were sacrificed 24, 48 and 72 hours following dosing for collection of bone marrow cells. Positive control animals were sacrificed at 48 hours for collection of bone marrow cells. All slides of the highest and intermediate doses were examined at any time point; of the lowest dose, only the 48–hour harvest slides were examined. One thousand polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The ratio of polychromatic to normochromatic erythrocytes was determined by counting and differentiating the first 1000 erythrocytes at the same time. The test chemical is to be considered positive in this assay if the following criteria are met: A biologically and statistically significant increase occurs in the frequency of micronucleated polychromatic erythrocytes (at any dose or at any sampling intervals) in the combined data for both sexes, or in the data for male or female groups separately.

Findings:
In the preliminary study, all animals in the 500, 400, 300, and 100 mg/kg groups (corn oil vehicle), were lethargic and had piloerection for 48 hours. After 48 hours, no signs of reaction to treatment were observed. In the groups with dimethylsulfoxide (DMSO) as the vehicle, mortality and clinical signs were observed as follows:

- 500 mg/kg bw: tachypnea, diarrhea, death of all animals (3 males, 3 females) within 48 hours,
- 400 mg/kg bw: lethargy, tachypnea, death of 3 males and 2 females in 72 hours,
- 300 mg/kg bw: tachypnea, diarrhea, lethargy, dacryorrhea, piloerection, death of 1 male and 3 females within 72 hours,
- 200 mg/kg bw: tachypnea, lethargy, piloerection, death of 2 females within 72 hours and 2 males within 120 hours.

In the definitive study, mortality occurred at the highest dose (4 males and 7 females from across three harvest times). There were no significant increases in the frequency of micronucleated polychromatic erythrocytes for any group, which received the test material at any harvest time for either sex. The incidence of micronuclei in the control animals was in the range of historical control data. Some groups treated with the test substance showed a decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a toxic effect on erythropoiesis. In the positive control group, a significant increase in the frequency of micronucleated polychromatic erythrocytes together with a decrease in the ratio of polychromatic to normochromatic erythrocytes was observed, confirming the validity of the assay.

Conclusion:
Dimethomorph does not have any chromosome-damaging (clastogenic) effect *in vivo* in the mouse micronucleus test.

CA 5.4.3 In vivo studies in germ cells

The results of the *in vitro* and *in vivo* studies demonstrate that dimethomorph has no genotoxic potential. Therefore, there was no necessity to evaluate the test substance in an *in vivo* study using germ cells.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Long-term dietary toxicity studies were conducted in rats and mice. In both species liver effects were observed at high doses. In the 2-year dietary toxicity study in Sprague-Dawley rats with dimethomorph, decreases in body weight gain and increased incidence of ”groundglass” foci of cellular alteration in the liver in females occurred at 750 ppm. The NOAEL for chronic toxicity in this study was 200 ppm, equal to approximately 9 mg/kg bw/day, based on food consumption data. Similarly, for the 2-year carcinogenicity study in Sprague-Dawley rats, the NOAEL for chronic toxicity was 200 ppm (equal to approximately 9 mg/kg bw/day), as based on a decrease in overall body weight gain for females at 750 ppm. In both 104-week dietary toxicity studies in rats, there was a borderline increase in the incidence of testicular tumors. However, the differences are not considered to be an oncogenic effect of dimethomorph because they were shown to be related to a longer life-span of the high dose animals compared to control . In the 104-week carcinogenicity study with dimethomorph in CD-1 mice, the data support a systemic toxicity NOAEL of about 10 mg/kg bw/day, based on reduced body weight gains in females at about 97 mg/kg bw/day.

Table 5.5-1: Summary of reviewed dimethomorph long-term and carcinogenicity studies

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
Chronic (2-year) dietary; Sprague-Dawley rats; 0, 200, 750, 2000 ppm	Males: 9.4, 36.2, 99.9 Females: 11.9, 57.7, 157.8	9	36	↓body weight gain; liver "ground glass" foci (females)	DK-427-006
Carcinogenicity (2-year) dietary; Sprague-Dawley rats; 0, 200, 750, 2000 ppm	Males: 8.8, 33.9, 94.6 Females: 11.3, 46.3, 132.5	9	34	↓body weight gain (females)	DK-428-005
Carcinogenicity (2-year) dietary; CD-1 mouse; 0, 10, 100, 1000 ppm	Males: 9.8, 98.0, 978.0 Females: 9.8, 96.8, 977.0	10	97	↓body weight gain (females)	DK-428-004

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the DAR/EFSA conclusion of dimethomorph (EFSA scientific report, 82, 1-69, 2006):

Long term toxicity and carcinogenicity	
Target / critical effect:	Liver; testes; decreased body weight
Lowest relevant oral NOAEL / NOEL:	2yr rat: (9 mg/kg bw/d)
Carcinogenicity	No carcinogenic potential

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
No further study was submitted.

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of dimethomorph and are provided under the respective chapters.

The conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is presented as follows:

Long-term toxicity and carcinogenicity (Regulation (EU) N°283/2013, Annex Part A, point 5.5)

Long-term effects (target organ/critical effect)	Rat: Reduced body weight gain, liver ground glass foci in females. Mouse: Reduced body weight gain in females	
Relevant long-term NOAEL	2-year chronic and 2-year carcinogenicity rat: 9 mg/kg bw/day 18-month carcinogenicity mouse: 10 mg/kg bw/day	
Carcinogenicity (target organ, tumour type)	Rat: No evidence of carcinogenicity. Mouse: No evidence of carcinogenicity. Dimethomorph is unlikely to pose a carcinogenic hazard to humans.	
Relevant NOAEL for carcinogenicity	2-year chronic rat and 2-year carcinogenicity rat: 94.6 mg/kg bw/day 18-month carcinogenicity mouse: 977 mg/kg bw/day	

Comparison with CLP Criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Chronic toxicity/carcinogenicity studies with dimethomorph were conducted in two species (rats and mice). There was no relevant treatment related increase in tumour formation in rats or mice.
In conclusion, based on the assessment of all available data dimethomorph is not subject to classification for carcinogenicity according to Regulation 1272/2008/EC.

Conclusion on classification and labelling

The available data on carcinogenicity of dimethomorph do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC and are therefore conclusive but not sufficient for classification.

[redacted] 1990, SAG 151: 104 week dietary toxicity study in rats (BASF DocID DK-427-006)

Dimethomorph was administered to groups of 20 male and 20 female Sprague-Dawley rats at dietary concentrations of 0, 200, 750, and 2000 ppm for 104 weeks. Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried pretrial and during weeks 51 and 102 on 10 males and 10 females from each dose group. Urinalysis, clinical chemistry and hematological examinations were carried out during weeks 25, 51, 77 and 102 of the study. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Findings:

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Mean test substance intake was 11.9, 57.7, 157.8 mg/kg bw/day for females and 9.4, 36.2, 99.9 mg/kg bw/day for males of the 200, 750, or 2000 ppm dose group.

Survival rates at study termination for the control, 200, 750 and 2000 ppm groups were 45%, 65%, 70% and 75%, respectively, for males, and 50%, 55%, 75% and 50%, respectively, for females. There were no clinical signs of toxicity related to dimethomorph treatment.

There was a marked reduction in overall body weight gain for the 2000 ppm males (33 %) which in terms of absolute body weight, attained statistical significance from weeks 65 – 104. Males treated at 200 and 750 ppm exhibited decreases in body weight gain during the 18-month to 2-year period of the study, as compared to controls, which corresponded to non-statistically significant decreased mean body weights at study termination. However, these reductions in body weight gain/mean body weights for males at 200 and 750 ppm were not considered treatment related because they occurred only at the end of the study, were not evident at 90 days, 1 year or 18 months, and no clear dose-response was evident. In addition, males at 200 and 750 ppm did not show a decreased weight gain as compared to control in the corresponding oncogenicity study, using a higher number of animals per dose group. For females at 2000 ppm, a moderate reduction in overall body weight gain (17 %) was observed, which in terms of absolute body weight, attained statistical significance from week 1 – 40 and 45 – 96. Females at 750 ppm showed reduced body weight gains compared to controls over the first 88 weeks of study, and these reductions were considered treatment-related.

Changes in red blood cell parameters indicative of a mild anemia occurred at 2000 ppm, including decreases in red blood cell count (males and females) and decreases in hemoglobin level and hematocrit (females). Females receiving 2000 ppm exhibited increased bone marrow cellularity, consistent with erythropoiesis, indicative of a compensatory effect related to the anemia.

Other treatment-related histopathological findings included a statistically significant increased incidence of hepatocellular hypertrophy and/or increased amount of pigment in hepatocytes for females at 2000 ppm. In addition, an increased incidence of ”ground-glass” foci of cellular alteration in the liver for both sexes was observed at 2000 ppm and for females at 750 ppm. However, in neither sex did this change attain statistical significance. The altered hepatocytes contained pale, granular, acidophilic cytoplasm and a variable content of glycogen.
There were increased incidences of dilated mesenteric blood vessels, arteritis and testicular interstitial cell proliferation in males at 2000 ppm. No treatment-related organ weight changes were observed for either males or females at study termination.

Conclusion:
Based on decreases in body weight gain an increased incidence of ”ground-glass” foci of cellular alteration in the liver in females at 750 ppm, the NOAEL for chronic toxicity in this study was 200 ppm. This concentration of 200 ppm is equal to approximately 9 mg/kg bw/day, based on food consumption data.

[redacted] 1990, SAG 151: 104 week dietary carcinogenicity study in rats (BASF DocID DK-428-005)

Dimethomorph technical was administered to groups of 50 male and 50 female Sprague-Dawley rats at dietary concentrations of 0, 200, 750, and 2000 ppm for 104 weeks. Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Differential blood counts were determined for all surviving animals during weeks 51/52, 78/79 and 103/104 of the study. After 24 months, the animals were subjected to detailed gross and histopathological examinations.

Findings:

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Mean test substance intake was 8.8, 33.9, and 94.6 mg/kg bw/day for males and 11.3, 46.3, and 132.5 mg/kg bw/day for females of the 200, 750, or 2000 ppm dose group.

There were no treatment-related effects on mortality or clinical signs of toxicity throughout the 104-week study period. Mortality rates were 56, 40, 32 and 46% (males) and 40, 38, 40 and 48% (females) at 0, 200, 750 and 2000 ppm, respectively. A slight decrease (7%) in absolute food consumption was noted for females fed 2000 ppm. Treatment related reductions in overall body weight gain were observed for males and females at 2000 ppm (14 and 38%, respectively) and for females at 750 ppm (23%) over a 2-year period, as compared to controls.

There were no treatment-related effects in differential blood counts at any time point. Non-neoplastic findings, similar to those observed in the 2-year dietary toxicity study in rats included a statistically significant increased incidence of dilated mesenteric blood vessels and arteritis in the abdominal vessels, predominantly in the pancreas, for males at 2000 ppm. There was a statistically significant increase in “ground-glass” foci of cellular alteration in the liver for males and females at 2000 ppm. The altered hepatocytes contained pale, granular, acidophilic cytoplasm and a variable content of glycogen. Furthermore there was a statistically significant increased incidence in hepatocellular pigmentation and hypertrophy and increased severity of bone marrow cellularity for females at 2000 ppm.

In both 104-week dietary toxicity studies in rats the incidences of testicular tumors in treatment groups were higher than incidences in control groups. However, for the following reasons dimethomorph is not considered to be oncogenic:

(1) Increased longevity in the chronic toxicity study (see Table 5.5-2)

For the 2-year chronic toxicity study, increased longevity observed for males at 2000 ppm likely predisposed these animals to increased incidences of benign interstitial cell tumors given that more animals in this group lived longer than animals in the other treatment groups or control group. Specifically, the survival rate at 104 weeks for males at 2000 ppm was 75%, as compared to 45, 65, and 70% for males at 0, 200, and 750 ppm, respectively. For premature male decedents, only one rat per each dietary concentration had a benign interstitial cell tumor. All other testicular tumors of this cell type were observed in males that survived until terminal sacrifice. In the 104 week carcinogenicity study the survival rate of male animals of the control group was also lower than the survival rates of male animals in the treatment groups. However, the differences are lower than in the chronic toxicity study.

Table 5.5-2: Mortality in male rats receiving dimethomorph for 104 weeks

Interval (week)		Dose level (ppm)		
	0	200	750	2000
Chronic toxicity study				
0 – 52	1/20 (5)[a]	1/20 (5)	0/20 (0)	0/20 (0)
53 – 78	3/19 (16)	2/19 (11)	0/20 (0)	2/20 (10)
79 – 104	7/16 (44)	4/17 (24)	6/20 (30)	3/18 (17)
0 - 104	11/20 (55)	7/20 (35)	6/20 (30)*	3/20 (25)
Carcinogenicity study				
0 – 52	1/50 (2)	0/50 (0)	3/50 (6)	2/50 (4)
53 – 78	5/49 (10)	6/50 (12)	7/47 (15)	5/48 (10)
79 – 104	22/44 (50)	14/44 (32)	6/40 (15)	16/43 (37)
0 - 104	28/50 (56)	20/50 (40)	16/50 (32)	23/50 (46)
Range historical mortality (0 – 104 weeks) 30 – 56 %				

a: the number in bracket presents % mortality, * $p < 0.05$

(2) No statistically significant oncogenic effects

There was no statistical significance in the incidence of testicular interstitial cell adenomas between the control group and any of the dimethomorph treated groups for either study ($p > 0.05$). Moreover, in a trend analysis, considered to be a more sensitive test to detect dose-related trends than a pair-wise comparison, no statistically significant positive trends were observed ($p > 0.05$). See Table 5.5-3

Table 5.5-3: Incidence of testicular interstitial cell adenoma in male rats receiving dimethomorph for 104 weeks

		Dose level (ppm)		
	0	200	750	2000
Chronic toxicity study				
	2/19[a] (10.5)[b]	5/20 (25)	4/20 (20)	6/20 (30)
Trend test p*	0.14			
Carcinogenicity study				
	5/50 (10)	7/49 (14)	8/50 (16)	10/50 (20)
Trend test p*	0.11			

*p values reported in this table were from Peto analysis

a number of animals with IC adenoma/number of animals examined

b number in bracket is percent value

(3) Incidence values for benign testicular interstitial cell tumors are within or just slightly higher the historical ranges for the testing laboratory (see Table 5.5-4). The range of the historical spontaneous incidence of testicular interstitial cell adenomas for carcinogenicity studies performed at [redacted] the laboratory which conducted the rat carcinogenicity study on dimethomorph, is from 4 % (minimal control incidence) to 20 % (maximal control incidence). Therefore, the 20 % incidence of interstitial cell adenoma of the testes in the high-dose group from the rat carcinogenicity study with dimethomorph is within the range of the historical control incidence of this laboratory. In addition, the incidence from the combined two studies for interstitial cell adenoma of the testes in the high-dose group is 16/70 (22.9%), just slightly higher the maximum historical incidence.

Table 5.5-4: Historical incidence of neoplastic and focal hyperplastic lesions in testicular interstitial cells of control Sprague-Dawley rats in 104 week studies at [redacted]

Study I.D.	Date	No examined	Incidence *		
			Adenoma	Hyperplasia	Combined
198	1986-1988	50	10 (20)[a]	0	10 (20)
507	1987-1989	50	9 (18)	1 (2)	10 (20)
827	1986-1988	48	4 (8.3)	9 (18.8)	13 (27.1)
261	1985-1987	49	2 (4.1)	7 (14.3)	9 (18.4)
717	1985-1987	48	5 (10.4)	3 (6.2)	8 (16.7)
671	1984-1986	50	6 (12)	1 (2)	7 (14)
729	1984-1986	50	4 (8)	0	4 (8)
802	1984-1986	50	6 (12)	0	6 (12)
Range			4.1 – 20 %	0 – 18.8 %	8 – 27.1 %

***There was no single case of malignant tumours recorded in these studies**
a Numbers in bracket represents incidence in %

(4) No progression of benign testicular adenomas to malignant carcinomas
There was no evidence of progression of benign interstitial cell adenomas to malignant interstitial cell carcinomas.

(5) No decreased latency for the benign testicular interstitial cell tumors from the high-dose group versus the controls.
There was no indication that testicular interstitial cell adenomas developed earlier in treated rats, as compared to controls (see Table 5.5-5).

Table 5.5-5: Observation of testicular IC tumours in premature decedents in male rats receiving dimethomorph

			Dose level (ppm)		
	0		200	750	2000
		Chronic toxicity study			
week	1/10[a] 94[b]		1/7 102	1/6 99	1/5 92
		Carcinogenicity study			
week	1/28 87		0/19	1/16 91	3/23 75, 100, 101

a number of animals with tumour/number examined
b week of study when incidence IC tumours were found

(6) Lack of oncogenic effects for the testes in the 2-year oncogenicity study in the mouse

(7) Absence of mutagenic or genotoxic activity of dimethomorph

(8) Very conservative size criterion was used on microscopic examination to diagnose benign testicular interstitial cell adenomas. Thus, some of these benign tumors may be downgraded to interstitial cell hyperplasia. The major criterion used for the differential diagnosis of benign testicular interstitial cell adenoma versus hyperplasia was based on size. The pathologists considered that any focal, expansile aggregates of interstitial cells of greater diameter than an average arteriole but smaller than a cross section of a seminiferous tubule were recorded as focal hyperplasia. Those greater in diameter than an average cross section of seminiferous tubule were classified as an adenoma. However, less conservative, present day diagnostic criteria for standardization purposes recommend that the diameter (or cross sections) of 3 or more seminiferous tubules be set as the arbitrary separation of interstitial cell neoplasia, as distinguished from focal hyperplasia. Thus, some of the benign interstitial cell adenomas may possibly downgraded to focal interstitial hyperplasia, using the present-day, less conservative size criterion.

Conclusion:
Based on a decrease in overall body weight gain for females at 750 ppm, the NOAEL for chronic toxicity for this study is 200 ppm, equivalent to approximately 9 mg/kg bw/day. Dimethomorph is not considered to be oncogenic.

[redacted] 1991, SAG 151: 104 week dietary carcinogenicity study in mice (BASF DocID DK-428-004)

Dimethomorph technical was administered to groups of 50 male and 50 female [redacted] CD-1 mice at adjusted nominal dietary dose levels of 0, 10, 100, and 1000 mg/kg bw/day for 104 weeks. The concentrations of the test material in the diet were adjusted according to body weights and dietary intake at weekly intervals for the first 13 weeks of the study and every 4 weeks thereafter, in order to maintain a constant test material intake. Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. A check of the general state of health of the animals was made at least daily. Additionally, the animals were examined in detail and palpated once a week. Differential blood counts were evaluated after 52, 78 and 104 weeks of treatment for all surviving animals in the control and 1000 mg/kg bw/day groups. A satellite group of 15 mice/sex received the test material at a dose level of 1000 mg/kg bw/day and a control satellite group of 4 males and 6 females were designed to provide differential blood counts, clinical chemistry, organ weight and liver histology data following 13 and 52 weeks of treatment. All satellite control animals and 8 satellite animals/sex in the 1000 mg/kg bw/day group were sacrificed following 13 weeks of treatment. All other satellite animals in the 1000 mg/kg bw/day group were sacrificed after 52 weeks of treatment (without concurrent controls).

Findings:
The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Mean test substance intake was 9.8, 98.0, and 978.0 mg/kg bw/day for males and 9.8, 96.8, and 977.0 mg/kg bw/day for females of the 10, 100, or 1000 ppm dose group.
Survival for males and females was unaffected by administration of the test material as adjusted survival rates for the treated groups were comparable to or greater than the controls. Specifically, adjusted survival rates at study termination for the control, 10, 100 and 1000 mg/kg bw/day groups were 54%, 78%, 50% and 74%, respectively for males, and 52%, 44%, 50% and 54%, respectively for females. No overt clinical signs were attributed to administration of the test material during the 24-month study period. Food consumption values for males and females at all treatment levels were comparable to controls for most measurement intervals during the 24-month treatment period. Statistically significant reductions in body weights were observed during weeks 17 through 84 and from weeks 92-104 for males at 1000 mg/kg bw/day when compared to controls. Overall body weight gains were reduced 17% for males at 1000 mg/kg bw/day when compared to controls. Although no significant differences in body weights were observed during the 24-month treatment period for females at any treatment level when compared to controls, overall body weight gains for females at 100 and 1000 mg/kg bw/day were reduced 11% and 21%, respectively, when compared to controls. The reductions in overall body weight gain for females at 100 and 1000 mg/kg bw/day were considered treatment-related.

Differential blood counts evaluated at approximately 52, 78 and 104 weeks of treatment in the control and 1000 mg/kg bw/day groups were unaffected by treatment with dimethomorph technical. After 13 weeks of treatment, alkaline phosphatase activity was significantly increased in males at 1000 mg/kg bw/day and aspartate aminotransferase activity was significantly increased for females in the 1000 mg/kg bw/day group (satellite animals) when compared to controls. In addition, slight but non-statistically significant increases in alanine aminotransferase activity (both sexes) and aspartate aminotransferase activity (males) was observed at 13 weeks for the 1000 mg/kg bw/day satellite group when compared to controls. Increases in absolute liver weights and liver-to-body weight ratios were observed for satellite male and female mice (1000 mg/kg bw/day) sacrificed after 13 weeks, when compared to concurrent controls. Absolute and relative liver weights remained elevated after 52 weeks of treatment when compared to historical controls; no concurrent control organ weight data were available for comparison. No dose-related microscopic liver findings were observed in the satellite animals at either the 13 or 52-week sacrifice. No treatment-related macroscopic or microscopic changes (neoplastic or non-neoplastic) were observed in any of the tissues evaluated from animals sacrificed following 13, 52 or 104 weeks of treatment or from any of the animals found dead or sacrificed moribund during the study. An increase in pulmonary tumors was observed at terminal sacrifice for males when compared to controls (see Table 5.5-6). However, this increase was not considered treatment-related for the following reasons:

(1) The incidence of pulmonary adenomas for males in the high-dose (1000 mg/kg bw/day) group (17 of 50) was not statistically significantly different from controls (13 of 50) by the Fisher Exact Test ($p < 0.05$). Thus, there was no dose-response relationship for the increased incidence of benign lung tumors for males because the high-dose level (1000 mg/kg bw/day), a dose level that is 10 times greater than the mid-dose level, showed no increase in this lesion.

(2) Males in the 1000 mg/kg bw/day group (highest dose tested) demonstrated a higher survival rate at termination as compared to the survival rate of males in the control and 100 mg/kg bw/day groups. The survival rate at termination (Weeks 104-105) for males at 1000 mg/kg bw/day was 74 % versus survival rates of 52 % and 50 % for males in the control and 100 mg/kg bw/day groups, respectively.

(3) There was no decreased time in appearance of benign pulmonary adenomas for either the mid-dose or high-dose males, when compared to controls.

(4) There was no evidence of any treatment-related increase in the incidence of pulmonary pre-neoplastic lesions (i.e., hyperplastic foci) or neoplastic lesions (adenomas or adenocarcinomas) in female CD-1 mice in the 24-month dietary oncogenicity study with dimethomorph technical.

Table 5.5-6: Incidence of Histology finding in males – Lungs (premature death and terminal kill combined).

	Control	10 mg/kg bw/day	100 mg/kg bw/day	1000 mg/kg bw/day
Number of animals	**50**	**50**	**50**	**50**
Pulmonary proliferative lesions				
One Bronchiolar/Alveolar hyperplastic focus	**2**	**1**	**2**	**3**
One pulmonary adenoma	**8**	**10**	**21**	**15**
Two pulmonary adenomas	**5**	**2**	**2**	**2**
Three pulmonary adenomas	**0**	**2**	**0**	**0**
One pulmonary adenomcarcinoma	**2**	**0**	**2**	**0**
Total with Hyperplastic focus	**2**	**1**	**2**	**3**
Total with benign tumour	**13**	**14**	**23**	**17**
Total with malignant tumour	**2**	**0**	**2**	**0**
Total with proliferative lesions	**15**	**14**	**26**	**19**

Conclusion:
Based on reduced body weight gains in females of more than 10% at 100 mg/kg bw/day (approximately 97 mg/kg bw/day, based on food consumption data), the data for this chronic oncogenicity study in the mouse support a systemic toxicity NOAEL of approximately 10 mg/kg bw/day, based on food consumption data. Dimethomorph is not considered to be oncogenic.

CA 5.6 Reproductive Toxicity

Studies evaluated in the draft monograph of Rapporteur Member State Germany

In the two-generation reproduction toxicity study conducted with Sprague-Dawley rats, dimethomorph technical did not affect reproductive performance. The NOAEL for parental toxicity was 300 ppm (equivalent to 20 mg/kg bw/day), based on reductions in pre-mating body weight gains for P1 and F1 females in the 1000 ppm group (highest concentration tested) and an increase in the percentage of females with pregnancy duration of 21 days. The NOAELs for the growth and development of offspring, as well as for fertility and reproductive function, were 1000 ppm (equivalent to 67 mg/kg bw/day), the highest concentration tested.

Developmental toxicity studies with dimethomorph, conducted in Sprague-Dawley rats and in New Zealand White rabbits, showed no evidence of teratogenic effects for fetuses, and no evidence of developmental toxicity in the absence of maternal toxicity.

In the rat developmental toxicity study, the NOAEL for maternal toxicity was 60 mg/kg bw/day based on decreased body weights, body weight gains, and food consumption at 160 mg/kg bw/day (highest dose tested). The NOAEL for developmental toxicity was also 60 mg/kg bw/day, based on a slightly increased number of total litter losses at 160 mg/kg bw/day.

In the rabbit developmental toxicity study, the NOAEL for maternal toxicity was 300 mg/kg bw/day based on decreased body weight gains and food consumption at 300 mg/kg bw/d and a slightly increased abortion rate at 650 mg/kg bw/day. The NOAEL for developmental toxicity was 300 mg/kg bw/day based on a slightly increased embryo-lethality presenting as abortion.

Thus, dimethomorph is neither a selective developmental toxicant nor a teratogenic agent in the Sprague-Dawley rat or the New Zealand White rabbit.

Table 5.6-1: Summary of reviewed dimethomorph reproductive toxicity studies

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
2-generation dietary reproductive toxicity; Sprague-Dawley rats;	0, 100, 300, 1000 ppm	Parental: 20 Reproductive: 67	Parental: 67	↓body weight gain; ↓duration of pregnancy	DK-430-001
Preliminary oral developmental toxicity; Sprague-Dawley rats	0, 50, 120, 300	Maternal: 300 Embryofetal: 300	-	No effects	DK-432-001
Preliminary oral developmental toxicity; Sprague-Dawley rats	150, 300	Maternal: ND Embryofetal: ND		Maternal: Intra-uterine death at all doses Embryofetal: Reduced mean fetal weight at 300 mg/kg bw/day	DK-432-006
Oral developmental toxicity; Sprague-Dawley rats	0, 20, 60, 160	Maternal: 60 Embryofetal: 60	Maternal: 160 Embryofetal: 160	Maternal: ↓body weight gain; ↓food consumption Embryofetal: slightly ↑early resorption rate	DK-432-002
Preliminary oral developmental toxicity; New Zealand White rabbits	0, 300, 600, 1000	 Embryofetal: 300	Maternal: 300 Embryofetal: 600	Maternal: ↓body weight gain; ↓food and water consumption Embryofetal: slightly ↑early resorption rate	DK-432-003
Oral developmental toxicity; New Zealand White rabbits	0, 135, 300, 650	Maternal: 300 Embryofetal: 300	Maternal: 650 Embryofetal: 650	Maternal: ↓body weight gain; ↓food consumption; slightly increased abortion rate Embryofetal: ↑prenatal lethality (abortion)	DK-432-004

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the DAR/EFSA conclusion of dimethomorph (EFSA scientific report, 82, 1-69, 2006):

Reproductive toxicity	
Reproduction target / critical effect:	No reproductive toxicity at parental toxic dose level
Lowest relevant reproductive NOAEL / NOEL:	Reproductive: 67 mg/kg bw/day Parental and offspring: 20 mg/kg bw/day
Developmental target / critical effect:	Slightly increased early resorption rate at very slight maternal toxic doses in rat and rabbit.
Lowest relevant developmental NOAEL / NOEL:	Developmental and maternal: 60 mg/kg bw/day (rat) 300 mg/kg bw/day (rabbit)

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of dimethomorph and are provided under the respective chapters. Evaluations provided are according to the opinion of the RMS of the last EU review. Additional data or argumentations provided for the already evaluated studies are marked in green.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

There is one extended 1-generation toxicity study in Wistar rats performed in 2014 that was not submitted in the former registration process ([see KCA 5.6.1/1 2014/1181670]). An extensive summary of this study is reported in the respective chapter.

Under the conditions of the present extended one-generation reproduction toxicity study the NOAEL (no observed adverse effect level) for general, systemic toxicity is 300 ppm, based on decreased food consumption and body weight/body weight gain, as well as clinical-chemical changes and pathological evidence of liver toxicity at 800 and/or 1600 ppm, in the F_0 parental animals and adult F_1 offspring.
The NOAEL for fertility and reproductive performance for the parental rats is 1600 ppm, the highest tested dose.
The NOAEL for developmental toxicity in the F_1 progeny is 300 ppm, due to the decrease in the pre-weaning pup body weights/pup weight gains, as well as decreased anogenital distance/index and delay of onset of puberty in males at 800 ppm.

Study	**Dosages (ppm)**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Main adverse effect at LOAEL**	**Reference (BASF DocID)**
Extended 1 generation Wistar rats	0, 300, 800, 1600.	Parental and offspring: 26 mg/kg bw/day (300 ppm) Reproductive: 144 mg/kg bw/day	Parental and offspring: 70 mg/kg bw/day (800 ppm)	Parental: effects on bw and bw gain. Liver toxicity in F_0 and F_1 Offspring: decrease in pre-weaning pup bw and bw gain. Decrease anogenital distance/index and delay of onset of puberty in males.	2014/1181670

Thus, the conclusion for relevant endpoints for the current re-registration is as follow:

Reproductive toxicity	
Reproduction target / critical effect:	No reproductive toxicity at parental toxic dose level
Lowest relevant reproductive NOAEL / NOEL:	Reproductive: 67 mg/kg bw/day Parental and offspring: 20 mg/kg bw/day
Developmental target / critical effect:	Slightly increased early resorption rate at maternal toxic doses in rat and rabbit.
Lowest relevant developmental NOAEL / NOEL:	Developmental and maternal: 60 mg/kg bw/day (rat) 300 mg/kg bw/day (rabbit)

Comparison with CLP criteria

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation 1272/2008/EC), substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.
Category 1: Known or presumed human reproductive toxicant
Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.
Category 1B: Presumed human reproductive toxicant
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
Category 2: Suspected human reproductive toxicant
Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Dimethomorph was tested in 2 reproductive toxicity studies in rats as well as in developmental toxicity studies in rats and rabbits. There are, however, no epidemiological data available regarding possible effects of Dimethomorph in humans, hence Dimethomorph cannot be placed in category 1A (CLP).
In the submitted 2-generation and extended 1-generation studies no findings with relevance for a classification for adverse effects on sexual function and fertility were reported up to the highest dose tested. Parental animals had slight effects on body weight and food consumption and did also show weak liver toxicity. Effects observed on the offspring were all weak and of transient nature: Slight weight effects on male sexual organs were observed in the extended 1-generation study, which can be partly explained by effects on body weight. The organ weight effects did not have a histopathological correlate and did not lead to any effect on sperm parameters. Consequently, no impairment of the fertility was associated with these slight changes. A weak effect on the onset of puberty was also noted in male and female animals that could not be fully attributed to body weight effects. However, these effects were not accompanied by any changes on male sexual hormones or sperm parameters in males or estrous cyclicity in females. No effects on fertility/reproduction were seen in the 2-generation study at similar doses. Therefore, no classification for effects on fertility/reproduction is proposed.

The prenatal developmental toxicity was investigated in rats and rabbits complying with international test guidelines and GLP. No malformations were observed in rats and in rabbits. Slight embryotoxicity was observed in both studies together with maternal toxicity. These effects were seen at the highest tested dose and considered to be due to marked maternal toxicity. An apparent increase of the incidence of early resorptions observed in high dose rats was shown to be due to total litter loss observed in two animals which had pronounced effects on food consumption and a marked body weight loss in the early phase of pregnancy. A similar situation was seen in the study in rabbits where in the high dose three abortions occurred. The affected dams all had a reduced food consumption early in pregnancy and lost body weight in the days preceding the abortions. Thus, the observed embryotoxicity was clearly secondary to pronounced maternal toxicity seen in individual dams and does not warrant a classification for developmental toxicity. Consequently, no classification was proposed during the first peer-review for Annex I inclusion.
Slight developmental effects were observed in the new extended 1-generation study. A decrease in the pre-weaning pup body weights/pup weight gains was seen with the animals recovering before reaching puberty at a high dose in the presence of slight maternal toxicity. A decreased anogenital distance/index in males was observed, but no effect on areola/nipple retention and also no effect on the sex ratio. These transient modifications on very sensitive developmental end-points are not related with altered physiological functions. Therefore, no classification is proposed for developmental toxicity.

CA 5.6.1 Generational studies

Report: CA 5.6.1/1
[redacted] 2014 a
Extended one-generation reproduction toxicity study in wistar rats - Administration via the diet
2014/1181670
Guidelines: OECD 443
GLP: yes

~~Remark: final study report will be sent in March 2016.~~

Executive Summary

Dimethomorph (BAS 550 F; Batch: COD-001646; Purity 99.7%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 300, 800, and 1600 ppm. The dietary concentrations of dimethomorph were adjusted to 0, 150, 400, and 800 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of dimethomorph administered to the rats during the entire study period was approx. 26 mg/kg bw/day in the 300 ppm group, approx. 70 mg/kg bw/day in the 800 ppm group and approx. 144 mg/kg bw/day in the 1600 ppm group.

There were no test substance-related mortalities or adverse clinical observations noted in any of the groups. In particular, regularly conducted detailed clinical observations revealed no effects at all.

Reduced food consumption was observed in the 1600 ppm dose groups in F0 females during lactation and in cohort 1A males throughout the study (800 and 1600 ppm). Body weights and weight changes were impaired in the F0 high dose animals (1600 ppm) and cohort 1A animals (800 and 1600 ppm). Treatment-related and adverse effects on liver weight together with centrilobular hepatocellular hypertrophy (females of high dose group) were observed in F0 and cohort 1A animals from 800 ppm onwards.

No indications of adverse effects on fertility or reproductive performance as well as no severe effects on development were observed. Only pup body weight development was affected in the 1600 ppm dose group (-13% after birth) but had no effect on pup survival.

A slight effect on anogenital distance/index in mid and high dose males as well as a significant delay in vaginal opening (female F1 offspring of 1600 ppm group) and preputial separation (male F1 offspring of 800 and 1600 ppm group) were observed and may be partly explained by the decreased body weight leading to slow general development. All effects on development observed after treatment with dimethomorph were of low severity and transient in nature. Hormone measurements (luteinizing hormone and testosterone) in male animals of cohort 1A did not reveal any changes.

Under the conditions of the present extended one-generation reproduction toxicity study the NOAEL (no observed adverse effect level) for general systemic toxicity is 300 ppm, based on decreased food consumption and body weight/body weight gain, as well as clinical-chemical changes and pathological evidence of liver toxicity at 800 and/or 1600 ppm, in the F_0 parental animals and adult F_1 offspring.
The NOAEL for fertility and reproductive performance for the parental rats is 1600 ppm, the highest tested dose.
The NOAEL for developmental toxicity in the F_1 progeny is 300 ppm, due to the decrease in the pre-weaning pup body weights/pup weight gains, as well as decreased anogenital distance/index in males and delay of puberty in males at 800 and 1600 ppm.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 550 F (Dimethomorph)
Description:	solid / beige
Lot/Batch #:	COD-001646
Purity:	99.7% (tolerance +- 1.0%)
Stability of test compound:	The test substance was stable over the study period at room temperature; (Expiry date: 31.01.2016)

2. Vehicle and/or positive control: rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar, Crl:WI(Han)
Sex:	Male and female
Age:	F_0 parental animals: 28 ± 1 days at delivery; 33 ± 1 days at beginning of treatment
Weight at dosing:	♂: 97.1 - 133.5 g, ♀: 89.8 - 112.5 g
Source:	[redacted]
Acclimation period:	6 days
Diet:	Kliba maintenance diet rat/mouse "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	drinking water from water bottles, ad libitum
Housing:	Groups of 5 animals/sex/group housed in Polysulfonate cages (H-Temp, supplied by TECHNIPLAST, Hohenpeßenberg, Germany) having a floor area of about 2065 cm², with the following exceptions:
	During the mating, gestation and lactation period the rats were housed individually in Makrolon type M III cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area of about 800 cm²), with the following exceptions:
	• During overnight mating male and female mating pairs were housed together in Makrolon type M III cages overnight

- pregnant animals and their litters housed together until PND 21 (end of lactation)

bedding: wooden, dust-free

enrichment: wooden gnawing blocks (Typ NGM E-022 supplied by Abedd® Lab. and Vet. Service GmbH, Vienna, Austria);

nesting material: cellulose wadding

Environmental conditions:

Temperature:	20 - 24°C (central air-conditioned rooms)
Humidity:	30 - 70% (central air-conditioned rooms)
Air changes:	15 times per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 25-Jun-2013 - 28-Jul-2015
(In-life dates: 02-Jul-2013 (start of administration of F_0 parental animals) to 11-Nov-2013 (sacrifice of F_0 parental animals) and 05 Nov-2013 (start of administration of F_1 animals) to 14-Jan-2014 (sacrifice of F_1 animals))

2. Animal assignment and treatment:

Dimethomorph was administered to groups of 25 male and 25 female rats at nominal dietary dose levels of 0 (ground diet), 300 (low dose), 800 (mid dose), and 1600 ppm (high dose). The animals used as F_0 parental animals were derived from different litters according to a written statement from the breeder. By this, sibling mating was avoided. The animals were randomly assigned to the test groups by means of computer generated randomization list based on body weights.

After the acclimatization period F_0 parental animals continuously received the test-substance throughout the entire study. Prior to sacrifice food was withdrawn overnight (approx. 16 hours).
At least 75 days after the beginning of treatment, male and female F_0 rats of the same dose groups were mated overnight (details see below).
Females were allowed to deliver and rear their pups (F_1 generation pups) until day PND 4 (standardization; see below) or PND 21. After weaning of F_1 pups the F_0 generation parental animals were sacrificed.

Mating procedure: Males and females were mated overnight at a 1:1 ratio for a maximum of 2 weeks. Throughout the mating period, each female was paired with a predetermined male from the same dose group. The animals were paired by placing the female in the cage of the male mating partner from about 4.00 pm until 7.00 - 9.00 am of the following morning. Deviations from the specified times were possible on weekends and public holidays, and were reported in the raw data.
A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".

Standardization of litters (F_1 generation pups): On PND 4, the individual litters were standardized in such a way that, where possible, each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If individual litters did not have 4 pups/sex it was proceeded in such a way that the most evenly distributed 8 pups per litter were taken for further rearing (e.g., 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups.
With the exception of those F1 generation pups, which were chosen as F1 rearing animals, all pups were sacrificed by decapitation under isoflurane anaesthesia after standardization or weaning. All culled pups, including stillborn pups and those that died during their rearing period, were subjected to a macroscopic (external and visceral) examination.
All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were further evaluated on a case-by-case basis (e.g., histopathological evaluation or special staining), depending on the findings noted.

F_1 rearing animals (Cohort 1A and Cohort 1B): After weaning, 40 male and 40 female F_1 pups in test groups 00, 01, 02, and 03 (0, 300, 800 and 1600 ppm), became F_1 rearing animals in test groups 10, 11, 12, and 13. These animals were assigned to 2 different cohorts (Cohort 1A [one male and one female/litter (20/sex/group)] and Cohort 1B [one male and one female/litter (20/sex/group)]). These animals were chosen by lot and each litter was taken into account as far as technically feasible. If fewer than 25 litters were available in a group or if one sex was missing in a litter, more animals were taken from the other litters of the respective test group to obtain the required number of paired animals.
All selected rearing animals were treated with the test substance at the same dose level as their parents, from post-weaning through adulthood.
The F_1 rearing animals were sacrificed about 70 days after weaning.
Since there were no indications for neurohistopathological alterations or immunotoxicity of Dimethomorph in previous studies (acute and subchronic neurotoxicity, and 28-day immunotoxicity studies), the inclusion of cohorts for developmental neurotoxicity or immunotoxicity into the present extended 1-generation study is not required.

3. Test substance preparation and analysis:
The required quantity of test substance was weighed in a beaker depending on the dose group and thoroughly mixed with a small amount of food. Then further amounts of food were added to this premix and thoroughly mixed for 1 minute. Afterwards, further amounts of food, depending on the dose group, were added to this premix in order to obtain the desired concentrations. Mixing of this final mix was carried out for about 10 minutes in a laboratory mixer. Details of the mixers used are retained with the raw data.

Dimethomorph concentrations in the diet of the F_0 females were reduced to 50% during the lactation period. This dietary adjustment, derived from historical body weight and food consumption data, maintained the dams at constant dose-levels of Dimethomorph during this period of increased food intake.

Analytical verifications of the stability of the test substance in the diet for a period of 49 days at room temperature were carried out via HPLC-UV before the study was initiated with a comparable batch (AC 9978-131, purity: 98.3%).

Homogeneity and concentration control analyses were carried out at the beginning of the premating phase, lactation period and towards the end of the post-weaning period. For homogeneity analysis of the diet preparations 3 samples were taken from the top, middle and bottom of the beaker for the low (300 ppm) and high dose level (1600 ppm). Concentration control analyses were carried out towards the end of the premating phase. Duplicate samples were kept in reserve and will be discarded after report finalization.
The sample preparation and analysis of the test substance in feed was carried out according to the valid control procedure 01/0271_02-01 and 01/0271_02-02, respectively.

Table 5.6.1-1: Analysis of diet preparations for homogeneity and test-item content

Nominal Dose level [ppm]	Sampling	Concentration [ppm]	% of nominal concentration	% of mean nominal concentration[#]	Relative standard deviation [%]
300	02.06.13	314.40 ± 3.85		104.8	1.2
	24.09.13	309.07	103.0		
	*01.10.13**	*147.07 ± 8.33*		*98.0*	*5.7*
	18.10.13	307.26 ± 3.03		102.4	1.0
	28.11.13	300.53	100.2		
	06.01.14	316.59 ± 7.05		105.5	2.2
	average[§]	**309.57** ± 6.32		**103.2**	**2.0**
800	02.06.13	863.37	107.9		
	24.09.13	795.77	99.5		
	*01.10.13**	*414.85*	*103.7*		
	18.10.13	795.53	99.4		
	28.11.13	834.82	104.4		
	06.01.14	879.95	110.0		
	average[§]	**833.89** ± 38.46		**104.2**	**4.6**
1600	02.06.13	1590.08 ± 41.60		99.4	2.6
	24.09.13	1639.59	102.5		
	*01.10.13**	*806.04 ± 26.62*		*100.8*	*3.3*
	18.10.13	1575.99 ± 39.54		98.5	2.5
	28.11.13	1632.77	102.0		
	06.01.14	1648.34 ± 53.41		103.0	3.2
	average[§]	**1617.35 ± 32.20**		**101.1**	**2.0**

[#] based on mean values of the three individual samples

* concentrations in the diet of the F_0 females were reduced to 50% during the lactation period due to increased food consumption.

[§] except the sample form 01.10.13 with reduced concentration

No test substance could be detected in the control feed samples.

The relative standard deviation in the range of 1.0% to 2.2% and of 2.6% to 3.2% for the 300 and 1600 ppm dose levels, respectively, reveals the homogeneous distribution of the test item in the diet preparations.

The mean and single values of BAS 550 F (Dimethomorph) in Ground Kliba maintenance diet mouse/rat „GLP“ meal were found to be in the acceptable range of 90 % – 110 % of the nominal concentrations, thus demonstrating the correctness of the concentration of the test item in the diet preparations.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Parameter	Statistical test
Statistics of clinical examinations	
Food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), number of mating days, duration of gestation, number of implantation sites, post-implantation loss and % post-implantation loss, number of pups delivered per litter	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Male and female mating indices, male and female fertility indices, gestation index, females delivering, females with live-born pups, females with stillborn pups, females with all stillborn pups	Pair-wise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Mating days until day 0 pc, % post-implantation loss	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided+) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Implantation sites, pups delivered, live pups day x, viability index, lactation index	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided-) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Number of cycles and Cycle Length (days 54 -74)	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians
Statistics of clinical pathology	
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. For parameters with unidirectional changes: Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Urinalysis parameters (apart from pH, urine volume, specific gravity, colour and turbidity)	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) for the hypothesis of equal medians
Urine pH, urine volume, specific gravity, colour and turbidity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. Urine colour and turbidity are not evaluated statistically
Sperm parameters	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians. If only the control and one dose group are measured, WILCOXON test (one-sided) without adjustment were used. For the percentage of abnormal sperms values < 6% were set to 6% (cut off 6%).
Statistics of pathology	
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.
DOFC (differential ovarian follicular count)	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) for the hypothesis of equal medians

C. Methods

1. Observations:

The animals, i.e. parental animals and pups, were examined for mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.

Observations for evident signs of toxicity were performed at least once daily.
The parturition and lactation behaviour of the dams was generally evaluated in the mornings in combination with the daily clinical inspection of the dams. Only special findings (e.g., disability to deliver) were documented on an individual dam basis. Except on weekends and public holidays, the parturition behaviour was additionally checked in the afternoons. The day of parturition was considered the 24-hour period from about 15.00 h of one day until about 15.00 h of the following day.
The live pups were examined daily for clinical symptoms (including gross morphological findings) during the clinical inspection of the dams. If pups showed particular findings, these were documented with the dam concerned.

All F_0 parental animals and F_1 animals in Cohorts 1A and 1B were subjected to detailed clinical observations (including palpation) outside their cages once per week (as a rule in the mornings), by the same trained technicians, whenever possible. For observation, the animals were removed from their cages and placed in a standard arena (50 x 37.5 cm with a lateral border of 25 cm) for at least 20 seconds/animal. The scope of examinations and the scoring of the findings were lexicon-based. Because not all potential observations were contained in the lexicon, free-field descriptions were also allowed. Examinations include but were not limited to the following parameters:

Detailed clinical observation (DCO) parameters	
1. abnormal behaviour during “handling”	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Body weight:

Body weight of parental F_0 animals and F_1 rearing animals was determined on the first day of the premating period and weekly thereafter at the same time of the day (in the morning), with following exceptions:

a. During pregnancy, body weight of the F_0 females with evidence of sperm was determined weekly for GD 0-7, 7-14, and 14-20.
b. F_0 females were not weighed during mating until there was a positive evidence of sperm in vaginal smears
c. Females without litter were not weighed during the lactation phase.
d. During lactation, body weight of the F_0 females, which gave birth to a litter was determined for PND 1-4, 4-7, 7-14, and 14-21.

Pup body weights were determined on the day after birth (PND 1) and on PND 4 (before standardization), 7, 14, and 21.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once a week (for a period of 6 days) for parental F_0 animals and F_1 rearing animals, with the following exceptions:

a. Food consumption was not determined after the 10th premating week (male F_0 animals) and during the mating period (male and female F_0 animals).
b. Food consumption of F_0 females during pregnancy was determined weekly for GD 0-7, 7-14, and 14-20
c. During the lactation period, food consumption of the F_0 females which gave birth to a litter was determined for PND 1-4, 4-7, 7-14, and 14-21.
d. No food consumption was determined during the mating period and for females without positive evidence of sperm and females without litter.

The mean daily intake of test substance (group means in mg/kg bw/day) was calculated based upon individual values for body weight and food consumption:

$$IT_x = \frac{FCx * C}{BWx}$$

IT_x = intake of test substance on day x [mg/kg bw/day]
BW_x = body weight on study day x [g]
FC_x= food consumption on day x [g]
C = concentration [ppm]

4. Oestrous cycle determination:

Oestrous cycle length was evaluated by daily analysis of vaginal smear for all F_0 female parental rats for a minimum of 3 weeks prior to mating. Determination was continued throughout the pairing period until the female exhibited evidence of copulation. At necropsy, an additional vaginal smear was examined to determine the stage of oestrous cycle for each F_0 female with scheduled sacrifice.
For all cohort 1B females, oestrous cycle length and normality was evaluated by preparing vaginal smears during a minimum of 3 weeks prior to necropsy.
In all cohort 1A females, vaginal smears were collected after vaginal opening until the first cornified smear (oestrous) was recorded. The oestrous cycle also was evaluated in cohort 1A females for 2 weeks around PND 75.

5. Male and female reproduction data:

Male reproduction data

The pairing partners, the number of mating days until vaginal sperm was detected in the female animals, and the gestational status of the females were recorded for F_0 breeding pairs.
For the F_0 males, mating and fertility indices were calculated for F_1 litters according to the following equations:

$$\text{Male mating index (\%)} = \frac{\text{number of males with confirmed mating} *}{\text{number of males placed with females}} \; x \; 100$$

*defined by a female with vaginal sperm or with implants in utero

$$\text{Male fertility index (\%)} = \frac{\text{number of males proving their fertility} *}{\text{number of males placed with females}} \; x \; 100$$

*defined by a female with implants in utero

Female reproduction and delivery data

The pairing partners, the number of mating days until vaginal sperm were detected, and gestational status were recorded for F_0 females.
For the F_0 females, mating, fertility and gestation indices were calculated for F_1 litters according to the following equations:

$$\text{Female mating index (\%)} = \frac{\text{number of females mated} *}{\text{number of females placed with males}} x\ 100$$

*defined by a female with vaginal sperm or with implants in utero

$$\text{Female fertility index (\%)} = \frac{\text{number of females pregnant} *}{\text{number of females mated} **} x\ 100$$

*defined by a female with implants in utero
**defined as the number of females with vaginal sperm or with implants in utero

$$\text{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant} *} x\ 100$$

*defined as the number of females with implants in utero

The total number of pups delivered and the number of live-born and stillborn pups were noted, and the live birth index was calculated for F_1 litters:

$$\text{Live birth index (\%)} = \frac{\text{number of live} - \text{born pups at birth}}{\text{total number of pups born}} x\ 100$$

The implantations were counted and the post-implantation loss (in %) was calculated. To determine the number of implantation sites, the apparently non-pregnant uteri were stained for about 5 minutes in 1% ammonium sulphide solution according to the method of SALEWSKI.

$$\text{Post-implantation loss (\%)} = \frac{\text{number of implantations} - \text{number of pups delivered}}{\text{number of implantations}} x\ 100$$

6. Litter data

All F_1 pups were examined as soon as possible on the day of birth to determine the total number of pups, the sex and the number of live-born and stillborn members of each litter. At the same time, the pups were also being examined for macroscopically evident changes. Pups, which died before the first examination on the day of birth, were designated as stillborn pups.
The number and percentage of dead pups on the day of birth (PND 0) and of pups dying between PND 1-4, 5-7, 8-14 and 15-21 (lactation period) were determined; however, pups, which died accidentally or had to be sacrificed due to maternal death, were not included in these calculations. The number of live pups/litter was calculated on the day after birth, and on lactation days 4, 7, 14, and 21. Furthermore, viability and lactation indices were calculated according to the following formulas:

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4} * \text{after birth}}{\text{number of live pups on the day of birth}} \; x \; 100$$

*before standardisation of litters (i.e. before culling)

$$\text{Lactation index (\%)} = \frac{\text{number of live pups on day 21after birth}}{\text{number of live pups on day 4} * \text{after birth}} \; x \; 100$$

*after standardisation of litters (i.e. after culling)

One the day after birth (PND 1) the sex of the pups was determined by observing the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups. The anogenital index was calculated according to the following formula:

$$\text{anogenital index} = \frac{\text{anogenital distance [mm]}}{\text{cubic root of pup weight [g]}}$$

Later, during the course of lactation, this initial sex determination was followed up by surveying the external appearance of the anogenital region and the mammary line. The sex of the pups was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 after birth using the following equation:

$$\text{Sex ratio} = \frac{\text{number of live male or female pups on day } 0/4}{\text{number of live male and female pups on day } 0/4} \; x \; 100$$

All surviving male pups were examined for the presence of nipple/areola complex on PND 12 and were re-examined on PND 20 before necropsy.

Date of sexual maturation of all F_1 pups was recorded.
All female F_1 pups selected to become the F_1 rearing females for Cohort 1A and Cohort 1B (20/group/cohort) were evaluated daily for vaginal patency beginning on PND 27. On the day of vaginal opening the body weights of the respective animals were determined.
All male F1 pups selected to become the F1 rearing males for Cohort 1A and Cohort 1B (20/group/cohort) were evaluated daily for preputial separation beginning on PND 38. On the day of preputial separation the body weights of the respective animals were determined.

7. Clinical pathology

Samples were withdrawn from 10 F_0 parental and Cohort 1A males and females per group at termination.

The assays of blood and serum parameters were performed under internal laboratory quality control conditions with reference controls to assure reliable test results.

The results of clinical pathology examinations were expressed in International System (SI) units.

Haematology and clinical chemistry:

Blood samples were taken from animals by puncturing the retro-bulbar venous plexus following isoflurane anaesthesia. Blood sampling and blood examinations were carried out in a randomized sequence. The list of randomization instructions was compiled with a computer.

Haematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time
✓	Haemoglobin (HBG)	✓	Neutrophils (differential)	✓	Platelet count
✓	Haematocrit (HCT)	✓	Eosinophils (differential)		Partial thromboplastin time
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		Thromboplastin time
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells (differential)		
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyltransferase (GGT)
✓	Potassium	✓	Globulin (by calculation)		
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Hormones:

Additionally to F_0 parental and Cohort 1A males and females, blood samples for hormone determination were withdrawn from 10 surplus (culled) PND 4 offspring (as far as possible of different litters) per sex and group. PND 4 samples were pooled per sex and litter if the available amount is not sufficient for a hormone analysis. Furthermore, blood samples were withdrawn from 10 surplus PND 22 offspring (as far as possible of different litters) per sex and group.

The blood samples was collected after decapitation (following isoflurane anaesthesia) from the Vena cava cranialis.

The concentrations of TSH and T_4 were determined with a radioimmunoassay (RIA) on a Gamma-Counter and ELISA, respectively.

Three days before sacrifice, in 10 male animals of cohort 1A blood samples has been taken from animals by puncturing the retrolobular venous plexus following isoflurane anesthesia. Blood sampling and blood examinations has been carried out in a randomized sequence. Animals were not fasted before blood sampling. The following hormones have been measured in the prepared serum samples:

- LH
- Testosterone

Urinalyses:

In the afternoon preceding the day of urinalysis, the animals were individually transferred into metabolism cages (no food or drinking water provided); on the following morning, the individual urine specimens were examined in a randomized sequence for the following parameters:

Urinalysis					
	Quantitative parameters:		*Semi-quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity (visual exam.)	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscop.exam.)
		✓	Ketones		Nitrite

Sperm parameter:

After the organ weight determination, the following parameters were determined in the right testis or right epididymis of all male F_0 parental animals and cohort 1A males sacrificed on schedule:

- sperm motility
- sperm morphology
- sperm head count (cauda epididymis)
- sperm head count (testis)

Sperm motility examinations were carried out in a randomized sequence. Sperm head count (testis and cauda epididymis) and sperm morphology were evaluated in control and high dose animals, only.

8. Sacrifice and pathology:

All F_0 parental animals and Cohorts 1A and 1B (PND 90) rearing animals were sacrificed by decapitation under isoflurane anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology with special attention given to the reproductive organs. A quantitative assessment of primordial and growing follicles in the ovaries was performed for all control and high-dose F1A rearing females. As there were no effects on oestrous cyclicity evident, no histopathological investigation was performed in cohort 1B animals.

Female # 101 (F_0, 0 ppm) had unscheduled mated with male no. 40 (F_0, 300 ppm). It was sacrificed prematurely and no further investigations were performed. Female animal (# 317; Cohort 1A, 0 ppm) died inter-currently. It was necropsied and assessed by gross pathology as soon as possible after its death.

On PND 4, all surplus F_1 pups as a result of standardization were sacrificed by decapitation under isoflurane anaesthesia and blood was sampled for determination of serum thyroid hormone concentrations. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically.
On PND 22, the surplus F_1 generation pups that were not used for the formation of the cohorts were sacrificed by decapitation under isoflurane anaesthesia in the pathology lab and blood was sampled for thyroid hormone analyses. Anaesthetised animals, brain, spleen and thymus were weighed. All gross lesions, brain, mammary gland, spleen, thymus and thyroid glands were fixed in 4% formaldehyde solution, but no histopathological investigation was performed.

All stillborn pups and all pups that died before weaning were examined externally, eviscerated and their organs were assessed macroscopically.
All pups without notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were evaluated on a case-by-case basis, depending on the type of finding noted.

Pathology:
The following organs were collected (column C), weighed (W: ✓: F_0 and Cohort 1A and 1B, #: F_0 and Cohort 1A) and examined histopathologically (H: ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
			adipose tissue	✓	#	#	kidneys	✓	✓	✓	seminal vesicles**
✓	#	✓	adrenals				lachrymal glands				skin
			aorta	✓	#	✓	liver	✓		#	spinal cord[§]
✓		#	bone marrow (femur)	✓		#	lungs	✓	#	#	spleen
✓	#	#	brain	✓	#	#	lymph nodes**				sternum w. marrow
✓		#	caecum	✓		#	mammary gland	✓		#	stomach[§]
✓		#	colon					✓	✓	✓	testes[##]
	✓	#	cauda epididymis	✓		#	muscle, skeletal	✓	#	#	thymus
✓		#	duodenum	✓		#	nerve (sciatic)	✓	#	#	thyroid/ parathyroid glands
✓	✓	✓	epididymides[##]	✓		#	oesophagus				tongue
✓		#	eyes with optic nerve	✓	✓	✓	ovaries / oviduct[#]				tonsils
			femur (with knee joint)	✓		#	pancreas	✓		#	trachea
			gall bladder	✓	✓	✓	pituitary	✓		#	urinary bladder
✓		✓	gross lesions	✓	✓	✓	prostate	✓	✓	✓	uterus with cervix
✓	#	#	heart	✓		#	rectum	✓		✓	vagina
✓		#	ileum				salivary glands	✓		✓	vas deferens
✓		#	jejunum (with Peyer's patches)				hind-limb (one)		✓		body (fasted animals)

* mesenteric and axillaris, Cohort 1A animals only; ** including coagulation glands; [#] oviduct not weighted, ovaries fixed in modified Davidson's solution; [##] left epididymis and testis was fixed in modified Davidson's solution; [§] fore-stomach and glandular stomach; [$] cervical-, thoracic- and lumbar cord;

The ovaries of animal no. 317 (Cohort 1A, 300 ppm) that died were fixed in 4% buffered formaldehyde solution. The left testis and left epididymis of all male F_0 parental and Cohort 1A animals sacrificed at scheduled dates were fixed in modified Davidson's solution, whereas the right testis and epididymis were used for sperm parameters.
The uteri of all cohabited female F_0 parental animals were stained according to SALEWSKI's method.
Special attention was given to stages of spermatogenesis in the male gonads.
Special attention was given to the synchrony of the morphology in ovaries, uterus, cervix, and vagina to the oestrous cycle status.
Auto-fluorescence examination of the liver was performed in animals No 102, 177, 302, and 362 to determine the presence of lipofuscin.
Reproductive organs of all low- and mid-dose F_0 parental animals suspected of reduced fertility were subjected to histopathological investigation.
A correlation between gross lesions and histopathological findings was performed.

A differential ovarian follicle count (DOFC) was conducted in test groups 10 and 13 (Cohort 1A females, control and high dose group) according to Plowchalk et.al. 1993. Therefore, both ovaries from each female were embedded in paraffin blocks. Each block was sectioned serially until the total ovary was laminated. Sections were prepared with 2 µm thickness and every 100 µm three serial sections (1A, 1B, 1C / 2A, 2B, 2C / 3A, 3B, 3C /…) were taken and mounted on glass slides:

- the first of the serial sections 1A, 2A, 3A…was stained with haematoxylin and eosin,
- the second section (e.g. 1B, 2B, 3B…) was used for immunohistochemistry (using MVH antibody [mouse vasa homolog], a protein expressed in all oocyte stages),
- the third section (e.g. 1C, 2C, 3C …) was taken as possible negative control for immunohistochemistry (the third serial section of the 14th serial sections (14C) was used as negative control).
-

The immunohistochemically stained slides were used for counting of primordial and growing follicles. Starting with "section 2B", each second section (section Nos. 2B, 4B, 6B …) was evaluated of both ovaries of each female.
Primordial follicles and growing follicles were counted by light microscope (magnification: 100x), according to the definitions given by Plowchalk et al., 1993.
To prevent multiple counting on serial sections, especially of the growing follicles, only follicles with an oocyte with visible chromatin were counted.
The number of each type of follicle was recorded individually for both ovaries of every animal, giving in summary the incidence of each type of follicle. Finally, the results of all types of follicles were summarized for all animals per group in test groups 10 and 13. As primordial follicles continuously develop into growing follicles, the assessment of the follicles was extended to the combined incidence of primordial plus growing follicles.
In general, the 10th section stained with haematoxylin and eosin from all females of test groups 10 and 13 was evaluated for histological findings.

II. RESULTS AND DISCUSSION

A. CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE PERFORMANCE

A.1 F0 GENERATION PARENTAL ANIMALS

A.1.1 OBSERVATIONS

Clinical signs of toxicity

No clinical signs of toxicity or changes of general behaviour, which may be attributed to the test substance, were detected in any of the **male F_0** parental animals in any of the groups.

There were no test substance-related clinical findings in all **F_0 females** of all dose groups during premating, gestation and lactation periods for F_1 litter. Three sperm positive females of 1600 ppm dose group (# 182, 196 and 197) and one sperm positive female of 300 ppm dose group (# 133) did not deliver F_1 pups. One further sperm positive female of 300 ppm dose group (# 146) did not deliver pups but had implants in the uterus.

F_0 animals in any Dimethomorph treated dose group did not show any abnormalities detectable during detailed clinical observations.

Mortality

There were no test substance-related or spontaneous mortalities in any of the groups.

A.1.2 PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

Body weights of all parental **F_0 males** were comparable to the concurrent control group throughout the entire study period.

Body weights of all parental **F_0 females** were comparable to the concurrent control group throughout the entire premating period.
Body weights of the high-dose F_0 females were statistically significantly below the concurrent control values during GD 14-20 (up to 6%) and during PND 14-21 (up to 5%) as shown in Table 5.6.1-2.
Body weights of the mid- and low-dose parental females were comparable to the concurrent control group throughout the entire study period.

Table 5.6.1-2: Body weight development of F_0 females during gestation and lactation phases

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight [g]								
Gestation phase	N = 24		N = 24		N = 25		N = 22	
GD 0	223.8	13.5	223.0	15.7	223.5	13.6	216.5	13.0
Δ% (compared to control)			-0.4		-0.1		-3.3	
GD 7	245.9	16.0	240.5	16.6	244.6	13.5	235.6	12.0
Δ% (compared to control)			-2.2		-0.5		-4.2	
GD 14	269.6	18.1	262.6	18.1	267.6	13.9	**256.4***	13.0
Δ% (compared to control)			-2.6		-0.8		-4.9	
GD 20	332.6	23.7	323.8	27.7	328.7	19.9	**313.7***	19.0
Δ% (compared to control)			-2.7		-1.2		-5.7	
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1	252.8	17.0	247.2	16.4	250.5	13.1	245.8	16.9
Δ% (compared to control)			-2.2		-0.9		-2.8	
LD 4	260.7	19.0	259.5	20.0	265.4	13.8	256.0	15.5
Δ% (compared to control)			-0.5		1.8		-1.8	
LD 7	262.7	16.1	264.3	11.2	265.1	13.0	257.0	9.2
Δ% (compared to control)			0.6		0.9		-2.2	
LD 14	289.9	19.1	282.8	19.1	288.6	12.1	**276.1***	14.2
Δ% (compared to control)			-2.4		-0.5		-4.8	
LD 21	279.7	14.4	278.2	18.4	277.6	10.5	**269.0***	15.0
Δ% (compared to control)			-0.5		-0.7		-3.8	

* = $p<0.05$; Dunnett test (two-sided)
GD = gestation day; LD = lactation day

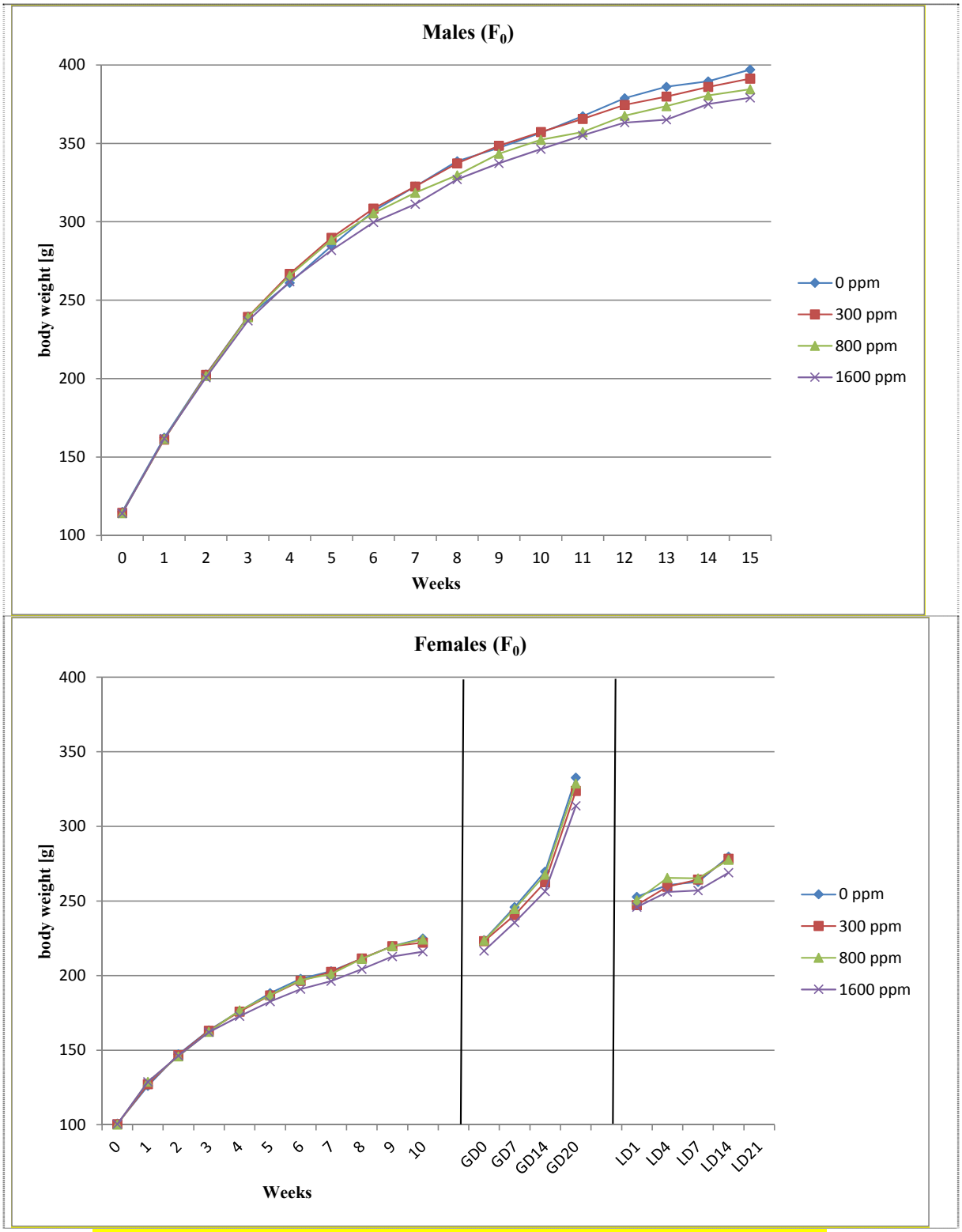


Figure 5.6.1-1: Body weight development of parental F_0 animals

The body weight change of the high-dose parental **F_0 males** was consistently below control during premating and mating, the difference gaining statistical significance during premating days 35 - 49 (up to 25% below control) [see Table 5.6.1-3]
The body weight change of the low- and mid-dose males was comparable to the concurrent control group throughout the entire study period.
Any other body weight changes in males, like the decreased body weight change of the mid-dose parental males during premating days 35 - 42 and 49 – 56, the statistically significantly increased body weight change in the mid- and low-dose males during premating days 56 - 63 and 21 - 28, respectively, and in the high-dose males during post-mating days 1 - 8 were considered to be spontaneous in nature and not treatment-related.

The body weight of the high-dose parental F0 females was below the concurrent control values during gestation, the difference to the control gained statistical significance on GD 7 - 14 and GD 0- 20 (about 12% and 11%, respectively) [see Table 5.6.1-3]

The body weight change of the low- and mid-dose females was generally comparable to the concurrent control group throughout the entire study.
Any other changes in females, like the decreased body weight change of the low-dose parental females during GD 0 - 7 and PND 7 – 14, and of the mid-dose parental females during premating days 7 – 14, as well as the increased body weight change in the mid-dose females during PND 1 – 4 were considered to be spontaneous in nature and not treatment related.

Table 5.6.1-3: Body weight development of F_0 animals during gestation and lactation phases

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight gain [g]								
F_0 males								
Premating phase	N = 25		N = 25		N = 25		N = 25	
Day 0-7	47.2	6.9	46.7	5.7	47.4	4.6	47.7	5.5
Δ% (compared to control)			-1.1		0.2		1.1	
Day 7-14	40.4	4.7	41.3	4.5	40.2	4.0	38.8	4.9
Δ% (compared to control)			2.2		-0.5		-3.7	
Day 14-21	36.9	5.1	37.0	5.2	37.4	5.4	36.2	5.8
Δ% (compared to control)			0.3		1.4		-2.2	
Day 21-28	21.6	12.5	**27.4***	4.6	26.6	5.4	25.0	6.7
Δ% (compared to control)			27.4		23.7		16.7	
Day 28-35	23.5	5.9	22.9	4.6	22.8	5.3	20.0	5.2
Δ% (compared to control)			-2.1		-3.0		-14.9	
Day 35-42	22.3	8.3	18.7	5.1	**16.9****	3.9	**17.7***	5.4
Δ% (compared to control)			-16.6		-24.2		-20.6	
Day 42-49	15.7	4.6	14.1	2.8	13.2	4.3	**11.7****	4.4
Δ% (compared to control)			-10.2		-15.9		-25.5	
Day 49-56	15.9	4.7	14.8	3.9	**11.2****	4.2	15.8	5.1
Δ% (compared to control)			-7.5		-30.0		-1.2	
Day 56-63	8.6	6.6	11.2	4.5	**13.6****	5.0	10.3	5.2
Δ% (compared to control)			30.2		59.3		18.6	
Day 63-70	9.7	12.5	8.8	3.6	8.8	3.6	9.0	3.9
Δ% (compared to control)			-8.3		-8.3		-5.2	
Day 0-70	241.7	32.1	242.9	31.8	238.1	30.3	232.2	36.1
Δ% (compared to control)			0.5		-1.5		-3.9	
F_0 females								
Gestation phase	N = 24		N = 24		N = 25		N = 22	
GD 0-7	22.2	5.3	**17.9***	4.3	21.1	5.9	19.2	4.1
Δ% (compared to control)			-20.8		-4.5		-13.6	
GD 7-14	23.7	3.6	21.5	4.2	23.0	4.5	**20.8***	3.3
Δ% (compared to control)			-6.8		-3.0		-12.2	
GD 14-20	62.9	12.8	61.2	14.2	61.2	10.5	57.3	9.7
Δ% (compared to control)			-2.9		-3.0		-9.0	
GD 0-20	108.8	15.3	100.8	18.0	105.2	13.6	**97.2***	9.9
Δ% (compared to control)			-7.4		-3.3		-10.7	

* = $p<0.05$; * = $p<0.01$; Dunnett test (two-sided)
GD = gestation day

A.1.3 PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of all **male F_0** rats in all dose groups was comparable to the concurrent control throughout the entire study period.

Food consumption of the high-dose **female F_0** rats was comparable to the concurrent control values throughout the premating and gestation periods. From PND 7 onwards throughout remaining lactation period, food consumption of the high-dose F_0 females was statistically significantly below (up to 8%) the concurrent control values [see Table 5.6.1-4**:**]. Food consumption of the female F_0 rats in the mid- and low-dose groups was comparable to the concurrent control values throughout the entire study.

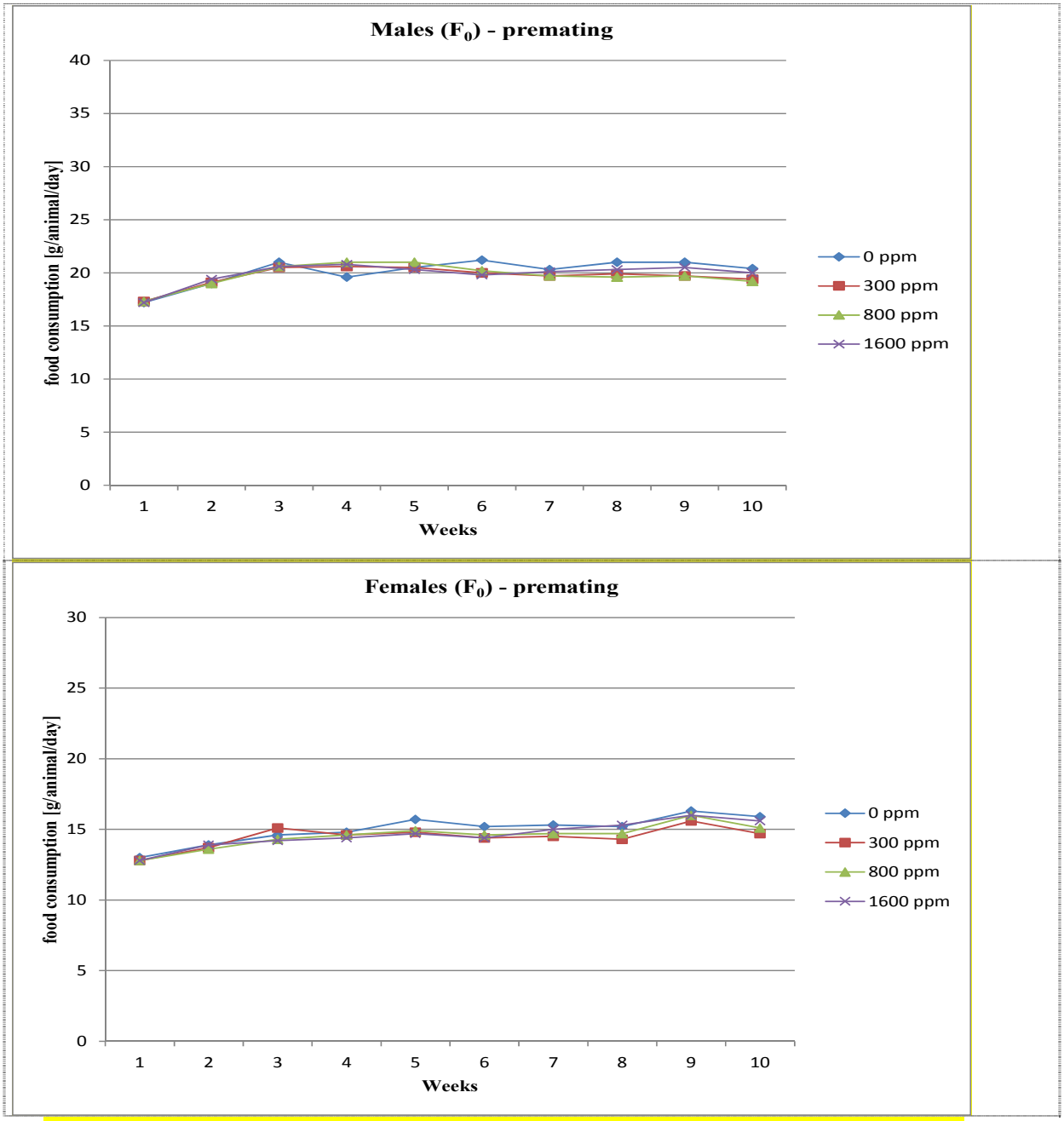


Figure 5.6.1-2: Food consumption of parental F_0 animals during premating

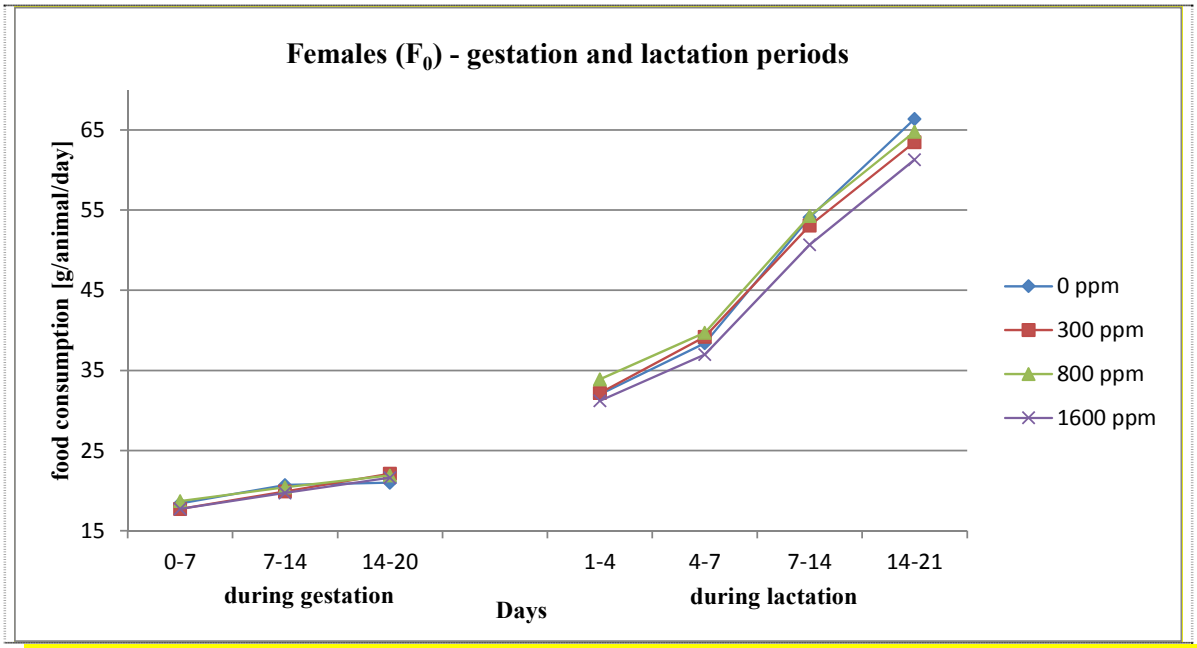


Figure 5.6.1-3: Food consumption of parental F_0 females during gestation and lactation periods

Table 5.6.1-4: Food consumption of parental F_0 females during lactation period

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Food consumption [g/rat/day]								
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1-4	32.0	4.0	32.2	4.8	33.9	4.4	31.2	5.3
Δ% (compared to control)			0.6		6.0		-2.4	
LD 4-7	38.4	2.9	39.2	3.1	39.7	3.2	37.0	2.7
Δ% (compared to control)			2.1		3.3		-3.7	
LD 7-14	54.1	4.5	53.1	5.2	54.3	3.4	**50.7***	5.6
Δ% (compared to control)			-2.0		0.2		-6.3	
LD 14-21	66.4	4.8	63.5	10.1	64.8	3.1	**61.3***	5.3
Δ% (compared to control)			-4.3		-2.3		-7.6	
LD 1-21	52.7	3.6	51.5	5.3	52.7	2.8	**49.4***	4.5
Δ% (compared to control)			-2.3		0.0		-6.2	

* = $p<0.05$; Dunnett test (two-sided)
LD = lactation day

For all test groups the intake of Dimethomorph correlated well with the desired target doses. The actual test substance intake was calculated on the basis of interpolated mean body weights of each test group. With regard to the target dose levels, the mean values and the minimum/maximum deviations of the actual test substance intake per time interval examined are shown in Table 5.6.1-5.

Table 5.6.1-5: Average Dimethomorph intake (mg/kg bw/day) in F_0 parental animals

Dose	300 / 150 ppm		800 / 400 ppm		1600 / 800 ppm	
Group & sex	average	min/max	average	min / max	average	min / max
F_0 males	23.4	16.5 / 37.7	63.0	44.3 / 100.3	128.4	93.9 / 199.8
F_0 females						
- premating	25.0	20.0 / 33.7	66.8	54.4 / 89.2	136.5	116.2 / 178.0
- gestation period	21.9	20.5 / 22.4	58.9	53.8 / 61.1	118.4	111.3 / 119.9
- lactation period	27.5	18.5 / 34.3	74.5	51.0 / 93.5	144.7	97.3 / 182.4

A.1.4 OESTROUS CYCLE DETERMINATIONS

Oestrous cycle data of the **F_0 females**, generated during the last 3 weeks prior to mating, revealed regular cycles in the females of all test groups including the control. The mean estrous cycle duration in the different test groups was comparable: 4.4 days in control, 4.4 in the low-dose group, 5.6 in the mid-dose group and 4.4 in the high-dose group.

A.1.5 MATING AND GESTATION DATA

1. Male reproductive performance

Copulation was confirmed for all F_0 parental males, which were paired with females to generate F_1 pups. Thus, the male mating index was 100% in all test groups.
Fertility was proven for most of the F_0 parental males within the scheduled mating interval for F_1 litter.
Three high-dose males (#. 82, 96 and 97) and one low-dose male (# 33) did not generate F_1 pups. One low-dose male (# 46) did not generate F_1 pups but implants in the uterus of low-dose female # 146. Thus, the male fertility index ranged between 88% (1600 ppm test group), 96% (300 ppm test group) and 100% (control and 800 ppm test group) without showing any relation to dosing. This reflects the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility.

Table 5.6.1-6: Reproductive data of F_0 males administered Dimethomorph

Parental generation	**F_0 males**			
Dose [ppm]	**0**	**300**	**800**	**1600**
Males placed with females	24	25	25	25
Mated [n]	24	25	25	25
Male mating index [%]	100	100	100	100
did not mate [n]	0	0	0	0
with females pregnant [n]	24	24	25	22
Male fertility index [%]	100	96	100	88
without females pregnant [n]	0	1	0	2
without females pregnant [%]	0	4	0	8

2. Sperm analysis

Concerning the motility of the sperms and the incidence of abnormal sperms in the cauda epididymis as well as the sperm head counts in the testis and in the cauda epididymis of F_0 males, no treatment-related effects were observed.

Table 5.6.1-7: Sperm parameters of F_0 males administered Dimethomorph

Parental generation	**F_0 males**			
Dose [ppm]	**0**	**300***	**800***	**1600**
Sperm count [10^6 / g]				
Testis	120			122
Cauda epididymis	676			641
Normal sperm [%]	93.5			92.9
Abnormal sperm [%]	6.5			7.1
Sperm motility [%]	85	87	85	86

*low and mid dose have not been evaluated.

3. Female reproductive performance

The female mating index calculated after the mating period for F_1 litter was 100% in all test groups.
The mean duration until sperm was detected (GD 0) varied between 2.2 and 2.7 days without any relation to the doses applied.
All female rats delivered pups or had implants in utero with the following exception:

- High-dose females # 182, 196 and 197 (mated with males # 82, 96 and 97, respectively) did not become pregnant.
- Low-dose female # 133 (mated with male # 33) did not become pregnant.

The fertility index varied between 88% in 1600 ppm test group, 96% in 300 ppm test group and 100% in control and 800 ppm test group. These values reflect the normal range of biological variation inherent in the strain of rats used for this study.
The mean duration of gestation values varied between 22.0 and 22.3 days in control, 300 and 800 ppm test groups without any relation to dosing. The mean duration of gestation was statistically significantly decreased in comparison to the concurrent control group in 1600 ppm test group ($p \leq 0.01$). The value (21.4 days) was marginally below the historical control range (21.5 to 22.3 days), while the concurrent control (22.3 days) was at the upper end of the historical control range. However, as this apparent shortening of gestation did not go along with any adverse effects on developmental status and well-being of the new-borns, it is considered to be as non-adverse and of no toxicological relevance.
The gestation index was 100% in the control, 800 and 1600 ppm test groups and 96% in 300 ppm test group. These values reflect the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility (91 - 100%).
Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test substance-treated groups and the controls, taking normal biological variation into account (12.1 / 11.5 / 12.2 and 11.7 implants/dam in control, 300, 800 and 1600 ppm groups, respectively). Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the post-implantation loss did not show any significant differences between the groups, and the mean number of F_1 pups delivered per dam remained unaffected (11.4 / 11.4 / 11.7 and 11.1 pups/dam in control, 300, 800 and 1600 ppm groups, respectively).
The rate of live-born pups was also not affected by the test substance, as indicated by live birth indices of 100% (high-, mid- and low-dose group) and 98.5% (control). Moreover, the number of stillborn pups was comparable between the groups.
Thus, Dimethomorph did not adversely affect reproduction and delivery of the F_0 generation parental females.

Table 5.6.1-8: Summary of F_0 female reproduction and delivery data

Parental generation		**F_0 females**			
Dose	**[ppm]**	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Animals per dose	[n]	25	25	25	25
Female fertility					
- placed with males	[n]	24	25	25	25
- mated	[n]	24	25	25	25
- mating index	[%]	100	100	100	100
- pregnant	[n]	24	24	25	22
- **Fertility index**	[%]	100	96	100	88
Duration of gestation	[days]	22.3	22.2	22.0	**21.4****
Implantation sites, total	[n]	290	276	305	258
- per dam	[n]	12.1	11.5	12.2	11.7
Post implantation loss	[n]	14	22	11	15
- per dam	[n]	0.6	0.9	0.4	0.7
- per litter	[mean %]	4.7	8.0	3.6	5.7
Females with live-born		24	23	25	22
- **Gestation index**	[%]	100	95.8	100	100
- with stillborn pups	[n]	2	0	0	0
- with all stillborn	[n]	0	0	0	0
Pups delivered	[n]	273	263	293	245
- per dam	[mean n]	11.4	11.4	11.7	11.1
- live-born	[n]	269	263	293	245
- stillborn	[n]	4	0	0	0
- **Live birth index**	[%]	98.5	100	100	100

** = p<0.01; Dunnett test (two-sided)

A.2 LITTER AND PUP DATA (F1 GENERATION)

A.2.1 Survival

The mean number of delivered F_1 pups per dam and the rates of live-born and stillborn F_1 pups were evenly distributed about the groups. The respective values reflect the normal range of biological variation inherent in the strain used in this study.

The viability index indicating pup mortality during early lactation (PND 0-4) varied between 97.6% (1600 ppm), 98.7% (800 ppm), 99.1% (300 ppm) and 100% (control).
The lactation index indicating pup mortality on PND 4-21 varied between 98.9% (1600 ppm), 99.6% (800 ppm), 100% (300 ppm) and 99.5% (control) without showing any association with the treatment.
Thus, the test substance did not influence pre-weaning pup survival in any of the treated groups.

Table 5.6.1-9: Summary of litter data

Pup generation	**F1**			
Dose [ppm]	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Number of litters	24	23	25	22
- with liveborn pups	24	23	25	22
- with stillborn pups	2	0	0	0
Pups live-born [n]	269	263	293	245
Pups stillborn [a] [n]	4	0	0	0
Pups died [n]	0	2	2	1
Pups cannibalized [n]	0	1	3	7
Pups culled day 4 [n]	77	76	91	67
Pups day 4 - pre-cull [n]	269	260	289	239
Viability index [%]	100	99.1	98.7	97.6
Pups day 4 - post cull [n]	192	184	198	172
Pups day 21 [n]	191	184	197	170
Lactation index [%]	99.5	100	99.6	98.9
Sex ratio [% live males]				
Day 0	53.8	50.5	50.5	46.1
Day 21	51.8	51.1	51.1	47.7
Male pup weight [g]				
PND 1 [g]	7.0	7.0	6.7	**6.2****
PND 4 [g]	10.8	10.3	10.3	**9.5****
PND 7 [g]	16.9	16.3	16.5	**15.3****
PND 14 [g]	34.0	32.8	33.7	**31.9****
PND 21 [g]	54.6	52.6	53.1	**49.5****
Male body weight gain				
PND 1 to 21 [g]	47.6	45.7	46.3	**43.3****
Female pup weight [g]				
PND 1 [g]	6.6	6.6	6.4	**5.8****
PND 4 [g]	10.2	10.0	10.0	**9.0****
PND 7 [g]	16.0	15.7	16.0	**14.6****
PND 14 [g]	32.7	32.0	32.9	30.9
PND 21 [g]	52.2	50.8	51.4	**47.8****
Female body weight gain				
PND 1 to 21 [g]	45.5	44.2	45.0	**42.0****

** = p<0.01; Dunnett test (two-sided)

A.2.2 Sex ratio

The sex distribution and sex ratios of live F1 pups on the day of birth and on PND 21 did not show substantial differences between the control and the test substance-treated groups; slight differences were regarded to be spontaneous in nature [see Table 5.6.1-9].

A.2.3 Pup clinical observations

The F_1 generation pups did not display any clinical signs until weaning.

A.2.4 Nipple/areola complex

The apparent number and percentage of male pups having areolae was not influenced by the test substance when examined on PND 12. Owing to the high background rate in control animals on this day, a recheck of all animals for nipples/areolae was performed on PND 20, one day prior to weaning. During the re-examination no areolae were detected in all male pups of all test groups [see Table 5.6.1-10].

Table 5.6.1-10: Nipple development in male lactation pup

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Nipple development [%]								
Lactation male pup	N = 24		N = 23		N = 25		N = 22	
Day 12	95	10	95	13	95	13	99	4
Day 20	0	0	0	0	0	0	0	0

A.2.5 Body weight

At 1600 ppm statistically significantly lower mean pup body weights were noted on postnatal day 1 (about 13% below control). High-dose pup weights remained lower until weaning (about 9% below control at PND 21).
No test compound-related influence on F_1 pup body weights was noted in the mid- and low-dose groups (800 and 300 ppm).

Mean body weight change of high-dose male and female pups was statistically significantly below the concurrent control towards the end of lactation (during PND 14–21, about 14% below control). This also had an impact on body weight change throughout lactation, which was decreased by 8% below control.
A very minor decrease of pup body weight change was also noted for the mid-dose pups (about 6% below control).
No test compound-related influence on F_1 pup body weight change was noted in the low-dose group.

A.2.6 Anogenital distance/ anogenital index

Anogenital (AG) distance of male pups was statistically significantly reduced at all dose levels; 2, 6 and 10% below control, respectively. When corrected for body weight (AG index) the reduction was 2, 4 and 5%, respectively.
For the low-dose male offspring, the AG distance and index are well covered by the historical control range of the test facility. Thus, these changes are considered to be incidental and not treatment-related. The AG distance of the mid- and high-dose males is below the range of historical control data. The AG index of the mid-dose males is in the lower range of historical control data, and of the high-dose males below the historical data range. Thus, these changes are considered to be treatment-related.

AG distance of all **female** treated pups was also statistically significantly decreased, by 2, 3 and 6%, respectively. When corrected for body weight (AG index) only the low and mid-dose pups were significantly below control; the reduction was 2% in both groups.
For the low-dose female offspring the AG distance is well covered by the historical control range of the test facility. Considering AG index, the apparent changes lack a dose-response and are all covered by the historical control range of the test. Thus, none of the apparent changes in females is considered to be treatment-related.

Table 5.6.1-11: Anogenital distance and index of lactation pup on PND1

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
PND 1								
male pup	N = 146		N = 129		N = 145		N = 112	
AG distance [mm]	3.15	0.19	**3.09***	0.23	**2.95****	0.19	**2.85****	0.25
historical control [mm]	2.99–3.15							
AG index	1.65	0.09	**1.62***	0.11	**1.58****	0.08	**1.57****	0.12
historical control	1.58–1.67							
female pup	N = 123		N = 131		N = 145		N = 131	
AG distance [mm]	1.53	0.13	**1.50***	0.08	**1.48****	0.11	**1.44****	0.12
historical control [mm]	1.48-1.67							
AG index	0.82	0.07	**0.80****	0.05	**0.80****	0.06	0.81	0.06
historical control	0.79-0.86							

* = p<0.05; ** = p<0.01; Dunnett test (two-sided)

A.2.7 Sexual maturation

Male and female F_1 pups selected to become F_1 rearing animals for Cohort 1A and Cohort 1B were examined for sexual maturation. Delay of puberty at 800 ppm (males) and 1600 ppm (both sexes) was observed.

Table 5.6.1-12: Sexual maturation of F_1 pups

Sex & parameter	Females / Vaginal opening				Males / Preputial separation			
Dose [m	0	300/150	800/400	1600/800	0	300/150	800/400	1600/800
pups examined	40	40	40	40	38	40	39	39
Days to criterion	31.4	31.9	32.0	**33.4****	42.0	41.8	**43.7****	**47.9****
Historical control range	30.0 - 32.1				39.7 - 42.5			
Body weight at criterion [g]	96.4	96.9	95.9	96.9	176.6	174.0	180.5	**199.5****
Historical control range	86.4 - 99.6				156.5 - 181.0			

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided)

Vaginal opening

The first day when vaginal opening was observed was PND 27, the last was PND 37. There was a statistically significant increase in the high-dose group for the days to criterion value that slightly exceeded the historical control range [see Table 5.6.1-12].]. In the absence of any effect on estrous cyclicity and without any effect on female sexual organs the biological relevance of this weak effect cannot be judged.

Preputial separation

The first day when preputial separation was observed was PND 39, the last was PND 47. There was a statistically significant increase in the mid- and high-dose groups for the days to criterion value that exceeded the historical control range [see Table 5.6.1-12]. Additionally, a statistically significant increase of the body weight at criterion that exceeded the historical control range was observed in the high-dose group.

A3 F_1 REARING ANIMALS

A.3.1 Observations

No clinical signs of toxicity, changes of general behaviour or other abnormalities, which may be attributed to the test substance, were detected in any of the male and female F_1 rearing animals in any of the groups during daily observations as well as during the detailed clinical observations.

On high-dose Cohort 1B male (# 468) showed a reddish encrusted right eye during in-life days 22 - 33, days 35 - 48 and days 51 - 59. This finding was not considered to be associated with the test substance.

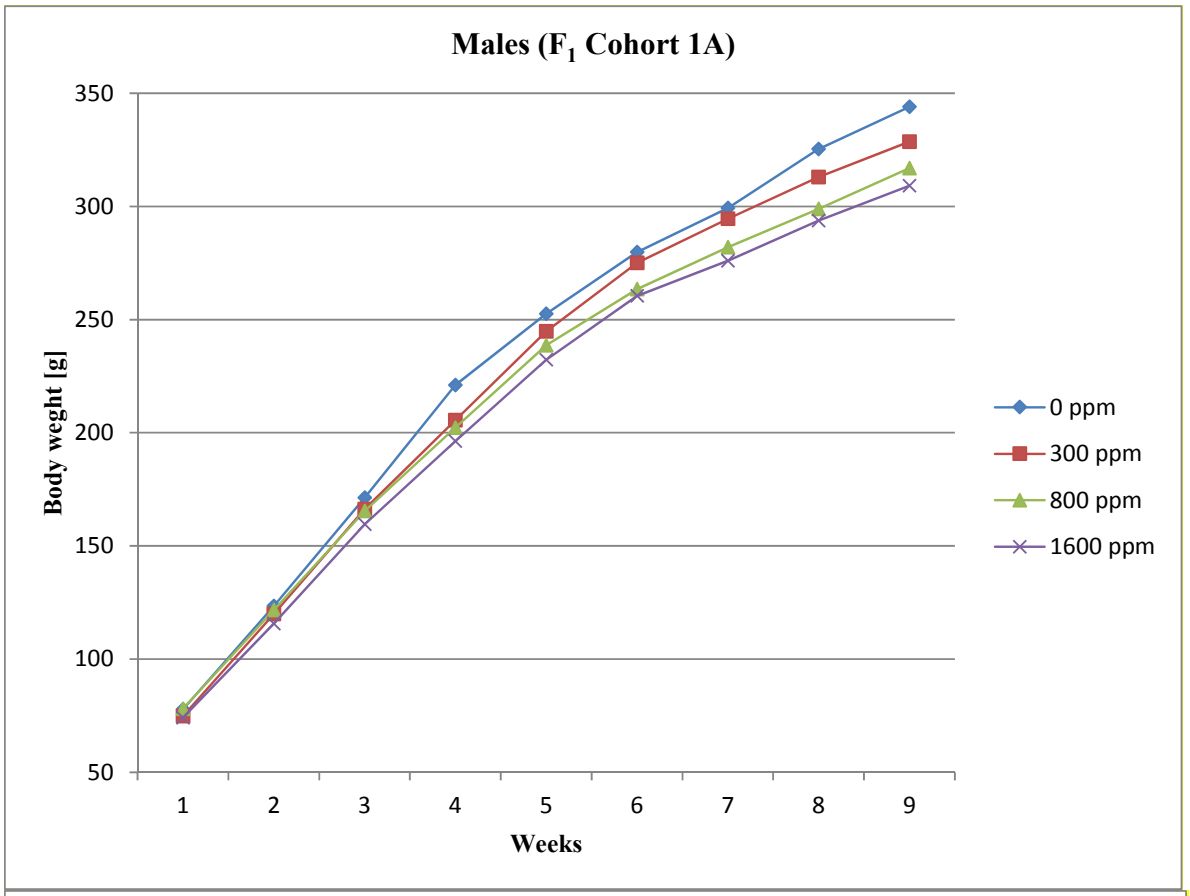
A.3.2 Mortality

There were no test substance-related mortalities in any of the Cohort 1A groups.
On control female (# 317) died accidentally on in-life day 50.

There were no test substance-related or spontaneous mortalities in any of the Cohort 1B groups.

A.3.3 Body weight and body weight gain

Body weights of 800 ppm and 1600 ppm **Cohort 1A males** were consistently below the concurrent control throughout the in-life period (up to 8% and 10%, respectively). The difference gained statistical significance from day 28 and 14 onwards, respectively. No significant differences to the control were noted for 300 ppm Cohort 1A males [see Figure 5.6.1-4 and Table 5.6.1-13].



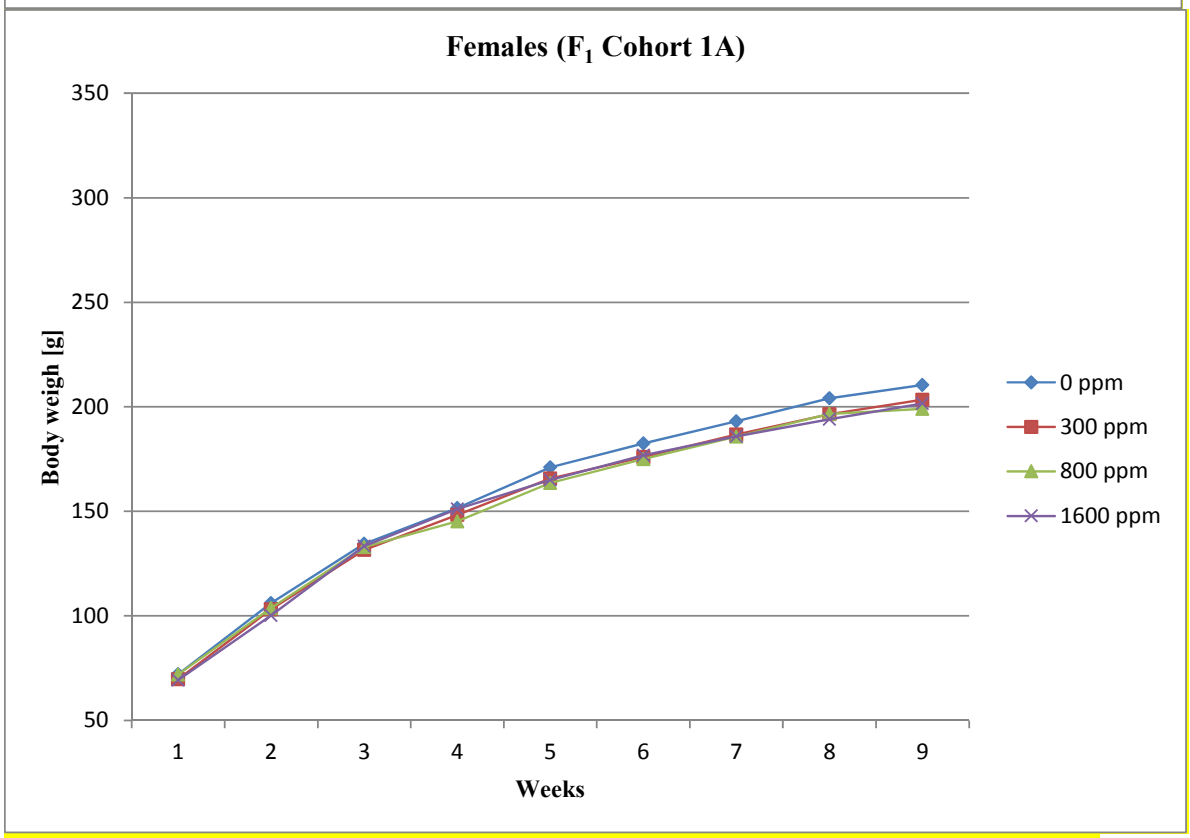


Figure 5.6.1-4: Body weight development of rearing F_1 animals of the Cohort 1A

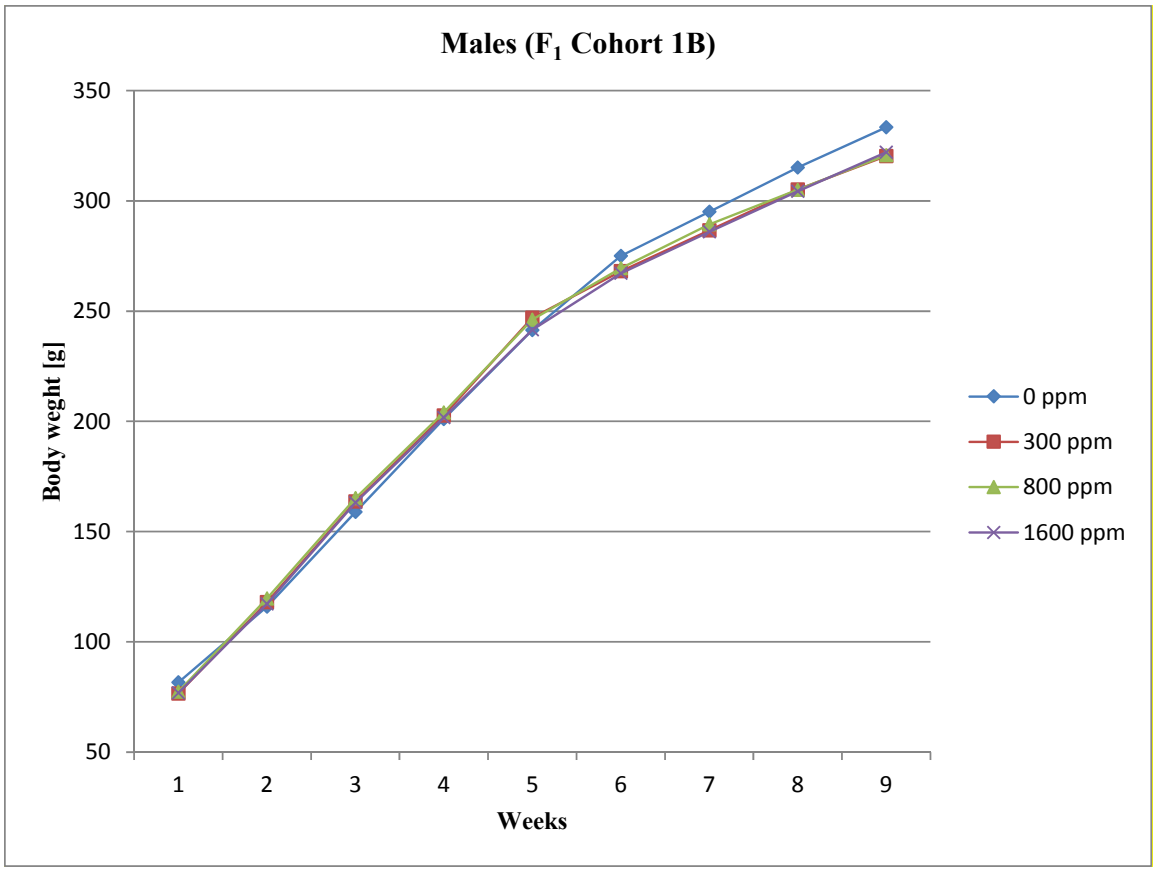
Body weights of all **Cohort 1A females** of all dose groups were basically comparable to the concurrent control values throughout the entire study period [see Figure 5.6.1-4].

Table 5.6.1-13: Body weight development of Cohort 1A males during in-life

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight gain [g]								
Cohort 1A males								
in-life phase	N = 20		N = 20		N = 20		N = 20	
Day 0	77.7	8.3	74.8	7.8	78.0	8.7	74.1	10.0
Δ% (compared to control)			-3.7		0.3		-4.6	
Day 7	123.4	10.1	119.9	10.5	121.7	14.1	115.7	12.6
Δ% (compared to control)			-2.9		-1.4		-6.3	
Day 14	171.3	12.8	166.2	11.9	165.4	16.5	**159.6***	12.3
Δ% (compared to control)			-3.0		-3.4		-6.8	
Day 21	211.1	14.6	205.5	14.0	202.3	18.0	**196.3****	13.9
Δ% (compared to control)			-2.7		-4.2		-7.0	
Day 28	252.6	17.9	244.8	16.3	**238.6***	21.7	**232.2****	14.5
Δ% (compared to control)			-3.1		-5.5		-8.1	
Day 35	279.8	19.3	275.1	20.1	**263.4***	23.0	**260.4****	15.3
Δ% (compared to control)			-1.7		-5.9		-6.9	
Day 42	299.3	22.7	294.5	23.9	**282.0***	23.0	**276.0****	18.4
Δ% (compared to control)			-1.6		-5.8		-7.8	
Day 49	325.4	25.1	312.9	26.0	**298.9****	24.6	**293.7****	22.1
Δ% (compared to control)			-3.9		-8.1		-9.7	
Day 56	344.1	27.2	328.6	27.2	**316.9****	25.9	**309.2****	23.0
Δ% (compared to control)			-4.5		-7.9		-10.1	

* = p<0.05; * = p<0.01; Dunnett test (two-sided)
GD = gestation day

Body weights of all **Cohort 1B males and females** of all dose groups (300, 800, and 1600 ppm) were generally comparable to the concurrent control values throughout the entire study period. Occasional statistically significant decreases or increases of body weight change were not considered to be treatment-related.



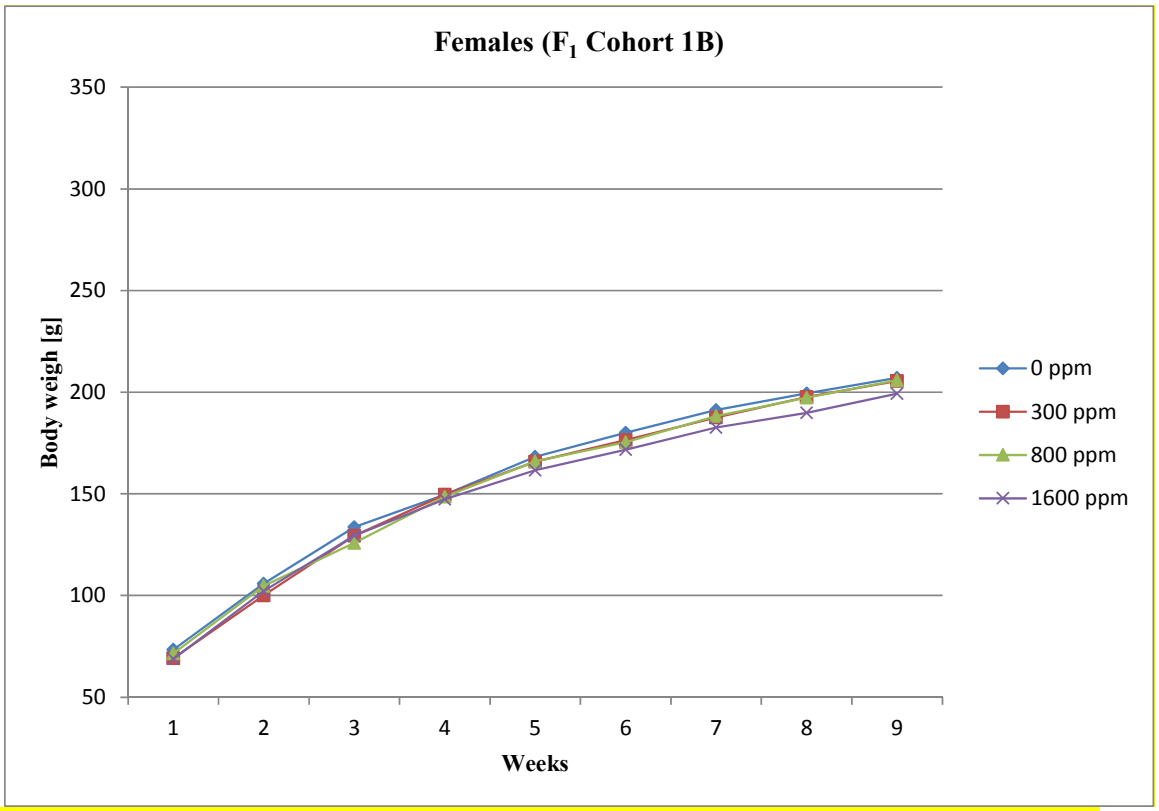


Figure 5.6.1-5: Body weight development of rearing F_1 animals of the Cohort 1B

The body weight change of 800 ppm and 1600 ppm **Cohort 1A males** was statistically significantly below the concurrent control values during major parts of the in-life period (about 35% and 32%, respectively). The males of these groups generally gained 10 and 12% less weight than the control throughout in-life, respectively
The body weight change of the low-dose Cohort 1A males was statistically significantly below the concurrent control values just on one occasion (in-life days 42 – 49, about 30%). The males of this group generally gained 5% less weight than the control throughout in-life, however this difference was not statistically significant [see Table 5.6.1-14].

The body weight change of 1600 ppm **Cohort 1A females** was statistically significantly below the concurrent control values during in-life days 0 - 14 and 21 - 28 (up to 17% and 29%, respectively). The females of this group generally gained 4% less weight than the control throughout in-life, however this difference was not statistically significant.
The body weight change of 800 ppm Cohort 1A females was statistically significantly below the concurrent control values during in-life days 14 - 21 and 49 - 56 (about 27% and 68%, respectively). The females of this group generally gained 8% less weight than the control throughout in-life, however this difference was not statistically significant.
The body weight change of the 300 ppm Cohort 1A females was comparable to the concurrent control values throughout the entire study period [see Table 5.6.1-14].

Body weight change of all **Cohort 1B males and females** of all dose groups (300, 800, and 1600 ppm) were generally comparable to the concurrent control values throughout the entire study period. Occasional statistically significant decreases or increases of body weight change were not considered to be treatment-related.

Table 5.6.1-14: Body weight change of Cohort 1A rearing F_1 animals during in-life

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight gain [g]								
Cohort 1A males								
in-life phase	N = 20		N = 20		N = 20		N = 20	
Day 0-7	45.7	4.1	45.1	3.8	43.8	5.7	**41.6****	3.3
Δ% (compared to control)			-1.3		-4.2		-9.0	
Day 7-14	47.9	4.6	46.2	4.2	**43.7****	4.5	**43.9****	3.4
Δ% (compared to control)			-3.5		-8.8		-8.4	
Day 14-21	39.8	3.7	39.3	4.0	**36.9***	3.1	**36.7***	4.0
Δ% (compared to control)			-1.3		-7.3		-7.8	
Day 21-28	41.5	4.6	39.4	4.7	**36.3****	5.4	**35.9****	3.1
Δ% (compared to control)			-5.1		-12.5		-13.5	
Day 28-35	27.2	7.1	30.2	7.1	24.8	5.5	28.2	4.8
Δ% (compared to control)			11.0		-8.8		3.7	
Day 35-42	19.6	6.3	19.4	6.5	18.7	4.3	15.5	5.4
Δ% (compared to control)			-1.0		-4.6		-20.9	
Day 42-49	26.1	6.9	**18.4****	5.0	**16.9****	4.7	**17.8****	5.5
Δ% (compared to control)			-29.5		-35.2		-31.8	
Day 49-56	18.7	4.8	15.7	4.3	18.0	3.9	15.5	4.1
Δ% (compared to control)			-16.0		-3.7		-17.1	
Day 0-56	266.4	23.1	253.8	25.8	**238.9****	20.7	**235.1****	18.0
Δ% (compared to control)			-4.7		-10.3		-11.7	
Cohort 1A females								
Gestation phase	N = 20		N = 20		N = 20		N = 20	
Day 0-7	34.0	2.9	33.5	3.0	31.8	3.7	**31.0****	3.0
Δ% (compared to control)			-1.5		-6.5		-8.8	
Day 7-14	28.4	5.8	28.4	5.8	29.0	5.6	**33.2****	6.9
Δ% (compared to control)			0.0		2.1		16.9	
Day 14-21	17.1	6.0	16.8	3.7	**12.4***	7.1	17.8	4.6
Δ% (compared to control)			-1.8		-27.5		4.1	
Day 21-28	19.4	4.4	17.4	4.8	18.4	6.6	**13.8****	4.8
Δ% (compared to control)			-10.3		-5.3		-28.9	
Day 28-35	11.5	5.6	10.3	6.9	11.4	4.6	11.9	3.8
Δ% (compared to control)			-10.4		-0.9		3.5	
Day 35-42	10.6	5.7	10.7	5.8	10.7	4.0	9.0	3.7
Δ% (compared to control)			0.9		0.9		-15.1	
Day 42-49	11.0	7.1	9.7	7.9	10.9	5.8	8.0	5.9
Δ% (compared to control)			-11.8		-0.9		-27.3	
Day 49-56	7.5	4.7	7.0	6.0	**2.4***	6.5	7.6	3.5
Δ% (compared to control)			-6.7		-68.0		1.3	
Day 0-56	138.3	13.0	133.7	15.0	127.0	13.3	132.3	17.6
Δ% (compared to control)			-3.3		-8.2		-4.3	

* = p<0.05; * = p<0.01; Dunnett test (two-sided)

A.3.4 Food consumption and substance intake

Food consumption of all treated **Cohort 1A males** was below the concurrent control values during in-life period, the difference to the control gained statistical significance during a number of study sections. The most consistent decrease was, however, noted towards the end of in-life. When summarized for the entire in-life period, the differences to the control were -5%, -9% and -7% in at the 300, 800 and 1600 ppm dose levels, respectively. However, as the reduction of food consumption did not correlate to body weight effects in the low-dose group, this was considered as a potentially treatment-related but non-adverse finding [see Table 5.6.1-15 and Figure 5.6.1-6].

Table 5.6.1-15: Food consumption of Cohort 1A rearing F_1 males during in-life

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Food consumption [g/animal/day]								
Cohort 1A males								
in-life phase	N = 20		N = 20		N = 20		N = 20	
Day 0-7	14.8	2.1	13.1	0.8	13.1	1.4	12.8	0.7
Δ% (compared to control)			-12.1		-11.8		-13.8	
Day 7-14	18.9	0.5	18.2	0.4	**17.6***	0.8	**17.7***	0.3
Δ% (compared to control)			-3.3		-6.8		-6.0	
Day 14-21	19.9	0.7	19.3	0.4	**18.8***	0.3	19.3	0.5
Δ% (compared to control)			-2.6		-5.4		-2.8	
Day 21-28	21.5	0.8	20.9	0.7	**20.2***	0.9	20.4	0.3
Δ% (compared to control)			-2.7		-6.2		-5.2	
Day 28-35	22.1	0.9	21.5	1.1	**20.6***	0.7	20.9	0.2
Δ% (compared to control)			-2.8		-7.0		-5.8	
Day 35-42	21.9	1.1	21.1	1.7	20.1	0.8	20.6	0.4
Δ% (compared to control)			-4.0		-8.1		-6.2	
Day 42-49	22.9	1.1	21.2	1.1	**19.8****	0.5	**20.4****	0.6
Δ% (compared to control)			-7.1		-13.7		-10.7	
Day 49-56	22.3	0.3	**20.9***	0.9	**20.1****	0.6	**20.2****	0.6
Δ% (compared to control)			-6.4		-9.6		-9.5	
Day 0-56	20.6	0.3	**19.5***	0.7	**18.8****	0.7	**19.0****	0.3
Δ% (compared to control)			-5.1		-8.5		-7.4	

* = p<0.05; * = p<0.01; Dunnett test (two-sided)

Food consumption of the **Cohort 1A females** in all treated groups was basically comparable to the concurrent control. Occasional statistically significant decreases or increases were not considered to be treatment-related [see Figure 5.6.1-6].

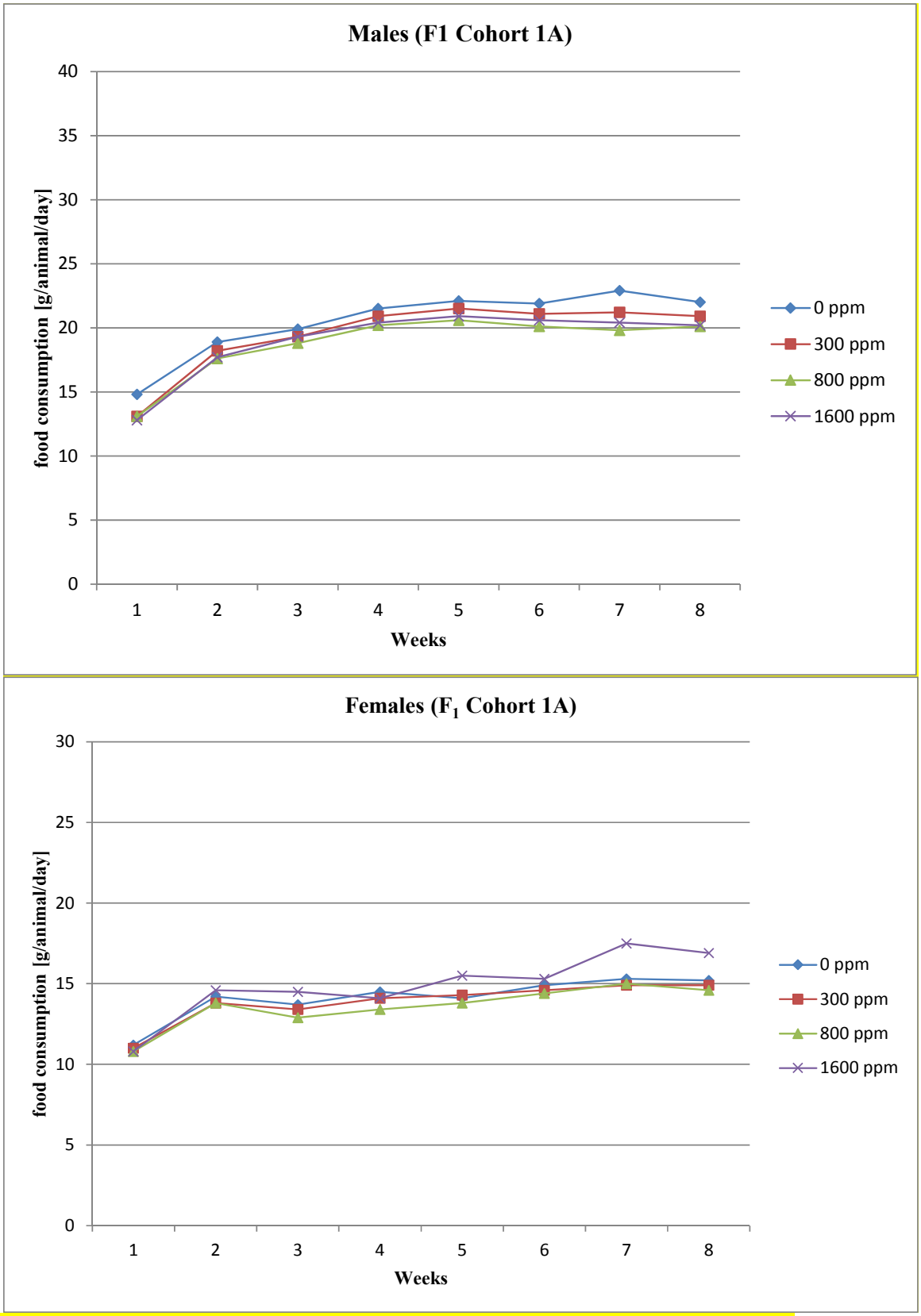
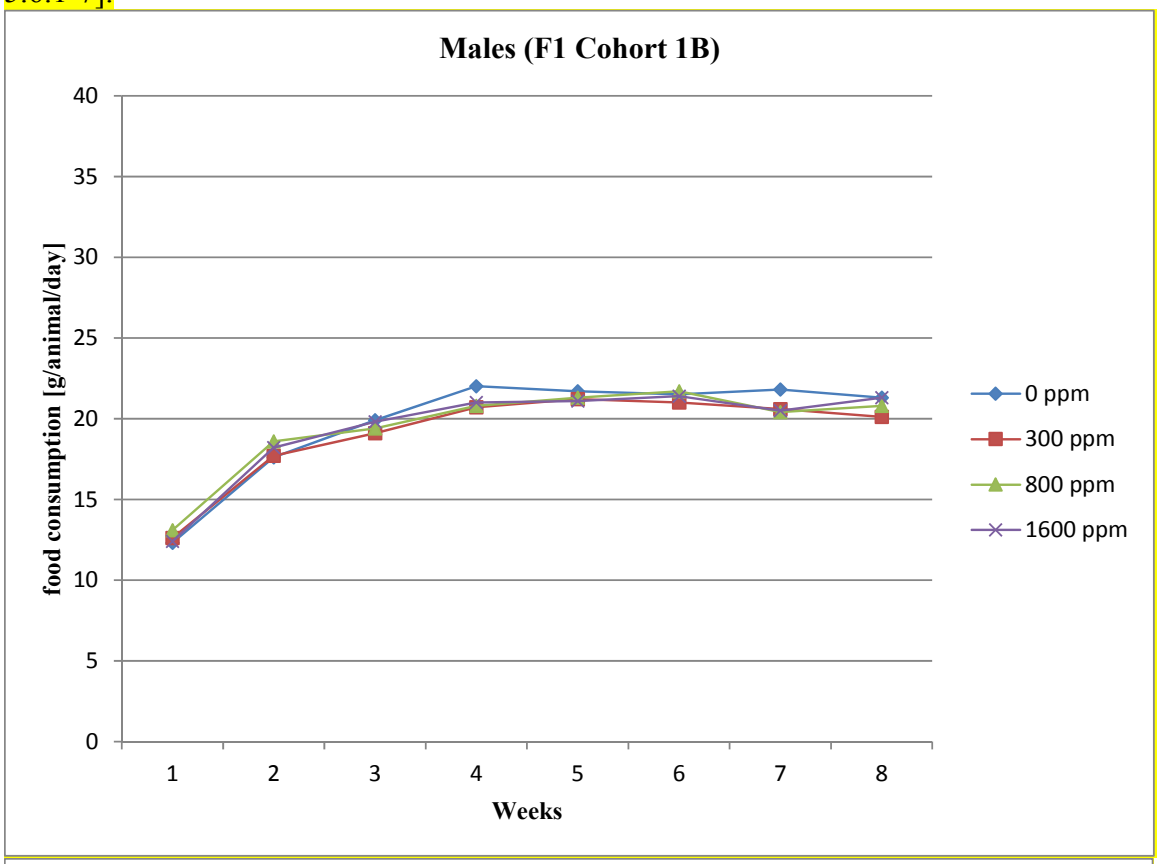


Figure 5.6.1-6: Food consumption of rearing F_1 animals of the Cohort 1A

Food consumption of all **Cohort 1B males and females** of all dose groups was generally comparable to the concurrent control values throughout the entire study period [see Figure 5.6.1-7].



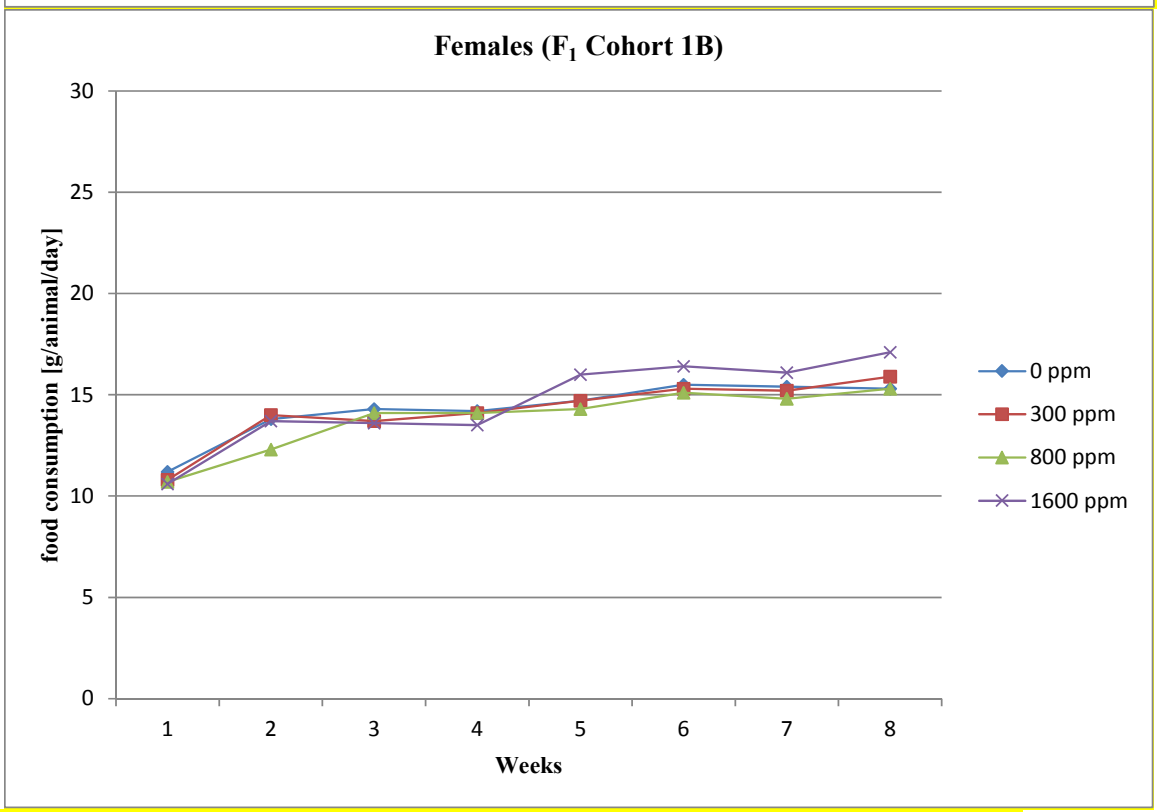


Figure 5.6.1-7: Food consumption of rearing F_1 animals of the Cohort 1B

For all test groups the intake of Dimethomorph correlated well with the desired target doses. The actual test substance intake was calculated on the basis of interpolated mean body weights of each test group. With regard to the target dose levels, the mean values and the minimum/maximum deviations of the actual test substance intake per time interval examined are shown in Table 5.6.1-16.

Table 5.6.1-16: Average Dimethomorph intake (mg/kg bw/day) in F_1 rearing animals

Dose	300 ppm		800 ppm		1600 ppm	
Group & sex	average	min/max	average	min / max	average	min / max
F_1 males (in-life)						
Cohort 1A	28.1	19.5 / 40.2	73.7	52.4 / 104.7	153.8	107.2 / 216.5
Cohort 1B	27.8	19.2 / 38.8	75.8	53.1 / 106.8	151.7	108.8 / 208.1
F_1 females (in-life)						
Cohort 1A	28.0	22.3 / 38.2	73.4	59.0 / 98.0	159.3	135.2 / 203.2
Cohort 1B	28.7	23.6 / 38.3	74.1	60.7 / 97.6	158.3	137.8 / 199.2

A.3.5 Oestrous cycle data

Oestrous cycle data revealed regular cycles in the **rearing F_1 Cohort 1A females** of all test groups including the control. The mean oestrous cycle duration in the different test groups was similar: 4.6 days in control, 5.0 days in the low-dose group, 5.4 days in the mid-dose group and 4.1 days in the high-dose groups.

Table 5.6.1-17: Oestrous cycle data of rearing F_1 females

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Cohort 1A females								
	N = 20		N = 20		N = 20		N = 20	
No. cycles	2.40	1.05	2.20	0.95	2.2	0.77	2.40	0.82
Cycles length (days)	4.61	2.16	4.95	2.56	5.43	2.55	4.10	0.63
Cohort 1B females								
	N = 20		N = 20		N = 20		N = 20	
No. cycles	4.15	0.67	3.75	1.12	3.85	0.93	4.00	0.92
Cycles length (days)	4.11	0.44	5.38	3.13	4.75	1.65	4.59	1.25

* = $p<0.05$; * = $p<0.01$; Dunnett test (two-sided)

Oestrous cycle data revealed regular cycles also in the **rearing F_1 Cohort 1B females** of all test groups including the control. The mean oestrous cycle duration in the different test groups was similar: 4.1 days in control, 5.4 days in the low-dose group, 4.8 days in the mid-dose group and 4.6 days in the high-dose groups.

A.3.6 Sperm analysis

Concerning the incidence of abnormal sperms in the cauda epididymidis as well as the sperm head counts in the testis and in the cauda epididymidis no treatment-related effects were observed.
At the end of the administration period in males of the 800 ppm test group, motility of the sperms was marginally reduced, but the effect was not dose-dependent. Therefore, this finding was regarded as incidental and not treatment-related.

Table 5.6.1-18: Sperm parameters of F_1 rearing males Cohort 1A on Day 90

F_1 rearing males		Cohort 1A			
Dose	[ppm]	0	300	800	1600
Sperm count	[10^6 / g]				
Testis		118	-	-	127
Cauda epididymis		685	-	-	703
Normal sperm	[%]	93.2			91.9
Abnormal sperm	[%]	6.8	-	-	8.1
Sperm motility	[%]	87	86	**81***	85

Urinalysis

No treatment-related changes of urinalysis parameters were observed.
In females of the 300 ppm test group urine pH value and urine volume (not statistically significant) were higher compared to controls and urine specific gravity was decreased. All mentioned parameters were not dose-dependently changed and therefore the alterations were regarded as incidental and not treatment-related.

Table 5.6.1-19: Urinalyses parameters of F_0 parental females

Parameter	Dose level [ppm]							
	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
pH value	5.4	0.5	**6.3****	0.5	5.8	0.8	5.6	0.7
Vol. [mL]	3.6	1.2	4.8	1.8	4.3	2.8	4.1	1.7
Spec. gravity [g/L]	1069	24	**1045****	8	1062	21	1063	19

* = p<0.05; * = p<0.01

B. CLINICAL PATHOLOGY

B.1 Clinical pathology parameters in F0 parental animals

Hematology

At the end of the administration period in males of 800 and 1600 ppm test groups, relative neutrophil cell counts were lower and relative lymphocyte cell counts were higher compared to controls without any change of the total white blood cell counts. The values of both cell fractions were within historical control ranges and therefore the changes were regarded as incidental and not treatment-related.
In males of the 800 ppm test group, relative monocyte counts were decreased and in males of the 300 ppm test platelet counts were increased, but both parameter were not dose dependently changed and therefore the alterations were regarded as incidental and not treatment-related.

Table 5.6.1-20: Haematology parameters of F_0 parental animals

Parameter	sex	specification	Dose level [ppm]							
			0		300		800		1600	
			mean	SD	mean	SD	mean	SD	mean	SD
WBC [giga/L]	♂	absolute	5.98	1.09	6.03	1.42	5.96	1.57	6.30	0.87
	♀	absolute	3.25	0.36	**3.98***	0.66	3.75	1.07	**5.08****	1.20
NEUT [giga/L]	♂	absolute	1.42	0.56	1.11	0.32	0.96	0.27	1.02	0.18
		relative	23.4	7.0	18.6	4.2	**16.4***	3.7	**16.3****	3.0
	HC	relative	15.0 - 25.4							
	♀	absolute	0.60	0.18	**0.76***	0.18	0.67	0.27	**0.92***	0.28
		relative	18.5	5.2	19.3	4.2	18.0	5.0	18.5	6.2
	HC	absolute	0.37 - 0.93							
LYMPH [giga/L]	♂	absolute	4.26	0.84	4.62	1.17	4.75	1.30	5.00	0.79
		relative	71.5	7.1	76.5	4.5	**79.5****	3.7	**79.2****	3.3
	HC	relative	69.8 - 81.2							
	♀	absolute	2.37	0.34	2.88	0.57	2.77	0.88	**3.83****	1.13
		relative	72.8	5.3	72.0	4.6	73.8	4.5	74.8	6.0
MONO [giga/L]	♂	absolute	0.14	0.03	0.14	0.05	0.10	0.05	0.12	0.04
		relative	2.3	0.4	2.4	0.8	**1.6****	0.4	1.9	0.5
	♀	absolute	0.09	0.02	0.10	0.04	0.09	0.04	0.12	0.04
		relative	2.7	0.7	2.5	1.1	2.5	0.8	2.4	0.8
PLT [giga/L]	♂	absolute	728	43	**836****	82	768	62	770	93
	♀	absolute	795	123	794	91	802	196	876	154

* = p<0.05; * = p<0.01
HC = historical control data

At the end of the administration period in females of the 1600 ppm test group, total white blood cell (WBC) counts and absolute lymphocyte and neutrophil cell counts were increased. The neutrophil cell counts were within the historical control range and therefore this change was regarded as incidental and not treatment-related.
In females of the 300 ppm test group total white blood cell (WBC) and absolute neutrophil cell counts were higher and relative basophil cell counts were lower compared to controls, but the changes were not dose-dependent and therefore these alterations were regarded as incidental and not treatment-related.

Clinical chemistry

No treatment-related, adverse changes of clinical chemistry parameters were observed.

At the end of the administration period in females of the 1600 ppm test group, total bilirubin values were decreased and calcium levels were increased. Without any signs of anaemia, a decrease of total bilirubin levels was most probably due to an increased conjugation rate and a subsequent accelerated excretion of bilirubin via the bile. This mechanism is regarded as adaptive and not adverse. The calcium mean of females in the 1600 ppm test group was within the historical control range and therefore, this alteration was regarded as incidental and not treatment-related.

Table 5.6.1-21: Clinical chemistry parameters of F_0 parental females

Parameter	Dose level [ppm]							
	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Bilirubin (total) [µmol/L]	2.18	0.37	2.07	0.26	1.94	0.55	**1.63****	0.29
Calcium [mmol/L]	2.51	0.06	2.51	0.07	2.51	0.07	**2.59****	0.04
	HC: 2.50 - 2.74							

* = p<0.05; * = p<0.01
HC = historical control data

B.2 Clinical pathology parameters in F1 rearing animals

At the end of the administration period in males and females of the 1600 ppm test group, WBC counts and absolute lymphocyte counts were increased.
Additionally, in males of the mentioned test group relative lymphocyte counts were increased and relative neutrophil and eosinophil cell counts were decreased.

Table 5.6.1-22: Haematology parameters of F_1 Cohort 1A animals

Parameter	sex	specification	Dose level [ppm]							
			0		300		800		1600	
			mean	SD	mean	SD	mean	SD	mean	SD
WBC [giga/L]	♂	absolute	5.83	0.99	6.00	1.11	6.21	1.21	**7.16****	1.03
	♀	absolute	4.53	0.96	4.35	0.72	4.71	0.80	**6.75****	1.64
NEUT [giga/L]	♂	absolute	1.14	0.38	1.16	0.27	0.98	0.31	0.93	0.18
		relative	19.4	5.2	20.0	6.2	15.7	3.5	**13.2****	3.2
	HC	relative	15.0 - 25.4							
	♀	absolute	0.81	0.28	0.68	0.20	0.62	0.10	0.96	0.34
		relative	17.8	4.4	16.2	5.5	13.4	0.01	14.0	2.9
LYMPH [giga/L]	♂	absolute	4.42	0.78	4.56	1.09	4.97	0.97	**5.97****	1.10
		relative	75.9	5.2	75.4	6.4	80.1	4.2	**83.0****	3.7
	HC	relative	69.8 - 81.2							
	♀	absolute	3.50	0.73	3.44	0.69	3.88	0.76	**5.54****	1.31
		relative	77.5	4.8	78.9	5.0	82.2	2.8	82.3	2.9
EOS [giga/L]	♂	absolute	0.09	0.02	0.10	0.03	0.08	0.03	0.07	0.02
		relative	1.6	0.4	1.6	0.4	1.4	0.4	**1.0****	0.3
	♀	absolute	0.07	0.02	0.07	0.02	0.07	0.02	0.07	0.02
		relative	1.5	0.4	1.7	0.4	1.5	0.5	1.2	0.5
MCH [fmol]	♂	absolute	1.06	0.04	1.05	0.04	1.07	0.04	1.04	0.03
	♀	absolute	1.10	0.04	1.10	0.03	1.10	0.04	**1.06****	0.03
MCHC [mmol/L]	♂	absolute	21.14	0.29	21.48	0.34	21.58	0.69	21.17	0.25
	♀	absolute	21.59	0.37	21.51	0.36	21.21	0.35	**21.06****	0.37

* = p<0.05; * = p<0.01
HC = historical control data

In females of the 1600 ppm test group, mean corpuscular haemoglobin content (MCH) and mean corpuscular haemoglobin concentration (MCHC) were lower compared to controls. Only these calculated red blood cell indices were altered without any change of the measured red blood cell parameters (i.e. haematocrit and haemoglobin values and red blood cell (RBC) counts. In females of the 800 ppm test group haematocrit levels were higher compared to controls, but the change was not dose-dependent. Therefore, the mentioned alterations were regarded as incidental and not treatment-related.

F_1 rearing Cohort1A animals

No treatment-related changes among clinical chemistry parameters were observed.

At the end of the administration period in females of the 1600 ppm test group, alanine aminotransferase (ALT) activities were higher compared to controls, but the mean was only marginally above the historical control range and therefore this change was not regarded as toxicological relevant. In males of the 1600 ppm test group urea and calcium levels were higher compared to controls and in females of this test group cholesterol and potassium levels were increased. Potassium levels were already higher in females of the 800 ppm test group. The values of the mentioned parameters were within historical control ranges. In males of the 800 ppm test group sodium levels were increased, but the changes were not dose-dependent. Therefore, all mentioned alterations were regarded as incidental and not treatment-related.

Table 5.6.1-23: Clinical chemistry parameters of F_1 Cohort 1A animals

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
ALT	♂	0.70	0.07	0.75	0.09	0.76	0.10	0.76	0.10
[µkat/L]	♀	0.62	0.09	0.63	0.10	0.07	0.14	0.83**	0.17
	HC	0.54 - 0.81							
Urea	♂	5.27	0.28	5.24	0.27	5.50	0.65	6.06**	0.72
[mmol/L]	HC	4.91 - 7.42							
	♀	5.81	0.61	5.53	0.69	5.96	0.79	5.91	0.62
Chol	♂	1.76	0.17	1.98	0.29	1.73	0.26	1.84	0.27
[mmol/L]	♀	1.56	0.27	1.48	0.30	1.77	0.32	1.84*	0.26
	HC	1.06 - 2.27							
K	♂	4.50	0.21	4.40	0.42	4.58	0.21	4.56	0.24
[mmol/L]	♀	4.08	0.29	4.19	0.30	4.36*	0.28	4.40*	0.24
	HC	3.76 - 4.40							
Ca	♂	2.52	0.03	2.48	0.04	2.55	0.05	2.57*	0.05
[mmol/L]	HC	2.49 - 2.67							
	♀	2.58	0.06	2.58	0.08	2.63	0.05	2.64	0.06
	HC	2.50 - 2.74							

* = $p<0.05$; * = $p<0.01$
HC = historical control data

Urinalyses (F_1 rearing Cohort1A animals)
No treatment-related changes among urinalysis parameters were observed.
In females of the 300 ppm test group specific gravity of the urine was decreased, but the mean was not dose-dependently changed. In males of the 300 and 1600 ppm test groups the incidences of crystals in the urine sediment were statistically higher compared to controls, but there was no relevant change of the crystals when regarding the individuals. Therefore, the mentioned alterations were regarded as incidental and not treatment-related.

Table 5.6.1-24: Urinalyses parameters of F_1 rearing Cohort 1A animals

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
F_0 parental animals									
Cryst.	♂	2	0	2*	1	2	0	2*	1
	♀	2	0	2	0	2	0	2	0
Spec. gravity [g/L]	♂	1052	16	1051	21	1054	20	1058	16
	♀	1056	25	1040	10	1064	30	1047	13

* = $p<0.05$; * = $p<0.01$

B.3 Hormone analysis in F0 and F1 animals

F_0 parental animals
At the end of the administration period no treatment-related changes of TSH and T_4 hormone levels were observed [see Table 5.6.1-25].

F_1 offspring (PND 4)
At PND4, in male and female F_1 pups no treatment-related change of TSH hormones was observed. T_4 levels in females of the 300 and 800 ppm test groups were lower compared to controls, but this change was not dose-dependent and it was not accompanied by an increase of TSH levels. Therefore, the T_4 level changes in the mentioned test groups were regarded as incidental and not treatment-related. T_4 levels in males were not altered [see Table 5.6.1-25].

F_1 offspring (PND 22)
At PND22, in male and female F_1 pups no treatment-related changes of TSH and T_4 hormones were observed [see Table 5.6.1-25].

F_1 rearing Cohort1A animals

At the end of the administration period no treatment-related changes of TSH and T_4 hormone levels were observed.

Table 5.6.1-25: Evaluation of hormone levels after Dimethomorph treatment

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
F_0 parental animals									
T_4	♂	73.01	11.80	80.86	14.76	77.44	10.33	80.33	8.37
[nmol/L]	♀	43.26	11.13	44.25	9.26	48.56	10.89	45.37	13.64
TSH	♂	8.01	1.93	9.59	3.41	8.43	2.31	9.10	2.84
[µg/L]	♀	6.94	2.11	5.90	0.79	6.60	1.48	7.29	1.21
F_1 pups (PND 4)									
T_4	♂	43.45	8.76	40.53	8.44	41.73	9.92	37.24	5.52
[nmol/L]	♀	46.73	8.96	**35.05***	10.78	**36.41***	7.04	39.68	9.10
TSH	♂	7.12	1.19	6.30	0.83	6.85	1.13	7.27	0.89
[µg/L]	♀	6.84	0.61	6.68	0.90	6.74	0.71	7.35	1.04
F_1 pups (PND 22)									
T_4	♂	88.30	12.12	83.77	15.40	85.14	13.05	85.14	11.17
[nmol/L]	♀	85.02	16.32	86.59	11.86	79.81	8.04	87.11	13.96
TSH	♂	5.22	0.96	4.36	0.78	4.85	1.05	4.61	1.35
[µg/L]	♀	4.38	0.81	4.25	0.49	4.83	1.01	4.88	0.94
F_1 rearing Cohort1A animals									
T_4	♂	104.46	12.12	96.84	16.36	103.33	14.17	102.95	17.02
[nmol/L]	♀	65.01	13.25	57.56	9.93	58.97	9.99	68.49	15.29
TSH	♂	9.90	3.36	11.09	2.42	12.81	4.29	8.92	2.86
[µg/L]	♀	7.65	2.71	6.57	2.50	7.09	2.55	8.77	2.76
LH [µg/L]	♂	2.04	0.62	2.83	1.01	1.76	0.78	2.38	1.45
Testo [nmol/L[	♂	15.38	8.47	19.49	13.01	10.15	4.85	14.69	8.44

* = p<0.05; * = p<0.01

Three days prior to sacrifice of the males, no treatment-related changes of LH and testosterone levels were observed.

C. PATHOLOGY

C.1 PATHOLOGY IN F0 PARENTAL ANIMALS

Organ weights

Organ weight determination in parental animals revealed a number of significant changes of absolute and/or relative organ weights [see Table 5.6.1-26]. All other mean absolute or relative weight parameters did not show significant differences when compared to the control group.

Table 5.6.1-26: Organ weights of F_0 male and female parental animals

Generation		F_0 Males				F_0 Females			
	Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Terminal weight [g]	0	379				232			
	300	374	(99)			228	(98)		
	800	369	(97)			229	(99)		
	1600	362	(96)			222	(96)		
Adrenal gland [mg]	0	55.96		0.015		75.21		0.032	
	300	56.88	(99)	0.015	(103)	74.16	(99)	0.033	(100)
	800	55.88	(97)	0.015	(102)	78.76	(105)	0.034	(106)
	1600	54.20	(96)	0.015	(102)	76.40	(102)	**0.035***	(106)
Heart [g]	0	1.054		0.278		0.908		0.392	
	300	1.069	(101)	0.286	(103)	**0.845***	(93)	**0.372****	(95)
	800	1.046	(99)	0.284	(102)	0.911	(100)	0.398	(101)
	1600	1.034	(98)	0.286	(103)	0.870	(96)	0.392	(100)
Kidneys [g]	0	2.324		0.615		1.733		0.749	
	300	2.349	(101)	0.628	(102)	1.712	(99)	0.753	(101)
	800	2.314	(100)	0.629	(102)	**1.838****	(106)	**0.802****	(107)
	1600	2.404	(103)	**0.665****	(108)	1.728	(100)	**0.778****	(104)
Liver [g]	0	8.144		2.152		6.317		2.729	
	300	8.277	(102)	2.206	(103)	6.307	(100)	2.773	(102)
	800	8.358	(103)	**2.268***	(105)	**7.046****	(112)	**3.069****	(112)
	1600	8.728	(107)	**2.405****	(112)	**6.906****	(109)	**3.103****	(114)
Pituitary gland [mg]	0	11.12		0.003		12.79		0.006	
	300	11.44	(103)	0.003	(105)	13.12	(103)	0.006	(104)
	800	11.36	(102)	0.003	(106)	12.84	(100)	0.006	(101)
	1600	11.96	(108)	0.003	(114)	12.20	(95)	0.006	(99)
Prostate [g]	0	1.001		0.267					
	300	0.970	(97)	0.261	(98)				
	800	0.952	(95)	0.259	(97)				
	1600	0.877**	(88)	0.243	(91)				
Seminal vesicle [g]	0	1.356		0.362					
	300	1.273	(94)	0.343	(95)				
	800	**1.221****	(90)	0.334	(92)				
	1600	**1.190****	(88)	0.330	(91)				
Spleen [g]	0	0.590		0.156		0.486		0.209	
	300	0.606	(103)	0.163	(104)	0.468	(96)	0.205	(98)
	800	0.589	(100)	0.160	(103)	0.501	(103)	0.218	(104)
	1600	0.596	(101)	0.165	(105)	**0.428***	(88)	**0.193***	(92)
Testes [g]	0	3.759		0.998					
	300	3.794	(101)	1.025	(103)				
	800	3.897	(104)	1.063	(107)				
	1600	3.948	(105)	1.100	(110)				
Ovaries [g]	0					119.1		0.051	
	300					**110.2***	(92)	**0.048***	(94)
	800					123.8	(104)	0.054	(105)
	1600					122.8	(103)	0.055	(107)

Table 5.6.1-26: Organ weights of F_0 male and female parental animals

Generation		F_0 Males				F_0 Females			
	Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Thyroid [mg]	0	23.96		0.006		18.96		0.008	
	300	25.20	(105)	0.007	(106)	20.28	(107)	0.009	(109)
	800	24.56	(103)	0.007	(105)	21.64	(114)	**0.009****	(115)
	1600	24.92	(104)	0.007	(108)	20.92	(110)	**0.009****	(115)
Thymus [mg]	0	258.4		0.068		334.2		0.144	
	300	267.0	(103)	0.072	(105)	324.7	(97)	0.142	(99)
	800	298.8	(116)	**0.080****	(118)	301.8	(90)	0.132	(91)
	1600	**303.2****	(117)	**0.085****	(124)	**278.1****	(83)	**0.125****	(87)

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))
& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

The increased absolute and relative liver weights in females of test groups 02 (800 ppm) and 03 (1600 ppm) were considered to be treatment-related.
The relative liver weights were significantly increased in males of test groups 02 (2.268%) and 03 (2.405%). Because the relative liver weights were within the range of historical control data (2.147% - 2.507%) and because there were no treatment-related histopathological findings, the increased relative liver weights in males of test groups 02 and 03 were related to the slightly, but not significantly decreased terminal body weight in these test groups (test group 02: -3%; test group 03: - 4%).
Because there was no dose-response relationship, the decreased absolute and relative weights of heart and ovaries in females of test group 01 (300 ppm) as well as the increased kidney weights in females of test groups 02 and 03 were regarded to be incidental. These organs did not show treatment-related findings, histopathologically.
The increased relative adrenal weight in females and the increased relative kidney weights in males of test group 03 were related to the slightly, but not significantly decreased terminal body weight (-4% in both sexes) in this test group. There were no histopathological correlates for the weight increase.
The absolute weight of the prostate (0.877 g) was significantly decreased in test group 03. The prostate weight was within the range of historical control data (0.796 g - 1.228 g) and there was no histopathological correlate. In addition, the relative prostate weight did not show a significant decrease. Therefore, the decrease of prostate weight was related to the slightly but not significantly reduced terminal body weight (-4%) in this test group.
The absolute weights of the seminal vesicles were significantly decreased in test groups 02 (1.221g) and 03 (1.190g). These weights were within the range of historical control data (0.905g - 1.426 g) and there were no histopathological correlates. In addition, the relative weights of the seminal vesicle did not show a significant decrease. Therefore, the decreased weights of the seminal vesicles were related to the slightly, but not significantly reduced terminal body weight in these test groups (test group 02: -3%; test group 03: - 4%).

The absolute and relative spleen weights were decreased in females of test group 03. Because there was no dose-response relationship, there were no histopathological findings, and there was no effect in F1 cohort 1A females, the weight decrease was considered to be incidental.
The thymus weight was increased in males of test groups 02 (relative) and 03 (absolute and relative). Because there were no histopathological correlates, these weight changes were regarded to be incidental. In females the absolute and relative thymus weights were decreased in test group 03. Because there were no histopathological correlates and there was no effect in F1 cohort 1A females, these weight changes were regarded to be incidental.
The relative weights of the thyroid glands were increased in females of test groups 02 and 03. The absolute weights were not significantly changed and there were no histopathological correlates. Therefore, the weight increases were considered to be incidental.

Gross lesions
All gross lesions occurred singularly or were similar in distribution pattern. All of them were considered to be spontaneous lesions in origin and were not related to treatment.
The female animals, which were not pregnant, as well as the male mating partners did not show gross lesions.

Histopathology
In the liver, a minimal or slight centrilobular hepatocellular hypertrophy was noted in 13 females of test group 03 (1600 ppm). Five females in this test group showed a minimal increase of apoptotic hepatocytes that was observed mostly in centrilobular areas. In addition, the severity of lymphoid infiltration was slightly increased in females of test group 03. All of these findings were considered to be treatment-related. The incidence and severity is given in the table below:

Table 5.6.1-27: Histopathology results in the liver of female animals

Liver	**Female animals**			
Test group [ppm]	**00**	**01 [300]**	**02 [800]**	**03 [1600]**
Organs examined	24	25	25	25
Hypertrophy, centrilobular	0	0	0	13
• Grade 1				11
• Grade 2				2
Apoptosis, increased	0	0	0	5
• Grade 1				5
Infiltration, lymphoid	24	25	25	25
• Grade 1	23	18	21	12
• Grade 2	1	7	4	7
• Grade 3				6

All other findings noted were single observations either, or were similarly in distribution pattern and severity in control rats compared to treatment groups. All of them were considered to be incidental and spontaneous in origin and without any relation to treatment.
The female animals, which were not pregnant, as well as their male mating partners did not show histopathological findings explaining the infertility.
Female No. 101 (test group 00) had unscheduled mated with male No. 40 (test group 01). It was sacrificed prematurely and no further investigations were performed.

C.2 PATHOLOGY IN F1 PUPS

Organ weights

The terminal body weight was significantly decreased in males of the 300 ppm test group as well as in males and females of the 1600 ppm test group, resulting in statistical significant secondary weight changes in various organs: decreased absolute spleen weight and increased relative weights of brain and thymus in males of the 300 ppm test group, as well as decreased absolute weights of brain, spleen and thymus and increased relative brain weight in males and females of the 1600 ppm test group. The increased relative weights of brain and thymus in females of the 300 ppm test group was related to the slightly but not significantly reduced terminal body weight (-2%) in this test group.

Table 5.6.1-28: Organ weights of F_1 pups on PND 22

Generation: F_1 pups		Males				Females			
Organ	Dose [mg/kg]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
Terminal body weight	0	56.63				54.67			
	300	**54.95***	-3			53.25	-2		
	800	55.71	-2			53.46	-2		
	1600	**52.13****	-8			**48.41****	-11		
Brain	0	1.57		2.78		1.5		2.77	
	300	1.56	0	**2.85***	3	1.52	1	**2.86***	3
	800	1.55	-1	2.79	0	1.49	0	2.80	1
	1600	**1.52****	-3	**2.94****	6	**1.47****	-2	**3.05****	10
Thymus[#]	0	255.81		0.45		262.08		0.48	
	300	269.93	6	**0.49****	9	274.59	5	**0.52***	8
	800	253.22	-1	0.45	0	262.37	0	0.49	2
	1600	**230.83****	-9	0.44	-2	**225.84****	-14	0.47	-3
Spleen	0	0.30		0.53		0.29		0.52	
	300	**0.28***	-6	0.51	-3	0.28	-2	0.52	0
	800	0.31	4	0.56	5	0.29	2	0.54	4
	1600	**0.27****	-10	0.52	-1	**0.25****	-14	0.51	-3

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis H and Wilcoxon-test (two-sided))
[#] thymus weight in mg
Values may not calculate exactly due to rounding of figures

Gross lesions

A few F_1 pups showed spontaneous findings at necropsy, such as discoloured liver lobe (pale), post mortem autolysis, discoloured left testis (red), malpositioned left testis (abdominal region) and abnormal liver lobation. These findings occurred without any relation to dosing and/or can be found in the historical control data at comparable or even higher incidences.

Thus, all these findings were not considered to be associated with the test substance.

Table 5.6.1-29: Incidence of gross necropsy observations in F_1 pups

Dose [ppm]		0	300 / 150	800 / 400	1600 / 800
		F_1 pups (males/females days 0 - 21)			
Litters evaluated		24	23	25	22
Pups evaluated (m/f)		50 /31	37 / 42	45 / 50	32 / 41
General (m/f)	[N]	3/1	0/1	2/2	2/4
	[%]	6.0/3.2	0.0/2.4	4.4/4.0	6.2/9.8
Post mortem autolysis (m/f)	[N]	3/1	0/0	1/0	0/0
	[%]	6.0/3.2	0.0/0.0	2.2/0.0	0.0/0.0
Historical control range(m+f)	[%]	0.0 - 2.7			
abnormal liver lobation (f)	[N]	0	0	0	1
	[%]	0.0	0.0	0.0	2.4
Historical control range(m+f)	[%]	0.0 - 0.4			
not assessed (m/f)	[N]	0/0	0/1	1/2	2/4
	[%]	0.0/0.0	0.0/2.4	2.2/4.0	6.2/9.8
liver lobe discoloured (m/f)	[N]	1/0	0/0	0/0	0/1
	[%]	2.0/0.0	0.0/0.0	0.0/0.0	0.0/2.4
Testes (m)	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
discoloured	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
malpositioned	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
Total pup necropsy observations					
animals with signs	[N]	4	1	2	2
affected pups	[%]	8.0	2.7	4.4	6.2
Historical control range(m+f)	[%]	0.0 - 3.7			

C.3 PATHOLOGY IN F1 REARING ANIMALS (COHORT 1A)

Some weight parameters were significantly changed in one or more dose groups and were listed in the following table.

Table 5.6.1-30: Organ weights of cohort 1A animals

Generation		F_1 Males (cohort 1A)				F_1 Females (cohort 1A)			
	Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Terminal weight [g]	0	333				201			
	300	320	(96)			195	(97)		
	800	**304****	(91)			193	(96)		
	1600	**297****	(89)			192	(96)		
Brain [g]	0	2.033		0.614		1.832		0.948	
	300	2.044	(101)	0.644	(105)	1.893	(100)	0.976	(103)
	800	**1.970****	(97)	**0.651***	(106)	1.851	(98)	0.960	(101)
	1600	1.994	(98)	**0.647****	(110)	1.866	(99)	0.978	(103)
Heart [g]	0	0.989		0.297		0.677		0.338	
	300	0.956	(97)	0.299	(101)	0.668	(99)	0.344	(102)
	800	**0.906****	(92)	0.298	(100)	0.662	(98)	0.343	(101)
	1600	**0.916****	(93)	0.307	(103)	0.696	(103)	**0.363****	(107)
Kidneys [g]	0	2.190		0.659		1.457		0.727	
	300	2.124	(97)	0.666	(101)	1.411	(97)	0.726	(100)
	800	2.070	(95)	0.681	(103)	1.460	(100)	0.755	(104)
	1600	**2.035****	(93)	0.686	(104)	1.499	(103)	**0.781****	(107)
Liver [g]	0	8.252		2.482		5.203		2.599	
	300	7.956	(96)	2.486	(100)	5.111	(98)	2.627	(101)
	800	7.598	(92)	2.496	(101)	**5.215**	(100)	2.697	(104)
	1600	8.093	(98)	**2.725****	(110)	**5.798****	(111)	**3.026****	(116)
Cauda epididymis [g]	0	0.388		0.117					
	300	0.382	(99)	0.121	(103)				
	800	0.373	(96)	0.123	(106)				
	1600	**0.344****	(89)	0.116	(100)				
Epididymides [g]	0	1.036		0.312					
	300	1.023	(99)	0.322	(103)				
	800	1.008	(97)	0.333	(107)				
	1600	**0.946****	(91)	0.320	(102)				
Prostate [g]	0	0.752		0.227					
	300	**0.669***	(89)	0.212	(93)				
	800	**0.643****	(86)	0.213	(94)				
	1600	**0.576****	(77)	0.194	(85)				
Seminal vesicle [g]	0	1.004		0.304					
	300	0.958	(95)	0.302	(99)				
	800	**0.831****	(83)	0.275	(91)				
	1600	**0.708****	(71)	**0.239****	(79)				
Testes [g]	0	3.637		1.096					
	300	3.564	(98)	1.123	(102)				
	800	3.766	(104)	1.244**	(113)				
	1600	3.746	(103)	1.269**	(116)				
Ovaries [g]	0					91.84		0.046	
	300					97.95	(107)	**0.050***	(110)
	800					94.95	(103)	0.049	(107)
	1600					103.20	(112)	**0.054****	(117)
Thyroid [mg]	0	21.05		0.006		16.42		0.008	
	300	21.35	(101)	0.007	(106)	17.15	(104)	0.009	(108)
	800	22.00	(105)	**0.007***	(114)	17.35	(106)	0.009	(109)
	1600	23.15	(110)	**0.008****	(123)	17.65	(107)	0.009	(112)
Thymus [mg]	0	383.9		0.115		345.9		0.173	
	300	372.6	(97)	0.116	(101)	355.1	(103)	0.181	(105)
	800	385.7	(100)	0.126	(110)	343.9	(99)	0.178	(103)
	1600	400.5	(104)	**0.134****	(117)	342.6	(99)	0.179	(104)

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))
& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

The increased absolute and relative liver weights in females of test group 13 (1600 ppm) were considered to be treatment-related. The terminal body weight was significantly decreased in males of test groups 12 and 13 resulting in statistical significant secondary weight changes in various organs: decreased absolute weights of the cauda epididymis, the epididymides and the kidneys in test group 13, in a decreased absolute brain weight in test group 12 (800 ppm), in decreased absolute weights of the heart, the prostate and seminal vesicles in test groups 12 and 13, in a decreased relative weight of the seminal vesicles in test group 13, increased relative weights of the liver and the thymus in test group 13, as well as increased relative weights of the brain, testes and thyroid glands in test groups 12 and 13.
The decreased absolute weights of the prostate in males of test groups 11 (300 ppm) as well as the increased relative heart and kidney weight in female animals of test group 13 were related to the slightly, but not significantly decreased terminal body weights (-4%/ -4%) in these test groups.
In all of these organs, there were no treatment-related histopathological findings.
Because there was no dose-response relationship and due to the lack of a histopathological correlate, the increased relative weights of the ovaries in females of test groups 11 (300 ppm) and 13 (1600 ppm) were regarded to be incidental.

Gross lesions
All gross lesions occurred singularly. All of them were considered to be spontaneous lesions in origin and were not related to treatment.

Histopathology
A minimal centrilobular hepatocellular hypertrophy was noted in 15 females of test group 13 (1600 ppm). In addition, the severity of lymphoid infiltration was slightly increased in females of this test group. All of these findings were considered to be treatment-related. The incidence and severity is shown in Table 5.6.1-31:

Table 5.6.1-31: Histopathology results in the liver of female animals

Liver	Female animals			
Test group [ppm]	00	01 [300]	02 [800]	03 [1600]
Organs examined	20	20	20	20
Hypertrophy, centrilobular	0	0	0	15
• Grade 1				15
Infiltration, lymphoid	19	19	20	20
• Grade 1	16	18	17	12
• Grade 2	3	1	3	5
• Grade 3				3

All other findings noted were single observations either, or were similarly in distribution pattern and severity in control rats compared to treatment groups. All of them were considered to be incidental and spontaneous in origin and without any relation to treatment. One control female died intercurrently but was without any histopathological findings.

Differential ovarian follicle count

The results of the differential ovarian follicle count (DOFC) – comprising the numbers of primordial and growing follicles, as well as the combined incidence of primordial plus growing follicles – did not reveal significant deviations between controls and animals of test group 13 (1600 ppm).

C.4 PATHOLOGY IN F1 REARING ANIMALS (COHORT 1B)

Some weight parameters were significantly changed in one or more dose groups and were listed in the following table.

Table 5.6.1-32: Organ weights of cohort 1B animals

Generation		F_1 Males (cohort 1B)				F_1 Females (cohort 1B)			
	Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Terminal weight [g]	0	318				200			
	300	311	(98)			198	(99)		
	800	**309***	(97)			199	(99)		
	1600	309	(97)			191	(95)		
Prostate [g]	0	0.737		0.232					
	300	**0.665***	(90)	0.217	(93)				
	800	**0.625****	(85)	**0.203****	(87)				
	1600	**0.542****	(74)	**0.176****	(76)				
Seminal vesicle [g]	0	0.949		0.300					
	300	0.899	(95)	0.293	(98)				
	800	**0.812****	(86)	**0.265***	(88)				
	1600	**0.721****	(76)	**0.234****	(78)				
Testes [g]	0	3.554		1.124					
	300	3.569	(100)	1.159	(103)				
	800	3.709	(104)	**1.203***	(107)				
	1600	**3.847****	(108)	**1.248****	(111)				

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))
& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

For all other organs mean relative weight parameters in males and all mean relative weight parameters in females did not show significant differences when compared to the control group 10. The decreased absolute and relative weights of prostate and seminal vesicle in test groups 12 (800 ppm) and 13 (1600 ppm) were considered to be treatment-related. Because the relative prostate weight in males of test group 11 (300 ppm) did not show a significant change, the decreased absolute prostate weight was related to the slightly, but not significantly decreased terminal body weights (-2%) in this test group. The testes weights were significantly increased in test groups 12 (relative) and 13 (absolute and relative). There was no comparable effect in F0 generation parental males. In Cohort 1A males, the relative testes weights were increased in test groups 02 and 03 but there was no histopathological correlate and the increased testes weights could be related to reduced terminal body weights. Therefore, a treatment-related effect seemed rather unlikely.

Gross lesions

All gross lesions occurred singularly. All of them were considered to be spontaneous lesions in origin and were not related to treatment.

III. CONCLUSIONS

Thus, under the conditions of the present extended one-generation reproduction toxicity study the **NOAEL** (no observed adverse effect level) **for general, systemic toxicity** is **300 ppm**, based on decreased food consumption and body weight/body weight gain, as well as clinical-chemical changes and pathological evidence of liver toxicity at 800 and/or 1600 ppm, in the F_0 parental animals and adult F_1 offspring.

The **NOAEL for fertility and reproductive performance** for the parental rats is **1600 ppm**, the highest tested dose.

The **NOAEL for developmental toxicity** in the F_1 progeny is **300 ppm**, due to the decrease in the pre-weaning pup body weights/pup weight gains, as well as decreased anogenital distance/index and delay of puberty in males at 800 and/or 1600 ppm.

[redacted] 1990, SAG 151: Two generation oral (dietary administration) reproduction toxicity study in the rat (two litters in the F1 generation) (BASF DocID DK-430-001)

Dimethomorph was fed daily to four groups of Sprague-Dawley rats at dietary concentrations of 0, 100, 300 and 1000 ppm. The P1 generation consisted of 30 male and 30 female rats per group while the F1 generation comprised 25 pairs per group. The pre-mating treatment periods for the P1 and F1 parental animals were 100 days. Treatment for both parental generations continued during the 21-day mating periods and post-mating periods until scheduled sacrifice. Following weaning of the F2a litters, the F1 parents were paired for a second 21-day mating period to produce the F2b litters. Mated P1 and F1 females continued to be treated during the ensuing gestation and lactation periods. All litters were culled to eight pups/litter on postnatal day 4.

Findings:

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Test substance intake values were similar for P1 and F1 females during gestation and increased during lactation. Equivalent mean test substance intake values, for the P1 and F1 parents, are 6.7, 20 and 67 mg/kg bw/day for the 100, 300 and 1000 ppm groups, respectively, based on nominal concentrations (WHO, 2000).

P1 and F1 parental animals

No treatment-related mortality occurred in either parental generation and no clinical signs of toxicity were observed at any dietary level. Food consumption, body weights and body weight gains for P1 and F1 males were unaffected by treatment with dimethomorph. Dose-related reductions in body weights and body weight gains were observed during the pre-mating treatment period for P1 females in the 1000 group. Body weight gains during the pre-mating treatment period for P1 females in the 1000 ppm group were reduced 14.7 % as compared to controls. Statistically significant reductions in food consumption, relative to controls, were only noted during the first five weeks of the pre-mating treatment period for P1 females in the 1000 ppm group. Although not statistically significant, a decrease in body weight gain of 6.8% was observed during the pre-mating treatment period for F1 females in the 1000 ppm group as compared to controls. No toxicologically significant changes in food consumption patterns were noted for F1 females in treated groups when compared to controls (see Table 5.6.1-33)

Table 5.6.1-33: Effects on bodyweights and food consumption.

Dose level (ppm)		M				F			
		0	100	300	1000	0	100	300	1000
F0	**Bodyweight** (g)								
	Week 5	362.5	360.7	356.2	368.2	222.5	221.0	217.0	206.8*
	Week 15	526.5	525.8	511.0	543.0	292.3	291.2	280.8	267.2*
	Weight gain (g)								
	Week 1-15	332.6	334.3	320.2	352.2	138.0	137.5	128.3	117.7*
	Food consumption (g)								
	Week 1	24.9	24.5	24.6	24.5	18.1	17.8	18.1	16.3*
	Week 2	26.7	25.8	25.6	26.2	18.2	18.2	17.9	17.0*
	Week 3	27.4	26.9	26.8	27.3	19.0	19.1	18.9	17.7*
	Week 4	28.6	27.4	27.5	28.0	19.4	19.5	19.2	18.0*
	Week 5	28.2	27.1	26.8	27.8	19.0	19.2	18.7	17.3*
	Week 1-14	27.4	26.3	26.0	27.0	18.3	18.5	18.3	17.5
F1	**Bodyweight** (g)								
	Week 15	521.6	520.0	507.2	520.2	292.4	285.2	281.0	277.4
	Weight gain (g)								
	Week 1-15	397.8	399.6	385.0	398.6	183.4	178.2	174.4	171.0
Intake (mg/kg bw/d)									
F0	**Pre-mate**	-	6.9	20.8	69.0	-	8.0	24.0	79.3
	Gestation					-	6.8	20.8	71.4
	Lactation					-	13.5	40.7	140.2
F1a	**Pre-mate**	-	7.9	23.7	78.6	-	8.9	27.0	89.2
	Gestation					-	7.0	21.5	74.1
	Lactation					-	14.6	46.2	151.8
F2b	**Pre-mate**	-	4.9	15.0	50.0	-	6.7	21.2	68.5
	Gestation					-	6.5	19.7	66.4
	Lactation					-	13.3	41.6	138.1

No adverse effects of treatment were indicated from the macroscopic postmortem evaluations of the P1 of F1 parental animals and no treatment-related microscopic changes were observed in any of the parental reproductive organs and tissues or pituitary glands.

No adverse effects at the 100, 300 or 1000 ppm dietary levels were evident for mating, fertility or gestation indices or for the parturition data during either litter interval. The number of females with duration of pregnancy of 21 days was increased (borderline statistical significance) in both the P1 and F1 generation in the 1000 ppm group (see Table 5.6.1-34).

Table 5.6.1-34: Summary of pregnancy duration.

Parameter		Dose level (ppm)			
		0	**100**	**300**	**1000**
Gestation duration (d)	F1	22.0 +/- 0.3	22.1 +/- 0.3	21.9 +/- 0.4	21.8 +/- 0.4
ND (#)		-	1	3	1
21 days (#)		1	-	3	4
22 days (#)		21	23	20	18
23 days (#)		1	2	1	-
Gestation duration (d)	F2A	21.9 +/1- 0.4	21.9 +/- 0.3	22.1 +/- 0.3	21.7 +/- 0.5
ND (#)		-	-	-	-
21 days (#)		2	2	-	7
22 days (#)		16	19	18	17
23 days (#)		1	-	2	-
Gestation duration (d)	F2B	21.9 +/- 0.2	22.2 +/- 0.5	21.9 +/- 0.3	21.8 +/- 0.4
ND (#)		-	-	-	-
21 days (#)		1	1	1	3
22 days (#)		18	12	11	15
23 days (#)		-	4	-	-

No details were provided on the statistical analysis in the DAR. A statistical re-evaluation has been performed for the AIR 3 dossier on this end point and gave the following results:

Table 5.6.1-35: Re-evaluation of the duration of pregnancy (Dunnett test (two-sided))

Statistical analysis		Dose level			
	F1	0	100	300	1000
Mean		22.00	22.08	21.92	21.82
SD		0.30	0.28	0.41	0.39
N		23	25	24	22
%DEV		0	0	-0	-1
SIG					
pvalue			0.7653	0.7489	0.1999
	F2A				
Mean		21.95	21.90	22.10	21.70
SD		0.40	0.30	0.31	0.47
N		19	21	20	23
%DEV		0	-0	1	-1
SIG					
pvalue			0.9696	0.4491	0.0900
	F2B				
Mean		21.95	22.18	21.92	21.83
SD		0.23	0.53	0.29	0.38
N		19	17	12	18
%DEV		0	1	-0	-1
SIG					
pvalue			0.1832	0.9930	0.6947

*p<=0.05 **p<=0.01

No statistical significance was observed for this end-point. It is therefore considered that this effects (if any) is not treatment-related.

F1 and F2 pups
Pup survival and mean pup weights for the F1, F2a and F2b offspring were comparable among all groups. However, in all groups, including controls, the pup mortality after postnatal day 4 was higher than it is considered normal for well-conducted studies. It must be assumed that the environmental conditions in this study were sub-optimal. In the 1000 ppm group, the percentage of pups in the F1, F2a and F2b generations which achieved incisor eruption was reduced on one or more days from postnatal days 9 - 11 when compared to controls and the differences were statistically significant. There were no treatment-related effects observed for any other developmental landmark (i.e., pinna unfolding, hair growth, or eye opening). The mean days of incisor eruption for the F1, F2a and F2b litters in the 1000 ppm group were 10.5, 10.5 and 10.7 days, respectively while the mean days of incisor eruption for control litters were 9.6, 9.6 and 9.7 days, respectively, for the same generations. Thus, incisor eruption in the 1000 ppm group was delayed by approximately one day. Rat pups begin to eat solid food in addition to milk at around postnatal day 15. By this time incisor eruption was complete in all groups. Thus, the delay in incisor eruption did not interfere with the development of feeding ability and is not considered an adverse finding (see Figure 5.6.1-8, Figure 5.6.1-9 and Figure 5.6.1-10)

Figure 5.6.1-8: F1a Generation – Incisor eruption

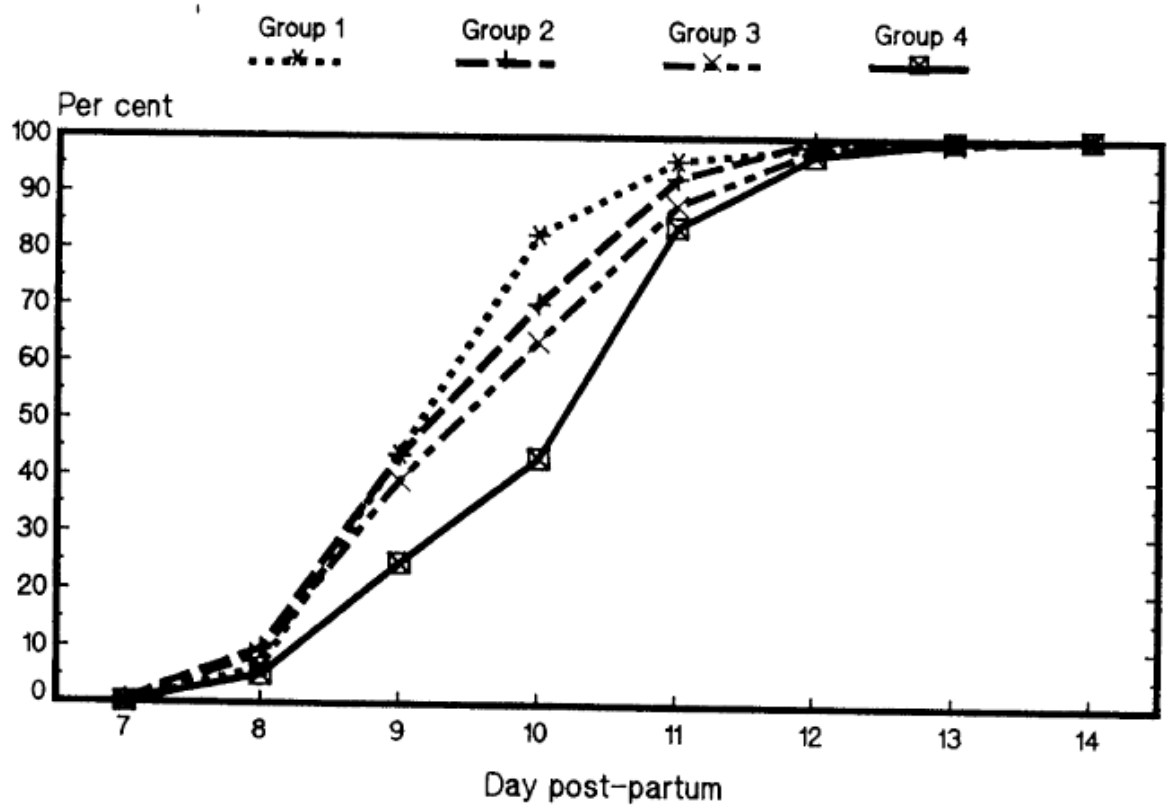


Figure 5.6.1-9: F2a Generation – Incisor eruption

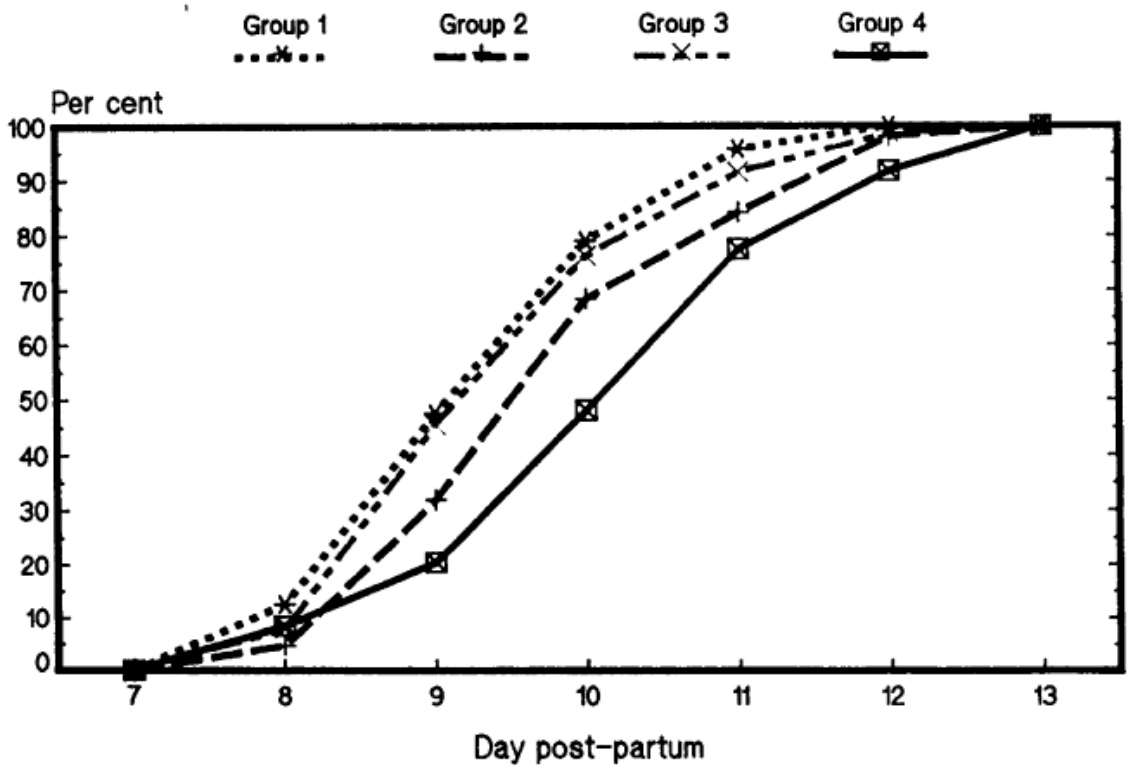
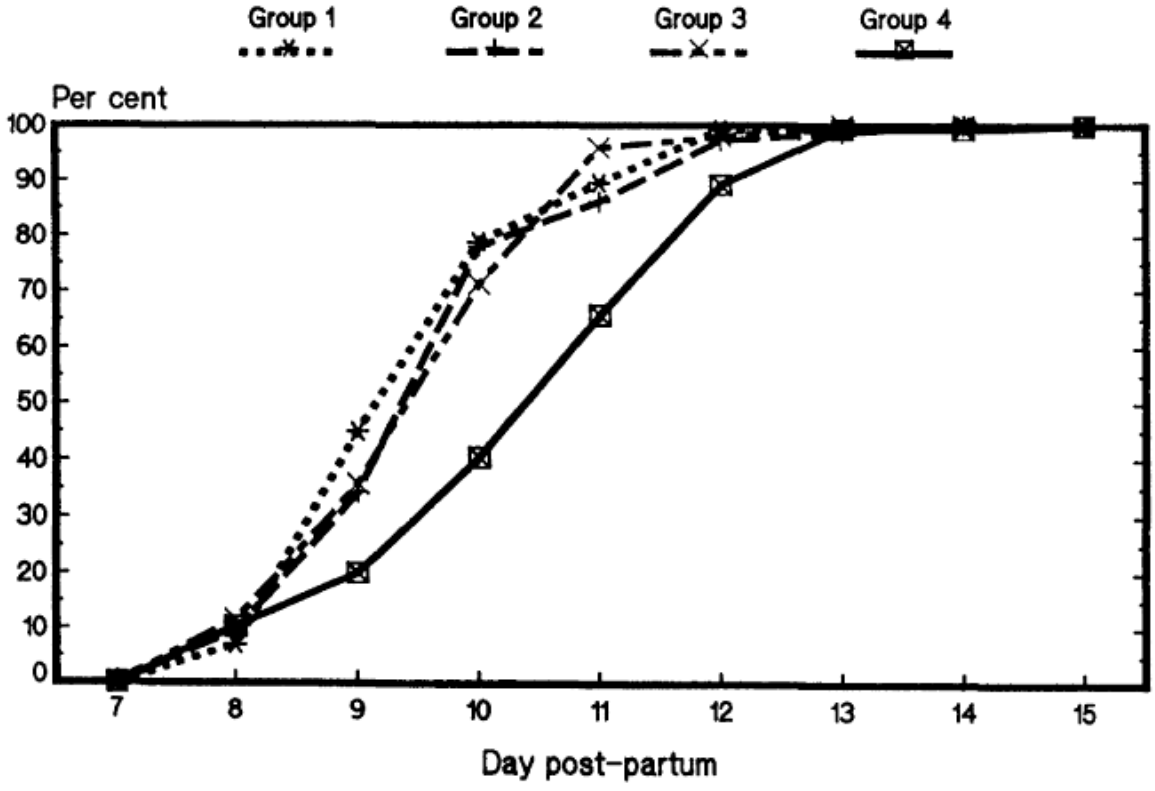


Figure 5.6.1-10: F2b Generation – Incisor eruption



All other physical development and reflex assessments were unaffected by prenatal exposure to dimethomorph at dietary concentrations up to and including 1000 ppm. No adverse effects of treatment with dimethomorph were indicated from the macroscopic postmortem evaluations of the F1, F2a or F2b offspring.

Conclusion:
Based on these results, the NOAEL for parental toxicity was 300 ppm (equivalent to 20 mg/kg bw/day), based on reductions in pre-mating body weight gains for P1 and F1 females in the 1000 ppm group. Because the delay in incisor eruption at 1000 ppm was not considered adverse, the NOAEL for the growth and development of offspring was 1000 ppm (equivalent to 67 mg/kg bw/day). The NOAEL for fertility and reproductive function was 1000 ppm (equivalent to 67 mg/kg bw/day), the highest concentration tested.

CA 5.6.2 Developmental toxicity studies

[redacted] 1989, SAG 151: Oral (gavage) teratogenicity study in the rat (BASF DocID DK-432-002)

Four groups of mated female Sprague-Dawley rats (30 females/group) were used in this study. Dimethomorph was administered by gavage as a suspension in distilled water containing 0.1% Tween 80. Animals were treated on gestation days 6 through 15 at dose levels of 0, 20, 60 and 160 mg/kg bw/day. On Day 20 post coitum, all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placentae). For each dam, corpora lutea were counted and the number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external findings. Thereafter, nearly one half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings.

Findings:

The stability and homogeneity distribution of the test substance in the water, as well as the correctness of the concentration, were confirmed by analysis. Only one death, resulting from gavage error, was noted in the 160 mg/kg bw/day group. All other animals survived to scheduled sacrifice and there were no treatment-related clinical signs or macroscopic pathology observed in any dose group. Statistically significant reductions in food consumption (gestation days 6-15) and body weights (gestation days 10, 15 and 20) were observed for females in the 160 mg/kg bw/day group as compared to controls.

Body weight gain during the treatment period (gestation days 6-15) was reduced by 28% for females in the 160 mg/kg bw/day group. This overall reduction, relative to controls, in body weight gain for the high-dose group, reflects a marked decrease (non-statistically significant) in body weight gain (7.0 grams versus 21.8 grams for controls) during the first half of the treatment period (gestation days 6-10). Body weight gains were comparable to controls for the second half of treatment (gestation days 10-15) as well as during the post-treatment period (gestation days 15-20) for the 160 mg/kg bw/day group.

Pregnancy rate for the 20 mg/kg bw/day group was 67% as compared to the control pregnancy rate of 83%; this finding was considered incidental as pregnancy rates in the 60 and 160 mg/kg bw/day groups (73% and 77%, respectively) were comparable to controls, and treatment was not initiated until after implantation had occurred.

Slight embryotoxicity, as evidenced by an increased number (non-statistically significant) of early resorptions was observed in the 160 mg/kg bw/day group as compared to controls. However, this increase reflects data from two females (Animals Nos. 80 and 83) in this group. The implantation data from Animal Nos. 80 and 83 indicated no viable fetuses for either female with a total of 15 and 14 early resorptions, respectively. A total litter loss was also observed in a female of the 60 mg/kg bw/day but in this female only one implantation was seen. The historical controls for total litter loss in other studies are between 0 and 1 (see Table 5.6.2-1). Therefore, the effect seen in the 60 mg/kg bw/day cannot be attributed to the treatment and due to the frequency of this finding in other studies, the effect seen at 160 mg/kg bw/day is doubtful, however as this effect is just outside the historical controls a treatment-related effect cannot be ruled-out. This effect can also be caused by maternal toxicity seen at this dose level.

The two high dose dams with total litter loss (Nos. 80 and 83) were specifically affected. During the early phase of pregnancy (GD 6-10) they had markedly reduced food consumption of below 50% compared to controls as well as body weight loss of -15 and -20 g, respectively. This is likely the cause of the early resorptions (total litter loss) observed in these dams. All other ovarian, uterine and fetal observations were unaffected by treatment at dose levels up to and including 160 mg/kg bw/day. No treatment-related malformations or variations were evident from the fetal external, visceral or skeletal examination data.

Table 5.6.2-1: Historical control data of teratogenicity studies in SD rat since 1984 – Total litter loss

Study No.	Number of females examined	Number of females with 100 % intra-uterine death
1	21	0
2	23	0
3	22	0
4	22	0
5	21	0
6	24	0
7	19	1
8	24	0
9	22	0
10	23	1
11	22	0
12	22	0
13	23	0
14	20	0
15	23	0
16	21	0
17	20	0
18	21	1
19	21	0
20	20	0
21	23	0
22	22	1
23	22	0

Table 5.6.2-2: Summary of relevant findings ([redacted], 1989)

Dose level (mg/kg bw/d)		**0**	**20**	**60**	**160**
Mated (#)		30	30	30	30
Pregnant (#)		25	20	22	23
Deaths (#l)		-	-	-	1
Total litter loss (#)		-	-	1	2
Litters (#)		25	20	21	20
Bodyweight (g)	**Day 0**	204.2	203.8	200.5	201.5
	Day 20	356.2	352.3	346.2	335.8**
Weight gain (g)	**Day 6-10**	21.8	22.2	19.3	7.0
	Day 6-15	51.8	50.6	49.3	37.3
	Day 0-20	152.0	148.5	145.7	134.3
Food consumption (g)	**Day 6-10**	23.2	23.1	22.0	16.5**
	Day 6-15	24.9	24.5	24.0	19.2
	Day 0-20	24.8	24.6	24.5	22.2
Implantations (#)		13.1	13.5	12.1	13.6
Post-implantation loss (#)		3.9	6.3	8.8	15.6
Litter size (#)		12.6	12.6	12.1	12.6
Foetal weight (g)		3.40	3.45	3.46	3.34
Litter weight g)		42.7	43.3	41.8	41.8
Total malformations (#)		1	1	-	2

**significantly different to controls (p<0.05); **p<0.01*

Conclusion:

Based on the reductions in food consumption, body weights and body weight gain in the 160 mg/kg bw/day group, the NOAEL for maternal toxicity was 60 mg/kg bw/day. Based on embryotoxicity (total litter loss in two animals) observed at 160 mg/kg bw/day, the NOAEL for developmental toxicity was 60 mg/kg bw/day. Thus, embryotoxicity was not observed at dose levels which were not maternally toxic. Dimethomorph is not teratogenic in the Sprague-Dawley rat at doses up to 160 mg/kg bw/day.

[redacted] 1986, ZHT 236Z50 – Preliminary oral (Gavage) embryotoxicity study in rats (BASF DocID DK-432-001)

Dimethomorph was administered by intragastric intubation to groups of eight sexually mature and mated female Sprague-Dawley rats at dose levels of 50, 120 or 300 mg/kg bw/day for ten consecutive days from Days 6 to 15 of gestation. A control group of eight rats received distilled water with admixture of 0.1 % Tween 80 for the same period of time. The test substance was prepared daily as an aqueous suspension; separate preparations were made for each dose level. The dose volume was 10 mL/kg, adjusted daily on the basis of the individual body weights. All animals were examined twice daily for signs of ill-health, toxicity and behavioral changes; daily mortality checks were also performed. Body weights were recorded on Days 0, 6, 10, 15 and 20 of gestation. On Day 20 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined and the following data recorded: number of corpora lutea, number and position of implantation (live fetuses, early intra-uterine deaths, early-late intra-uterine deaths and late intra-uterine deaths), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

Findings:

No treatment-related clinical changes or necropsy findings were observed with dimethomorph. Also, there were no treatment-related effects on maternal body weight gain, on pregnancy incidence, pre-implantation loss or post-implantation loss. No treatment-related effects on number, weight or sex of the fetuses. No incidence of external malformations in the fetuses was recorded.

Conclusion:

In this preliminary range-finding study, the administration of dimethomorph by oral gavage at dose level up to 300 mg/kg bw/day, did not elicit maternal toxicity or embryolethality.

[redacted], 1987, CME 151 – Letter Report: Treatment of pregnant Sprague-Dawley rats with CME 151 (BASF DocID DK-432-006)

In a second range-finding study with dimethomorph, pregnant Sprague Dawley rats were treated with dimethomorph by oral gavage, in a constant dosing volume of 10 mL/kg, during the period of organogenesis (day 6 to 15 of gestation). Four pregnant rats received 150 mg/kg dimethomorph and four other pregnant rats received 300 mg/kg dimethomorph. Details regarding maternal or foetal gross and histopathological examinations were not provided in this letter report.

Findings:

One of four females treated with 150 mg/kg/day showed 100 percent intra-uterine deaths. In addition, one to three early resorptions in each of the further three females receiving 150 mg/kg/day was observed. Two out of three females receiving 300 mg/kg/day showed 100 percent intra-uterine deaths. Pups from the other female receiving 300 mg/kg/day had reduced mean fetal weight.

Conclusion:

No conclusions were made in this letter report. However, the findings from this study were used in combination with other acute studies to select doses used in the definitive oral teratogenicity study in the rat.

[redacted] 1989, SAG 151: Oral (gavage) teratogenicity study in the rabbit (BASF DocID DK-432-004)

Four groups of inseminated female New Zealand White rabbits (22 does/group) were used in this study. Dimethomorph was administered by gavage as a suspension in distilled water containing 0.1% Tween 80. Animals were treated on gestation days 6 through 18 at dose levels of 0, 135, 300 or 650 mg/kg bw/day. On Day 29 post insemination, all surviving females were sacrificed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each dam, corpora lutea were counted and the number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

Findings:

The stability and homogeneity distribution of the test substance in the water, as well as the correctness of the concentration, were confirmed by analysis. In the definitive study, a few accidental deaths occurred as a result of gavage errors, which were noted in all groups. There were no mortalities that were attributed to the test material. A slightly increased abortion rate in the 650 mg/kg bw/day group (3 of 22 does versus 1 of 22 does in the control group) was attributed to the test material. It has to be noted that does No. 59, 60, and 71 had a marked loss of body weight early in pregnancy of -0.3 to -0.5 kg between gestational days 6 and 12 when compared to a body weight gain of 0.1±0.1 kg for the control group. Food consumption of these three animals ranged between 33 to 96 g/day compared to a control mean food consumption of 207 g/day. These animals had abortions between gestational days 17 and 21, which is likely due to their poor general state.

There were no treatment-related clinical signs or macroscopic pathology findings observed in any dose group.

Because of several individual body weight losses early in the treatment period (gestation days 6-12), mean body weight gains for the entire treatment period (gestation days 6-18) were reduced in the 650 mg/kg bw/day group as compared to controls but the differences were not statistically significant. Body weight gains during the post-treatment period (days 18-24) were slightly increased for the 650 mg/kg bw/day group as compared to controls. These data indicate that most animals in the high-dose group were able to compensate for the earlier weight losses once treatment was terminated. Statistically significant reductions in absolute (g/day) food consumption values during the treatment period were observed in the 650 mg/kg bw/day group when compared to controls.

Pregnancy rates were comparable in all groups. Ovarian, uterine and fetal observations were unaffected at all dose levels. No treatment-related malformations or variations were evident from the fetal external, visceral or skeletal examination data.

Table 5.6.2-3: Summary of relevant findings ([redacted], 1989)

Dose level (mg/kg bw/d)		**0**	**135**	**300**	**650**
Inseminated (#)		22	22	22	22
Pregnant (#)		20	17	18	20
Deaths (#l)		1	-	2	4
Abortion (#)		1	1	-	3
Total resorption (#)		1	2	-	1
Litters (#)		17	14	16	12
Bodyweight (kg)	**Day 0**	3.5	3.3	3.4	3.5
	Day 28	4.3	4.0	4.1	4.1
Weight gain (kg)	**Day 6-12**	0.1	0.1	0.1	0.0
	Day 6-18	0.3	0.2	0.2	0.1
	Day 0-28	0.8	0.6	0.7	0.5
Food consumption (g/d)	**Day 6-12**	206.8	183.2	177.1	147.0**
	Day 6-18	192.7	170.5	167.4	134.8**
	Day 0-28	187.1	169.2	174.4	156.6**
Post-implantation loss (%)		10.0	4.6	5.3	11.3
Litter size (#)		6.1	6.3	6.9	7.2
Foetal weight (g)		40.9	38.1	37.4	37.5
Litter weight g)		232.4	226.6	238.1	260.9
Malformations (#)		4	1	4	4

**significantly different to controls (p<0.05); **p<0.01*

Conclusion:
Based on a slightly increased abortion rate and reductions in food consumption and body weight gain in the 650 mg/kg bw/day group, the NOAEL for maternal toxicity was 300 mg/kg bw/day. The NOAEL for developmental toxicity was 300 mg/kg bw/day based on a slightly increased embryolethality presenting as abortion. Dimethomorph is neither a developmental toxicant nor a teratogenic agent in the New Zealand White rabbit.

[redacted] 1987, CME 151 – Preliminary Oral (Gavage) Embryotoxicity Study in the Rabbit (BASF DocID DK-432-003)

Dimethomorph was administered by oral gavage to groups of eight sexually mature and mated female New Zealand White rabbits at dose levels of 300 or 600 mg/kg bw/day and to a group of nine female New Zealand White rabbits at 1000 mg/kg bw/day for 13 consecutive days from Days 6 to 18 post-coitum. A control group of eight rabbits received distilled water with admixture of 0.1% Tween 80 for the same period of time. The test substance was prepared daily as an aqueous suspension; separate preparations were made for each dose level. Formulations were prepared under darkroom condition immediately prior to dosing and used within two hours. The dose volume was 10 mL/kg, adjusted daily on the basis of the individual body weights. All animals were examined twice daily for signs of ill-health, toxicity and behavioral changes and mortality. Body weights were recorded on Days 0, 6, 12, 18, 24 and 28 post-coitum. On Day 28 post-coitum, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined and the following data recorded: number of corpora lutea, number and position of implantation (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

Findings:

In Group 4 (1000 mg/kg bw/day), one animal was killed on Day 7 post-coitum because of the left hindlimb injury and this injury was considered to be incidental. However, six animals aborted their litters between Days 16 – 23; in three of these animals, necropsy revealed findings in the liver and/or spleen. Also, some clinical signs (e.g., reduced food and/or water consumption) during or after the treatment period were observed in all nine animals. Two animals were severely emaciated when they aborted their litters, one further animal showed 100 per cent intra-uterine deaths at necropsy. In Group 2 (300 mg/kg bw/day), one animal aborted its litter on Day 20 post-coitum. A reduction in maternal body weight gain was noted in the 300 and 600 mg/kg bw /day treatment groups. There were no treatment-related effects on pregnancy incidence or implantations. In Group 4 (1000 mg/kg bw/day), only two animals were available for implantation evaluation; these values were comparable with the control group. Post-implantation loss was increased only in Group 4 (1000 mg/kg bw/day).

No treatment-related effects were observed in the number and sex of fetuses, mean fetal weights and fetal defects in Groups 2 and 3 (300 and 600 mg/kg bw/day, respectively). As only seven fetuses of one litter were available for evaluation in Group 4 (1000 mg/kg bw/day), no statistics on fetal parameters were performed.

Conclusion:

Based on the results of this study, the administration of dimethomorph by oral route at a dose level of 1000 mg/kg bw/day caused maternal toxicity (reduced food consumption and body weight gain) and embryotoxicity (high rate of abortions and increased number of intra-uterine deaths). Due to the low number of fetuses, teratogenic potential could not be evaluated. At a dose level of 600 mg/kg bw/day, maternal toxicity (reduced body weight gain) and embryotoxicity (reduced fetal weight) were observed, but no teratogenicity. The administration of 300 mg/kg bw/day elicited slight maternal toxicity (slightly reduced body weight gain), but no embryotoxicity and teratogenicity. Thus, dose levels not exceeding 600 mg/kg bw/day were recommended for a subsequent teratology study.

CA 5.7 Neurotoxicity Studies

No studies were submitted for the first application.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
There is an acute and a subchronic oral neurotoxicity study in Wistar rats that were performed after the last registration process.

Table 5.7-1: Summary of not peer-review neurotoxicity studies

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
Acute oral neurotoxicity; Wistar rats	0, 250, 500, 2000	-	250	FOB and motor activity decreased on the day of administration.	2010/1054130
Subchronic oral neurotoxicity; Wistar rats 0, 300, 800, 2400 ppm	0, 21.7, 58.7, 177.9 in males 0, 25.7, 69.6, 204.0 in females	58.7 for systemic toxicity 177.9 for neurotoxicity	177.9 for systemic toxicity	Systemic toxicity: reduced food consumption, impaired body weight gain and food efficiency. No neurotoxicity.	2004/1013810

In the acute neurotoxicity study, slight effects on FOB and decreased motor activities were observed on the first day of administration at any dose tested, in this study a LOAEL of 250 mg/kg bw/day was determined.

In the subchronic neurotoxicity study, no neurotoxicity was observed at any dose level, the NOAEL is 2400 ppm (177.9 and 204.0 mg/kg bw/kg in males and females respectively). Systemic toxicity was observed at 2400 ppm for each sex: reduced food consumption, impaired body weight gain and food efficiency. The NOAEL for systemic toxicity is 800 ppm (58.7 and 69.9 mg/kg bw/day in males and females respectively).

Thus, the conclusion for relevant endpoints for the current re-registration is as follow

Acute neurotoxicity	Acute neurotoxicity in rat by gavage. LOAEL 250 mg/kg bw based on slight behavioural effects without neurohistopathological correlate.
Delayed neurotoxicity	90-day oral in rat via the diet. No neurotoxicity observed at the highest tested dose: NOAEL 177.9 mg/kg bw/day. Systemic toxicity observed at the highest tested dose: NOAEL 58.7 mg/kg bw/day.

CA 5.7.1 Neurotoxicity studies in rodents

Report:	CA 5.7.1/1 [redacted]., 2011 a BAS 550 F (Dimethomorph) - Acute oral neurotoxicity study in Wistar rats - Administration via gavage 2010/1054130
Guidelines:	EPA 870.6200, OECD 424, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.43
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The aim of this study was to determine the effect of BAS 550 F (Dimethomorph) on neurotoxicity in rats after a single administration via gavage and an observation time of 14 days. The test substance (in 1% aqueous CMC) was administered to groups of 10 male and female Wistar rats at doses of 250, 500, and 2000 mg/kg bw at a dose volume of 10 mL/kg. FOB and MA measurement revealed test substance-related effects at 250, 500 and 2000 mg/kg bw on the day of test substance administration, i.e. impairment of gait, reduced area exploration, reduced motor activity in both sexes. Rearing was significantly decreased on study day 0 in both sexes of test groups 2 and 3 (500 and 2000 mg/kg bw) as well as in females of test group 1 (250 mg/kg bw). These findings were reversible and not observed on study days 7 and 14. The light microscopy examinations of the central and peripheral nervous system did not reveal any test substance-dependent neuropathological lesions in the organ samples examined. Based on these findings the no observed adverse effect level (NOAEL) was below 250 mg/kg bw under the conditions of the study.

(DocID 2010/1054130)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F (Dimethomorph)

Description:	solid / white
Lot/Batch #:	AC9978-131
Purity:	97.5%
Stability of test compound:	The test substance preparation was stable for a period of 7 days, as determined in an earlier experiment (Study code: PCP06240).

2. Vehicle and/or positive control: aqueous CMC solution (1%)

3. Test animals:

Species:	Rats
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	49±1 days (start of administration)
Weight at dosing:	males: 200-245 g females: 144-185 g
Source:	[redacted]
Acclimation period:	approx. 2 weeks
Diet:	ground Kliba maintenance diet (rat/mouse/hamster pellets), Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in Makrolon cages type M III

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes per hour:	10
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 03-Nov-2009 - 07-Oct-2010
(In life dates: 17-Nov-2009 (start of administration) to 04-Dec-2009 (necropsy)

2. Animal assignment and treatment:
BAS 550 F was administered once to groups of 10 male and 10 female rats at doses of 250, 500, and 2000 mg/kg bw by gavage.

3. Test substance preparation and analysis:
The test substance was applied as a suspension. To prepare the suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then the vehicle (drinking water containing 1% carboxymethylcellulose) was filled up to the desired volume, subsequently mixed using a magnetic stirrer. During administration of the test substance, preparations were kept homogeneous using a magnetic stirrer. The test substance preparations were made once before the first administration.
The stability of a similar batch of the test substance in M4, OECD- and tap water at room temperature over a period of a maximum of 7 days was proven before start of the study (Study code: PCP06240). This stability investigation is also applicable for the test substance preparation within the current study, for which a solution of 1% carboxymethylcellulose in water was used as a vehicle.
Homogeneity analyses of the test substance preparations were performed in samples of all concentrations administered at the beginning. These samples also served for concentration control analysis.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further parameters and statistical tests are listed below.

Parameters	Statistical test	Markers in the tables	References
body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491
feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York
Weight parameters (neuropathology)	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON-test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference, Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. If animals were in a moribund state, they were sacrificed. All animals were checked daily for any clinically abnormal signs. Abnormalities and changes were documented for each animal.

2. Body weight:

The body weight was determined before the first neurofunctional test in order to randomize the animals. During the conduct of the study, the body weight was determined on the days when functional observational batteries were carried out (study days –7, 0, 7 and 14). The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food and water consumption:

Individual food and water consumption was determined daily by visual inspection.

4. Clinical assessment of neurotoxicity:

Functional observational battery

The functional observational batteries (FOB) started at about 10.00 h with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. In order to guarantee the blind status of the observer, the cages were randomly distributed in the racks at least 30 minutes prior to the examinations, and the cage labels (indicating the test group) were turned. Thus, the observer was only able to identify the animal numbers but not the allocation of the animals to the different test groups. Moreover, the examinations were carried out in randomized order.

For *home cage observations* attention was paid to: posture, tremor, convulsions, abnormal movements, impairment of gait, and other findings.

Open field observations:

The animals were transferred to a standard arena (50×50 cm with sides of 25 cm high) and observed for at least 2 minutes. Following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

Sensorimotor tests/reflexes:

The animals were removed from the open field and subjected to following sensorimotor and reflex tests:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hindlimbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

Motor activity assessment

Motor activity (MA) measurements were carried out in all animals on study days -7, 0, 7 and 14. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the animals were placed in new clean polycarbonate cages for the time of measurement. Eighteen beams were allocated per cage. During the measurement the animals were kept in polycarbonate cages with absorbent material. The animals were put into the cages in a randomized order. The measurements started at about 14.00 h. The number of beam interrupts was counted over 12 intervals, each lasting 5 minutes. Measurement did not commence at the same instant for all cages; the period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes later. During the measurements the animals received no food and no water.

4. Neuropathology:

The first 5 surviving animals per test group and sex were subjected to deep anesthesia with Isoflo® (Essex GmbH, München, Germany) and sacrificed by perfusion fixation. SOERENSEN phosphate buffer was used as a rinsing solution and a fixation solution according to KARNOVSKY was used as a fixative. The animals fixed by perfusion were necropsied with regard to the question of neuropathology, and the visible organs or organ sections were assessed by gross pathology as accurately as is possible after a perfusion fixation.

Weight determinations

The following organ/tissue was weighed after its removal but before each further preparation:

1. Brain (without olfactory bulb)

For determination of the relative organ weights the terminal body weights were used.

Organ/tissue preservation list

Paraffin embedding, sectioning, staining and preservation:

Organ samples from:	Test groups			
	0	1	2	3
Central nervous system:		F5*	F5*	
Brain (cross sections):				
• Frontal lobe	A5			A5
• Parietal lobe with diencephalon	A5			A5
• Midbrain with occipital and temporal lobe	A5			A5
• Pons	A5			A5
• Cerebellum	A5			A5
• Medulla oblongata	A5			A5
• Eyes with retina and optical nerve	A5	F5	F5	A5
Spinal cord (cross and longitudinal sections):				
• Cervical cord (C3–C6)	A5	F5	F5	A5
• Lumbar cord (L1–L4)	A5	F5	F5	A5
Peripheral nervous system:				
• Gasserian ganglia with nerve	A5	F5	F5	A5
• Gastrocnemius muscle	A5	F5	F5	A5
All gross lesions	A2	A2	A2	A2

F = Preservation in 4% formaldehyde

A = Paraplast embedding, sectioning and staining with hematoxylin-eosin (HE)

* = Fixation in toto

5 = All perfused animals per test group and sex

2 = All animals affected from the test group fixed by perfusion

Plastic embedding, sectioning, staining and storage:

Organ samples from:	Test groups			
	0	1	2	3
Peripheral nervous system:				
• Dorsal root ganglion, (3 of C3–C6)	T5	P5	P5	T5
• Dorsal root fiber (C3-C6)	T5	P5	P5	T5
• Ventral root fiber (C3-C6)	T5	P5	P5	T5
• Dorsal root ganglion, (3 of L1-L4)	T5	P5	P5	T5
• Dorsal root fiber (L1-L4)	T5	P5	P5	T5
• Ventral root fiber (L1-L4)	T5	P5	P5	T5
• Proximal sciatic nerve	T5	P5	P5	T5
• Proximal tibial nerve (at knee)	T5	P5	P5	T5
• Distal tibial nerve (at lower leg)	T5	P5	P5	T5

P = Storage of fixed specimen in buffer solution

T = Plastic embedding (epoxy resin), semithin sectioning and staining

5 = All perfused animals per test group and sex

In addition the following organs/tissues (after trimming) were preserved in neutrally buffered 4% formaldehyde solution:

1. Brain (remaining material after trimming)
2. Spinal cord (sections from cervical and lumbar cords)
3. All gross lesions

The remaining animal body was stored in neutrally buffered 4% formaldehyde solution.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (95.9, 85.5, and 99.1% of nominal concentrations of the 250, 500, and 2000 mg/kg bw dose groups).

B. OBSERVATIONS

1. Clinical signs of toxicity

The female animal of test group 3 (No. 85; 2000 mg/kg bw) that was found dead on study day 2 showed hypothermia, reduced nutritional condition (grade: slight), poor general condition (grade: moderate) and piloerection on day 1.

2. Mortality

One female animal of test group 3 (No. 85; 2000 mg/kg bw) was found dead on study day 2.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No test substance-related findings on body weight data were found. The significantly increased body weight change value for female animals of test group 3 (2000 mg/kg bw) was assessed as being incidental and not related to treatment.

Table 5.7.1-1: Mean body weight and body weight change of rats administered BAS 550 F for 14 days

	Males				Females			
Dose level [mg/kg bw]	0	250	500	2000	0	250	500	2000
Body weight [g]								
day -7	185	185	184	185	137	137	140	138
day 0	226	227	227	225	157	159	160	157
day 7	257	263	259	252	171	176	177	170
day 14	284	290	287	281	180	183	187	186
Body weight change [g] (% control)								
day 0-14	57	63 (9)	60 (4)	56 (-2)	22	24 (8)	27 (22)	29* (33)

* = $p \leq 0.05$

D. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals, these observations were considered to have been incidental. Beside this, the FOB was performed in different examination segments, which have to be assessed individually:

Home cage observations:

No significant deviations from controls were observed.

Open field observations:

One male and 2 female animals of test group 3 (2000 mg/kg bw) and 1 female animal of test group 2 (500 mg/kg bw) did not move during observation on study day 0. Seven male and 4 female animals of test group 3 (2000 mg/kg bw) and 9 male animals of test group 2 (500 mg/kg bw) showed a reduced exploration of the area on study day 0. The above-mentioned findings were assessed as being related to the test substance administration by gavage.

Quantitative parameters:

Rearing was significantly decreased in both sexes of test groups 2 and 3 (500 and 2000 mg/kg bw) and in females of test group 1 (250 mg/kg bw) on study day 0. The above-mentioned findings were assessed as being related to the test substance administration by gavage.

Table 5.7.1-2: Summary F.O.B. - Males

		0/M	1/M	2/M	3/M
Feces	Mean	0.7K	0.6	1.2	0.6
[N]	S.D.	1.5	1.0	1.8	1.1
Day 0	N	10	10	10	10
	Deviation vs control		-14.3	71.4	-14.3
Rearing	Mean	1.0W	0.4	0.0*	0.3*
[N]	S.D.	1.2	0.7	0.0	0.9
Day 0	N	10	10	10	10
	Deviation vs control		-60.0	-100.0	-70.0
GS F	Mean	5.31 K	4.78	5.09	5.22
[Newton]	S.D.	1.10	0.96	0.93	0.79
Day 0	N	10	10	10	10
	Deviation vs control		-9.98	-4.14	-1.69
GS H	Mean	3.12 K	3.05	3.19	3.39
[Newton]	S.D.	0.66	0.47	0.81	0.74
Day 0	N	10	10	10	10
	Deviation vs control		-2.24	2.24	8.65
FST	Mean	9.38 K	8.88	9.24	9.20
[cm]	S.D.	0.97	1.29	0.77	1.15
Day 0	N	10	10	10	10
	Deviation vs control		-5.38	-1.55	1.97

Statistic Type = Kruskal-Wallis + Wilcoxon test (two-sided), * p<=0.05, ** p<=0.01

K: Kruskal-Wallis W: Wilcoxon

Table 5.7.1-3: Summary F.O.B. – Females

		0/F	1/F	2/F	3/F
Feces	Mean	0.0 K	0.0	0.7	0.0
[N]	S.D.	0.0	0.0	1.5	0.0
Day 0	N	10	10	10	10
	Deviation vs control				
Rearing	Mean	6.0W	1.7*	1.0**	0.1**
[N]	S.D.	4.6	2.4	2.5	0.3
Day 0	N	10	10	10	10
	Deviation vs control		-71.7	-83.3	-98.3
GS F	Mean	3.70 K	3.71	3.91	4.05
[Newton]	S.D.	0.47	0.46	0.47	1.04
Day 0	N	10	10	10	10
	Deviation vs control		0.27	5.68	9.46
GS H	Mean	2.82 K	2.60	2.56	2.62
[Newton]	S.D.	0.39	0.43	0.31	0.45
Day 0	N	10	10	10	10
	Deviation vs control		-7;80	-9.22	-7.09
FST	Mean	9.34 K	8.88	9.08	8.62
[cm]	S.D.	0.57	0.78	0.31	0.60
Day 0	N	10	10	10	10
	Deviation vs control		-4.87	-2.73	-7.61

Statistic Type = Kruskal-Wallis + Wilcoxon test (two-sided), * p<=0.05, ** p<=0.01

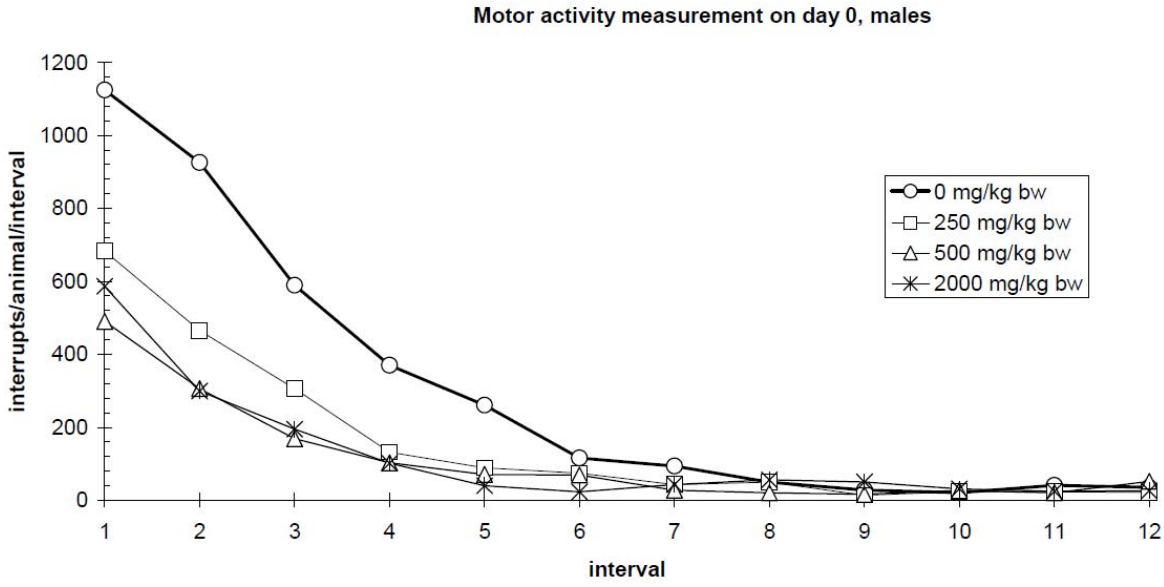
K: Kruskal-Wallis W: Wilcoxon

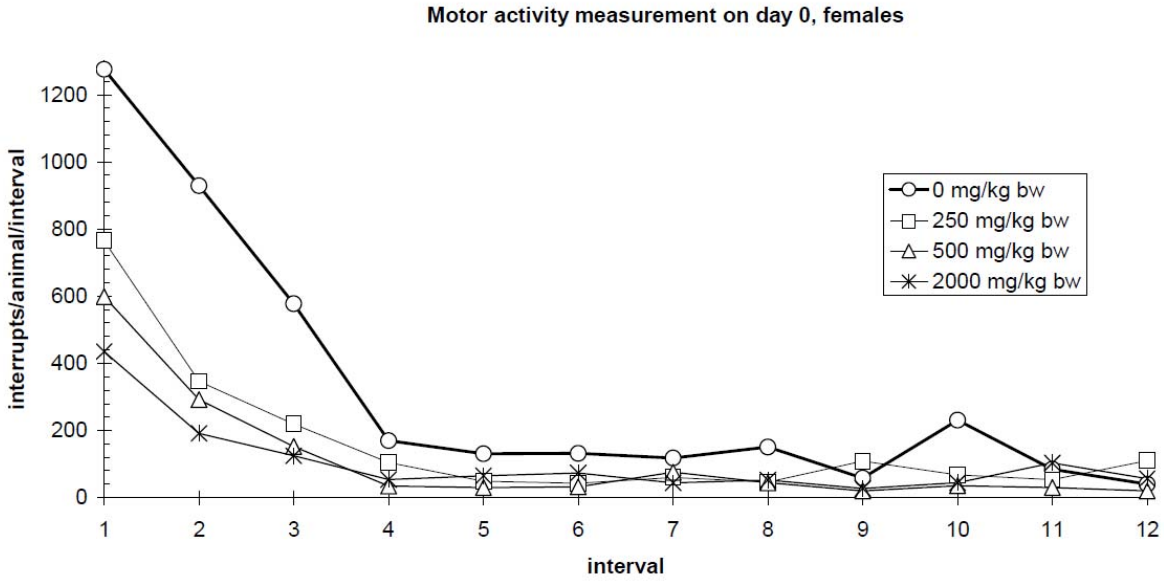
Grip strength of forelimbs (GSF) was significantly increased in females of test group 2 (500 mg/kg bw) on study day 7. Due to the isolated occurrence and the lack of dose-response relationship, this finding was assessed as spontaneous in nature and, therefore, not test substance-related.

Motor activity assessment

Study day 0:

Regarding the overall motor activity (MA), the values in both sexes of all test groups were significantly reduced. Comparing the single intervals with the control groups, the following significantly decreased values were measured: interval 1-5 in all males of test groups 1-3 (250, 500 and 2000 mg/kg bw), interval 1-3 in all females of test groups 1-3 (250, 500 and 2000 mg/kg bw) and additionally interval 4 of test group 2 (500 mg/kg bw). The above-mentioned findings were assessed as being related to the test substance administration by gavage.





Study day 7:

Interval 6 in test group 1 (250 mg/kg bw), interval 5 and 6 in test group 2 (500 mg/kg bw) were significantly increased, interval 2 in test group 3 (2000 mg/kg bw) was significantly reduced in female animals. The above-mentioned findings at isolated time points only were assessed as spontaneous in nature and, therefore, not test substance-related.

Study day 14:

Interval 11 in test group 2 (500 mg/kg bw) was significantly increased in female animals. The above-mentioned isolated finding was assessed as spontaneous in nature and, therefore, not test substance-related.

D. NEUROPATHOLOGY

No significant changes were noted for absolute or relative organ weights, and no gross lesions were recorded.

A discrete, single “axonal degeneration” was recorded in the proximal sciatic, proximal tibial, and the distal tibial nerve each for single control animals and single animals of test group 3 (0 and 2000 mg/kg bw). Based on the occurrence in the control group at comparable incidences this finding was regarded as incidental and not related to treatment.

III. CONCLUSIONS

The single administration of BAS 550 F (250, 500, and 2000 mg/kg bw) had adverse neurobehavioral effects in male and female Wistar rats at dose levels of 250 mg/kg bw and above on the day of test substance administration. No test substance-related effects were observed in the neurohistopathology investigation at any dose level. The no observed adverse effect level (NOAEL) under the conditions of this study was below 250 mg/kg bw.

Report: CA 5.7.1/2
[redacted]., 2004 a
BAS 550 F - Subchronic neurotoxicity study in Wistar rats - Administration in the diet for 3 months
2004/1013810
Guidelines: EPA 870.6200, OECD 424
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary
The aim of this study was to determine the effects of BAS 550 F (Dimethomorph) on neurotoxicity in rats after administration of 300, 800, and 2400 ppm test substance via the diet over 90 days. The mean daily intake was 21.7, 58.7, and 177.9 mg/kg bw/day for males and 25.7, 69.6, and 204.0 mg/kg bw/day for females in the 300, 800, and 2400 ppm dose group, respectively. In the high dose groups reduced food consumption, impaired body weight gain and food efficiency was observed. FOB and MA measurement revealed no test substance-related effects at any dose level. Furthermore, neither necropsy nor the light microscopy examinations of the central and peripheral nervous system did reveal any test substance-dependent neuropathological lesions in the organ samples examined. Based on these findings the no observed adverse effect level (NOAEL) was 800 ppm for general systemic toxicity and 2400 ppm for neurotoxicity under the conditions of this study, respectively.
(DocID 2004/1013810)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F (Dimethomorph)

Description:	solid, powder / white
Lot/Batch #:	AC9978-131
Purity:	98.3%
Stability of test compound:	proven by reanalysis

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rats
Strain:	CrlGlxBrlHan:WI
Sex:	Male and female
Age:	49±1 days (start of administration)
Weight at dosing:	Males: 165-211 g Females: 121-164 g
Source:	[redacted]
Acclimation period:	approx. 2 weeks
Diet:	ground Kliba maintenance diet (rat/mouse "GLP"), Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in type DK III (Becker&Co., Castrop-Rauxel, Germany)

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not specified (fully air-conditioned room)
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 08-July-2003 - 24-Mar-2004
(In life dates: 21-July-2003 (start of administration) to 23-Oct-2003 (necropsy)

2. Animal assignment and treatment:
BAS 550 F was administered to groups of 10 male and 10 female rats at concentrations of 300, 800, and 2400 ppm in the diet. On the day of arrival an acclimatization period started, in which the animals were accustomed to housing and diet. The animals were randomly assigned to the groups based upon body weight and separated by sex prior to the first functional observational battery. At the start of the administration period (day 0) the rats were 49±1 days old. The weight variation of the animals did not exceed 20 percent of the mean weight of each sex. The list of randomization instructions was compiled by a computer. In order to balance the groups for functional observational batteries and motor activity, the study was conducted with several subsets (Section A males and Section A females = first 5 animals of each dose group; Section B males and Section B females = second 5 animals of each dose group). For functional observational batteries and measurements of motor activity, animals of section A and B, were tested again in randomized order.

3. Test substance preparation and analysis:
For each concentration, the test substance was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer. The test substance preparations were mixed monthly.
The stability of the test substance in the diet over a period of 49 days at room temperature was verified analytically before the start of the study.
Homogeneity analyses of the test substance preparations were performed in samples of all concentrations on the first week of the administration period. These samples also served for concentration control analyses. Additional concentration control analyses were performed with samples of all concentrations at the end of the administration period.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further parameters and statistical tests are listed below.

Parameters	Statistical test	Markers in the tables	References
body weight, body weight change, food consumption, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491
feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York
Weight parameters (neuropathology)	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON-test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference, Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays.

2. Body weight:

The body weight was determined before the first neurofunctional test in order to randomize the animals. During the conduct of the study, the body weight was determined on day 0 and thereafter in weekly intervals. In addition the body weight was determined on the days when functional observational batteries were carried out. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food and water consumption, food efficiency and compound intake:

Individual food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. Individual water consumption was determined daily by visual inspection.
Food efficiency was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \text{ x } 100$$

with BW_x and BW_y body weight (g) on day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration in food on day x (mg/kg = ppm) and BW_x as body weight on day x of the study (g).

4. Ophthalmoscopy:

Prior to the start of the administration period the eyes of all animals, and to the end the eyes of the control and high dose animals were examined for any changes using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after administration of a mydriatic (Chauvin, ankerpharm GmbH, Rudolfstadt, Germany).

5. Clinical assessment of neurotoxicity:

Functional observational battery

FOBs were performed in all animals before the administration (day -7) and on days 1, 22, 50 and 85. The FOBs were performed each time from about 10.00 a.m. onwards, started with passive observations without disturbing the animals, followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians which performed positive control studies as part of their training. The findings were ranked according to the degree of severity, if applicable. In order to guarantee the blind status of the observer, the cages were randomly distributed in the racks at least 30 minutes prior to the examinations, and the cage labels (indicating the dose group) were turned. Thus only the animal number, but not the allocation of the animals to the different dose groups could be identified by the observer. Moreover, the examinations were carried out in randomized order. The findings and values obtained were documented by another technician knowing the identification of the animals.

For *home cage observations* attention was paid to: posture, tremor, convulsions, abnormal movements, and impairment of gait.

Open field observations:

The animals were transferred to a standard arena (50×50 cm with sides of 25 cm high) and observed for at least 2 minutes. Following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

Sensorimotor tests/reflexes:

The animals were removed from the open field and subjected to following sensorimotor and reflex tests:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hindlimbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

Motor activity assessment

Motor activity was measured with all animals of Section A and B on the same day as FOB was performed. The measurement was performed in the dark using the Multi-Varimex-System (Columbus Instruments Int. Corp., Ohio, USA) with 4 infrared beams per cage. During the measurement the animals were kept in Polycarbonate cages with absorbent material. The cages were cleaned prior to each use. The animals were put into the cages in a randomized order. Motor activity measurements were from 2.00 a.m. onwards and the number of beam interrupts was counted over 12 intervals, each lasting 5 minutes. Measurement did not commence at the same instant for all cages; the period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes thereafter. During the measurements the animals received no food and no water.

6. Neuropathology:

The five surviving animals per sex and test group that are selected for neuropathology, were deeply anesthetised (Narcoren®, about 4 mL/kg body weight) at the end of the study and sacrificed by perfusion fixation.

SOERENSEN's phosphate buffer served as rinsing solution and the fixation solution according to KARNOVSKY served as fixative.

The sacrificed animals were necropsied and the visible organs assessed by gross pathology as thoroughly as possible for perfused animals. The organ/tissue samples listed in paragraphs below were carefully removed.

Weight determinations

Weight assessment of the brain (without olfactory bulb) was carried out on all perfused animals after removal of the brain but before any further preparation.

Organ/tissue preservation list

Paraffin embedding, sectioning, staining and preservation:

Organ samples from:	Test groups (dose)			
	0 control	**1** 300 ppm	**2** 800 ppm	**3** 2,400 ppm
Brain (cross sections):		F5*	F5*	
– Frontal lobe	A5			A5
– Parietal lobe with *diencephalon*	A5			A5
– Midbrain with occipital and temporal Lobe	A5			A5
– Pons	A5			A5
– Cerebellum	A5			A5
– Medulla oblongata	A5			A5
Brain-associated organs/tissues				
– Eyes with retina and optical nerve	A5	F5	F5	A5
Spinal cord (cross and longitudinal sections):				
– Cervical swelling (C3-C6)	A5	F5	F5	A5
– Lumbar swelling (L1-L4)	A5	F5	F5	A5
Peripheral nervous system:				
– Gasserian ganglia with nerve	A5	F5	F5	A5
– Gastrocnemius muscle	A5	F5	F5	A5

METHODS/SCOPE OF EXAMINATIONS:

A = Paraffin embedding (paraplast), sectioning and staining with hematoxylin-eosin (H & E)
F = Preservation in 4% formaldehyde
5 = all perfused animals per group and sex
* = in toto

Plastic embedding, sectioning, staining and storage:

Organ samples from:	Test groups (dose)			
	0 control	1 300 ppm	2 800 ppm	3 2,400 ppm
Peripheral nervous system:				
– Dorsal root ganglion, 3 of (C3-C6)	T5	P5	P5	T5
– Dorsal root fiber (C3-C6)	T5	P5	P5	T5
– Ventral root fiber (C3-C6)	T5	P5	P5	T5
– Dorsal root ganglion, 3 of (L1-L4)	T5	P5	P5	T5
– Dorsal root fiber (L1-L4)	T5	P5	P5	T5
– Ventral root fiber (L1-L4)	T5	P5	P5	T5
– Proximal sciatic nerve	T5	P5	P5	T5
– Proximal tibial nerve (at knee)	T5	P5	P5	T5
– Distal tibial nerve (at lower leg)	T5	P5	P5	T5

METHODS/SCOPE OF EXAMINATIONS:

T = Plastic embedding (epoxy resin), semithin sectioning and staining with Azure II-Methylene blue-basic Fuchsin (AMbf)
P = Storage of fixed specimen in buffer solution
5 = all perfused animals per group and sex

In addition the following organs/tissues (after trimming) were preserved in neutrally buffered 4% formaldehyde solution:

1. Brain (remaining material after trimming)
2. Spinal cord (sections from cervical and lumbar cords)
3. All gross lesions

The remaining animal body was stored in neutrally buffered 4% formaldehyde solution.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (97.6-104.1% of nominal concentrations).

B. OBSERVATIONS

1. Clinical signs of toxicity

One male animal of group 0 showed unpalpable left testis in scrotum from day 49 to the end of the study. One male animal of group 3 showed sparse hair on the ventral region and on both forelimbs from day 78 to the end of the study. The above-mentioned isolated findings were spontaneous in nature and therefore not substance-related.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

In male animals of group 3, body weight was reduced over the whole study period, statistically significantly on most days, up to -14.4 % on day 84. In male animals of group 3, body weight change was reduced statistically significantly over the whole study period up to -33.2 % on day 7.

In female animals of group 3, body weight change was reduced over the whole study period, statistically significantly on day 21 and from day 42 until the end of the study period, up to -27.5 % on day 42. The above-mentioned impaired body weight data were assessed as related to the treatment with the test substance.

Table 5.7.1-4: Mean body weight and body weight change of rats administered BAS 550 F for 90 days

	Males				Females			
Dose level [ppm]	0	300	800	2400	0	300	800	2400
Body weight [g]								
day 0	190	187	186	183	137	139	137	140
day 56	356	341	335	313**	204	202	201	194
day 91	390	377	366	340**	219	214	213	206
Body weight change [g] (% control)								
day 0-91	199	190 (-5)	179 (-10)	157** (-21)	81	76 (-6)	75 (-6)	66* (-18)

* = p≤0.05; ** = p≤0.01

D. FOOD CONSUMPTION AND FOOD EFFICIENCY

In male animals of group 3, food consumption was reduced over the whole study period, statistically significantly on days 7, 42, 70 and 84 up to -12.7 % on day 7. This finding was assessed as substance-related.

In female animals of group 1, food consumption was statistically significantly reduced on day 42 (-8.7 %). This isolated finding was clearly not substance-related. Whereas, in female animals of group 3 food consumption was substance-related reduced over the whole study period, statistically significantly on days 7, 35, 42, 56, 63 and 70 up to -20.9 % on day 7.

In male animals of group 3, food efficiency was statistically significantly impaired on days 7 and 35. This finding was assessed as related to the test compound. In female animals of group 1 food efficiency was statistically significantly impaired on day 28. This isolated finding was clearly not substance-related, whereas food efficiency was substance-related impaired in female animals of group 3 (statistically significant on days 28 and 42).

E. TEST SUBSTANCE INTAKE

The approximate mean daily test substance intake in mg/kg body weight/day over the entire study period is shown in the following table:

Table 5.7.1-5: Test substance intake over the study period

Test group	Concentration in the diet (ppm)	Mean daily substance intake (mg/kg bw/day)	
		males	females
1	300	21.7	25.7
2	800	58.7	69.6
3	2400	177.9	204.0

F. OPHTHALMOSCOPY

No substance-related effects were obtained. All findings were spontaneous in nature and equally distributed between treated animals and controls.

G. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals, these observations were considered to have been incidental.

Home cage observations:

No significant deviations from controls were observed.

Open field observations:

One male animal of group 0 showed unpalpable left testis in scrotum on days 22, 50 and 85. One male animal of group 3 showed sparse hair on the ventral region and on both forelimbs on day 85. These findings were spontaneous in nature and therefore not substance-related.

Quantitative parameters:

No significant deviations from controls were observed.

Sensorimotor tests/reflexes

No significant deviations from controls were observed.

Motor activity assessment

Regarding the overall motor activity (summation of intervals 1-12), a statistically significant reduced value was only obtained in female animals of dose group 1 on day 85. This single occurrence was clearly fortuitous. The comparison of the single intervals resulted in the following deviations:

Group 1: In female animals at interval 11 on day 85.

Group 2: In female animals at intervals 10 and 11 on day 50.

Group 3: In female animals at interval 2 on day - 7 as well as at interval 11 on day 85.

However, due to the isolated occurrences and alternating results in the sense of increased as well as decreased motor activity and moreover, as all findings were without any dose-response relationship, the above-mentioned deviations were assessed as being incidental and not related to treatment.

H. NEUROPATHOLOGY

No significant changes were noted for absolute or relative organ weights, and no gross lesions were recorded.

The only lesion found was a single "axonal degeneration" in the peripheral nerves in 2 control and 1 top dose test animal. This lesion is regarded to be spontaneous in nature and not related to treatment.

III. CONCLUSIONS

The administration of BAS 550 F at concentrations of 300, 800, and 2400 ppm in the diet revealed signs of toxicity, such as reduced food consumption and impaired body weight gain in the high dose group. No test substance-related effects were observed concerning clinical observations, FOB, motor activity or in the neurohistopathology investigation at any dose level. The no observed adverse effect level (NOAEL) under the conditions of this study was 800 ppm for general systemic toxicity and 2400 ppm for neurotoxicity, respectively.

Literature data:

Regueiro J. et al, 2015. Toxicity evaluation of new agricultural fungicides in primary cultured cortical neurons. Envir. Res. 140, 37-44. [see KCA 5.7.1/3 2015/1278188]

Abstract: Fungicides are crucial for food protection as well as for the production of crops of suitable quality and quantity to provide a viable economic return. Like other pesticides, fungicides are widely sprayed on agricultural land, especially in wine-growing areas, from where they can move-off after application. Furthermore, residues of these agrochemicals can remain on crops after harvest and even after some food processing operations, being a major exposure pathway. Although a relatively low toxicity has been claimed for this kind of compounds, information about their neurotoxicity is still scarce. In the present study, nine fungicides recently approved for agricultural uses in the EU — ametoctradin, boscalid, cyazofamid, dimethomorph, fenhexamid, kresoxim-methyl, mepanipyrim, metrafenone and pyraclostrobin — have been evaluated for their toxicity in primary cultured mouse cortical neurons. Exposure to 0.1–100 mM for 7 days in vitro resulted in a dose-dependent toxicity in the MTT cell viability assay. Strobilurin fungicides kresoxim-methyl (KR) and pyraclostrobin (PY) were the most neurotoxic compounds (lethal concentration 50 were in the low micromolar and nanomolar levels, respectively) causing a rapid raise in intracellular calcium [Ca2þ]i and strong depolarization of mitochondrial membrane potential. KR- and PY-induced cell death was reversed by the calcium channels blockers MK-801 and verapamil, suggesting that calcium entry through NMDA receptors and voltage-operated calcium channels are involved in KR- and PY-induced neurotoxicity. These results highlight the need for further evaluation of their neurotoxic effects in vivo.

Analysis: In this publication, no toxicity was seen with dimethomorph after 24h of exposure to 100 µm and a slight cytotoxicity was observed after 7 days of continuous exposure with an EC50 of 58 µm. In guideline studies no effects were seen histologically after acute or sub-chronic 90-day exposure and dimethomorph is not considerd as a neurotoxic substance. This study is only considered as supplementary data.

CA 5.7.2 Delayed polyneuropathy studies

As there was no indication for neurotoxicity and/or neuropathy from any of the studies conducted and as dimethomorph does not belong to a chemical class suspected to induce delayed neuropathies, no study is considered to be necessary and thus no further study was conducted.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Within the past few years, metabolites in food of plant and animal origin were getting more into focus within the regulatory evaluation process. In 2012, EFSA published a Scientific Opinion on approaches to evaluate the toxicological relevance of metabolites and degradates of pesticides (see: EFSA Journal 2012;10(07): 2799. [187 pp.] doi:10.2903/j.efsa.2012.2799). The opinion shows a scheme for the evaluation of the relevance of metabolites for dietary risk assessment and identifies the threshold of toxicological concern (TTC) concept as an appropriate screening tool. On the basis of this Scientific Opinion, metabolites of dimethomorph have been assessed.

While in plant, metabolism of dimethomorph is limited, in animals (rat and livestock) dimethomorph is extensively metabolised, resulting in numerous metabolites identified. A list of all metabolites from plant and livestock metabolism and the confined rotational crop studies is shown in Table 5.8.1-1.

The following main metabolic steps have been identified for dimethomorph:

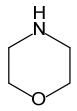
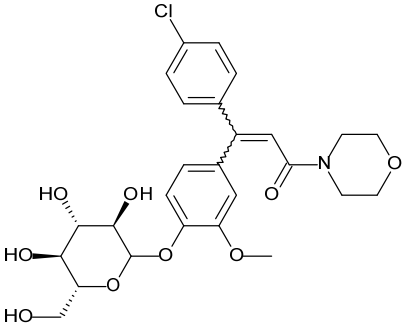
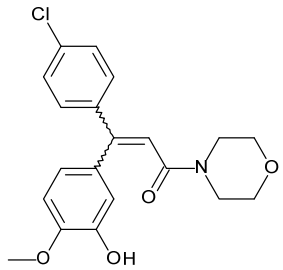
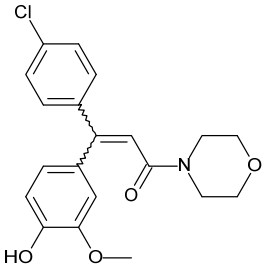
- Demethylation at the dimethoxyphenyl ring and subsequent conjugation
- Hydroxylation of the morpholine ring
- Opening of the morpholine ring and further modifications (degradation, conjugation)
- Cleavage/hydrolysis and release of the intact morpholine ring

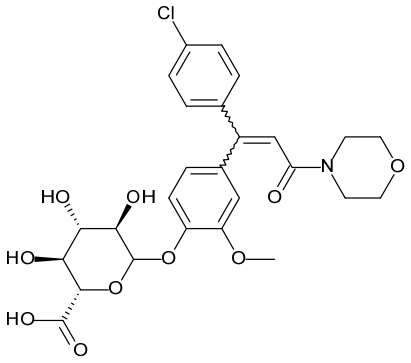
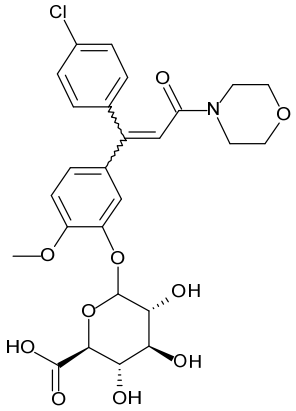
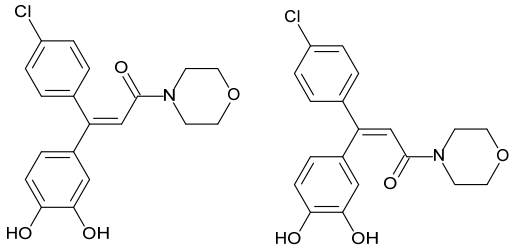
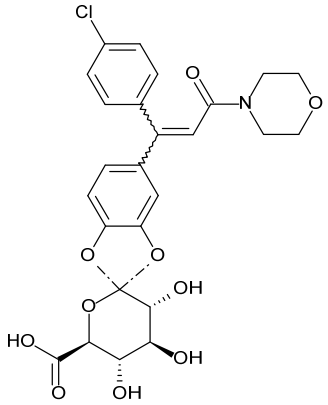
Based on similarity of structure and metabolic transformation steps, the plant and livestock metabolites have been assigned into the following groups. Details of this grouping can be found in M-CA 6.7 and M-CA 6.9.

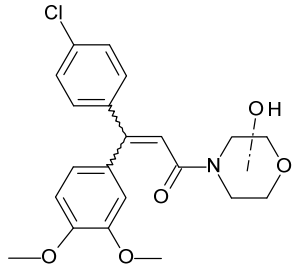
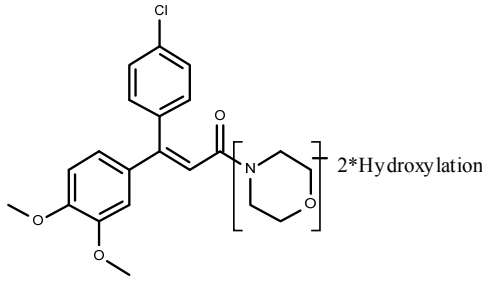
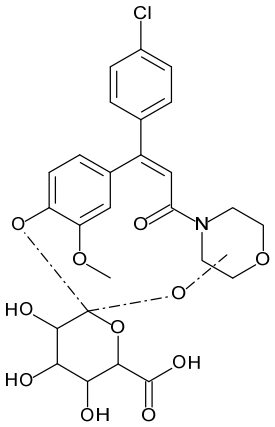
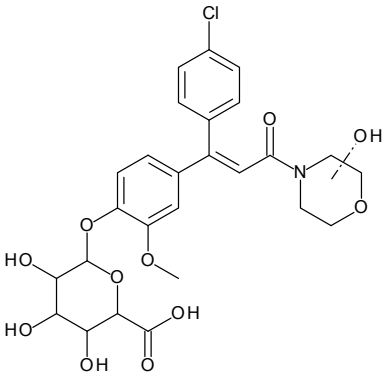
- Group 0: Cleavage (free morpholin)
- Group 1A: Demethylation and conjugation
- Group 1B: Hydroxylation of morpholine ring
- Group 2: Morpholine ring opening and modification (opening at the oxygen atom)
- Group 3: Morpholine ring opening and modification (opening at the nitrogen atom)
- Group 4: Complete loss of morpholine ring (free nitrogen)
- Group 5: Complete loss of morpholine ring (cleavage, no free nitrogen)

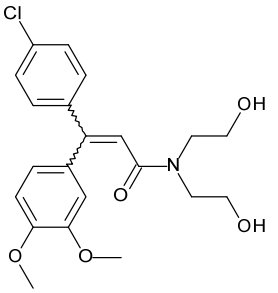
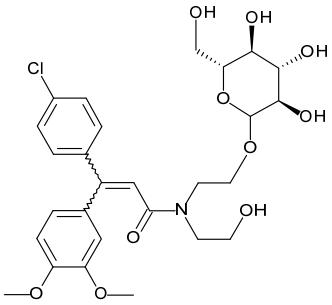
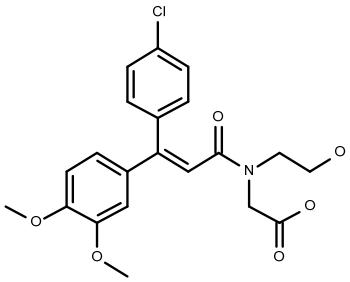
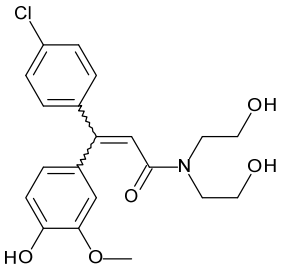
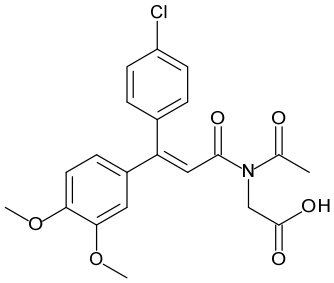
To decide for which group the toxicological endpoints (ADI, ARfD) of the parent molecule can be applied, the plant and livestock metabolites were compared with the rat metabolites.
Usually, if a representative metabolite is present in a rat metabolism study with more than 10% of the applied dose for these metabolites and their respective groups the toxicological endpoints of the parent molecule can be applied. Depending on the detected metabolite quantities (expressed as mean % dose) either the weight of evidence approach can be selected or additional investigations can be initiated. In the case of dimethomorph, an extensive metabolisation occurs in rat and also in livestock and plant. For metabolites in groups 1 to 4, some of them are not present in rat at levels of more than 10 %, however, using a weight of evidence approach, in each group there is always one metabolite that can be considered as a major rat metabolite (approx. 10% dose) or an intermediate metabolite which can be found in another group. Therefore, it is considered that all metabolites in groups 1 to 4 are assessed accordingly to the EFSA guidance and can be considered as covered by the parent m.toxicity studies with parent. Thus for an exposure assessment for groups 1 to 4, the reference values of dimethomorph parent are applied. For free morpholine (group 0) and for M550F003 (group 5), there are toxicity studies available allowing a derivation of substance specific reference values (see pp. 83 and 93), which are used in the risk assessment for these metabolites.

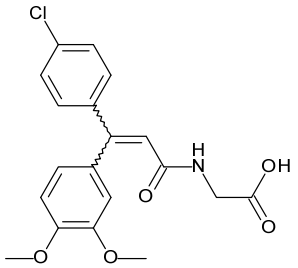
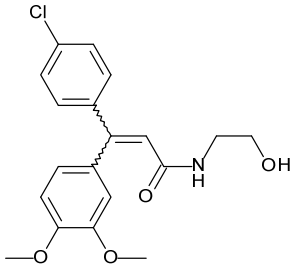
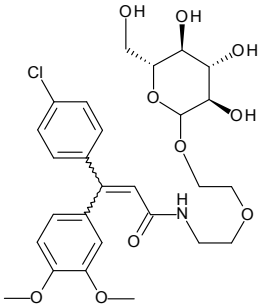
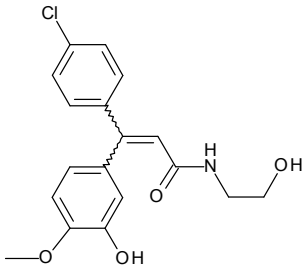
Table 5.8.1-1: List of identified dimethomorph metabolites in plant, confined rotcrop and livestock metabolism

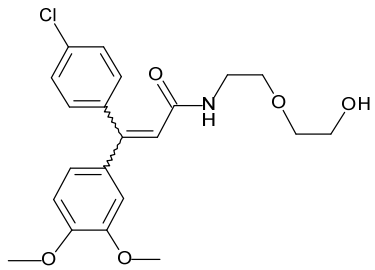
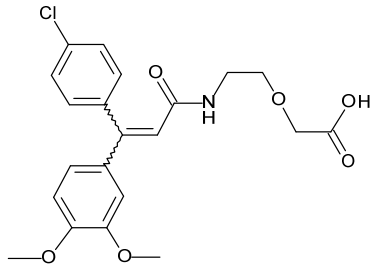
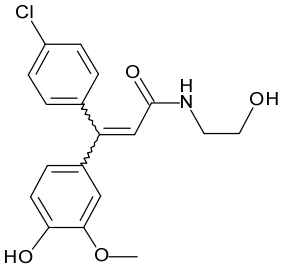
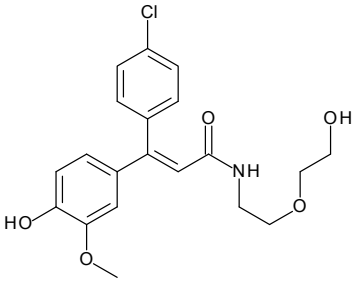
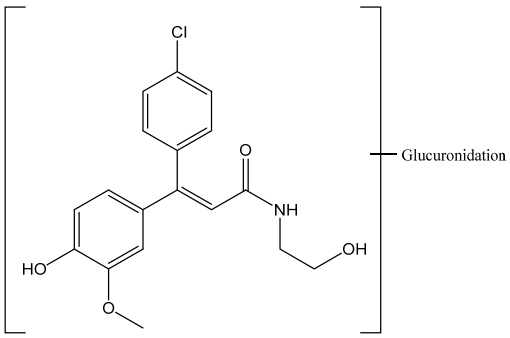
Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
Group 0: Cleavage (free morpholine)			
M550F021		Plants: Grapes (fruit, leaves), lettuce Rotational Crops: Radish (roots, leaves) lettuce, wheat (forage) Livestock: Goat (whole and skim milk, cream, liver, kidney) Hen (liver, kidney, egg yolk and fat)	Urine 0.95%, bile 0.34%, also in plasma, kidney <0.1% dose and liver <0.7% dose
Group 1A: Demethylation and conjugation			
M550F002		Plants: Grapes (fruit, leaves) Rotational Crops: Radish (leaves), lettuce, wheat (forage)	Aglycon M550F007 in urine 1.8% dose, in Feces 16% dose, in bile 6.8%, in liver 0.6%; Glucuronide M550F013 in urine max. 2.4% dose and in bile max. 40% dose (main metabolite in bile)
M550F006		Plants: Lettuce Potato peel Livestock: Goat (liver) Hen (liver, kidney, egg yolk)	Feces 7.2% dose, bile 0.5% dose, its glucuronide M550F015 in bile max.11.6% dose
M550F007		Plants: Grapes (fruit, leaves) potato peel Rotational Crops: Radish (roots, leaves) lettuce, wheat (forage) Livestock: Goat (liver, kidney) Hen (liver, egg yolk)	Urine 1.8% dose, in Feces 16% dose, in bile 6.8%, in liver 0.6%; its glucuronide M550F013 main metabolite in bile (max. 40% dose)

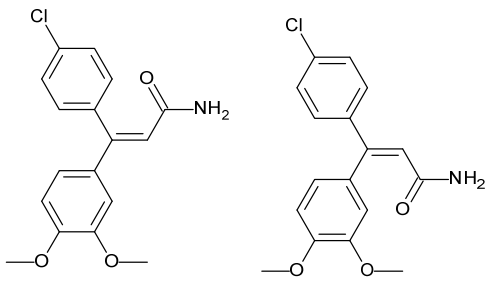
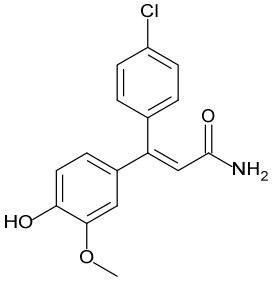
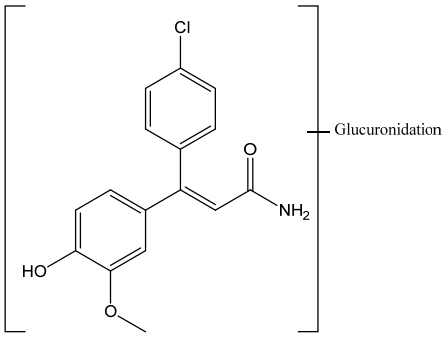
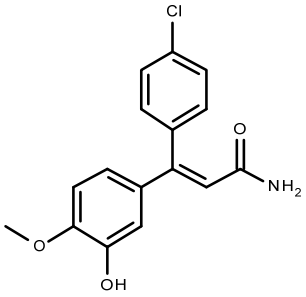
Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
M550F013		Livestock: Goat (liver, kidney) Hen (liver)	in urine max. 2.4% dose and in bile max. 40% dose (main metabolite in bile)
M550F015		Livestock: Goat (liver, kidney)	Urine 1% dose, feces 2.9% dose, in bile max.11.6% dose
M550F016		Livestock: Goat (liver)	1% in urine, 21.8% in faeces
M550F029		Livestock: Goat (liver, kidney)	3.3% dose in urine, 3.9% dose in feces, in bile max. 44% dose

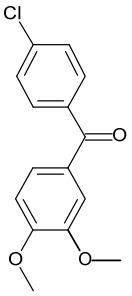
Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
Group 1B: Hydroxylation of the morpholine ring			
M550F018		Plants: Grapes (fruit, leaves) Rotational Crops: Radish (roots, leaves) wheat (forage) Livestock: Goat (liver, kidney, cream) Hen (egg yolk)	Faeces 11.2%, Plasma 0.1%, Liver 0.4%, Kidney <0.1%, in bile ca. 6.8% Glucuronide M550F083 urine 1%, bile 9.2%, in feces 17.3%
M550F028		Livestock: Goat (liver) Hen (liver, egg yolk)	Urine 1.8% dose, in bile 6.8% dose, in feces 16.2% dose, in kidney<0.1% and in liver 0.6%
M550F074		Livestock: Hen (liver)	In urine 1%, in bile max.18%
M550F076		Livestock: Hen (liver)	Urine 0.9%, in bile max. 11.5%, in feces 4.1%

Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
Group 2: Morpholine ring opening and modification (opening at the oxygen atom)			
M550F009		Livestock: Goat (liver, cream)	in urine 1.1%, in feces 21.5%, liver 0.16%, in bile 9.2% Its Glucuronide M550F087 1% in urine
M550F017 (one isomer)		Plants: Grapes (fruit, leaves) Rotational Crops: Radish (leaves), wheat (forage)	Its aglycon M550F009 in urine 1.1%, in feces 21.5%, liver 0.16%, in bile 9.2% Its Glucuronide M550F087 1% in urine
M550F028 (other isomer)		Livestock: Goat (liver) Hen (liver, egg yolk)	Urine 1.8% dose, in bile 6.8% dose, in feces 16.2% dose, in kidney<0.1% and in liver 0.6%
M550F035		Livestock: Hen (liver)	In urine 1.1% dose, in feces 6.6% dose
M550F053		Livestock: Goat (cream)	Faeces 30.9%, Urine 1.7%, Bile max. 6.8% dose

Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
<u>Group 3:</u> Morpholine ring opening and modification (opening at the nitrogen atom)			
M550F008		<u>Livestock:</u> Goat (liver, kidney, cream, whole milk), hen (liver, kidney)	Feces 1.1% dose demethylated metabolite M550F045 ca. 3.9% in feces; demethylated glucuronide M550F070 in urine 1.4%, in bile 11.9% dose
M550F011		<u>Livestock:</u> Goat (liver, kidney, cream, whole milk), hen (liver, egg yolk)	1.8% in urine, 12.1% in feces, 4.3% in bile, <0.1% in kidney, 0.6% in liver
M550F017 (other isomer)		<u>Plants</u>: Grapes (fruit, leaves) <u>Rotational Crops:</u> Radish (leaves), wheat (forage)	aglycon M550F030 in urine 1.8%, in feces 12.7%, liver 0.42%, bile 2.4%; acetylated form M550F060 in faeces 3.5%, demethylated glucuronide M550F079 in urine 0.34%, in bile max.11.5% dose
M550F022		<u>Livestock:</u> Hen (liver, kidney, egg yolk)	In feces 6.6% dose, its glucuronide M550F068 3.1% in bile

Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
M550F030		Livestock: Goat (liver)	in urine 1.8%, in feces 12.7%, liver 0.42%, bile 2.4%;
M550F031		Livestock: Goat (liver, kidney, cream)	In feces 9.1% dose, bile 0.48%, kidney, plasma 0.01%, liver 0.21%
M550F033		Livestock: Hen (liver, egg yolk)	Urine 1.1%, bile 16.7%, feces 19.6%, traces in kidney
M550F049		Livestock: Hen (liver)	In urine 1% dose, in feces 18% dose, its glucuronide M550F079 in urine 0.34%, in bile max.11.5% dose
M550F069		Livestock: Hen (liver)	12.6% in bile, 2.9% in faeces, 0.9% in urine

Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
Group 4: Complete loss of morpholine ring (free nitrogen)			
M550F012		Livestock: Goat (liver, cream), hen (liver, kidney, egg yolk)	7.2% in faeces; demethylated metabolite M550F038 3.9% in faeces, demethylated glucuronide M550F062 in urine 2.4%, faeces 1.7%, in bile 33.5%
M550F038		Livestock: Hen (liver, egg yolk)	3.9% dose in feces
M550F062		Livestock: Hen (liver)	in urine 2.4%, faeces 1.7%, in bile 33.5%
M550F091		Livestock: Hen (egg yolk)	Not identified in rat; *p*-demethylated isomer M550F038 in feces 3.9% dose, its glucuronide M550F062 in urine 2.4%, faeces 1.7%, in bile 33.5%

Metabolite code	Metabolite structure	Occurrence	Occurrence in rat
<u>Group 5:</u> Complete loss of morpholine ring (cleavage, no free nitrogen)			
M550F003		<u>Plants</u>: Lettuce, potato (leaves)	Not identified in rat

1- QSAR analysis of metabolites:
   a. QSAR evaluation of metabolites

The presence of potential structural alerts was evaluated with different SAR/QSAR models and compared with Dimethomorph. Models used, were the OECD toolbox, OASIS TIMES and VEGA.
Based on the close structural relationship of these metabolites with dimethomorph, the predicted alerts for the metabolites were similar to parent. Dimethomorph was shown to be non-genotoxic in a comprehensive set of in vitro and in vivo studies. Based on the fact that the QSAR evaluations for the metabolites were comparable to parent, all metabolites are considered to have a genotoxic potential comparable to Dimethomorph, i.e. they are considered to have no genotoxic potential.

   b. Material and methods

The majority of metabolites was tested in the different models. For some of them, the structure was not totally identified (M550F017, M550F018, M550F028, M550F029), in this case different hypothesis of structure have been proposed and tested. For the metabolites M500F062, M550F069, M550F074, M550F076 which are conjugates of other metabolites many structures were possible. The respective aglycone part of these metabolites was tested in the different models. It is not expected that conjugation can have an influence on genotoxicity of these compounds, therefore, they were not tested but considered to be covered by the results obtained with the aglycones metabolites.

   c. OECD Toolbox (profiling module)

The OECD toolbox version 3.2 as downloadable via link on the ECHA webpage [http://www.qsartoolbox.org/download.html] was used for the evaluation. The outcome of the OECD toolbox profiling conducted for Dimethomorph and the metabolites was exported and collected [see DocID 2015/1224347 and DocID 2015/1240224, 2015/1240225, 2015/1240226, 2015/1240227, 2015/1240229, 2015/1240230, 2015/1240232, 2015/1240233, 2015/1240234, 2015/1240235, 2015/1240236, 2015/1240241, 2015/1240242, 2015/1240243, 2015/1240244, 2015/1240245, 2015/1240246, 2015/1240247, 2015/1240248, 2015/1240249, 2015/1243914, 2015/1249728]. The profiling module provided structural alerts for different endpoints. Of particular interest were the modules dealing with DNA-binding capacity as well as genotoxicity predictions. It should be noted that the profiles just provide structural alerts without consideration on probability that these alerts may become active or inactive due to chemical reactivity and/or sterical hindrance.

d. OASIS TIMES

OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of Dettenbiotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test and *in vitro* chromosome aberration were considered and therefore predictivity is limited to these test systems only. The reports for the evaluations made are available under DocID 2015/1224345, DocID 2015/1240250, DocID 2015/1243920 for Ames mutagenicity and DocID 2015/1224346, DocID 2015/1240251, DocID 2015/1243921, DocID 2015/1249730, DocID 2015/1249734 for prediction of chromosomal aberration *in vitro*.

The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set having clear interpretation for the molecular mechanism causing the ultimate effect included in the model. The mechanistic interrelation between alerts and related parametric ranges generalizing the effect of the rest of the molecules on the alert is also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighborhood of atom-centered fragments is used to determine this similarity. The training set consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.

The derived model is combined with metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.

This OASIS QSAR system is also included in the OECD Toolbox (but not in combination with TIMES), in order to make use of (Q)SAR approaches also in the assessment of chemicals under REACH (OECD, 2008). The BASF-internal version has the advantage that it is capable to consider metabolic transformation.

e. VEGA

Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.vega-qsar.eu/], four independent statistical prediction models for mutagenicity (Ames) were selected. The data obtained for dimethomorph and the metabolites can be found in DocID 2015/1224348, 2015/1240220, 2015/1240222 for dimethomorph and DocID 2015/1240252, 2015/1240253, 2015/1240254, 2015/1240255, 2015/1243916, 2015/1243917, 2015/1243918, 2015/1243919 2015/1249729, 2015/1249731, 2015/1249732, 2015/1249736 for the metabolites. These reports includes both models used (CAESAR, SarPy, ISS and KNN).

f. CAVEAT on reliability of QSAR modules implied

With regard to the QSAR evaluations as implied in the OECD toolbox, in OECD TIMES and in VEGA$_{CAESAR}$ it should be noted that for nearly all analysis the algorithm reported an out of structural domain error. Each of this QSAR models is built on a set of chemicals that forms its chemical domain, space or applicability domain. That means that the prediction is best if a structure of interest is represented in the original baseline dataset. Substances outside of the dataset are evaluated in comparison to the chemical space, and only in case that the chemical space adequately covers all structural elements or the queried structure, the prediction is considered to be adequately covered by experimental data. Predictions outside of the applicability domain have far lower predictability. In addition all mentioned QSAR models check for structural alerts, like those identified by the Benigni-Bossa rules that have been implicated in mutagenic actions.

As a consequence, the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (OECD toolbox and VEGA) or not appropriately (OASIS TIMES) taking into account possible functional group interaction and stereochemical hindrance. It is well established that structure elements have to be evaluated within the context of a structure.

To evaluate the predictivity of each QSAR model, the results were compared with dimethomorph results of genotoxicity tests. The genotoxic alerts identified in the QSAR models, that did not result in positive experimental genotoxicity results are considered to be false positive.
In general, the predictivity of various QSAR models for genotoxicity equivalent to the Ames test has been considered to be reasonable accurate. Predictivity rates expressed as accuracy and specificity are usually >80%. This is in particular true, if information from more than one QSAR model is combined.

QSAR models for chromosome aberration however are far less well established. One of the underlying limitations is that typical *in vitro* assays for chromosome aberration, like chromosome aberration in V79 cells or the in vitro micronucleus assay have false discovery rates of approximately 30%. This means that three out of ten molecules are falsely categorized. In addition a high number of in vitro positive substances are negative in adequate *in vivo* assays. The later is often a function of the underlying mode of action and the kinetic behavior of a substance. Both are not adequately covered by QSAR predictions.

Predictions for chromosome damage were performed with OASIS Times and OECD Toolbox. The prediction model used in the OECD toolbox is similar to that of OASIS Times, with the major difference being the different underlying database of reference compounds and the combination with the metabolism generator in OASIS Times.

BASF has tested the valididy of the predictions for chromosome aberration of the OASIS Times. For this > 100 chemicals with in vitro experimental data on chromosome aberration were evaluated with OASIS Times. It is important to note that all chemicals from this subset were within the applicability domain of the algorithm. The concordance between the predicted values and the experimental values was below 50%. This was even worse for a second set of chemicals, which was outside of the applicability domain of the QSAR model.

g. Results

Table 5.8.1-2 gives a summary of the QSAR predictions and Table 5.8.1-3 the actual results of the mutagenicity testing of Dimethomorph. The positive outcome observed for CA with Dimethomorph with OASIS TIMES and TOOLBOX and for mutagenicity with TOXTREE was contradicted by the test results. For the metabolites (except morpholine) the same alerts were observed for all metabolites in all models except CAESAR. All positive outcomes in CAESAR are out of the domain of application and considered as false positives. Overall, the structural similarities of the metabolites, the results obtained in the different QSAR model and the experimental results obtained with dimethomorph permit to consider that these metabolites have the same genotoxic properties than dimethomorph.

Table 5.8.1-2: Summary of results

	TIMES AMES	TIMES CA	TOOLBOX AMES	TOOLBOX CA	CAESAR	SarPy	ISS	KNN
BAS 550 F	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F002	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg OfD	neg ID	neg OfD
M550F003	neg OfD	pos OfD	neg OfD	pos OfD	neg OfD	neg ID	neg ID	pos ID
M550F006	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F007	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F008	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F009	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg ID	neg ID
M550F011	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F012	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F013	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg OfD	neg ID
M550F015	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg OfD	neg OfD	neg ID
M550F016	neg OfD	neg OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F017 hyp 1	neg OfD	pos OfD	neg OfD	pos OfD	neg OfD	neg ID	neg ID	neg ID
M550F017 hyp 2	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg ID	neg ID
M550F018 hyp 1	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	pos OfD	neg ID	neg ID
M550F018 hyp 2	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F021	neg ID	neg ID	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F022	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F028 hyp 1	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg ID	neg ID
M550F028 hyp 2	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	pos OfD	neg ID	neg ID
M550F028 hyp 3	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	pos OfD	neg ID	neg ID
M550F028 hyp 4	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F029 hyp 1	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg OfD	neg OfD	neg ID
M550F029 hyp 2	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg OfD	neg OfD	neg ID

	TIMES AMES	TIMES CA	TOOLBOX AMES	TOOLBOX CA	CAESAR	SarPy	ISS	KNN
M550F030	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F031	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F033	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F035	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg ID	neg ID
M550F038	neg OfD	pos OfD	neg OfD	pos OfD	neg OfD	neg ID	neg ID	neg ID
M550F049	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID
M550F053	neg OfD	pos OfD	neg OfD	pos OfD	pos OfD	neg ID	neg ID	neg ID
M550F091	neg OfD	pos OfD	neg OfD	pos OfD	neg ID	neg ID	neg ID	neg ID

neg: negative
pos: positive
OfD: out of domain
ID: In domain

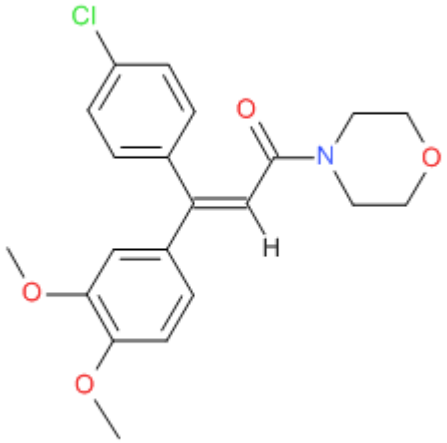
Table 5.8.1-3: Summary of experimental results with dimethomorph

Study/strain/species	Test conditions	Results	Reference (BASF DocID)
Ames mutagenicity test; S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538 E. coli WP2 uvrA$^-$	All six strains received 5000, 2000, 1000, 500, 250, 125, 62.5, and 31.25 µg/mL with and without S9	Negative; Negative	DK-435-018; DK-435-011 DK-435-010
V79/HGPRT mutagenicity test	133, 180, 237 and 333 µg/mL with S9; 33, 100, 133, and 180 µg/mL without S9	Negative; Negative	DK-435-014
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells (Prestudy)	13, 60, and 170 µg/mL with S9; 12, 60 and 160 µg/mL without S9 (200 metaphases scored per dose group	Slight increase in aberrations at 160 µg/mL without S9 and 170 µg/mL with S9 (marked cytotoxicity) at 7 hours and at 160 µg/mL without S9 at 18 hours	DK-435-004
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells (Main study)	170 µg/mL with S9; 160 µg/mL without S9 (400 metaphases scored per dose group)	Slight increase in aberrations at 7 h fixation time at 170 µg/mL with S9 (marked cytotoxicity); Negative without S9 (including 18 hours)	DK-435-006
In vitro cytogenetics: chromosome aberration in cultured human peripheral lymphocytes	Up to 422 µg/mL with S9; Up to 333 µg/mL without S9	Negative, except for positive results at a high, precipitating and strongly cytotoxic concentration (422 µg/mL with S9)	DK-435-013
In vitro UDS, rat hepatocytes	0, 2.5, 10, 25, 100, and 250 µg/mL	Negative	DK-435-002
In vitro cell transformation assay with Syrian hamster embryo cells	Up to 50 µg/mL without S9, 6 and 48 h Up to 265 µg/mL with S9, 6 h	Negative	DK-435-005
In vivo chromosome aberration: Mouse micronucleus test	0, 20, 100, and 200 mg/kg bw (intraperitoneal)	Negative	DK-435-012

Study/strain/species	Test conditions	Results	Reference (BASF DocID)
Study/strain/species	Test conditions	Results	Reference (BASF DocID)
Ames mutagenicity test; S. typhimurium TA 98, TA 100, TA 1535, TA 1537, E. coli WP2 uvrA-	All six strains received 5000, 1580, 1000, 500, 158, 50 and 15, µg/mL with and without S9. Additional dose of 5 µg/mL with S9.	Negative; Negative	DK-435-001
V79/HGPRT mutagenicity test	15.4, 30, 100, 250 and 300 µg/mL with S9; 23, 100, 200, and 230 µg/mL without S9	Negative; Negative	DK-435-003
In vitro cytogenetics: chromosome aberration in Chinese hamster V79 cells	120, 170 and 250 µg/mL with S9; (200-1000 metaphases scored per dose group)	Negative	DK-435-007
In vitro cytogenetics: chromosome aberration in CHL	93.8 to 1500 µg/mL with S9; 11.7 to 187 µg/mL without S9	Negative; Negative	DK-435-015
In vitro cytogenetics: chromosome aberration in CHL	93.8, 187.5, 375, 750 and 1500 µg/mL with and without S9;	Negative; Negative	DK-435-016
DNA repair	20, 50, 100, 200, 500, 1000 µg/plate with and without S9.	Negative	DK-435-017
In vivo chromosome aberration: Mouse micronucleus test oral route	5000 mg/kg bw	Negative	DK-435-009

h. Individual results by molecules

1. Dimethomorph (other denominator: BAS 550 F)



QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see DocID 2015/1224347 doc JCA]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones
Acylations >> Direct Acylation Involving a Leaving group >> Acetate
H-acceptor-path3-H-acceptor
Acrylamide Reactive Functional Groups

In this model dimethomorph is out of domain. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/34 2015/1224345]

There were no Ames mutagenicity alerts for dimethomorph or in-silico generated metabolites. In this model dimethomorph is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/35 2015/1224346]

In this model dimethomorph is out of domain.

An alert for chromosomal aberration is reported for Dimethomorph and for some in-silico generated hydroxylated metabolites. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see molecule 1 of report DocID 2015/1224348 - Doc JCA]

Dimethomorph is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report DocID 2015/1224348 - Doc JCA]

Dimethomorph is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/36 2015/1240220]

Dimethomorph is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/37 2015/1240222]

Dimethomorph is in the model applicability domain. The prediction is “non-mutagen”.

Summary and discussion:

Dimethomorph does not induce mutagenic effects in in vitro and in vivo studies. All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox was positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). The alert for chromosome aberration is not reflected by the available experimental data for dimethomorph and metabolites of similar structures and therefore is rejected. CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, dimethomorph was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms are not reflected by the experimental database of Dimethomorph.

2. M550F002

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/38 2015/1240224]

In the OECD toolbox some alerts were observed:
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F002 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F002 or in-silico generated metabolites. In this model M550F002 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F002 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F002. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F002 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. M550F002 is a conjugate of M550F007 which is predicted to be “non-mutagen” in CAESAR. In the report, compounds with mutagenic properties cannot be related to M550F002, moreover it cannot be expected that conjugation of a metabolite will induce mutagenic properties, therefore this alert is rejected. (It must be noted that some other conjugates are also predicted positive in CAESAR whereas their aglycones are not. This is specific to CAESAR modelling and therefore all these alerts are rejected).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

M550F002 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F002 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F002 is out of the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F002

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity, M550F002 was in the domain of applicability only for ISS model.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictios obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore it can be considered that M550F002 has the same genotoxic properties as dimethomorph, i.e. no genotoxicty is expected.

3. M550F003

For M550F003 some toxicological studies, including genotoxicity tests has been performed and are reported in the respective section in this document.

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/70 2015/1243914]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F003 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on M550F003 and dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.17 6; Mutagenicity S-9 activated v11.11) [see KCA 5.8.1/64 2015/1243920]

There were no Ames mutagenicity alerts for M550F003 or in-silico generated metabolites. In this model M550F003 is out of domain.

OASIS TIMES (MIX V.2.27.17 6; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/67 2015/1243921]

In this model M550F003 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F003. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on M550F003 or dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.13) [see KCA 5.8.1/66 2015/1243916]

M550F003 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.7) [see KCA 5.8.1/65 2015/1243919]

M550F003 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/69 2015/1243917]

M550F003 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/68 2015/1243918]

M550F003 is in the model applicability domain. The prediction is “mutagen”. However 2 out of 4 molecules in the data set have negative experimental results and experimental results on M55F003 are negative. This alert is rejected.

Overall toxicological evaluation of M550F003

These results demonstrate that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms are not reflected by the experimental database of M550F003 and Dimethomorph, therefore it can be considered that M550F003 has the same genotoxic properties than dimethomorph. No genotoxicty is expected.

4. M550F006

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/39 2015/1240225]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F006 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F006 or in-silico generated metabolites. In this model M550F006 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F006 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F006. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F006 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F006 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F006 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F006 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F006

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F006 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F006 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

5. M550F007

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/40 2015/1240226]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F007 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F007 or in-silico generated metabolites. In this model M550F007 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F007 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F007. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F007 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F007 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F007 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F007 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F007

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F007 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F007 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

6. M550F008

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/41 2015/1240227]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F008 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F008 or in-silico generated metabolites. In this model M550F007 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F008 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F008. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F008 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F008 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F008 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F008 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F008

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F008 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F008 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

7. M550F009

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/42 2015/1240229]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F009 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F009 or in-silico generated metabolites. In this model M550F002 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F009 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F009. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F009 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. The underlying database of VEGA contains five closely related structures (CAS 458-37-7; similarity 0.83, CAS 49562-28-9; similarity 0.813, CAS 569-57-3; similarity 0.812, CAS 42017-89-0; similarity 0.807, CAS 104775-36-2; similarity 0.804) that like dimethomorph have negative experimental values. Therefore, the prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

M550F009 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F009 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F009 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F009

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F009 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F009 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

8. M550F011

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/43 2015/1240230]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F011 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F011 or in-silico generated metabolites. In this model M550F0011 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F011 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F011. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F011 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F011 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F011 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F011 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F011

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F011 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F0011 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

9. M550F012

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/44 2015/1240232]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F012 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F012 or in-silico generated metabolites. In this model M550F0012 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F012 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F012. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F012 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F012 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F012 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F012 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F012

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F012 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F012 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

10. M550F013

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/45 2015/1240233]

In the OECD toolbox some alerts were observed:
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F013 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F013 or in-silico generated metabolites. In this model M550F013 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F013 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F013. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F013 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. M550F013 is a conjugate of M550F007 which is predicted to be “non-mutagen” in CAESAR. In the report, compounds with mutagenic properties cannot be related to M550F013, moreover it cannot be expected that conjugation of a metabolite will induce mutagenic properties, therefore this alert is rejected. (It must be noted that some other conjugates are also predicted positive in CAESAR whereas their aglycones are not. This is specific to CAESAR modelling and therefore all these alerts are rejected).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

M550F013 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F013 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F013 is out of the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F013

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity, M550F013 was out of the domain of applicability only for ISS model.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F013 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

11. M550F015

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/46 2015/1240234]

In the OECD toolbox some alerts were observed:
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F015 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F015 or in-silico generated metabolites. In this model M550F015 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F015 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F015. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F015 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. M550F015 is a conjugate of M550F006 which is predicted to be “non-mutagen” in CAESAR. In the report, compounds with mutagenic properties cannot be related to M550F015, moreover it cannot be expected that conjugation of a metabolite will induce mutagenic properties, therefore this alert is rejected. (It must be noted that some other conjugates are also predicted positive in CAESAR whereas their aglycones are not. This is specific to CAESAR modelling and therefore all these alerts are rejected).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

M550F015 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F015 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F015 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F015

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity, M550F015 was in the domain of applicability only for KNN model.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F015 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

12. M550F016

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/47 2015/1240235]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F016 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F016 or in-silico generated metabolites. In this model M550F016 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F016 is out of domain.

No alert for chromosomal aberration is reported for M550F016 or in-silico generated metabolites.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F016 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F016 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F016 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F016 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F016

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test) or for CA (unlike DMM). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F016 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F016 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

13. M550F017

Two structures are possible for this metabolite. Both have been tested in the different models.

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/48 2015/1240236]

In the OECD toolbox some alerts were observed for hypothesis 1:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

And an additional alert for hypothesis 2
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines

In this model both hypothesis of M550F017 are out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for both hypothesis of M550F017 or in-silico generated metabolites. In this model M550F017 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model both hypothesis of M550F017 are out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for both M550F017. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F017 is out of the model applicability domain for both hypothesis. The prediction is "non-mutagen" for hypothesis 1 and "mutagen" for hypothesis 2 with no structural alert associated. M550F017 is a conjugate of M550F009 which is also predicted to be "mutagen" in CAESAR. In the report, compounds with mutagenic properties cannot be related to M550F017 or M550F009, moreover it cannot be expected that conjugation of a metabolite will induce mutagenic properties, therefore this alert is rejected. (It must be noted that some other conjugates are also predicted positive in CAESAR whereas their aglycones are not. This is specific to CAESAR modelling and therefore all these alerts are rejected).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

Both hypothesis of M550F017 are in the model applicability domain. The prediction is "non-mutagen".

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

Both hypothesis of M550F017 are in the model applicability domain. The prediction is "non-mutagen".

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

Both hypothesis of M550F017 is in the model applicability domain. The prediction is "non-mutagen".

Overall toxicological evaluation of M550F017

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive for one hypothesis but it was rejected and both hypothesis were out of the domain of application. SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F017 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F017 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

14. M550F018

Two structures are possible for this metabolite. Both have been tested in the different models.

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/49 2015/1240241]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model both hypothesis of M550F018 are out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for both M550F018 or in-silico generated metabolites. In this model M550F018 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model both M550F018 are out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F018. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F018 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

One hypothesis of M550F018 is in the model applicability domain. The prediction is “non-mutagen”.

The second hypothesis of M500F018 is out of the model applicability ans is predicted “mutagen”. This is due to Frag SM98. The most similar substance in the model dataset (similarity 0.751) has negative experimental results. However another substance in the model dataset (similarity 0.749) has positive experimental results. Both hypothesis are hydroxylation of dimethomorph on the morpholin moiety. It can be expected that metabolisation of the parent structure will not lead to a genotoxic compound. Therefore the alert on hypothesis 2 is rejected.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

Both hypothesis of M550F018 are in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

Both hypothesis of M550F018 are in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F018

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, ISS and KNN predicted no mutagenicity and in these QSAR, M550F018 was in the domain of applicability. An alert was identified in SarPy for hypothesis 2 but was rejected, Hypothesis 2 was out of the domain of application in SarPy, whereas hypothesis 1 was in the domain of application.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F018 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

15. M550F021

For M550F021 some toxicological studies, including genotoxicity tests has been performed and are reported in the respective section in this document.

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/50 2015/1240242]

In the OECD toolbox some alerts were observed:
Schiff base formers>>Chemicals activated by P450 to glyoxal>>ethanolamines (including morpholine)
H-acceptor-path3-H-acceptor

In this model M550F021 is in domain. Different alerts are reported compared to dimethomorph but a complete database for genotoxicity is available on M550F021 (morpholine), see Table 5.8.1-7

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F021 or in-silico generated metabolites. In this model M550F021 is in the domain of application.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F021 is out of domain.

No alert for chromosomal aberration is reported for M550F021 or in-silico generated metabolites.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F021 is in the model applicability domain. The prediction is “non-mutagen”. M550F021 is in the model database. Experimental results are negative.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

M550F021 is in the model applicability domain. The prediction is “non-mutagen”. M550F021 is in the model database. Experimental results are negative.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F021 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F021 is in the model applicability domain. The prediction is “non-mutagen”. M550F021 is in the model database. Experimental results are negative.

Overall toxicological evaluation of M550F021

The only QSAR analysis models predicting positive outcome was out of domain and are thus of low predictivity. Analysis by the OECD Toolbox for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test) or chromosomal aberrations. CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F021 was in the domain of applicability.

This summary demonstrates that the positive association to chromosome damage identified by the OECD toolbox algorithms are not reflected by the experimental database of M550F021. No genotoxicty is expected.

16. M550F022

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/51 2015/1240243]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F022 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F022 or in-silico generated metabolites. In this model M550F022 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F022 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F022. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F022 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F022 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F022 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F022 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F022

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F022 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F022 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

17. M550F028

Four structures are possible for this metabolite. Both have been tested in the different models.

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/52 2015/1240244]

In the OECD toolbox some alerts were observed for all hypothesis:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model both hypothesis of M550F028 are out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for both M550F028 or in-silico generated metabolites. In this model all hypothesis of M550F028 are out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model both M550F028 are out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F028. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

Three hypothesis of M550F028 is in the model applicability domain. The prediction is “non-mutagen” (hypothesis 2, 3 and 4). For hypothesis 1 the prediction is “mutagen” and ouf of the applicability domain. However the underlying database of VEGA contains six closely related structures (CAS 458-37-7; similarity 0.829, CAS 569-57-3; similarity 0.803, CAS 104775-36-2; similarity 0.8, CAS 42017-89-0; similarity 0.798, CAS 49562-28-9; similarity 0.798 and CAS 522-40-7; similarity 0.784 that like dimethomorph have negative experimental values. Therefore, the prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see KCA 5.8.1/63 2015/1240255]

Two hypothesis of M550F028 (1 and 4) are in the model applicability domain. The prediction is “non-mutagen”.

The two other hypothesis of M500F028 are out of the model applicability and are predicted “mutagen”. This is due to Frag SM98. Two similar substances in the model dataset have negative experimental results. However another substance in the model dataset has positive experimental results. Both hypothesis are hydroxylation of dimethomorph on the morpholin moiety. It can be expected that metabolisation of the parent structure will not lead to a genotoxic compound. Therefore the alert on hypothesis 2 and 3 are rejected.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

Both hypothesis of M550F028 are in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

Both hypothesis of M550F028 are in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F028

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). ISS and KNN predicted no mutagenicity and in these QSAR, all hypothesis of M550F028 was in the domain of applicability. An alert was identified in SarPy for hypothesis 2 and 3 but was rejected, Hypothesis 2 and 3 was out of the domain of application in SarPy, whereas hypothesis 1 and 4 was in the domain of application. An alert was identified in CAESAR for hypothesis 1, and this hypothesis was out of the domain of application and rejected, the three other hypothesis were in the domain of application and negative.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F028 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

18. M550F029

Two structures are possible for this metabolite. Both have been tested in the different models.

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/53 2015/1240245]

In the OECD toolbox some alerts were observed for both hypothesis:
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model both hypothesis of M550F029 are out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for both hypothesis of M550F029 or in-silico generated metabolites. In this model M550F029 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model both hypothesis of M550F029 are out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for both M550F029. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F029 is out of the model applicability domain for both hypothesis. The prediction is “mutagen” for both hypothesis with no structural alert associated. M550F029 is a conjugate of M550F016 which is predicted to be “non-mutagen” in CAESAR. In the model database, compounds with mutagenic properties cannot be related to M550F029 or M550F016, moreover it cannot be expected that conjugation of a metabolite will induce mutagenic properties, therefore this alert is rejected. (It must be noted that some other conjugates are also predicted positive in CAESAR whereas their aglycones are not. This is specific to CAESAR modelling and therefore all these alerts are rejected).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

Both hypothesis of M550F029 are out of the model applicability domain. The prediction is "non-mutagen".

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

Both hypothesis of M550F029 are out of the model applicability domain. The prediction is "non-mutagen".

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

Both hypothesis of M550F029 are in the model applicability domain. The prediction is "non-mutagen".

Overall toxicological evaluation of M550F029

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive for both hypothesis but it was rejected and both hypothesis were out of the domain of application. SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F029 was in the domain of applicability for KNN only.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F029 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

19. M550F030

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/54 2015/1240246]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F030 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F030 or in-silico generated metabolites. In this model M550F030 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F030 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F030. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F030 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F030 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F030 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F030 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F030

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F030 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F030 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

20. M550F031

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/55 2015/1240247]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F031 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F031 or in-silico generated metabolites. In this model M550F031 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F031 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F031. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F031 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F031 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F031 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F031 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F031

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F031 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F031 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

21. M550F033

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/56 2015/1240248]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F033 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F033 or in-silico generated metabolites. In this model M550F033 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F033 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F033. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F033 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F033 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F033 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F033 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F033

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F033 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F033 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

22. M550F035

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/71 2015/1249728]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F035 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.17 6; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/76 2015/1249734]

There were no Ames mutagenicity alerts for M550F035 or in-silico generated metabolites. In this model M550F035 is out of domain.

OASIS TIMES (MIX V.2.27.17 6; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/73 2015/1249730]

In this model M550F035 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F035. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.13) [see KCA 5.8.1/72 2015/1249729]

M550F035 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. The underlying database of VEGA contains six closely related structures (CAS 458-37-7; similarity 0.837, CAS 49562-28-9; similarity 0.813, CAS 42017-89-0; similarity 0.808, CAS 569-57-3; similarity 0.804, CAS 104775-36-2; similarity 0.798, CAS 30418-38-3; similarity 0.792) that like dimethomorph have negative experimental values. Therefore, the prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.7) [see KCA 5.8.1/77 2015/1249736]

M550F035 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/75 2015/1249732]

M550F035 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/74 2015/1249731]

M550F035 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F035

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F035 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F035 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

23. M550F038

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/71 2015/1249728]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F038 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.17 6; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/76 2015/1249734]

There were no Ames mutagenicity alerts for M550F038 or in-silico generated metabolites. In this model M550F038 is out of domain.

OASIS TIMES (MIX V.2.27.17 6; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/73 2015/1249730]

In this model M550F038 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F038. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.13) [see KCA 5.8.1/72 2015/1249729]

M550F038 is out of the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.7) [see KCA 5.8.1/77 2015/1249736]

M550F038 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/75 2015/1249732]

M550F038 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/74 2015/1249731]]

M550F038 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F038

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F038 was in the domain of applicability (except for CAESAR).

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F038 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

24. M550F049

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/57 2015/1240249]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F049 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.16 8; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/58 2015/1240250]

There were no Ames mutagenicity alerts for M550F049 or in-silico generated metabolites. In this model M550F049 is out of domain.

OASIS TIMES (MIX V.2.27.16 8; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/59 2015/1240251]

In this model M550F049 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F049. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see KCA 5.8.1/60 2015/1240252]

M550F049 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report KCA 5.8.1/63 2015/1240255]

M550F049 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/61 2015/1240253]

M550F049 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/62 2015/1240254]

M550F049 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F049

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F049 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F049 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

25. M550F053

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/71 2015/1249728]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
SN1 >> Iminium Ion Formation >> Aliphatic tertiary amines
H-acceptor-path3-H-acceptor

In this model M550F053 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.17 6; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/77 2015/1249736]

There were no Ames mutagenicity alerts for M550F053 or in-silico generated metabolites. In this model M550F053 is out of domain.

OASIS TIMES (MIX V.2.27.17 6; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/73 2015/1249730]

In this model M550F053 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F053. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.13) [see KCA 5.8.1/72 2015/1249729]

M550F053 is out of the model applicability domain. The prediction is “mutagen” with no structural alert associated. The underlying database of VEGA contains six closely related structures (CAS 458-37-7; similarity 0.83, CAS 569-57-3; similarity 0.807, CAS 42017-89-0; similarity 0.804, CAS 49562-28-9; similarity 0.802, CAS 104775-36-2; similarity 0.797, CAS 13676-54-5; similarity 0.791) that like dimethomorph have negative experimental values. Therefore, the prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.7) [see KCA 5.8.1/77 2015/1249736]

M550F053 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/75 2015/1249732]

M550F053 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/74 2015/1249731]

M550F053 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F053

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR prediction was positive but the results of this prediction is rejected. SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F053 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F053 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

26. M550F091

QSAR predictions:

OECD Toolbox (Version: 3.3.2) [see KCA 5.8.1/71 2015/1249728]

In the OECD toolbox some alerts were observed:
Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Hydroquinones
H-acceptor-path3-H-acceptor

In this model M550F091 is out of domain. Less alerts are reported compared to dimethomorph. The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

OASIS TIMES (MIX V.2.27.17 6; Mutagenicity S-9 activated v10.10) [see KCA 5.8.1/77 2015/1249736]

There were no Ames mutagenicity alerts for M550F091 or in-silico generated metabolites. In this model M550F091 is out of domain.

OASIS TIMES (MIX V.2.27.17 6; Chromosomal Aberration S-9 activated v10.10) [see KCA 5.8.1/73 2015/1249730]

In this model M550F091 is out of domain.

An alert for chromosomal aberration is reported for some in-silico generated hydroxylated metabolites but not for M550F091. The alert is due to “substituted phenol” moiety with a mechanism involving interaction with topoisomerases/proteins.

The alerts reported by the model are not confirmed by experimental results on dimethomorph, therefore the predictions are rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.13) [see KCA 5.8.1/72 2015/1249729]

M550F091 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (SarPy; version 1.0.7) [see KCA 5.8.1/77 2015/1249736]

M550F091 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (ISS; version 1.0.2) [see KCA 5.8.1/75 2015/1249732]

M550F091 is in the model applicability domain. The prediction is “non-mutagen”.

VEGA: Mutagenicity model (KNN; version 1.0.0) [see KCA 5.8.1/74 2015/1249731]

M550F091 is in the model applicability domain. The prediction is “non-mutagen”.

Overall toxicological evaluation of M550F091

All QSAR analysis models predicting positive outcomes were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox and TIMES for chromosomal aberration were positive but the results of this prediction are rejected. OASIS Times predicted no mutagenicity in bacteria (Ames test). CAESAR, SarPy, ISS and KNN predicted no mutagenicity and in these QSAR, M550F091 was in the domain of applicability.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms is comparable to the predictions obtained for parent which are not reflected by the experimental database of Dimethomorph. Therefore, it can be considered that M550F091 has the same genotoxic properties as dimethomorph, i.e. no genotoxicity is expected.

2- Proposal of grouping for metabolites:

   a. Rationale for grouping

Based on the structure of metabolites and metabolic scheme in rat, metabolites found in livestock and plants are grouped according the following rationale. All metabolites in the same group are supposed to have an equivalent toxicity, while for the different groups toxicity might vary due to diverse chemical groups.

Figure 5.8.1-1: Metabolic scheme in rat

Figure 1: Proposed Metabolic Pathway of BAS 550 F in Rats

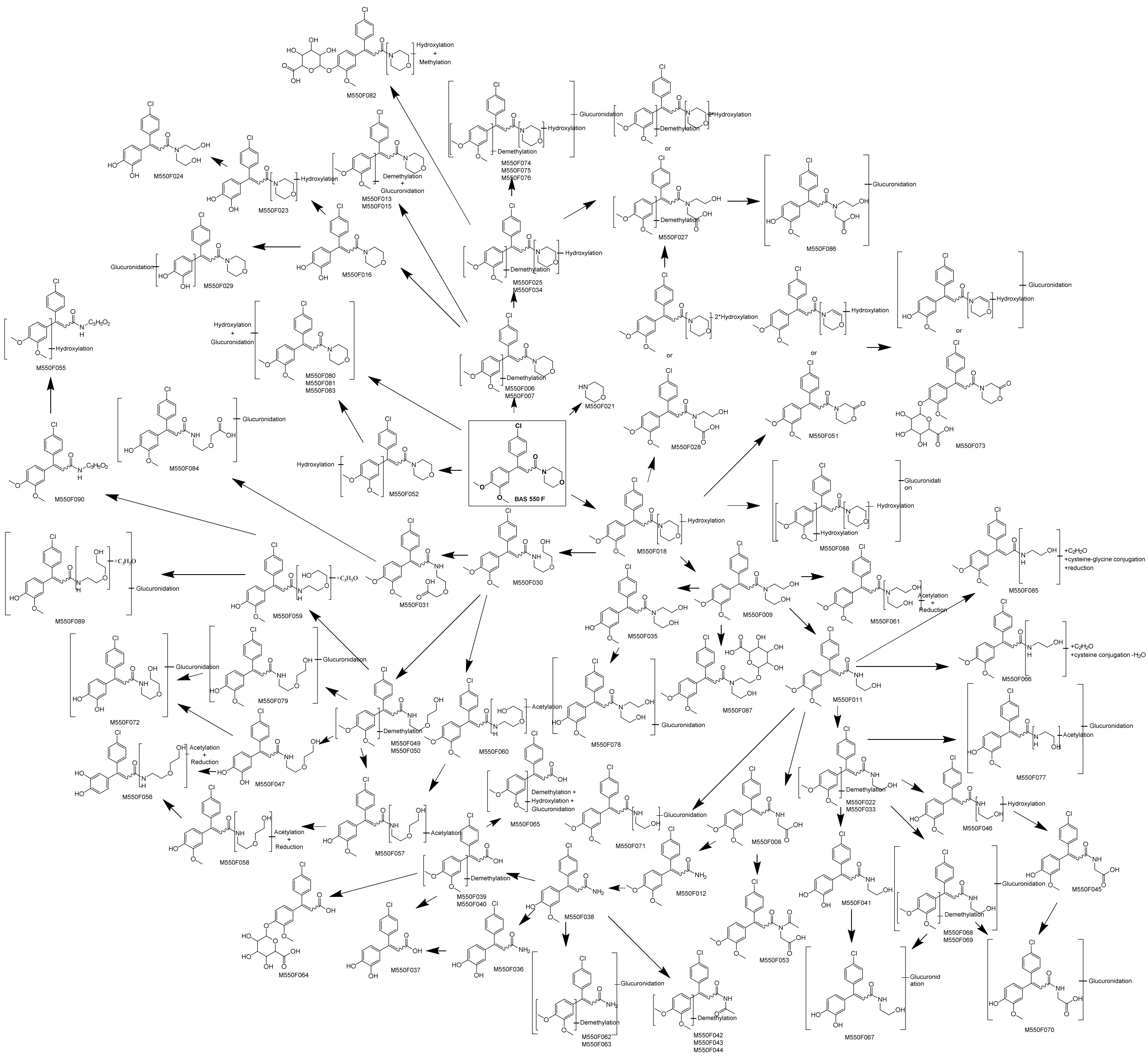
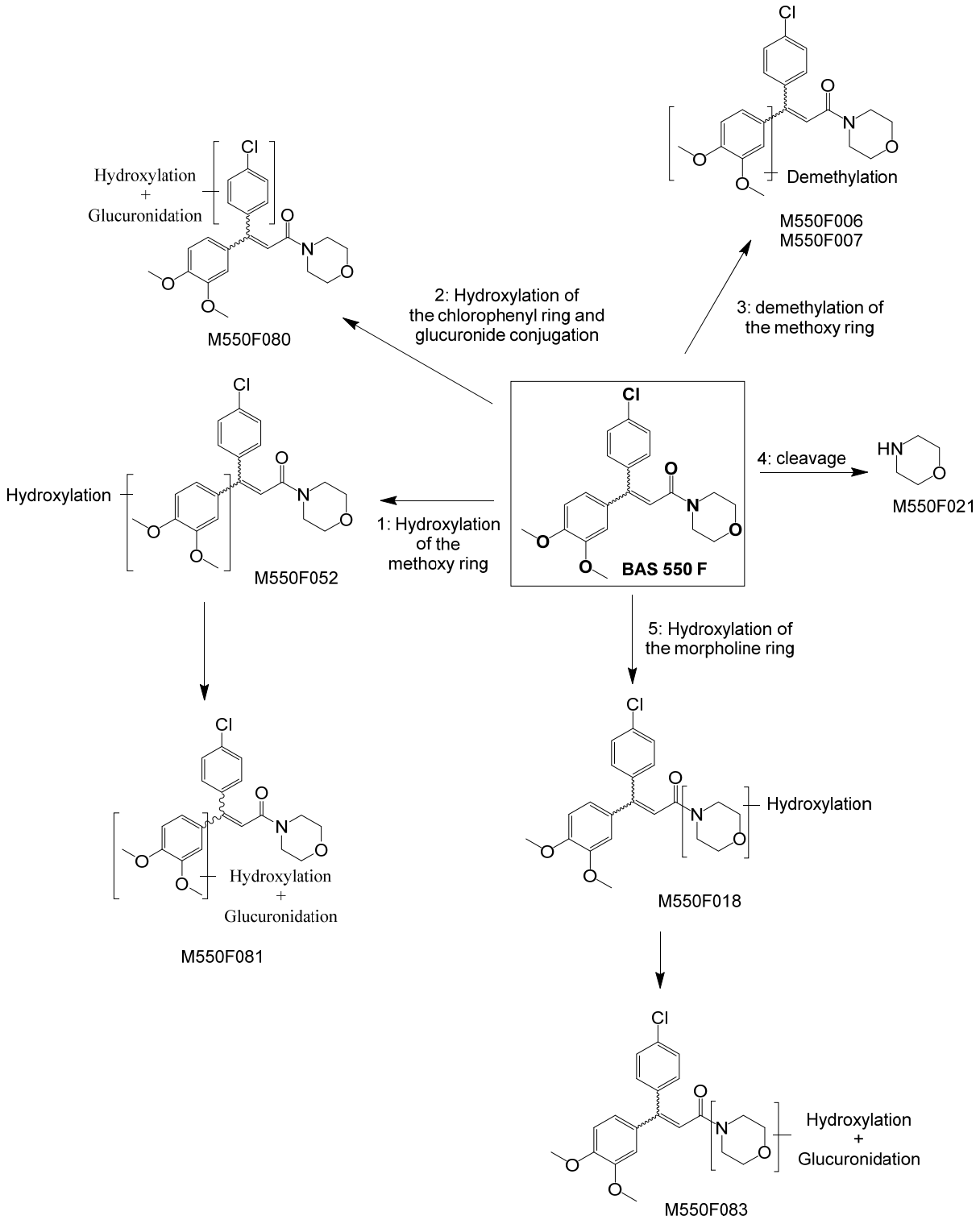


Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial 5 metabolic steps



Steps 3 and 5 have further metabolic steps, as shown in the following figures.

Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 3: Demethylation of the methoxy ring

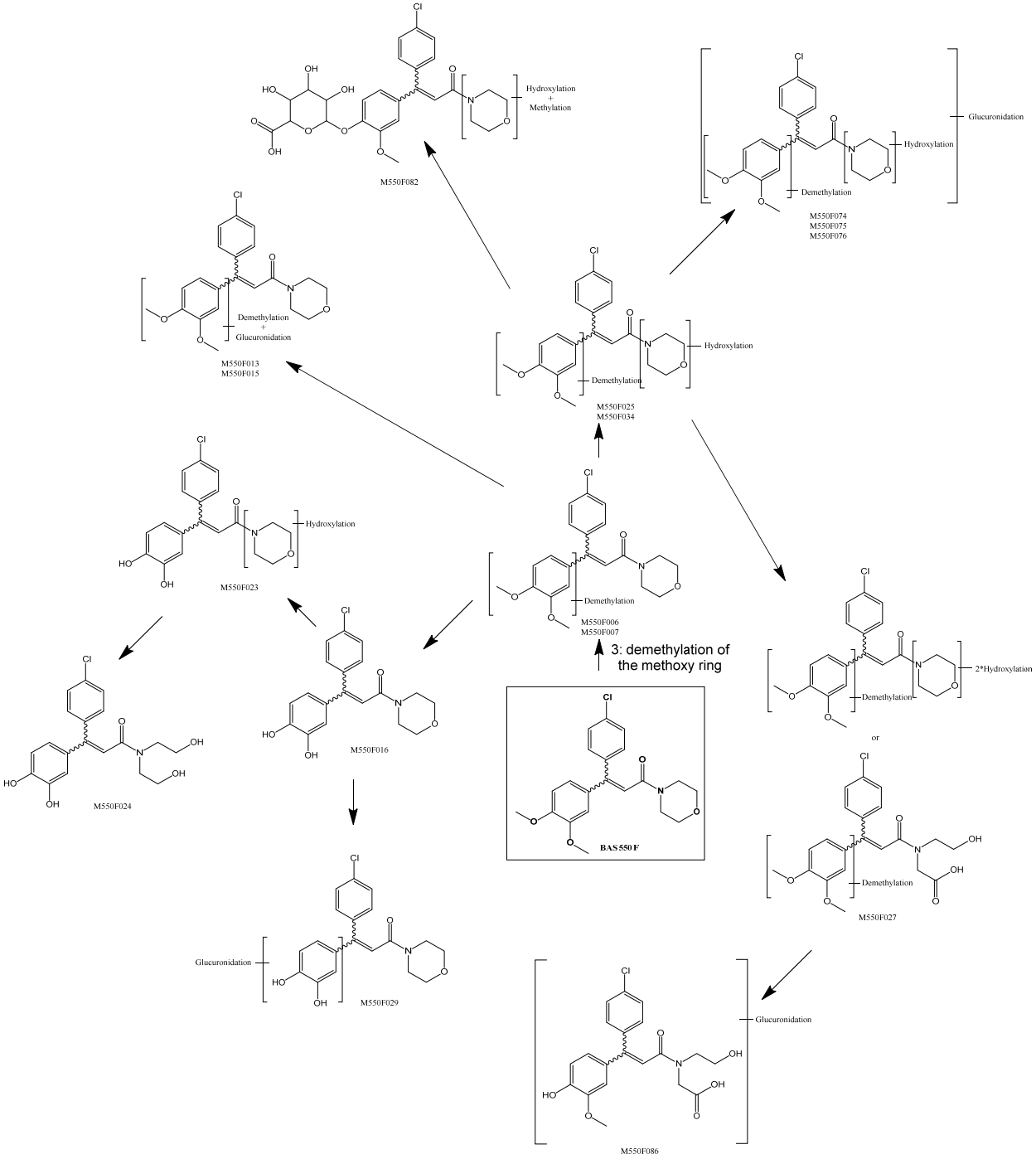


Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring

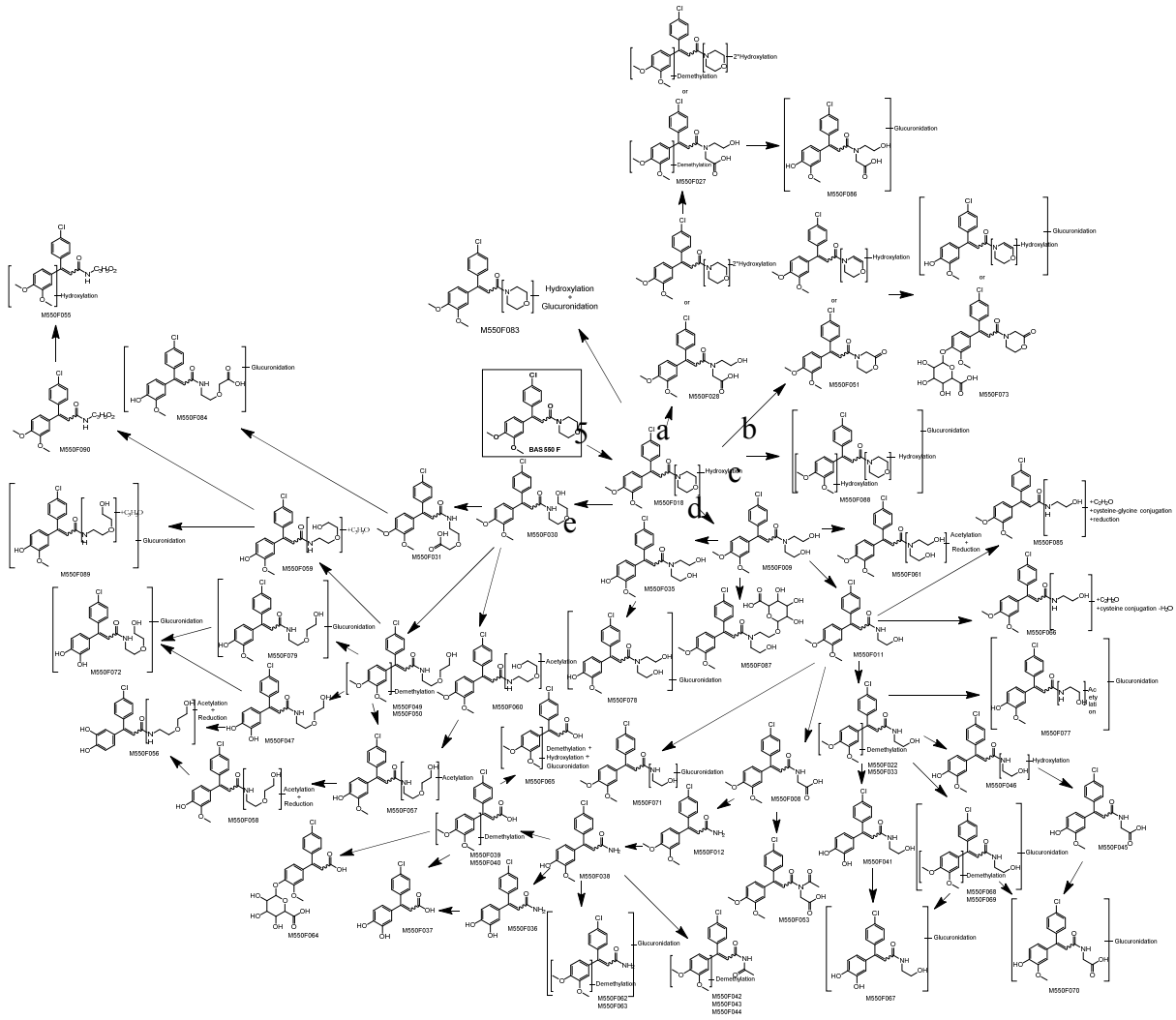


Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-steps a, b and c:

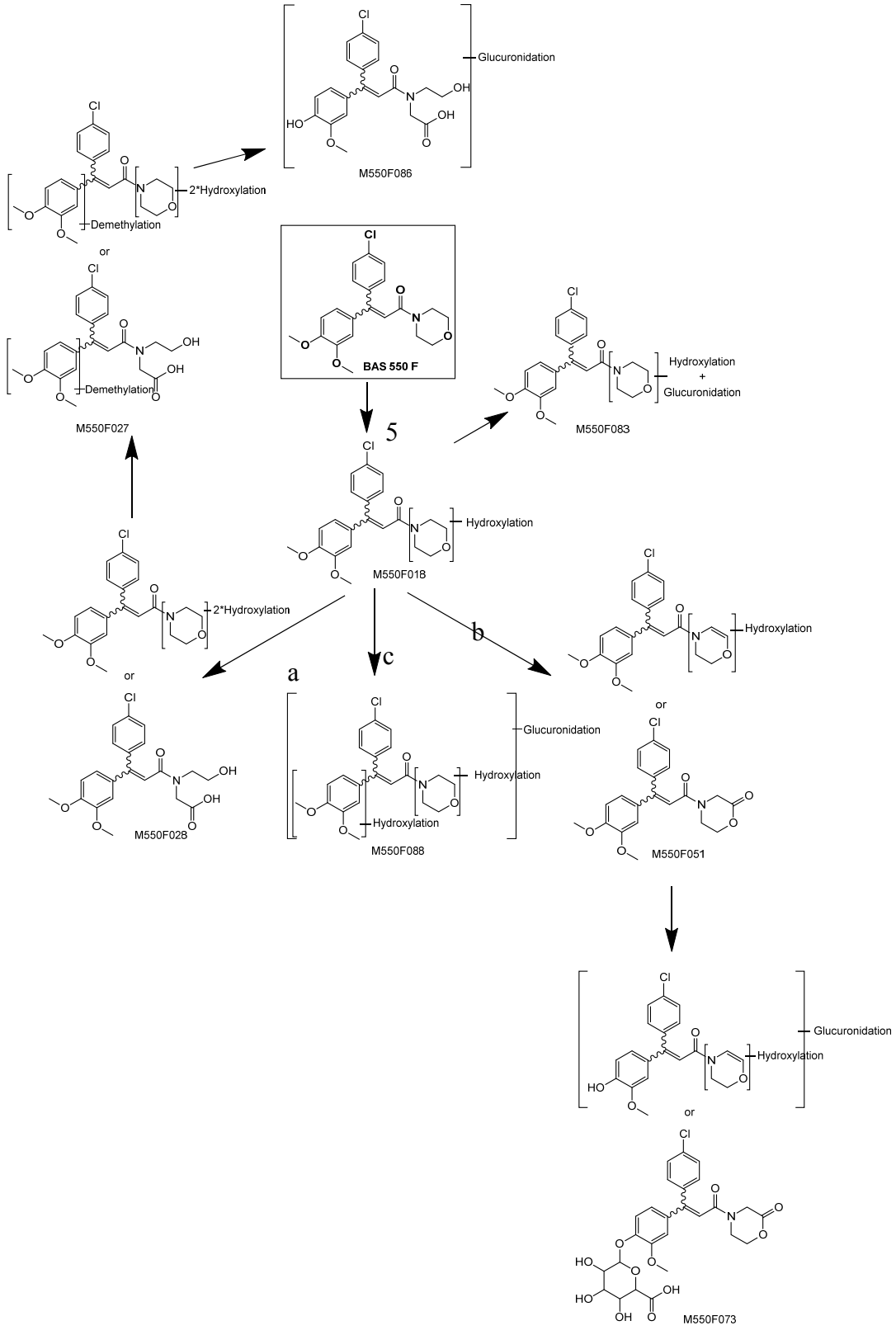
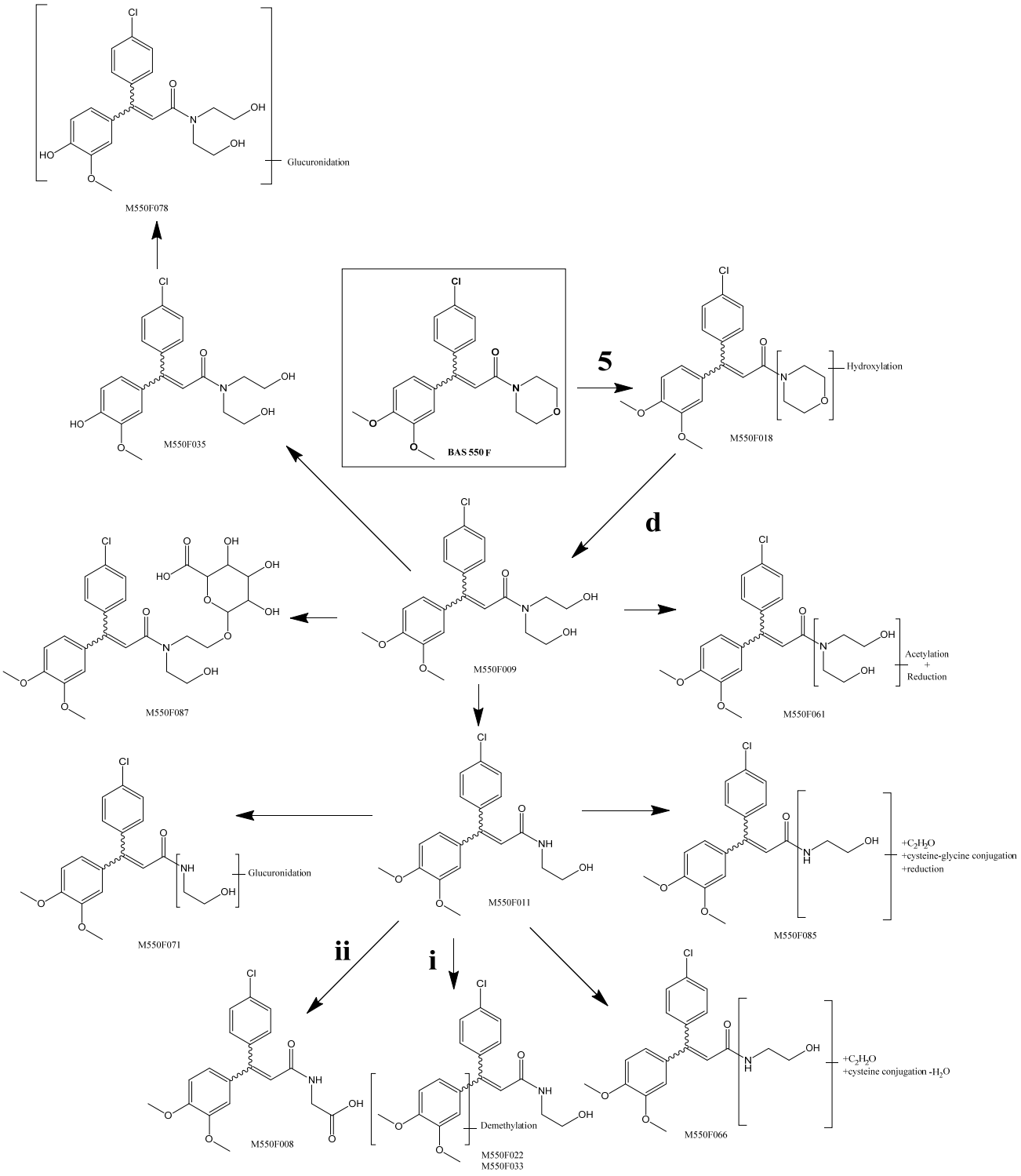


Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d



Steps 5 d i and 5 d ii have further metabolic steps, as shown in the following figures.

Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d i

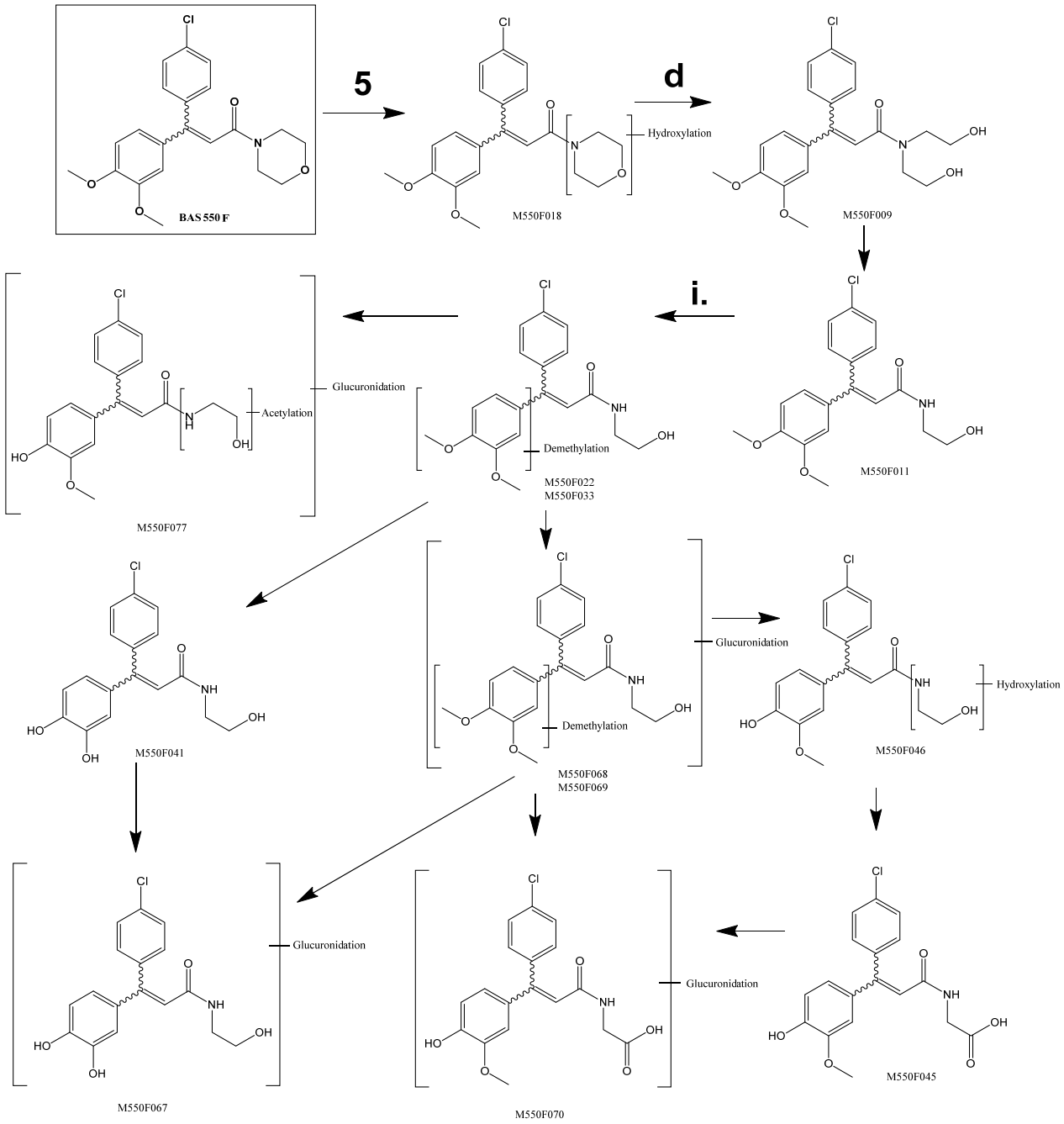
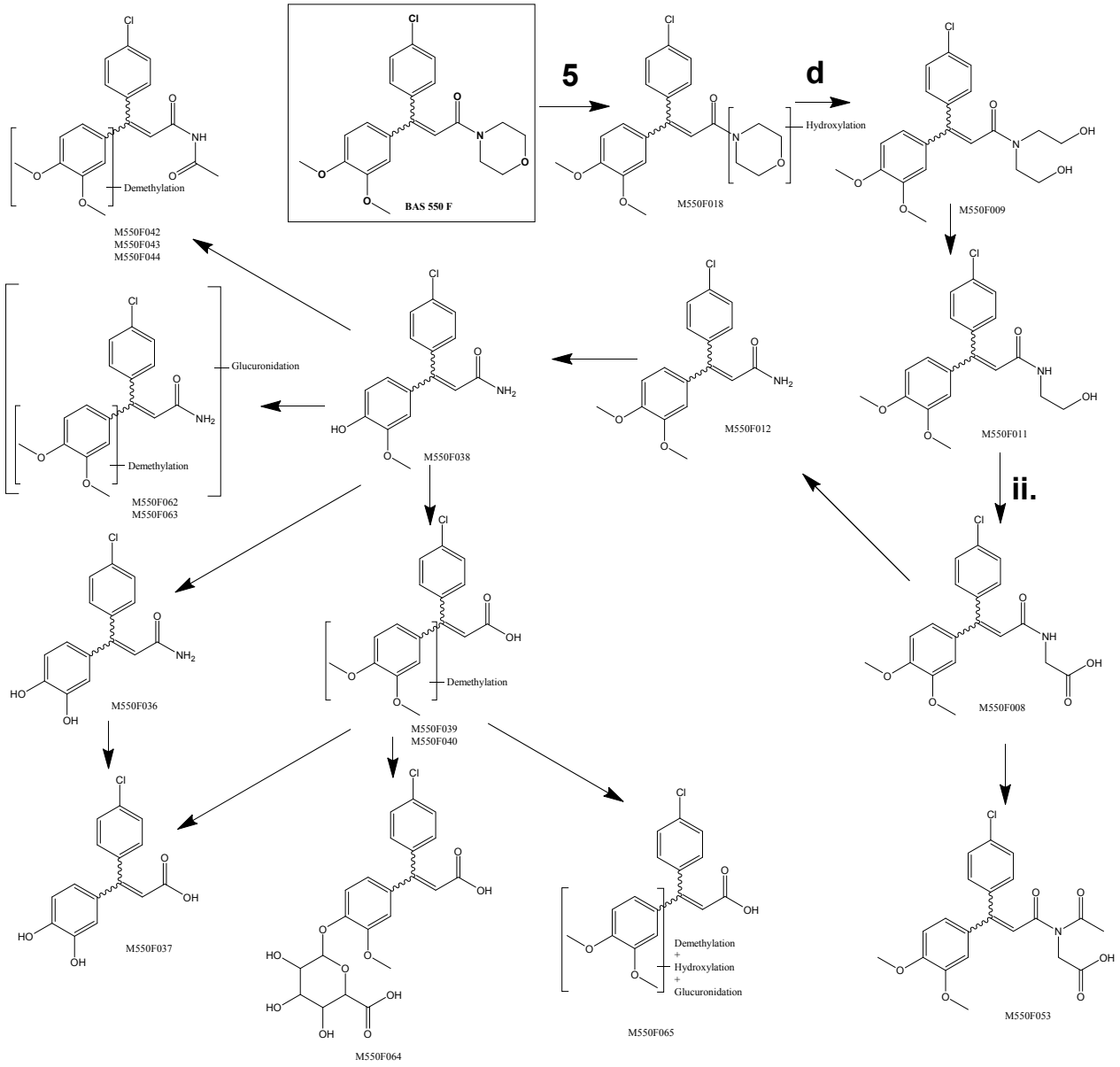


Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

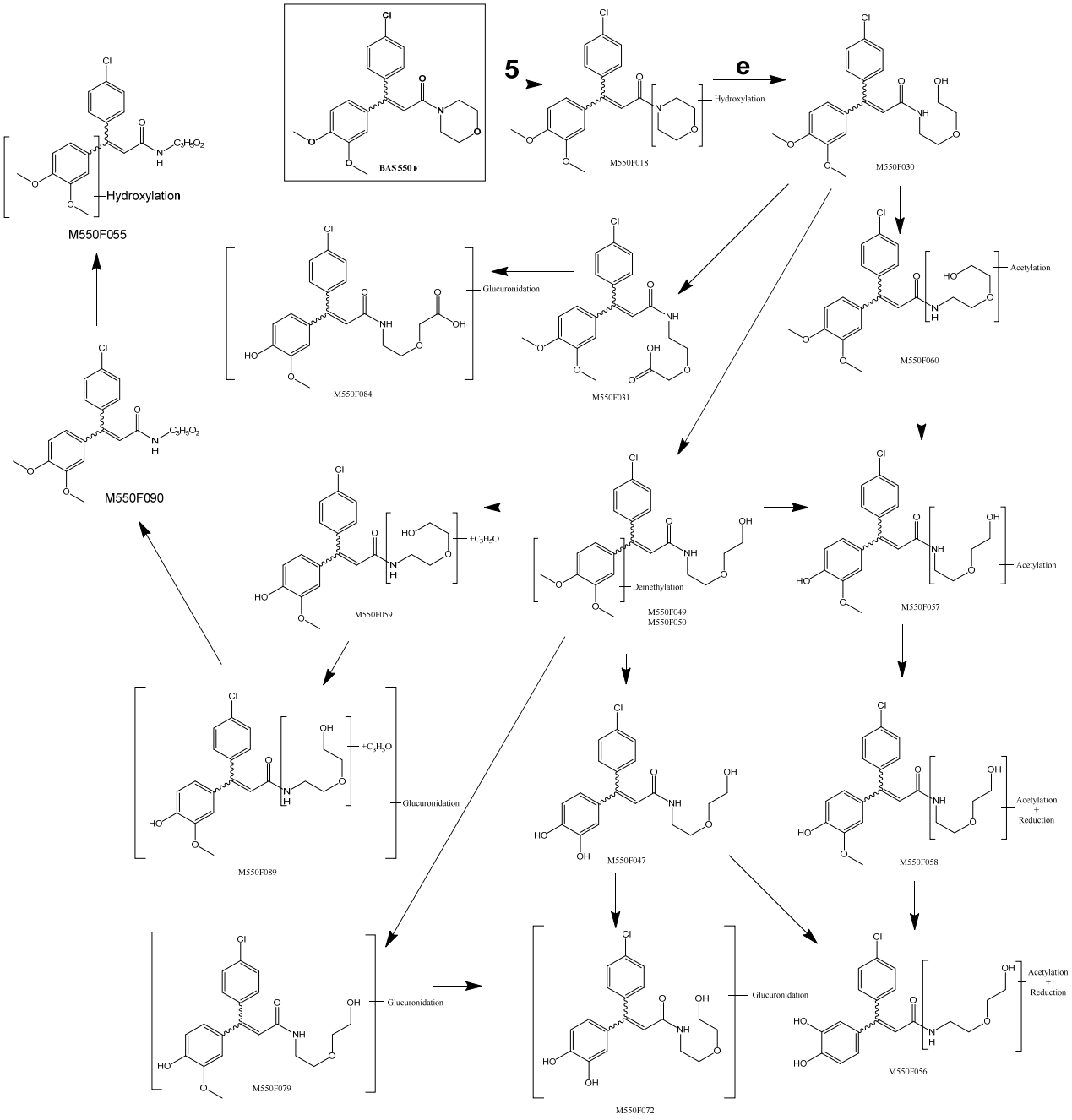
Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d ii[9]



[9] Please note the metabolites M550F039, M550F040, M550F037, M550F064 and M550F065 could also occur as products of the Cleavage pathway (Step 4)

Figure 1 Proposed Metabolic Pathway of BAS 550 F in Rats (continued)

Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step e



Group 0: morpholine moiety. This structure is unique in the rat metabolism. It is also a chemical registered under REACH with a comprehensive data package. This substance will not be grouped.

Group 1: Metabolisation of dimethomorph with no modification of the morpholine moiety. This represents a first step of metabolisation in rat. This group can be divided into 2 sub-groups:

1a: demethylation of the benzyl methoxy moiety and subsequent modifications (conjugation)

1b: hydroxylation of the morpholine moiety only

This group contains the following metabolites:
Group1a:
M550F007: Demethylation of benzyl methoxy group
M550F006: Demethylation of benzyl methoxy group
M550F013: Conjugation of M550F007
M550F002: Conjugation of M550F007
M550F016: Demethylation of both benzyl methoxy group
M550F015: Conjugation of M550F006
M550F029: Conjugation of M550F016

Group1b:
M550F018: Hydroxylation of morpholin moiety
M550F028: Hydroxylation of morpholin moiety
M550F074: Hydroxylation of morpholin moiety of M550F007 and conjugation
M550F076: Hydroxylation of morpholin moiety of M550F006 and conjugation

Group 2: Metabolisation by opening of the morpholine moiety leading to a terciary amine with or without further modifications (conjugation, oxidation). This group contains the following metabolites:

M550F009: Opening of the morpholin moiety
M550F017: Conjugation of M550F009
M550F028: Oxydation of M550F009
M550F035: Demethylation of benzyl methoxy group of M550F009
M550F053

Group 3: Metabolisation by opening of the morpholine moiety leading to a secondary amine with or without further modifications (conjugation, oxidation). This group contains the following metabolites:

M550F011
M550F008: oxidation of M550F011
M550F017: conjugation of M550F011
M550F022: equivalent of M550F011 with M550F006
M550F033: equivalent of M550F011 with M550F007
M550F030:
M550F031: oxidation of M550F030
M550F049: equivalent of M550F30with M550F007
M550F069: glucuronide of M50F033

Group 4: Metabolisation by opening of the morpholine moiety leading to an amide with or without further conjugation. This group contains the following metabolites:

M550F012
M550F062: conjugation of M550F012
M550F038: Demethylation of benzyl methoxy group of M550F012
M550F091: Demethylation of benzyl methoxy group of M550F012

Group 5: Further step of metabolisation. No carboxamide group anymore. Only one metabolite is in this group that does not appear in the rat metabolism:

M550F003.

b. Toxicological assessment of individual metabolite groups

1. Group 0: Morpholine

M550F021 (morpholin). A very small amount has been detected in the plant metabolism. Moreover, the detected peak can not only be attributed to morpholin but is instead the sum of very polar molecules derived from morpholin. The distinction between these molecules is technically not feasible. It is considered that toxicological properties of morpholin are representative for this group of molecules.

Morpholin is a chemical substance registered under the REACH regulation. A comprehensive set of toxicity studies is available for this substance. In the table below the relevant studies which can be used to assess morpholin as a plant metabolite of DMM (studies performed by oral route and genotoxicity studies) are summarized.

Table 5.8.1-4: List of toxicity studies relevant to assess morpholine

Test	Experimental conditions	Results	Reference
LD50 oral	Rat/male and female	1900 mg/kg bw	[redacted], 1967
LD50 oral	Rat/male	1680 mg/kg bw	[redacted], 1981a
LD50 oral	Rat/female	1050 mg/kg bw	[redacted] 1954
LD50 oral	Rat/unspecified	1600 mg/kg bw	[redacted] 1939
Repeated-dose toxicity, oral route, 91d	Mouse/male and female	NOAEL = 400 LOAEL = 700	[redacted], 1987a
Repeated-dose toxicity, oral route, 92w	Mouse/male and female	LOAEL = 500 (female)	[redacted] 1987b
Repeated-dose toxicity, oral route, 56d	Rat/female	LOAEL = 500	[redacted] 1969
Repeated-dose toxicity, oral route, 30d	Rat/male and female	LOAEL = 160	[redacted], 1939
Repeated-dose toxicity, oral route, 30d	Guinea pig/male and female	LOAEL = 90	[redacted] 1939
Ames	*S. typhimurium* *E. coli*	Weak positive with or without activation	Glatt, 1981
Ames	*S. typhimurium* *S. cerevisiae*	Negative with and without activation	Huntsman, 1979a
Ames	*S. typhimurium*	Negative with and without activation	Haworth, 1983
Ames	*S. typhimurium*	Negative with and without activation	Ishidate, 1984

Test	Experimental conditions	Results	Reference
Mammalian gene mutation	Mouse lymphoma L5178Y cells	Weak positive without activation; negative with activation	Huntsman, 1979
Mammalian cell transformation	BALB/3T3 mouse cells	Negative	Litton, 1982
Mammalian cell transformation	BALB/3T3 mouse cells	Positive	Huntsman, 1979c; Myhr, 1979
Sister chromatid exchange	Chinese hamster ovary cells	Negative with and without activation	Huntsman, 1980
UDS	Rat hepatocytes	Negative without activation	Huntsman, 1982; Conaway, 1984b; Naylor Dana Institute, 1982
Chromosome aberration	Chinese hamster lung cells	Negative without activation	Ishidate, 1984
Chromosome aberration and micronucleus	Hamster embryos	Negative	Inui, 1979
Developmental toxicity	Rat	Maternal NOAEL: 75 mg/kg bw/d Developmental NOAEL: 750 mg/kg bw/d.	[redacted], 2009a

Morpholin has recently been peer-review by OECD in the framework of HPV substance assessment. The conclusions of the relevant end-points are summarized below. Summaries for all studies can be found in section 5.8.2.3

In some studies in this section, a morpholine salt was tested to avoid damage to the gastrointestinal tract following oral (gavage or drinking water) administration due to the caustic mode of action.

Testing the salt also provides the ability to distinguish between symptoms caused by local effects and those due to systemic toxicity.

ADME:
Absorption of morpholine by oral route is expected be about 90%. Morpholine is well distributed mainly to the kidney, intestine, and muscle. The highest concentrations were found in the kidney. The major routes of metabolism of morpholine involve various oxidative processes, including N-oxidation and dealkylation followed by deamination and conjugation, and other enzyme-catalyzed reactions leading to detoxification and excretion. However, most of the administered dose is excreted in its non-metabolized form. The primary excretory pathway for morpholine is urinary excretion.

Acute Toxicity oral route:
In four studies, acute toxicity of morpholine was determined. Oral LD50 values were between 1050 and 1900 mg/kg bw in rats. Clinical signs reported include breathing abnormalities, oral-nasal wetness and/or staining, effects on gait, postural abnormalities, and eye closure. Site of contact effects (irritation/corrosion) in the gastrointestinal tract were the only findings noted at gross necropsy. Based on the oral toxicity studies, females may be more sensitive than males.

Table 5.8.1-5: Summary of acute oral toxicity of morpholine

Test Species/Sex	Result (LD50)	Reference
Rat/male and female	1900 mg/kg bw	[redacted] 1967
Rat/male	1680 mg/kg bw	[redacted] 1981a
Rat/female	1050 mg/kg bw	[redacted], 1954
Rat/unspecified	1600 mg/kg bw	[redacted] 1939

Repeated dose toxicity oral route:
In mice exposed to morpholine oleic acid salt in drinking water (~ 0, 140, 200, 400 and 700 mg/kg bw/day) for 91 days, cloudy swelling of the proximal tubules of the kidneys was observed at 700 mg/kg bw/day in drinking water. The NOAEL for oral systemic toxicity for morpholine in mice was 400 mg/kg bw/day.
In mice exposed to morpholine oleic acid salt in drinking water (~ 0, 0.4 and 1.5 g/kg bw/day for males; ~ 0, 0.5 and 1.5 g/kg bw/day for females) for 96 weeks, followed by 8 weeks of tap water, reduction in body weight was observed in both sexes given 1.5 g/kg bw/day and in females given 0.5 g/kg bw/day. Water consumption was also decreased in both sexes at 1.5 g/kg bw/day compared to controls consuming tap-water. Significant increases in blood-urea nitrogen concentrations were only observed in the 1.5 g/kg bw/day male group. A NOAEL was not identified based on a reduction in body weights in females at 0.5 g/kg bw/day and both sexes at 1.5 g/kg bw/day.
Moderate adiposis of the liver was observed in rats administered 500 mg/kg bw/day morpholine (only dose tested) in the diet for 56 days; this was the established LOAEL.
In rats administered morpholine by gavage (0, 160, 320 and 800 mg/kg bw/day) for 30 days, swelling, congestion, necrosis and/or desquamation of the liver, kidneys, lungs and stomach were observed; the LOAEL was 160 mg/kg bw/day.
In guinea pigs administered morpholine by gavage (0, 90, 180 or 450 mg/kg bw/day) for 30 days, clinical signs of toxicity included prostration, sneezing and couging. Effects on the kidney (cloudy swelling, congestion, necrotic tubules), liver (cloudy swelling, congestion, necrosis and fatty degeneration), spleen and stomach (necrosis) were seen at all treatment levels; the LOAEL was 90 mg/kg bw/day.

Table 5.8.1-6: Summary of repeated-dose oral toxicity studies:

Species/sex	Exposure route, doses and duration	NOAEL/LOAEL (mg/kg bw/day)	Reference
Mouse/male and female	Drinking water, 70, 140, 200, 400 and 700 mg/kg bw/day morpholine (0.15 ; 0.3 ; 0.6 ; 1.25 ; 2.5 % morpholine oleic acid salts), 91 days	NOAEL = 400 LOAEL = 700	[redacted], 1987a
Mouse/male and female	Drinking water, 400 and 1500 mg/kg bw/day for males and 500 and 1500 mg/kg bw/day for females (0.25 ; 1 % morpholine oleic acid salts), 96 weeks	LOAEL = 500 (female)	[redacted] 1987b
Rat/female	Diet, 500 mg/kg bw/day, 56 days	LOAEL = 500	[redacted] 1969
Rat/male and female	Gavage, 160, 320 and 800 mg/kg bw/day, 30 days	LOAEL = 160	[redacted] 1939
Guinea pig/male and female	Gavage, 90, 180 and 450 mg/kg bw/day	LOAEL = 90	[redacted] 1939

Mutagenicity

Morpholine did not increase reverse mutations in *E. coli* or *S. cerevisiae* or in three studies with *S. typhimurium* (including one study conducted with morpholine fatty acid salt) *in vitro* (all similar to OECD TG 471). However, weak positive results for gene mutations were noted in another study in *S. typhimurium* (Ames test) and in an *in vitro* study of mammalian (mouse lymphoma) cells (similar to OECD TG 476) at high and/or cytotoxic doses. One negative and one positive result were observed in two *in vitro* mammalian (BALB/3T3 mouse) cell transformation assays (EU Method B.21). No increases in the frequency of sister chromatid exchanges (CHO cells; similar to OECD TG 479) or unscheduled DNA synthesis (rat hepatocytes; similar to OECD TG 482) were observed in *in vitro* studies of mammalian cells. Morpholine fatty acid salt did not induce chromosome aberrations in mammalian (CHL cells; no guideline specified) cells *in vitro*.

In an *in vivo* study, morpholine did not induce chromosomal aberrations or micronuclei in hamster embryos (no guideline specified).

Based on the weight of evidence, with special regard to the equivocal findings of the gene mutation studies conducted at high doses *in vitro*, and the negative results for clastogenicity *in vitro* and *in vivo*, morpholine is not considered to be genotoxic.

In two publications, the formation of nitrosomorpholine (NMOR) was observed *in vitro* and *in vivo* in particular conditions: with presence of high quantities of nitrates or with high fat diet. N-Nitroso compounds (including nitrosomorpholine) are commonly formed from food constituents (amines that are part of the protein) with presence of nitrates. No nitrosomorpholine has been found in plants or livestock. According to the negative results seen in the mutagenicity studies and the carcinogenicity studies performed on morpholine, it can be assumed that if the level of morpholine in residues is below the proposed ADI, the level of NMOR formed is not an issue.

Table 5.8.1-7: Results of *in vitro* and *in vivo* mutagenicity studies.

Test	Test system	Result	Reference
In vitro studies			
Ames	*S. typhimurium* *E. coli*	Weak positive with or without activation	Glatt, 1981
Ames	*S. typhimurium* *S. cerevisiae*	Negative with and without activation	Huntsman, 1979a
Ames	*S. typhimurium*	Negative with and without activation	Haworth, 1983
Ames	*S. typhimurium*	Negative with and without activation	Ishidate, 1984
Mammalian gene mutation	Mouse lymphoma L5178Y cells	Weak positive without activation; negative with activation	Huntsman, 1979
Mammalian cell transformation	BALB/3T3 mouse cells	Negative	Litton, 1982
Mammalian cell transformation	BALB/3T3 mouse cells	Positive	Huntsman, 1979c; Myhr, 1979
Sister chromatid exchange	Chinese hamster ovary cells	Negative with and without activation	Huntsman, 1980
UDS	Rat hepatocytes	Negative without activation	Huntsman, 1982; Conaway, 1984b; Naylor Dana Institute, 1982
Chromosome aberration	Chinese hamster lung cells	Negative without activation	Ishidate, 1984
In vivo studies			
Chromosome aberration and micronucleus	Hamster embryos	Negative	[redacted] 1979

Carcinogenicity oral route
Carcinogenicity studies conducted via oral route (no guideline specified) indicate morpholine is not carcinogenic.

Toxicity for reproduction
In a prenatal developmental toxicity study (OECD TG 414), pregnant rats were administered morpholine HCl by oral route (gavage) at doses of 0, 75, 250 and 750 mg/kg bw/day for GD6-19. The maternal NOAEL was 75 mg/kg bw/day based on hematological changes. There were no effects on gestational parameters and fetal examinations revealed no effects on sex distribution of the fetuses, fetal body weights or placenta weights. Fetal findings in this study were primarily limited to skeletal variations (slight increase in the delayed ossification) in the mid- and high-dose groups, and are considered to be transient in nature, and secondary to maternal toxicity. These findings were regarded to be of no toxicological relevance and are not considered adverse. NOAEL for prenatal developmental toxicity was 750 mg/kg bw/day (highest dose tested).

Derivation of reference values

ADI:
Most of the repeated dose toxicity studies available did not identify a clear NOAEL (see Table 5.8.1-6). Based on these results it seems that the guinea-pig is the most sensitive species. In the 30-day study in guinea-pig a LOAEL of 90 mg/kg bw/d was determined. To derive the ADI, a standard safety factor of 100 is applied to take into account intra- and inter-species variability. As this study is a short term study an additional safety factor of 6 is also applied to extrapolate to a long term study. As no NOAEL was identified, another additional safety factor of 3 was applied. Overall a safety factor of 1800 (100 x 6 x 3) is applied on the LOAEL of 90 mg/kg bw/day leading to an **ADI of 0.05 mg/kg/day**.
This value is considered very conservative due to the additional safety factors applied and the nature of the effects seen in all short-, medium-, long term studies. Moreover a margin of safety of at least 3200 is observed compared to other studies.

ARfD:
The most appropriate study to derive the Acute Reference Dose is the developmental toxicity study where effects on dams are observed within one week after the first administration. In this study a clear NOAEL of 75 mg/kg bw/day is observed for maternal toxicity. A standard safety factor of 100 is deemed sufficient to take into account intra- and inter-species extrapolation. This leads to an **ARfD of 0.75 mg/kg.**

2. Group 1: metabolites derived from dimethomorph with morpholine moiety intact

Group1a:
M550F007: Demethylation of benzyl methoxy group
M550F006: Demethylation of benzyl methoxy group
M550F013: Conjugation of M550F007
M550F002: Conjugation of M550F007
M550F016: Demethylation of both benzyl methoxy group
M550F015: Conjugation of M550F006
M550F029: Conjugation of M550F016

Group1b:
M550F018: Hydroxylation of morpholin moiety
M550F028: Hydroxylation of morpholin moiety
M550F074: Hydroxylation of morpholin moiety of M550F007 and conjugation
M550F076: Hydroxylation of morpholin moiety of M550F006 and conjugation

For group 1 metabolites, the structure is close to the structure of dimethomorph, only demethylation, hydroxylation or conjugation occur. Group 1 metabolites are present in the rat metabolism as intermediate metabolites and their amount is considered high enough to be covered by the toxicity studies performed on dimethomorph. Therefore, for consumer risk assessment it can be assumed that their toxicity will not be higher than the toxicity of dimethomorph and that the reference values of parent can be applied:

ADI: 0.05 mg/kg/d
ARfD: 0.6 mg/kg

3. Group 2: Opening of the morpholine moiety (leading to terciary amines)

M550F009: Opening of the morpholin moiety
M550F017: Conjugation of M550F009
M550F028: Oxydation of M550F009
M550F035: Demethylation of benzyl methoxy group of M550F009
M550F053

For group 2 metabolites, the only change compared to dimethomorph is the opening of the morpholine moiety and conjugation of the alcohol moiety formed at the morpholin moiety. M550F009 is found at considereable amounts in the rat metabolism study the other four metabolites are structurally closely related. All metabolites of group 2 are intermediate metabolites which will be further metabolized. Finally, it can be considered that toxicity of group 2 metabolites is covered by dimethomorph toxicity studies and therefore reference values of dimethomorph can be used:

ADI: 0.05 mg/kg/d
ARfD: 0.6 mg/kg

4. Group 3: Opening of the morpholine moiety (leading to secondary amine), oxidation and conjugation

M550F011
M550F008: oxidation of M550F011
M550F017: conjugation of M550F011
M550F022: equivalent of M550F011 with M550F006
M550F033: equivalent of M550F011 with M550F007
M550F030:
M550F031: oxidation of M550F030
M550F049: equivalent of M550F30with M550F007
M550F069: glucuronide of M50F033

M550F011 is a major rat metabolite, the other metabolites of group 3 are derivates of this major metabolite formed by common detoxification reactions and thus their toxicity is not expected to be higher than for M550F011. Metabolites of group 3 can therefore be considered to be covered by toxicity studies performed with dimethomorph and reference values can be considered to be equivalent:

ADI: 0.05 mg/kg/d
ARfD: 0.6 mg/kg

5. Group 4:

M550F012
M550F062: conjugation of M550F012
M550F038: Demethylation of benzyl methoxy group of M550F012
M550F091: Demethylation of benzyl methoxy group of M550F012

M550F062 is a major rat metabolite found in bile, M550F012 is equivalent to M550F062 without conjugation. M550F038 and M550F091 are formed by simple demethylation of M550F012 which is not considered to result in higher toxicity compared to M550F012 or M550F062. Thus, all metabolites of group 4 are considered to be covered by the toxicity studies performed with dimethomorph and reference values can be considered to be equivalent:

ADI: 0.05 mg/kg/d
ARfD: 0.6 mg/kg

6. Group 5: M550F003

Toxicological tests have been performed with M550F003.

Table 5.8.1-8: Summary of study performed with M550F003

Test	Experimental conditions	Results	Reference
LD50 oral	Rat/male and female	> 5000 mg/kg	DK-470-009
LD50 oral	Rat/male and female	> 5000 mg/kg	DK-470-011
LD50 dermal	Rat/male and female	> 2000 mg/kg	DK-470-011
Dermal Irritation	Rabbit/male and female	No irritation	DK-470-011
Ocular Irritation	Rabbit/male and female	No irritation	DK-470-011
Skin Sensitization (M&K)	Guinea Pig/male and female	No sensitization	DK-470-011
Repeated Dose Toxicity – 28 days	Rat/male and female 0 – 15 – 60 – 250 – 1000 mg/kg bw/day	NOAEL: 60 mg/kg bw/day LOAEL: 250 mg/kg bw/day Target organs: liver, kidney, caecum	DK-470-014
AMES	S.Typhimurium TA98, TA100, TA1535, TA1537 – E.Coli 1 – 5000 µg/plate +/- S9	Negative	DK-470-010 DK-470-026
AMES	S.Typhimurium TA98, TA100, TA1535, TA1537, TA 1538 - E.Coli 15 – 2000 µg/plate +/- S9	Negative	DK-470-012
Chromosome Aberration	CHO 45 – 225 – 450 µg/mL without metabolic activation 50 – 250 – 500 µg/mL with metabolic activation	Negative	DK-470-013
Developmental study (preliminary)	Rat 0 – 60 – 250 – 1000 mg/kg bw/day	NOAEL maternal: 250 mg/kg bw/day NOAEL development: 1000 mg/kg bw/day Effects in dams: slight decrease of bw gain at 1000 mg/kg bw/day.	DK-470-025

M500F003 is of low acute toxicity via oral or dermal route. No dermal or ocular irritation was observed in standard tests. M550F003 is not a skin sensitizer. In a 28-day study performed in Fischer 344 rats, treatment-related findings were noted for the liver, kidneys, and caecum including amongst others enlargement of the organs. Only in the kidney histopathological correlates were found including moderate proximal tubular degenerative and regenerative lesions in the renal cortex of rats of both sexes at the high dose level and proximal tubular regenerative foci at 250 (only females) and 1000 mg/kg bw/day. Based on the changes of the liver, kidneys and caecum and the histopathological findings in the kidney that were observed at 250 and 1000 mg/kg bw/day a no observed adverse effect level (NOAEL) was set at 60 mg/kg bw/day for both sexes.
M550F003 is negative in two Ames tests and one chromosomal aberration test *in vitro*.
In a preliminary oral embryotoxicity in the rat, there were no treatment-related mortalities, clinical observation or necropsy findings detected in any dose group. In the 1000 mg/kg bw/d group, a slight decrease in body weight gain was observed from PMD6 to PMD9, this finding was considered treatment-related. There were no effects on implantation and post-implantation loss in any dose group. Number, sex and weight of the fetuses were not affected by the treatment. No fetal defects were observed at external examination.
The NOAEL for maternal effects can be set at 250 mg/kg bw/d due to slight decrease body weight gain at 1000 mg/kg bw/d.
The NOAEL for development is considered to be 1000 mg/kg bw/d, the highest tested dose, because no effects were observed in the fetuses.

According to these results, M550F003 has a different toxicological profile than dimethomorph in the repeated dose toxicity studies. Target organs are different in the 28-day study performed in rats. In the preliminary developmental study in rats, M550F003 is of low toxicity both in parents and in fetuses compared to dimethomorph.

Derivation of reference values:

As no acute toxicity is observed in these studies, especially a slight maternal toxicity and no developmental toxicity in the developmental study, the derivation of an ARfD is not deemed necessary.

For the Acceptable Daily Intake, a NOAEL of 60 mg/kg bw/day is observed in a 28-day study in rats. A standard safety factor (SF) of 100 is applied to take into account intra- and inter-species variability. An extra safety factor of 6 is also considered to extrapolate from a sub-acute to a chronic study.
Overall, a SF of 600 is applied to the NOAEL of 60 mg/kg bw/day leading to an **ADI of 0.1 mg/kg bw/day** for M550F003.

ADI: 0.1 mg/kg bw/day
ARfD: not necessary

3- Studies performed with metabolites
   a. Morpholine

Toxicokinetics, metabolism and distribution

Report: CA 5.8.1/1
[redacted] 1978 a
Excretion and distribution of Morpholine salts in rats
1978/1001644
Guidelines: none
GLP: no

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine (purity >99%), morpholine HCl, morpholine palmitate, ^{14}C-morpholine HCl; Specific activity of test substance (morpholine + ^{14}C-morpholine) was 1.02 x 10^4 dpm/mg.
Test animals: male Wistar rats
Male Wistar rats (3/group) were dosed with ^{14}C-morpholine following oral (200 mg/kg bw) or i.v. administration (150 mg/kg bw), ^{14}C-morpholine HCl following oral (500 mg/kg bw) or i.v. administration (250 mg/kg bw) or ^{14}C-morpholine palmitate following oral administration (400 mg/kg bw). For determination of excretion the rats were held in metabolism cages and urine and faeces were collected every 24 h. In the distribution study the animals were killed at 2, 6 and 12 h intervals after treatment with ^{14}C-morpholine. Organs and tissues were removed and radioactivity was determined by LSC.

Findings:
Following both oral and i.v. administration of morpholine or morpholine salts, approximately 90% of the administered dose was absorbed. The highest percentages of ^{14}C-morpholine were found in muscle and intestine and the lowest percentages were found in the adipose tissue, regardless of route of administration. Two hours after oral administration of morpholine HCl, 29% of the radioactivity was found in the intestine and 26% in muscle tissue, and 2 hours after i.v. injections, 19% and 27% of the dose was found in the intestine and muscle tissue, respectively. Morpholine was rapidly excreted after oral or intravenous administration. In all cases, over 85% of the total dose was excreted in urine within 24 hours. A further portion, up to 5%, was excreted during the next three days. The elimination pattern for ^{14}C-Morpholine palmitate was similar, but slightly more slowly. For all groups, 0.08 - 0.14% of the total dose was excreted in the faeces.

Conclusion:
Morpholine was largely excreted unchanged in the urine and a rapid elimination of organs, tissues and blood was observed. Based on the study results, the bioaccumulation potential of morpholine is assessed to be low.

Report: CA 5.8.1/2
[redacted] 1981 a
Distribution and disposition of Morpholine in the rabbit
1981/1001601
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine (purity 100%), ^{14}C-morpholine (radiochem. purity > 98%)
Test animals: male New Zealand White rabbit
Seven male NZW rabbits received an intravenous injection of ^{14}C-morpholine at a dose level of 435 mg/kg bw. After 30 minutes, the distribution of radioactivity in the blood and soft tissue of these animals was determined. Other rabbits received similar injections and blood was collected at 1, 2, 3 or 4 hours after administration. Urine was collected from the bladder of these animals at 4 hours. Animals used for the assessment of the renal clearance received a continuous intravenous infusion. The glomerular filtration rate was measured by the clearance of labelled inulin.

Findings:
The radioactivity was preferentially distributed to the kidney. The distribution of radioactivity after 30 minutes showed the highest concentrations in the renal medulla (3136 mg/kg bw) and cortex (252 mg/kg bw), followed by the lung (444 mg/kg bw), liver (410 mg/kg bw) and blood (200 mg/L). 63% of the dose was excreted in the bile and 43% in the urine during the first 3 hours. Most of the radioactivity excreted was in the form of unchanged morpholine, indicating the stability of morpholine *in vivo*. Morpholine was considered as neither significantly metabolized nor being bound to plasma proteins. The morpholine clearance exceeded the inulin clearance by a factor of 2, pointing towards the involvement of active tubular transport in renal elimination of morpholine. Only 0.0008% of the total dose was eliminated as carbon dioxide by exhalation

Conclusion:
Morpholine is distributed preferentially to the kidney, indicating the kidney be a significant organ for its elimination. The excretion was mainly complete within 3 hours after application via bile and urine, with morpholine being mostly unchanged and not bound to plasma proteins.

Report: Naylor Dana Institute, 1983
Metabolism and Disposition of Morpholine.
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

(Study report not available)

Material and Methods:
Test material: Morpholine (purity not specified), N-methylmorpholine (purity not specified)
Test systems: subcellular liver fractions of rats, guinea pigs and humans
To characterize the enzyme(s) catalysing the N-oxidation of N-methylmorpholine, an in vitro incubation in the presence of methimazole, SKF 525 -A (a specific inhibitor of cytochrom P450) as well as using liver microsomes obtained from animals pre-treated with cytrochrome P450 and P448 inducers phenobarbital and 3-methylcholanthrene was performed. Liver microsomes were obtained from untreated, phenobarbital treated (80 mg/kg bw/d, 3 days) or 3-methylcholanthrene (20 mg/kg bw, once, 24 h prior to sacrifice) treated guinea pigs. In vitro assays of N-methylmorpholine N-oxidation and N-hydroxylation were carried out with microsomal protein and substrate of 3 mg and 1 µmol per incubation mixture, respectively.

Findings:
In vitro assays in rat cells indicate that Flavin-dependent monooxygenase (FMO) was exclusively involved in the N-oxidation of N-methylmorpholine and at least partially so in the N-hydroxylation of morpholine and was evidence against the involvement of cytochrome P450 (CYP) in these reactions. No detectable morpholine N-methylase activity was found in the cytosol of human liver cells. When the formation of N-methylmorpholine-N-oxide from N-methylmorpholine was monitored, microsomes from human liver were found to be active in carrying out this reaction, though the activity was lower than that observed with microsomes from rat liver. In addition, studies using enzyme modifiers showed that the reaction was inhibited 70% by methimazole, indicating that the FMO was also involved in the human liver in carrying out this reaction. Determination of morpholine hydroxylase indicated that the human liver microsomes were able to N-hydroxylate morpholine, with an activity of ~ 0.26 nmol/min/mg protein.

Conclusion:
Guinea pig liver microsomes showed the most metabolic activity in vitro. FMO was exclusively involved in the N-oxidation of N-methylmorpholine and at least partially so in the N-hydroxylation of morpholine and there was an evidence against the involvement of CYP in these reactions. Of the species examined, human liver most resembled that of the rat in its ability to metabolize morpholine.

Report: CA 5.8.1/3
[redacted], 1982 a
Metabolism and disposition of Morpholine in the rat, hamster and guinea pig
1982/1001911
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine (purity 99.2%), ^{14}C-morpholine (radiochem. purity > 98%, spec. activity 20 mCi/mmol)
Test animals: male Sprague Dawley rats, male Syrian golden hamsters, male strain II guinea pigs
Urinary and plasma clearance, and urinary metabolites were determined in 3 males of each of three rodent species following a single intraperitoneal injection of ^{14}C-morpholine at 125 mg/kg bw (50 µCi/animal) in 0.9% NaCl with an application volume of 1 mL. Sample analyses were performed by means of chromatography and mass spectrometry.

Findings:
After i.p. administration of morpholine, the blood plasma half-lives in the rat, hamster and guinea pig were 115, 120 and 300 min, respectively. In all three species, approximately 80% of the radioactivity was excreted in the urine within 24 hours. However, while non-metabolized morpholine constituted up to 99% of the urinary radioactivity in the rat and hamster, a significant portion of the dose (approximately 20%) appeared in guinea pig urine as N-methylmorpholine-N-oxide.

Conclusion:
Marked differences were found between the guinea pig and other two rodent species rat and hamster regarding metabolism of morpholine. However, the excretion rate was comparable in all three species examined.

Report: CA 5.8.1/4
[redacted] 1997 a
Carcinogenicity of Methylurea or Morpholine in combination with sodium nitrite in a rat multi-organ carcinogenesis bioassay
1997/1008521
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material:
Test material: Morpholine (purity > 97%), $NaNO_2$ (purity > 97%)
Test animals: male Fischer 344 rats ([redacted])

Summary of a metabolite study
Fourteen male F344 rats received 2% morpholine (38.3 µmol) in the diet and 50 mg/mL/kg bw $NaNO_2$ in distilled water (191 µmol) intra-gastrically (i.g.) 1 hour after dietary application. Five animals received 2% morpholine (38.3 µmol) in the diet and distilled water 1 hour thereafter. A control group consisted of 14 males received ground diet and distilled water only. To demonstrated formation of N-nitroso morpholine (NMOR) in the stomach, the rats were sacrificed under ether anaesthesia at 1 and 2 hours after i.g. administration of $NaNO_2$ or distilled water.
NMOR was found in the stomach of rodents exposed to morpholine and nitrite in feed or water, respectively. Animals administered morpholine and $NaNO_2$ had a 600-fold increase in the urinary excretion of the metabolite of NMOR (N-nitroso(2-hydroxyethyl)glycine) compared with rats treated with morpholine alone ([redacted], 1984).
In the presence of nitrite, morpholine can be nitrosated to the carcinogenic N-nitrosomorpholine. According to the estimation of [redacted], 1984, 12% of the administered morpholine was nitrosated in rats.

Summary of the carcinogenicity study

Eight groups of ten or 20 male F344 rats were administered carcinogens targeting different organs. To achieve wide-spectrum initiation, animals in groups 1-6 underwent the following treatments: A) single intraperitoneal administration of 100 mg/kg bw diethylnitrosamine (DEN) at the commencement of the experiment, B) single gavage administration of 100 mg/kg bw N-methyl-N-nitro-N-nitrosoguanidine on day 14; C) four subcutaneous injections of 40 mg/kg bw 1,2-dimethylhydrazine on days 19, 22, 25 and 28, D) 0.01 % ethylnitrosourethane plus 0.05 % N-butyl-N-(4-hydroxybutyl)nitrosamine in drinking water for the first two weeks, E) 0.1% 2,2-dihydroxy-di-n-propylnitrosamine in drinking water for the subsequent two weeks. Then after a one-week interval without any treatment, animals were given 0.1 % methylurea or 0.5 % morpholine (>97% purity; ca. 250 mg/kg bw/day, see dossier for estimation detail) in diet and/or 0.15% sodium nitrite in drinking water for 23 weeks. Groups 1-6 were administered methylurea plus sodium nitrite (group 1), morpholine plus sodium nitrite (group 2), methylurea (group 3), morpholine (group 4), sodium nitrite (group 5) or basal diet alone (group 6). Rats in groups 7 and 8 were given methylurea plus sodium nitrite and morpholine plus sodium nitrite without the initiation regimen. The total observation period of the experiment was 28 weeks. The combination of initiation followed by sodium nitrite and morpholine caused an increase in the number and area of GST-P-positive (glutathione S-transferase, placental form) liver foci, as compared to the group that was only initiated. This indicates that following initiation, morpholine plus sodium nitrite, but not morpholine alone, has a tumour promoting effect. However, treatment with morpholine or morpholine plus sodium nitrite following tumor initiation did not lead to an increased tumour incidence. No tumours were induced by morpholine plus sodium nitrite in the absence of initiation.

Report: CA 5.8.1/5
Ziebarth D. et al., 1997 a
N-nitrosation of medicinal drugs catalysed by bacteria from human saliva and gastro-intestinal tract, including Helicobacter pylori
1997/1008541
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine (purity not specified)
Test organism: Microorganisms commonly present in human saliva
Following incubation of 1 mmol morpholine with salivary bacteria at pH 7.2 in the presence of nitrate for up to 24 hours with the molar ratio of morpholine/nitrate being 1:4, the yield of N-nitroso compounds was quantified by HPLC with post-column derivatisation.

Findings:
Morpholine (1 mmol) underwent bacteria-catalysed nitrosation yielding 0.2 µmol N-nitroso compound.

Conclusion:
This *in vitro* study have demonstrated that nitrosation of morpholine is possible in human saliva.

Report: CA 5.8.1/6
[redacted] 1968 a
The metabolism of N-triphenylmethylmorpholine in the dog and rat
1968/1000221
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: [G-^{3}H]morpholine hydrochloride (purity not specified, specific radioactivity 1 µCi/mg)
Test animals: rats (C.F.E. strain maintained as a specific pathogen free colony of the performing laboratory)
Three male and female rats received 50 mg [G-^{3}H]morpholine in 1 mL water (~290 mg/kg bw) as a single oral dose by gavage. Urine, faeces and expired gases were collected every 24 hours. After 4 days rats were sacrificed and the skin (plus muzzle, feet and tail), alimentary canal, carcass and remaining viscera were examined for radioactivity to determine the routes of excretion and metabolite distribution.

Findings:
The elimination of ^{3}H-morpholine in rats occurred mainly through the urine (72 - 83.2%), followed by 2.4 - 5.9% in expired air, and 1.2 - 1.9% in the faeces. The majority of the total dose was eliminated through the urine within 24 hours (57.7 - 73.5%).

Conclusion:
Morpholine is rapidly absorbed and excreted in a largely unchanged form in the urine, up to 90% being excreted within 24 hours.

Acute Toxicity

Report:	CA 5.8.1/7 Anonymous, 1967 a Morpholine - Industrial hygiene orientating investigation - BASF 1967 1967/1000301
Guidelines:	none
GLP:	no
Deviations:	Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine (purity 99.2%)
Test animals: male and female Sprague-Dawley rats, (male weight: 108-288 g; female weight: 156-226 g)
Groups of male and female Sprague-Dawley rats were administered morpholine (99.2% purity) by gavage. There were five animals/sex at 1600, 2000 and 3200 mg/kg bw and ten animals/sex at 2500 mg/kg bw. Animals were observed for mortality and clinical signs of toxicity for 14 days following dosing.

Findings:
All ten animals died at 3200 mg/kg bw; 4/10 males and all ten females died at 2500 mg/kg bw; 1/5 males and all females died at 1600 mg/kg bw; and 1/5 males and 1/5 females died at 2000 mg/kg bw, respectively. The results indicate that females may be more sensitive than males to morpholine administered orally. At 3200 and 2500 mg/kg bw, clinical signs included squatting posture, ruffled fur, abdominal position, shallow and irregular respiration, and closed eyes. After 24 hours, the surviving animals showed red crusted eyes and noses, and trembling gait with delayed motion of the hind limbs. At 1600 and 2000 mg/kg bw, clinical signs included fast respiration, squatting posture, red crusted noses, and exaggerated gait. There were no findings at gross necropsy for surviving animals. The oral LD_{50} for the combined sexes was 1900 mg/kg bw; the oral LD_{50} for females would be lower than this.

Conclusion:
Morpholine was moderately toxic after oral administration to Sprague-Dawley rats (LD_{50} approximately 1900 mg/kg bw), with females being more sensitive.

Report: [redacted] 1981
Metabolism and Disposition of Morpholine - Final Progress Report
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

(Study report not available)

Test material: Morpholine (purity 99.2%)
Test animals: male Sprague Dawley rats
In this acute oral toxicity study similar to OECD TG 401, groups of 4 male Sprague-Dawley rats were administered morpholine by gavage at doses of 250, 500, 1000, 2000, and 4000 mg/kg bw. Animals were observed for 3 days following dosing.

Findings:
At 4000 mg/kg bw all animals died within 3 days. At the end of the observation period 3/4 males received 2000 mg/kg bw were dead. No mortalities occurred in animals administered 250, 500 and 1000 mg/kg bw morpholine. Based on occurred mortalities, LD_{50} was calculated according to the method described by Weil (1952) and was 1680 mg/kg bw.

Conclusion:
The oral LD_{50} for male rats was estimated to be 1680 mg/kg bw.

Report: CA 5.8.1/8
[redacted], 1954 a
Range-finding toxicity data
1954/1000041
Guidelines: none
GLP: no

Summary
In this study similar to OECD TG 401, female Carworth-Wistar rats (5/dose) were administered morpholine (purity not specified) by gavage (in water, corn oil or 1% Tergitol Penetrant 7). A logarithmic series of dose levels were administered at a dose volume of 1 to 10 mL/rat, and animals were observed for 14 days following dosing. There were no details provided regarding mortalities, clinical signs, body weight or findings at gross necropsy. The oral LD_{50} in female rats was estimated to be 1050 mg/kg bw.

Report: CA 5.8.1/9
[redacted] 1939 a
The acute and sub-acute toxicity of Morpholine
1946/1001601
Guidelines: none
GLP: no

Summary of acute toxicity

In this acute oral toxicity study, **albino rats and guinea pigs** were administered morpholine (≥ 98% purity) undiluted or in water by gavage at doses ranging from 100 to 10000 mg/kg bw. The number of animals/group and sex was not specified. Animals were observed for mortality and clinical signs for 7 days following dosing. Mortality incidence was not reported; all deaths in rats occurred within 2-3 days of dosing. In both species, gastrointestinal bleeding and haemorrhaging of the stomach was observed. Clinical signs in guinea pigs included collapse, prostration, and diarrhea; details regarding clinical signs in rats or body weight in either species were not provided. **The oral LD_{50} in rat was 1600 mg/kg bw; the minimum lethal dose in guinea pigs was 900 mg/kg bw.**

Repeated dose toxicity

Report: CA 5.8.1/10
[redacted] 1986 a
13-week subchronic toxicity study with morpholine oleic acid salt administered to B6C3F1 mice
1987/1002774
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine oleic acid salt (MOAS; purity 92%), pale yellow, viscous liquid
Test animals: Male and female B6C3F1 mice (6 weeks old) from [redacted]
The animals were housed in groups (5 per cage) in polycarbonate cages on hardwood chip bedding in an environment-controlled room maintained at 20-24°C and artificially illuminated for 12 h each day. Male and female B6C3F1 mice (10/sex/dose) were exposed to morpholine oleic acid salt at doses of 0%, 0.15%, 0.3%, 0.6%, 1.25%, and 2.5% in drinking water (~ 0, 70, 140, 200, 400, and 700 mg morpholine/kg bw/day) during 91 days.

Findings:
Reduced weight gains were noted in both sexes in the 700 mg/kg bw/day group as compared to controls, but reductions were not significant (the magnitude of the decreased weight gain was not reported). Water consumption was lower and showed a dose-related tendency in both females and males (no additional information provided). Urine analysis showed significant elevation of specific gravity in the males in the 200, 400 and 700 mg/kg bw/day groups and the females in the 400 and 700 mg/kg bw/day groups. Significant elevation of plasma urea nitrogen was observed in males of the 400 and 700 mg/kg bw/day groups and in females of the 200, 400 and 700 mg/kg bw/day groups. The relative weight of the kidneys showed a dose-dependent increase that was statistically significant for the 400 and 700 mg/kg bw/day groups in both sexes. Except for cloudy swelling of the proximal tubules of the kidneys in mice on the 700 mg/kg bw/day regimen, no treatment-related histopathological alterations were observed in organs of either sex. The NOAEL for oral systemic toxicity was 400 mg/kg bw/day based on cloudy swelling of the proximal tubules observed at 700 mg/kg bw/day.

Conclusion:
After dietary exposure of B6C3F1 mice to morpholine oleic acid salt a NOAEL for oral systemic toxicity was 400 mg/kg bw/day deduced based on cloudy swelling of the proximal tubules observed at 700 mg/kg bw/day.

Report: CA 5.8.1/11
[redacted]., 1987 a
Combined chronic toxicity and carcinogenicity studies of Morpholine oleic acid salt in B6C3F1 mice
1987/1002775
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Morpholine oleic acid salt (MOAS; purity not reported), pale yellow, viscous liquid
Test animals: Male and female B6C3F1 mice (6 weeks old) from [redacted]
The animals were housed in groups (5 per cage) in polycarbonate cages on hardwood chip bedding in an environment-controlled room maintained at 20-24°C and artificially illuminated for 12 h each day. Male and female B6C3F1 mice (50/sex/dose) were exposed to morpholine oleic acid salt at doses of 0%, 0.25%, and 1% in drinking water (~ 0, 400 and 1500 mg/kg bw/day for males; ~ 0, 500 and 1500 mg/kg bw/day for females) for 96 weeks and then received normal tap-water for additional 8 weeks.

Findings:
No treatment-related changes in mortality were reported. Both sexes received 1500 mg/kg bw/day and females treated with 500 mg/kg bw/day showed a reduction in body weight. Mice administered 1500 mg/kg bw/day consumed less water than the control mice given normal tap-water. Food consumption was similar in both control and treated groups. At termination, significant increases in blood-urea nitrogen concentrations were only observed in males of the 1500 mg/kg bw/day dose group. Changes in absolute/relative organ weights were not attributed to MOAS. A NOAEL could not be identified based on a reduction in body weight in females at 500 mg/kg bw/day.

The incidence of squamous-cell hyperplasia of the fore-stomach epithelium was significantly higher in the males treated with 1.0% MOAS as compared to the controls. The male mice received 0.25% MOAS had a significantly reduced incidence of hepatocellular carcinoma in comparison with the control group and this trend was indicated also in the 1.0% dose group. This experiment did not demonstrate any carcinogenic effect of MOAS in mice at levels up to 1% in the drinking water.

Conclusion:
After dietary exposure of B6C3F1 mice to morpholine oleic acid salt a NOAEL was not identified, as a reduction of body weight was observed in the females at 500 mg/kg bw/day, the lowest dose group tested. The test item was not carcinogenic under conditions of this study.

Report: CA 5.8.1/12
[redacted]., 1969 a
Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines
1969/1000321
Guidelines: none
GLP: no
Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Summary
Female Sprague-Dawley rats (seven/dose) received morpholine (purity not reported) in the diet at 0 or 500 mg/kg bw/day for 56 days, and then sacrificed after 270 days. At sacrifice, body weight was measured and gross and microscopic examinations were conducted. No mortalities occurred during the course of the study. Moderate adiposis of the liver was observed resulting in a LOAEL of 500 mg/kg bw/day.

Genotoxicity

Report:	CA 5.8.1/13 Glatt H.R., 1981 a AMES Test for Morpholine 1981/1001581
Guidelines:	none
GLP:	no
Deviations:	Not applicable

Acceptability: The study is considered to be supplementary.

Summary

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 uvr A were exposed to morpholine (99.8% purity): Experiment 1: 0, 15.8, 50, 158, 500, 1580, 5000, 15800, and 50000 µg/plate in the presence and absence of S9 mix (from liver homogenate of Aroclor 1254 treated rats); Experiment 2: 0, 15800, 50000, 100000, and 200000 µg/plate in the presence and absence of S9 mix; Experiment 3: 0, 15800, 31600, and 50000 µg/plate in the presence of S9 mix; and Experiment 4: 0.5, 1.0, 1.5 and 2.5 mg/20 L using the desiccator method in the presence and absence of S9 mix. Appropriate positive and solvent (water) controls were included in the assay, and results indicated the test was valid. Cytotoxicity was observed at >50000 µg/plate. In the first experiment, a slight increase in the number of revertants was found at the 50000 µg dose in TA 100 without metabolic activation (increased by a factor of 1.57) and was reproduced in the second experiment (factor: 1.8). Using metabolic activation, a slight increase in WP2 uvrA revertants was seen (factor: 2) in the first experiment; it was not reproduced in the second experiment, but in a third experiment it was only marginally increased (factor: 1.9) at the 50000 µg dose. Morpholine was not mutagenic using the desiccator method. Results indicate that morpholine was weakly mutagenic in the Ames test at a concentration of 50000 µg/plate; however, the observed increase in the number of mutants at this relatively high dose was not more than 2-fold. This test result is considered weakly positive with *S. typhimurium* and negative with *E. coli*.

Huntsman 1979a (Study report not available)

S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to morpholine (purity not reported) at concentrations of 0, 0.005, 0.01, 0.1, 1, 5, and 10 µg/plate in the presence and absence of S9 mix (from liver homogenate of Aroclor 1254 treated rats). The positive controls induced the appropriate responses in the corresponding strains. There was no evidence of a concentration-related positive response of induced mutant colonies over the background. This test is considered negative.

Report: CA 5.8.1/14
Haworth S. et al., 1983 a
Salmonella mutagenicity test results for 250 chemicals
1983/1002418
Guidelines: none
GLP: no

Summary
S. typhimurium strains TA98, TA100, TA1535, and TA1537 were exposed to morpholine ("practical" grade) at concentrations of 0, 109, 363, 1090, 3633, and 10900 µg/plate in the presence and absence of S9 mix (from liver homogenate of Aroclor 1254 treated rats) using the pre-incubation procedure. The high dose of 10900 µg/plate was slightly cytotoxic. The positive controls induced the appropriate responses in the corresponding strains. There was no evidence of a concentration-related positive response of induced mutant colonies over the background. This test is considered negative.

Report: CA 5.8.1/15
Ishidate M. et al., 1984 a
Primary mutagenicity screening of food additives currently used in Japan
1984/1002262
Guidelines: none
GLP: no

Summary of the Ames test
Salmonella typhimurium strains TA92, TA1535, TA100, TA1537, TA 94 and TA98 were exposed to morpholine fatty acid salt (purity not reported) at concentrations up to 10000 µg/plate for the pre-incubation test, in the presence and absence of metabolic activation system (from liver homogenate of PCB-treated rats). Appropriate concurrent vehicle (DMSO) and positive controls were included and the expected responses were observed. No cytotoxicity was observed in the mutation study and morpholine was negative for mutagenicity.

Summary of the in vitro chromosome aberration test
Chinese hamster fibroblast lung cells (CHL) were exposed to morpholine fatty acid salt (purity not reported) at three different doses for 24 and 48 hr. No metabolic activation system was used. The maximum dose was selected by a preliminary test in which the dose needed for 50% cell-growth inhibition was estimated using a cell densitometer. The maximum dose tested was 0.25 mg/mL in physiological saline. The incidence of polyploid cells at 48 hr after treatment was 3.0% and the incidence of cells with structural chromosomal aberrations at 48 hr after treatment was 3.0%. Morpholine was considered to be negative for the induction of chromosomal aberration in CHL cells.

Report: CA 5.8.1/16
Myhr B.C., 1979 a
Mutagenicity evaluation of Morpholine 7H-4892/LOS-0575 in the mouse lymphoma forward mutation assay
1979/7001752
Guidelines: none
GLP: no

Summary
Mouse lymphoma L5178Y cells were exposed to morpholine (purity not reported) in the absence of metabolic activation: Experiment 1: 0.078, 0.5, 0.625, 1.0 and 1.25 µL/mL; Experiment 2: 0.5, 0.625, 0.75, 1.0 and 1.25 µL/mL; and Experiment 3: 0.75, 1.0 and 1.2 µL/mL, and the presence of S9 mix (liver S9 from Aroclor 1254-induced rats): Experiment 1: 0.156, 0.5, 0.625, 1.0 and 1.25 µL/mL; Experiment 2: 0.5, 0.625, 0.75, 1.0 and 1.25 µL/mL; and Experiment 3: 0.75 and 1.0 µL/mL. The target gene was thymidine kinase, and section medium contained 100 µg/mL of BrdU or 3 µg/mL of TFT. Appropriate positive, solvent (DMSO) and negative (untreated) controls were included in the assay and results indicated the test was sensitive and valid. The test material induced small increases in the mutation frequency over the applied concentration range of 0.625 to 1.25 µL/mL in the absence of metabolic activation. These treatments were highly toxic and the mutant frequency increases (approximately 2.5 -fold) were at the limit of detectability for this assay. In the presence of metabolic activation, concentrations up to 1.0 - 1.25 µL/mL were moderately toxic and not detectably mutagenic; concentrations from 1.2 - 2 µL/mL were excessively lethal. Under the conditions of this study, morpholine was considered to be weakly mutagenic in the assay without metabolic activation. This test is considered weakly positive.

Report: CA 5.8.1/17
Myhr B.C., 1979 b
Evaluation of Morpholine 7H-4892/LOS-0575 in the in vitro transformation of Balb/3T3 cells assay
1979/7001753
Guidelines: none
GLP: no

Summary
BALB/3T3 mouse cells were exposed to morpholine (purity not reported) at concentrations of 0, 0.000122, 0.00195, 0.0156, 0.125, and 0.25 µL/mL. No information was provided regarding presence or absence of metabolic activation. Appropriate positive and solvent (culture medium) controls were included in the assay and results indicated the test was valid. In a range-finding study conducted up to 1 µL/mL, cytotoxicity was observed starting at concentrations of 0.5 µL/mL and higher and it was determined that the optimum testing range was up to 0.25 µL/mL. The frequency of transformed foci was increased in a dose-related manner, with significant increases compared to the negative control at 0.125 and 0.25 µL/mL. This test is considered positive.

Report: CA 5.8.1/18
Myhr B.C., 1979 c
Evaluation of 4236-26-15 (Morpholine) in the in vitro transformation of BALB/3T3 cells assay
1979/7001756
Guidelines: none
GLP: no

Summary
BALB/3T3 mouse cells were exposed to morpholine (purity not reported) at concentrations of 0, 0.001, 0.25, 0.1, 0.2 and 0.3 µL/mL. No information was provided regarding presence or absence of metabolic activation. Appropriate positive and solvent (culture medium) controls were included in the assay and results indicated the test was valid. In a range-finding cytotoxicity study conducted up to 1 µL/mL, cytotoxicity (< 50% colony survival relative to control) was observed starting at concentrations of 0.5 µL/mL and higher and it was determined that the optimum testing range was up to 0.3 µL/mL. The frequency of transformed foci was significantly increased in a dose-related manner over the applied concentration range of 0.001 to 0.3 µL/mL, with a corresponding colony surviving of 78% to 52%. Therefore, this test is considered positive.

Litton, 1982 (Study report not available)

BALB/3T3 mouse cells were exposed to morpholine (purity not specified) as follows: Non-activation assays: Trial I: Control (medium), positive control (MCA), and 35.0, 175.0, 350.0, 700.0 and 1400.0 nl/ml and Trial II: Control (medium), positive control (MCA), and 15.0, 75.0, 150.0, 300.0 and 600.0 nl/ml. for the activation assay: Control (medium), positive control (PC), and 17.5, 87.5, 175.0, 350.0 and 700.0 nl/ml. Morpholine did not induce significant increases in transformed foci over the tested concentration ranges of 15.0 to 1400.0 nl/ml and 17.5 to 700.0 nl/ml in the non-activation and activation assay, respectively. The tested concentrations corresponded to approximately 20% to nearly 100% survival in the non-activation and activation preliminary cytotoxicity tests. Therefore, the test material is considered to be inactive in the Balb/3T3 and Balb/3T3 rat liver cell-mediated *in vitro* cell transformation assays.

Report: CA 5.8.1/19
Galloway S.M., 1980 a
Mutagenicity evaluation of Morpholine 4236-26-15 in the sister chromatid exchange assay with Chinese Hamster Ovary (CHO) cells
1980/7001750
Guidelines: none
GLP: yes

Summary
Chinese Hamster Ovary (CHO) cells were exposed to morpholine (purity not reported) in the presence and absence of metabolic activation (liver S9 of Aroclor 1254-treated rats) at concentrations of 0, 3.13, 6.25, 12.5, 25, 50 and 100 nL/mL. Appropriate positive, solvent (DMSO) and negative controls were included in the assay and results indicated that the test was valid. Cytotoxicity was not reported. The maximum increases in sister chromatid exchange noted were 25% and 22% with and without S9 mix, respectively and did not meet the criteria for a positive response. In the absence of a positive dose response these results indicated a very weak, or negative, response. Under the conditions of this assay, the increase in sister chromatid exchange is not considered significant.

Report: CA 5.8.1/20
Tong C., 1982 a
The hepatocyte primary culture/DNA repair assay on compound 4236-48-10 using rat hepatocytes in culture
1982/7001784
Guidelines: none
GLP: no

Summary
Hepatocytes from adult male Fischer 344 rats were exposed to morpholine (batch and purity not specified) without metabolic activation at concentrations ranging from 0.0001 to 10 mg/mL. Appropriate positive (B[a]P) and negative (pyrene) as well as solvent (DMSO) and culture (medium) controls were included in the assay and provided the expected results. Cytotoxicity occurred at > 0.1 mg/mL, therefore only slides exposed to concentration of 0.1 mg/mL and lower were evaluated. Morpholine did not induce DNA repair over the background up to the highest non-toxic dose tested.

Report: CA 5.8.1/21
Conaway C.C. et al., 1984 a
Evaluation of morpholine, 3-morpholine, and N-substituted morpholines in the rat hepatocyte primary culture/DNA repair test
1984/1002242
Guidelines: none
GLP: no

Summary
Hepatocytes from adult male Fischer 344 rats were exposed to morpholine (99.2%) without metabolic activation at concentrations of 0, 0.0001, 0.001, 0.01, 0.1 and 1 mg/mL. Appropriate positive and solvent controls were included in the assay and provided the expected results. Cytotoxicity occurred at 1 mg/mL, therefore the counting of slides began with the slides exposed at 0.1 mg/mL and lower. Morpholine did not induce DNA repair over background at the highest non-toxic doses and lower doses.

Report: CA 5.8.1/22
[redacted] 1979 a
Transplacental mutagenesis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine
1979/1001783
Guidelines: none
GLP: no

Summary
Hamster embryos were exposed *in utero* to sodium nitrite and morpholine or morpholine alone (0 or 500 mg/kg bw; purity not specified) administered by gavage in water to pregnant Syrian Golden hamsters (number not specified) on the 11th or 12th day of pregnancy. Twenty-four hours after exposure, the embryos were removed and examined for chromosomal aberrations, micronucleus formation, morphological or malignant transformation, and drug resistance mutations. Cells exposed *in utero* to morpholine showed no increases in the numbers of chromosomal aberrations, micronuclei, 8-azaguanine- or ouabain-resistant mutants, or transformation rates. The number of resistant colonies was markedly increased after administration of sodium nitrite and morpholine, showing that morpholine alone has no mutagenic effect *in vivo* under the conditions of this study. This study is considered negative.

Developmental toxicity

Report:	CA 5.8.1/23 [redacted] 2009 a Morpholine hydrochloride - Prenatal developmental toxicity study in Wistar rats - Oral administration (gavage) 2009/7010945
Guidelines:	EEC 2004/73, OECD 414, EPA 870.3700
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Morpholine hydrochloride (Batch JB125, Purity 97%) was administered daily to groups of 25 presumably pregnant Crl:WI (Han) Wistar rats by gavage during gestation days 6-19 at dose levels of 0 (drinking water), 75, 250 and 750 mg/kg bw with an application volume of 10 ml/kg bw. Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day. On GD 20, blood was taken from all females for haematological and clinic-chemical evaluations, and the dams were subsequently sacrificed and assessed by gross pathology. Organ weights of liver, kidneys, thyroids, the unopened uterus and the placentae were determined. For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated by resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for external findings. Thereafter, one half of the foetuses of each litter were examined for soft tissue findings and the remaining foetuses for skeletal (inclusive cartilage) findings.

At 750 mg/kg bw/day, dams revealed transient reduction of food consumption and body weight gain, increased liver weight with corresponding clinic-chemistry findings as well as clinic-chemistry signs of mild anaemia. No test substance-related adverse effects were observed in the foetuses.
At 250 mg/kg bw/day, dams revealed increased liver and clinic-chemistry signs of mild anaemia. No test substance-related adverse effects were observed in the foetuses.
At 75 mg/kg bw/day, no test substance-related adverse effects on dams, gestational parameters or foetuses were observed.

Thus, based on the results of this study the **developmental NOAEL** was at least **750 mg/kg bw/day**.
Based on the liver effects of the dams received 250 and 750 mg/kg bw/d, evident by increased organ weights with corresponding clinic-chemistry findings (only at 750 mg/kg bw/d) and haematology findings (mild anaemia) as well as transient reduction of food consumption and body weight gain at 750 mg/kg bw/d, the **maternal NOAEL** was considered to be **75 mg/kg bw/day.**

(DocID 2009/7010945)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material Morpholine hydrochloride (4451403)

Description:	Solid / white
Lot/Batch #:	JB125
Purity:	97%
Stability of test compound:	The stability of the test substance over the study period under the storage conditions (under N_2, at room temperature) was revealed by re-analysis. The Expiry Date was Oct 2008.

2. Vehicle control: drinking water

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Female
Age:	Time-mated rats of about 10-12 weeks of age
Weight at dosing:	143.5 to 197.9 g (on day 0 of the study)
Source:	[redacted]
Acclimation period:	5 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Individual housing in type M III Makrolon cages (Becker & Co, Castrop-Rauxel, Germany), floor area about 800 cm² with Lignocel FS 14 fibres as dust-free bedding (SSNIFF, Soest) and wooden gnawing blocks (Typ NGM E-022) enrichment (Abedd® Lab. and Vet. Service GmbH, Vienna, Austria)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 30-Oct-2007 to 13-Feb-2009
(In-life dates: 05-Nov-2007 (Start of treatment at gestation day (GD) 6) to 20-Nov-2007 (Sacrifice of the second cohort))

2. Animal assignment and treatment:

The animals were paired by the breeder and supplied on Day 0 post coitum (= detection of vaginal plug / sperm). The animals were acclimated to the laboratory conditions and assigned at random to the treatment groups.
Morpholine hydrochloride was administered to groups of 25 "time-mated", presumably pregnant Wistar rats by gavage at dose levels of 0 (drinking water), 75 (low dose), 250 (mid dose) and 750 mg/kg bw/day (high dose) with an application volume of 10 mg/kg bw during days 6 to 19 of gestation (GD). The calculation of the administration volume was based on the most recent individual body weight.

3. Test substance preparation and analysis:

Prior to study initiation the stability of the test substance in drinking water at room temperature was confirmed for at least 7 days. Thus, the preparation of the test substance solutions were prepared at the beginning of administration period and in a weekly intervals thereafter.
For the preparation of the solutions, appropriate amounts of the test substance were weighed in a beaker, topped up with drinking water and subsequently thoroughly mixed using a magnetic stirrer.
Due to the fact, that the test substance is completely miscible with water, solutions were considered to be homogenous without further analysis.
The correctness of the test-item content in drinking water preparations was determined at the start and towards the end of the application period by capillary electrophoresis with indirect UV detection using internal standard.

Analysis of drinking water preparations for test-item content

Concentration [g/100 mL] (Dose level [mg/kg])	Sampling	Concentration [mg/100 mL]	% of Nominal[§]
0.75 g/100 mL	05.11.2007	0.73	97.3
(75 mg/kg)	12.11.2007	0.75	100.0
Average		**0.74**	**98.7**
2.5 mg/100 mL	05.11.2007	2.4	96.0
(250 mg/kg)	12.11.2007	2.5	100.0
Average		**2.45**	**98.0**
7.5 mg/100 mL	05.11.2007	7.4	98.7
(750 mg/kg)	12.11.2007	7.5	100.0
Average		**7.45**	**99.3**

[§] not exact values due to rounding

The actual analysed test-item concentrations were in the range of 96.0 to 100% of the nominal concentrations.

4. Statistics:

Where relevant, means and standard deviations of each test group were calculated. Statistical analyses were performed according to the following tables:

Parameter	Statistical test
Statistics for clinical and foetal examinations	
Food consumption [a)], body weight, body weight change, corrected body weight gain (net maternal body weight change), carcass weight, weight of unopened uterus, number of corpora lutea, number of implantations, number of resorptions, number of live foetuses, proportions of pre-implantation loss, proportions of post-implantation loss, proportions of resorptions, proportion of live foetuses in each litter, litter mean foetal body weight, litter mean placental weight	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Female mortality, females pregnant at terminal sacrifice, number of litters with foetal findings	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test (one-sided) for the hypothesis of equal proportions
Proportions of foetuses with malformations, variations and/or unclassified observations in each litter	Pairwise comparison of the dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Statistics for pathology and clinical pathology	
Clinical pathology parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using the WILCOXON test (two-sided) for the equal medians
Pathology (weight) parameters	Main groups: Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians. Recovery groups: A pair wise comparison of the high dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.

[a)] For the parameter food consumption the "mean of means" was calculated and can be found in the relevant summary tables. The "mean of means" values allow a rough estimation of the total food consumption during different time intervals (pre-treatment, treatment and post-treatment period); they are not exactly precise values, because the size of the intervals taken for calculation differs. For the "mean of means" values no statistical analysis was performed.

C. METHODS

1. Observations

The animals were examined for mortality twice daily on working days and once daily on weekends and public holidays. Cage side examinations for signs of morbidity, pertinent behavioural changes and overt toxicity were performed at least once daily. If such signs occurred, the animals were examined several times daily (GD 0-20).

2. Body weight

All animals were weighed on GD 0, 1, 3, 6, 8, 10, 13, 15, 17, 19, and 20. The body weight change of the animals was calculated from these results. In addition, the corrected (net) body weight gain was calculated after terminal sacrifice (terminal body weight on GD 20 minus weight of the unopened uterus minus body weight on GD6.).

3. Food consumption

With the exception of day 0 p.c., the consumption of food was determined on the same days as was body weight.

4. Haematology and clinical chemistry

Blood was drawn in the morning from non-fasted, isoflurane anesthetized animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Thrombocyte count (PLT)
✓	Haemoglobin (HGB)	✓	Neutrophils (differential)		
✓	Haematocrit (HCT)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes		Large unstained cells		
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyl transferase (γ-GT)
	Potassium	✓	Globulin (by calculation)		
	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

5. Sacrifice

On GD 20, the dams were sacrificed after blood sampling in randomized order by cervical dislocation (after isoflurane anaesthesia). Dams were subsequently assessed by gross pathology in randomized order to minimize bias. The following organs of the dams were weighed and fixed in 4% neutral buffered formaldehyde:

- Thyroid glands (including parathyroid glands)
- Liver
- Kidney

After dams had been sacrificed, the uteri and the ovaries were removed and the following data were recorded:

- Weight of the unopened uterus
- Number of corpora lutea
- Number and distribution of implantation sites classified as
  - live foetuses or
  - dead implantations
    a. early resorptions (only decidual or placental tissues visible or positive staining according to SALEWSKI of uteri from apparently non-pregnant animals and the empty uterus horn in the case of single-horn pregnancy)
    b. late resorptions (embryonic or foetal tissue in addition to placental tissue visible)
    c. dead foetuses (hypoxemic foetuses which did not breathe spontaneously after the uterus had been opened)

Based on the above the following parameters were calculated:

- Conception rate [%]: $\frac{number\ of\ pregnant\ animals}{number\ of\ fertilized\ animals} \times 100$
- Pre-implantation loss [%]:
$$\frac{number\ of\ corpora\ lutea - number\ of\ implantation}{number\ of\ corpora\ lutea} \times 100$$
- Post-implantation loss [%]:
$$\frac{number\ of\ implantations - number\ of\ live\ fetuses}{number\ of\ implantations} \times 100$$

6. Examination of foetuses

At necropsy each foetus was weighed and examined macroscopically for any external findings. The sex was determined by observing the distance between the anus and the base of the genital tubercle and was later confirmed in all foetuses fixed in Harrison's fluid by internal examination. If there were discrepancies between the "external" and the "internal" sex of a foetus, the foetus was finally sexed according to the internal sex.

Furthermore, the viability of the foetuses and the condition of the placentae, the umbilical cords, the foetal membranes, and fluids were examined. Individual placental weights were recorded. Subsequently the foetuses were sacrificed by s.c. injection of pentobarbital (Narcoren®; dose: 0.1 mL/foetus).

After these examinations, approximately one half of the foetuses per dam were eviscerated, skinned and placed in ethyl alcohol, the other half was placed in Harrison's fluid for fixation and further evaluation.

Soft tissue examination of the foetuses

The foetuses fixed in Harrison's fluid were examined for any visceral findings according to the method of Barrow and Taylor. After this examination these foetuses were discarded.

Skeletal examination of the foetuses

The skeletons of the foetuses fixed in ethyl alcohol were stained according to a modified method of Kimmel and Trammell. Thereafter, the skeletons of these foetuses were examined under a stereomicroscope. After this examination the stained foetal skeletons were retained individually.

Evaluation criteria for assessing the foetuses

Foetal morphology findings were described using the glossary of Wise et al. (1997) as far as possible. Classification of these findings was based on the terms and definitions proposed by Chahoud et al. (Chahoud et al. 1999; Solecki et al. 2001 and 2003; for detailed references see study report):

Malformation	A permanent structural change that is likely to adversely affect the survival or health
Variation	A change that occurs also in foetuses of control animals and is unlikely to adversely affect the survival or health. This includes delays in growth or morphogenesis that has otherwise followed a normal pattern of development.

Moreover, the term "**unclassified observation**" was used for those foetal findings, which could not be classified as malformations or variations (e.g. focal liver necrosis in foetuses).

II. RESULTS AND DISCUSSION

Please note: Only pregnant dams were used for the calculations of mean maternal food consumption, body weight and body weight change. Only pregnant dams with scheduled sacrifice on GD 20 were taken for the calculation of mean gravid uterine weights, mean net maternal body weight change (corrected body weight gain) and summary of reproduction data.

All females were included in the above mentioned calculations

A. TEST SUBSTANCE ANALYSES

See Section B 3. above.

B. OBSERVATIONS

1. Mortality

There were no substance-related or spontaneous mortalities in any of the groups.

2. Clinical signs of toxicity

All mid and high-dose dams (250 and 750 mg/kg bw/d) excreted a yellowish discoloured urine from GD 7 onwards until terminal sacrifice (GD 20). This urine discoloration mirrors the systemic availability of the test substance rather than being an adverse effect. It is most likely due to the excreted test compound or its metabolite(s).
Furthermore 8/25 dams received 250 mg/kg bw/d showed transient salivation from GD 12 onwards that persisted in the respective females for a few minutes immediately after each administration. After cessation of treatment on GD 19, salivation was no longer observed in these rats. This effect is considered to be treatment-related. It was likely to be induced by the unpleasant taste of the test substance or by local irritation of the upper digestive tract. It is not considered to be a sign of systemic toxicity.

No clinical symptoms were noted in the low-dose group (75 mg/kg bw/d).

C. BODY WEIGHT AND FOOD CONSUMPTION

1. Food consumption

At 750 mg/kg bw/d, food consumption was statistically significant reduced (up to about 13% below the concurrent control value) during the initial phase of exposure on GD 6-10. However, on the following days the food consumption of the high-dose rats recovered and was comparable to control [see Table 5.8.1-9] and thus the effect was not seen if the food consumption is calculated for the period of GD 6-19.

Food consumption in females received 250 or 75 mg/kg bw/d was unaffected by the treatment and did not show any statistically significant or biologically relevant differences in comparison to the controls.

2. Body weight and body weight gain

Body weight of females received morpholine hydrochloride was not affected by the treatment.

Table 5.8.1-9: Food consumption and body weight development in rat administered morpholine hydrochloride on GD 6 to 19

Dose level [mg/kg]	0	75	250	750
Food consumption [g/animal/day]				
Day 0 to 6	14.6 ± 2.3	14.4 ± 2.7	14.9 ± 2.0	14.4 ± 2.4
Δ%		- 1.4	2.1	- 1.4
Day 6 to 19	17.5 ± 1.1	18.4 ± 1.3	18.2 ± 1.4	17.3 ± 2.5
Δ%		5.1	4.0	- 1.1
Day 0 to 20	16.7 ± 2.0	17.2 ± 2.5	17.4 ± 2.3	16.5 ± 2.6
Δ%		3.0	4.2	- 1.2
Body weight [g]				
Day 0	166.4 ± 10.3	168.8 ± 12.2	170.4 ± 10.1	167.5 ± 9.0
Δ%		1.4	2.4	0.7
Day 6	194.6 ± 10.8	196.0 ± 12.3	198.8 ± 9.6	195.1 ± 9.8
Δ%		0.7	2.2	0.3
Day 19	259.1 ± 16.0	267.2 ± 19.9	269.2 ± 12.6	260.4 ± 16.0
Δ%		3.1	3.9	0.5
Day 20	269.8 ± 17.3	278.6 ± 21.7	281.2 ± 14.7	272.1 ± 16.7
Δ%		3.3	4.2	0.9
Body weight gain [g]				
Day 0 to 6	28.1 ± 4.6	27.2 ± 4.0	28.4 ± 4.0	27.5 ± 5.2
Δ%		-3.2	1.1	-2.1
Day 6 to 19	64.5 ± 10.7	71.2 ± 10.8	70.4 ± 8.5	65.3 ± 11.0
Δ%		10.4	9.1	1.2
Day 0 to 20	103.4 ± 15.2	109.8 ± 12.7	110.8 ± 12.2	104.6 ± 15.2
Δ%		6.2	7.2	1.2

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)

As a consequence of reduced food consumption, females received 750 mg/kg bw/d showed statistically significant reduction of body weight gain during the initial phase of the treatment (GD 6-8, about 41% below the concurrent control value). However, if calculated for the entire treatment phase (GD 6-19), no reduction in mean body weight gain of these rats was seen.
At 250 mg/kg bw/d, the statistically significantly increased body weight gain on GD 13-15 was observed, however considered to be not of biological relevance.
At 75 mg/kg bw/d, body weight gain was unaffected by the treatment and was comparable to the control throughout the study period.

D. NECROPSY OBSERVATIONS

1. Corrected (net) body weight gain

No treatment-related effects on carcass and corrected (net) body weight gain were observed. The statistically significantly higher carcass weights of females treated with 250 mg/kg bw/d was assessed being not of biological relevance.

Table 5.8.1-10: Mean gravid uterus weights and net body weight change of pregnant rats administered morpholine hydrochloride on GD 6 to 19

Dose level [mg/kg bw/d]	0	75	250	750
Gravid uterus (g)	45.2 ± 12.8	49.7 ± 10.2	47.0 ± 8.7	45.3 ± 8.2
Carcass (g)	224.5 ± 12.4	228.9 ± 16.2	**234.2*** ± 12.1	226.8 ± 13.5
Net weight change from day 6 (g)	30.0 ± 9.1	32.9 ± 9.2	35.3 ± 9.6	31.7 ± 9.0

* $p < 0.05$, ** $p < 0.01$ (Dunnett test, two-sided)

The mean gravid uterus weights of the animals of all test groups were not influenced by the test substance. The differences between these groups and the control group revealed no dose-dependency and were assessed to be without biological relevance. Considering the fluctuations in the mean number of live foetuses/dam [see Table 5.8.1-14], they reflect the normal degree of variation for rats of the strain used in this study

2. Haematology

Females received 750 mg/kg bw/d revealed statistically significant decreases of red blood cell counts as well as the haemoglobin and haematocrit values. Additionally, relative as well as the absolute eosinophil counts were slightly, but statistically significantly decreased. Furthermore, increased relative reticulocyte counts were noted.

Females received 250 mg/kg bw/d revealed statistically significant decreases of red blood cell counts as well as the haemoglobin and haematocrit values. Furthermore, increased relative reticulocyte counts were noted.

Thus, the haematology changes observed in animals of the mid- and top-dose represent a mild anaemia, which was still of regenerative type.

Haematology parameters of females treated with 75 mg/kg bw/d were not affected.

Table 5.8.1-11: Selected haematology findings of pregnant rats administered morpholine hydrochloride on GD 6 to 19 (group means on GD 20)

Dose	**[mg/kg bw/d]]**	**0**	**75**	**250**	**750**
RBC	[10^9/L	6.16 ± 0.3	6.04 ± 0.3	**5.94*** ± 0.32	**5.93*** ± 0.27
Hgb	[mmol/L]	7.2 ± 0.4	7.1 ± 0.3	**7.0*** ± 0.3	**6.9*** ± 0.2
Hct	[L/L]	0.312 ± 0.016	0.307 ± 0.016	**0.302*** ± 0.013	**0.299*** ± 0.011
Reti	[%]	2.1 ± 0.8	2.1 ± 0.6	**2.7*** ± 0.8	**2.7**** ± 0.5
Eosinophils	[%]	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	**1.0**** ± 0.4
	[10^9/L]	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	**0.05*** ± 0.02

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Clinical chemistry

Females received 750 mg/kg bw/d revealed liver effects as evident by statistically significantly increased ALT activity as well as urea, total bilirubin, and the cholesterol values. The ALT activity increase indicates a liver cell membrane leakage. The increase of the total bilirubin levels is due to an obstruction of intra-hepatic bile flow because of a liver cell swelling. The affected liver cell metabolism is shown by the increase of the urea and cholesterol levels. The cholesterol concentration increase as well as the decrease of the eosinophil counts in the rats of the 750 mg/kg bw/d dose group can be related to a stress situation of these animals.

The clinic-chemical parameters of females treated with 250 or 75 mg/kg bw/d were not affected.

Table 5.8.1-12: Selected clinical chemistry findings of pregnant rats administered morpholine hydrochloride on GD 6 to 19 (group means on GD 20)

Dose	**[mg/kg bw/d]**	**0**	**75**	**250**	**750**
ALT	[µkat/L]	0.88 ± 0.11	0.86 ± 0.13	0.93 ± 0.09	**1.13**** ± 0.18
Urea	[mmol/L]	7.18 ± 0.92	7.16 ± 0.76	7.20 ± 0.92	**7.80*** ± 0.76
Bilirubin, total	[µmol/L]	1.74 ± 0.69	1.58 ± 0.57	1.69 ± 0.60	**2.12*** ± 0.62
Cholesterol	[mmol/L]	1.92 ± 0.22	2.04 ± 0.26	1.97 ± 0.28	**2.26**** ± 0.37

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

4. Gross necropsy observations

No gross necropsy observations were made at Cesarian section.

5. Organ weights

Absolute liver weights were slightly, but significantly increased in all treated groups. In the 250 and 750 mg/kg bw/d groups, this increase was correlated to haematological (anaemia) findings. Additionally, the 750 mg/kg bw/d group showed clinical pathological findings (increased ALT, urea, bilirubin, cholesterol) indicative of an affection of liver cells and liver cell metabolism. Neither such correlates nor any corroborative gross lesions were noted in the 75 mg/kg bw/d group, thus the rather marginal increase of liver weight in this dose group is not considered as an adverse effect of systemic toxicity.

Table 5.8.1-13: Absolute (g or mg) and relative (% of carcass weight) organ weights of pregnant rats administered morpholine hydrochloride on GD 6 to 19

Dose	[mg/kg bw/d]	0	75	250	750
Liver	[g]	10.2 ± 1.1	**10.9*** ± 1.2	**11.2**** ± 0.8	**11.2**** ± 1.3
	[%][#]	4.5	4.8	4.8	4.9
Thyroid	[mg]	15.7 ± 2.3	17.2 ± 3.3	**18.0**** ± 2.4	16.1 ± 2.6
	[%][#]	0.007	0.008	0.008	0.007

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
[#] no statistical evaluation was performed for the relative organ weight values

Furthermore, at 250 mg/kg bw/d statistically significant increase of the thyroid weight was observed. However, due to lack of a dose-response relationship, this finding is considered to be not of biological relevance.

E. CESAREAN SECTION DATA

All dams were pregnant, and thus the conception rate reached 100% in all test groups including controls. There were no test substance-related and/or biologically relevant differences between the different test groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the post-implantation losses, the number of resorptions and viable foetuses. Generally, gestational parameters in the various test groups were within the normal range for animals of this strain and age.

Table 5.8.1-14: Caesarean section data

Dose level [mg/kg bw/d]		0	75	250	750
Pregnancy status					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	25	25	25
conception rate	[%]	100	100	100	100
- aborted	[n]	0	0	0	0
- premature birth	[n]	0	0	0	0
- dams with viable foetuses	[n]	25	25	25	25
- dams with all resorptions	[n]	0	0	0	0
- mortality		0	0	0	0
- pregnant terminal sacrifice	[n]	25	25	25	25
Cesarean section data[a]					
- Corpora lutea	[n]	10.3 ± 1.4	10.8 ± 1.3	10.4 ± 1.2	10.2 ± 1.5
total number	[n]	258	270	260	254
- Implantation sites	[n]	9.2 ± 2.3	9.8 ± 1.8	9.6 ± 1.4	9.5 ± 1.8
total number	[n]	229	246	241	237
- Pre-implantation loss	[%]	11.8 ± 16.4	8.9 ± 12.1	7.2 ± 9.7	6.6 ± 10.8
- Post-implantation loss	[%]	10.8 ± 15.6	7.6 ± 11.5	10.5 ± 10.1	8.4 ± 10.5
- Resorptions	[n]	0.8 ± 1.1	0.7 ± 1.0	1.0 ± 0.9	0.8 ± 1.1
total number	[n]	20	17	24	21
- Early resorptions		0.6 ± 1.0	0.6 ± 0.9	0.8 ± 0.9	0.8 ± 1.1
total number	[n]	15	14	21	21
- Late resorptions		0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0
total number	[n]	5	3	3	0
- Dead foetuses	[n]	0	0	0	0
- Live foetuses	[n]	8.4 ± 2.8	9.2 ± 2.2	8.7 ± 1.8	8.6 ± 1.7
total number	[n]	209	229	217	216
- Total live female foetuses	[n]	4.4 ± 2.3	4.8 ± 1.8	5.0 ± 2.1	4.6 ± 1.5
total number	[n]	111	119	125	115
Mean	[%]	46.4 ± 20.0	48.6 ± 15.9	50.7 ± 17.1	49.8 ± 16.6
- Total live male foetuses	[n]	3.9 ± 2.0	4.4 ± 1.8	3.7 ± 1.6	4.0 ± 1.9
total number	[n]	98	110	92	101
Mean	[%]	42.8 ± 18.3	43.8 ± 14.8	38.8 ± 16.3	41.8 ± 16.4
- Percent live females		53.1	52.0	57.6	53.2
- Percent live males		46.9	48.0	42.4	46.8
Placental weights	[g]	0.42 ± 0.08	0.40 ± 0.06	0.41 ± 0.06	0.39 ± 0.05
- male foetuses	[g]	0.44 ± 0.11	0.41 ± 0.9	0.42 ± 0.7	0.41 ± 0.05
- female foetuses	[g]	0.40 ± 0.07	0.39 ± 0.05	0.40 ± 0.06	0.38 ± 0.05
Mean foetal weight	[g]	3.7 ± 0.5	3.6 ± 0.3	3.6 ± 0.3	3.5 ± 0.2
- males	[g]	3.8 ± 0.5	3.7 ± 0.4	3.7 ± 0.4	3.6 ± 0.3
- females	[g]	3.5 ± 0.4	3.6 ± 0.3	3.5 ± 0.2	3.4 ± 0.2

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

The sex distribution of the foetuses in test groups 75, 250 and 750 mg/kg bw/d was comparable to the control foetuses. Observable differences were without biological relevance. Furthermore, the mean placental weights were comparable between the treated groups and the corresponding control. Differences observed in comparison to the control were neither statistically significant nor biologically relevant.
The mean foetal body weights all test groups were not influenced by the test substance and were comparable to the corresponding control values.

F. EXTERNAL, VISCERAL AND SKELETAL EXAMINATION OF FETUSES

1. External examination

No external variations or unclassified observations were observed in any foetus of any group in this study.

Regarding external malformations, the one sole malformation (i.e. cleft palate) was recorded for 3/217 foetuses from 2/25 litters in the 250 mg/kg bw/d group. Considering the missing dose-response relationship and the presence of this finding in the historical control data, these abnormalities were considered to be spontaneous in nature and without a relation to treatment. The incidences of total external malformations were comparable to the historical control data [see Table 5.8.1-15].

Table 5.8.1-15: Incidence of external malformations and variations

Dose level [mg/kg bw/d]	**0**	**75**	**250**	**750**
Litters Evaluated	25	25	25	25
Foetuses Evaluated	208	229	217	216
Live	208	229	217	216
Dead	0	0	0	0
Total external malformations				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	3 (1.4)	0 (0.0)
- Litter incidence [a]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.	0.0	1.5	0.0
Historical control data				
Foetuses (% Mean range)	0.0 - 1.0			
Litter(% Mean range)	0.0 - 8.7			
Affected foetuses/litter (% Mean range)	0.0 - 1.0			
Individual external malformations				
- Cleft palate				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	3 (1.4)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.	0.0	1.5	0.0
Historical control data				
Foetuses (% Mean range)	0.0 - 0.5			
Litter(% Mean range)	0.0 - 4.3			
Affected foetuses/litter (% Mean range)	0.0 - 0.6			

2. Visceral examination

Two soft tissue malformations (i.e. short or interrupted spinal cord) were recorded in one foetus each of control and 250 mg/kg bw/d groups. Although these particular findings are not in the historical control data, they were considered to be spontaneous in nature and without a relation to treatment, because there is no dose-response relationship. The incidences of soft tissue malformations were comparable to the historical control data.

Three soft tissue variations (dilated cerebral ventricle, uni- or bilateral dilation of renal pelvis and ureter) were detected in each group including the controls, without any dose-response relationship. The incidences of soft tissue variations were comparable to the historical control data.

No unclassified soft tissue observations were recorded.

Table 5.8.1-16: Incidence of visceral (soft tissue) malformations and variations

Dose level [mg/kg bw/d]	**0**	**75**	**250**	**750**
Litters Evaluated	25	25	25	25
Foetuses Evaluated	101	106	103	103
Live	101	106	103	103
Dead	0	0	0	0
Total visceral malformations				
- Foetal incidence [No. (%)]	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
- Litter incidence [a]	1 (4.0)	0 (0.0)	1 (4.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.6	0.0	0.8	0.0
Historical control data				
Foetuses (% Mean range)	0.0 - 1.1			
Litter (% Mean range)	0.0 - 4.3			
Affected foetuses/litter (% Mean range)	0.0 - 1.1			
Individual visceral malformations				
- short spinal cord				
- Foetal incidence [No. (%)]	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.06	0.0	0.0	0.0
- interrupted spinal cord				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.0	0.0	0.8	0.0
Total visceral variations				
- Foetal incidence [No. (%)]	6 (5.9)	11 (10)	4 (3.9)	9 (8.7)
- Litter incidence [No. (%)]	5 (20)	7 (28)	4 (16)	7 (28)
- Affected foetuses/litter (Mean %)	5.6	10.0	4.7	9.2
Historical control data				
Foetuses (% Mean range)	1.0 - 10.9			
Litter (% Mean range)	4.2 - 38.1			
Affected foetuses/litter (% Mean range)	0.8 - 11.9			

Table 5.8.1-16: Incidence of visceral (soft tissue) malformations and variations

Dose level [mg/kg bw/d]	0	75	250	750
Individual visceral variations				
- Dilated cerebral ventricle				
- Foetal incidence [No. (%)]	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.6	0.0	0.0	0.0
- Dilated renal pelvis				
- Foetal incidence [No. (%)]	5 (5.0)	11 (10)	4 (3.9)	9 (8.7)
- Litter incidence [No. (%)]	4 (16)	7 (28)	4 (16)	7 (28)
- Affected foetuses/litter (Mean %)	5.1	10.0	4.7	9.2
Historical control data				
Foetuses (% Mean range)	1.0 - 10.9			
Litter (% Mean range)	4.2 - 38.1			
Affected foetuses/litter (% Mean range)	0.8 - 11.9			
- Dilated ureter				
- Foetal incidence [No. (%)]	4 (4.0)	7 (6.6)	3 (2.9)	8 (7.8)
- Litter incidence [No. (%)]	3 (12)	6 (24)	3 (12)	6 (24)
- Affected foetuses/litter (Mean %)	4.4	6.2	3.7	8.2
Historical control data				
Foetuses (% Mean range)	0.0 - 7.3			
Litter (% Mean range)	0.0 - 25.0			
Affected foetuses/litter (% Mean range)	0.0 - 9.8			

3. Skeletal examination

Skeletal malformations were noted in single foetuses of the control as well as 250 and 750 mg/kg bw/d groups. Although some of these findings are not present in the historical control data, each of them affected individual foetuses and neither statistically significant differences between the test groups nor a dose-response relationship were observed. The incidences of total skeletal malformations were comparable to the historical control data.

For all test groups, skeletal variations of different bone structures were observed, with or without effects on corresponding cartilages. The observed skeletal variations consisted primarily of transient delays/disturbances of ossification. The majority of the skeletal variations are equally distributed among the different test groups including controls, if normal biological variation is taken into account.

Two isolated findings – incomplete ossification of parietal with unchanged cartilage and wavy ribs – were statistically significantly increased, outside the historical control data, at mid- and high-dose levels (250 and 750 mg/kg bw/d), although not in a dose-related manner. The former findings, which was also significantly increased but within historical control data at the low-dose level (75 mg/kg bw/d), is a delay of ossification which is reversible and does not affect the morphology of the parietal as it becomes obvious by the unchanged underlying cartilage. Wavy ribs represent a minor delay and disturbance of ossification which is frequently found in rat foetuses and which is completely repaired during postnatal development. Taking all this into consideration, these findings were regarded to be of no toxicological relevance and are not classified as adverse events. Three other skeletal variations, such as supraoccipital hole(s), incomplete ossification of skull and unossified sternebra (all with unchanged underlying cartilages) were noted at significantly higher incidences. Of them, only the increased incidence for incomplete ossification of skull in the high-dose group slightly exceeded the historical control range. Such slight retardations of the ossification process occur very frequently in gestation day 20 rat foetuses of this strain. An increase of this spontaneously high incidence of skeletal variations is often noted in the presence of maternal toxicity or maternal stress, as has been substantiated in this study for mid- and high-dose dams displaying anaemia, affection of liver cells and liver cell metabolism. Furthermore, these types of minor ossification delays are regarded as transient phenomena that are fully reversible during the postnatal period. Thus, these findings are considered secondary to maternal toxicity and not relevant in terms of developmental toxicity.

The total incidences of skeletal variations were comparable to the historical control data.

Table 5.8.1-17: Incidence of skeletal malformations and variations

Dose level [mg/kg]	0	75	250	750
Litters Evaluated	25	25	25	25
Foetuses Evaluated	108	123	114	113
Live	108	123	114	113
Dead	0	0	0	0
Total skeletal malformations				
- Foetal incidence [No. (%)]	1 (0.9)	0 (0.0)	2 (1.8)	2 (1.8)
- Litter incidence [a]	1 (4.0)	0 (0.0)	1 (4.0)	2 (8.0)
- Affected foetuses/litter (Mean ± SD) [%]	0.7	0.0	2.0	1.5
Historical control data				
Foetuses (% Mean range)	0.0 - 3.7			
Litter (% Mean range)	0.0 - 18.2			
Affected foetuses/litter (% Mean range)	0.0 - 4.8			
Individual skeletal malformations				
- bipartite ossification of basisphenoid				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	2 (1.8)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.0	0.0	2.0	0.0
- absent presphenoidal bone				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
- Affected foetuses/litter (Mean %)	0.0	0.0	0.0	0.8
- malpositioned and bipartite sternebra, unchanged cartilage				
- Foetal incidence [No. (%)]	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.9)
- Litter incidence [No. (%)]	1 (4.0)	0 (0.0)	0 (0.0)	1 (4.0)
- Affected foetuses/litter (Mean %)	0.7	0.0	0.0	0.8
Historical control data				
Foetuses (% Mean range)	0.0 - 0.9			
Litter (% Mean range)	0.0 - 4.3			
Affected foetuses/litter (% Mean range)	0.0 - 1.1			
Total skeletal variations				
- Foetal incidence [No. (%)]	108 (100)	120 (98)	111 (97)	112 (99)
- Litter incidence [No. (%)]	25 (100)	25 (100)	25 (100)	25 (100)
- Affected foetuses/litter (Mean %)	100.0	96.5	97.1	99.3
Historical control data				
Foetuses (% Mean range)	90.6 - 100.0			
Litter (% Mean range)	100.0 - 100.0			
Affected foetuses/litter (% Mean range)	89.1 - 100.0			
Individual skeletal variations[#]				
- supraoccipital hole(s)				
- Foetal incidence [No. (%)]	24 (22)	36 (29)	30 (26)	44 (39)
- Litter incidence [No. (%)]	18 (72)	19 (76)	15 (60)	20 (80)
- Affected foetuses/litter (Mean %)	20.1	30.6	24.9	**37.6****
Historical control data				
Foetuses (% Mean range)	13.2 - 48.8			
Litter (% Mean range)	33.3 - 95.7			
Affected foetuses/litter (% Mean range)	12.3 - 49.0			

Table 5.8.1-17: Incidence of skeletal malformations and variations

Dose level [mg/kg]	**0**	**75**	**250**	**750**
Litters Evaluated	25	25	25	25
Foetuses Evaluated	108	123	114	113
Live	108	123	114	113
Dead	0	0	0	0
- incomplete ossification of parietal, unchanged cartilage				
- Foetal incidence [No. (%)]	15 (14)	32 (26)	39 (34)	40 (35)
- Litter incidence [No. (%)]	10 (40)	16 (64)	**22**** (88)	**20**** (80)
- Affected foetuses/litter (Mean %)	13.5	**27.1***	**34.9****	**34.9****
Historical control data				
Foetuses (% Mean range)	9.1 - 29.9			
Litter (% Mean range)	22.2 - 68.2			
Affected foetuses/litter (% Mean range)	9.0 - 27.6			
- incomplete ossification of skull, unchanged cartilage				
- Foetal incidence [No. (%)]	2 (1.9)	9 (7.3)	11 (9.6)	15 (13)
- Litter incidence [No. (%)]	2 (8.0)	**8*** (32)	6 (24)	**13**** (52)
- Affected foetuses/litter (Mean %)	2.8	**7.2***	10.3	**13.1****
Historical control data				
Foetuses (% Mean range)	0.0 - 11.3			
Litter (% Mean range)	0.0 - 39.1			
Affected foetuses/litter (% Mean range)	0.0 - 11.0			
- unossified sternebra, unchanged cartilage				
- Foetal incidence [No. (%)]	3 (2.8)	13 (11)	7 (6.1)	10 (8.8)
- Litter incidence [No. (%)]	3 (12)	5 (20)	5 (20)	**10*** (40)
- Affected foetuses/litter (Mean %)	2.6	10.0	6.2	**8.5***
Historical control data				
Foetuses (% Mean range)	1.8 - 35.7			
Litter (% Mean range)	8.3 - 70.8			
Affected foetuses/litter (% Mean range)	1.7 - 39.0			
- wavy rib				
- Foetal incidence [No. (%)]	9 (8.3)	15 (12)	25 (22)	27 (24)
- Litter incidence [No. (%)]	7 (28)	8 (32)	**15*** (60)	13 (52)
- Affected foetuses/litter (Mean %)	11.9	12.4	**24.3***	**24.5***
Historical control data				
Foetuses (% Mean range)	1.0 - 18.1			
Litter (% Mean range)	4.8 - 54.2			
Affected foetuses/litter (% Mean range)	1.0 - 18.0			
Total skeletal unclassified observations				
- Foetal incidence [No. (%)]	52 (48)	61 (50)	68 (60)	51 (45)
- Litter incidence [No. (%)]	23 (92)	23 (92)	23 (92)	22 (88)
- Affected foetuses/litter (Mean %)	53.3	50.2	60.4	46.2
Historical control data				
Foetuses (% Mean range)	16.7 - 64.9			
Litter (% Mean range)	47.8 - 95.8			
Affected foetuses/litter (% Mean range)	17.0 - 64.5			

Table 5.8.1-17: Incidence of skeletal malformations and variations

Dose level [mg/kg]	0	75	250	750
Litters Evaluated	25	25	25	25
Foetuses Evaluated	108	123	114	113
Live	108	123	114	113
Dead	0	0	0	0
Individual skeletal unclassified observations				
- notched cartilage between basisphenoid and basioccipital				
- Foetal incidence [No. (%)]	0 (0.0)	2 (1.6)	0 (0.0)	2 (1.8)
- Litter incidence [No. (%)]	0 (0.0)	1 (4.0)	0 (0.0)	2 (8.0)
- Affected foetuses/litter (Mean %)	0.0	1.6	0.0	1.8
Historical control data				
Foetuses (% Mean range)	0.0 - 4.3			
Litter (% Mean range)	0.0 - 16.7			
Affected foetuses/litter (% Mean range)	0.0 - 4.7			
- hole in cartilage between basisphenoid and basioccipital				
- Foetal incidence [No. (%)]	1 (0.9)	0 (0.0)	2 (1.8)	0 (0.0)
- Litter incidence [No. (%)]	1 (4.0)	0 (0.0)	2 (8.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.8	0.0	1.5	0.0
Historical control data				
Foetuses (% Mean range)	0.0 - 1.0			
Litter (% Mean range)	0.0 - 4.8			
Affected foetuses/litter (% Mean range)	0.0 - 1.0			
- bipartite processus xiphoideus				
- Foetal incidence [No. (%)]	52 (48)	61 (50)	65 (57)	49 (43)
- Litter incidence [No. (%)]	23 (92)	23 (92)	23 (92)	21 (84)
- Affected foetuses/litter (Mean %)	53.3	50.2	58.1	44.4
Historical control data				
Foetuses (% Mean range)	13.6 - 64.9			
Litter (% Mean range)	39.1 - 95.8			
Affected foetuses/litter (% Mean range)	12.2 - 64.5			
- branched rib cartilage				
- Foetal incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)
- Affected foetuses/litter (Mean %)	0.0	0.0	0.8	0.0
Historical control data				
Foetuses (% Mean range)	0.0 - 0.9			
Litter (% Mean range)	0.0 - 4.5			
Affected foetuses/litter (% Mean range)	0.0 - 1.0			

* $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's exact- or Wilcoxon-test, one-sided);
# only findings that are statistically significant different to the control are summarised

Additionally, some isolated cartilage findings without impact on the respective bony structures, which were designated as unclassified cartilage observations, occurred in all treatment groups including the controls. The observed unclassified cartilage findings were related to the skull, the ribs and the sternum and did not show any relation to dosing. The percentages of affected foetuses per litter are within the overall historical control range and did not show any relation to dosing. Thus, a toxicological relevance for these findings is not assumed.

3. Total malformations and variations

The overall assessment of the occurred foetal malformations after application of morpholine hydrochloride to pregnant rats during GD 6 -19 revealed no dose-response relationship observable if all the different types of malformations are summarised and the findings did not show a consistent pattern, since various morphological structures of different ontogenic origin were affected. Furthermore, the overall incidences were comparable to the historical control data. Therefore, these findings were considered to be of spontaneous origin and not induced by the treatment. No test substance-induced effects on foetal morphology occurred at the low-dose level (75 mg/kg bw/d).

Table 5.8.1-18: Total incidence of foetal malformations and variations

Dose level [mg/kg bw/d]	**0**	**75**	**250**	**750**
Litters Evaluated	25	25	25	25
Foetuses Evaluated	209	229	217	216
Live	209	229	217	216
Dead	0	0	0	0
Total foetal malformations				
- Foetal incidence [No. (%)]	2 (1.0)	0 (0.0)	4 (1.8)	2 (0.9)
- Litter incidence [a]	2 (8.0)	0 (0.0)	3 (12)	2 (8.0)
- Affected foetuses/litter (Mean %)	0.7	0.0	1.9	0.7
Historical control data				
Foetuses (% Mean range by study)	0.0 - 2.9			
Litter(% Mean range by study)	0.0 - 18.2			
Affected foetuses/litter (% Mean range by study)	0 0 - 3-0			
Total foetal variations				
- Foetal incidence [No. (%)]	114 (55)	131 (57)	115 (53)	121 (56)
- Litter incidence [No. (%)]	25 (100)	25 (100)	25 (100)	25 (100)
- Affected foetuses/litter (Mean %)	54.4	56.4	53.3	56.5
Historical control data				
Foetuses (% Mean range by study)	50.5 - 57.1			
Litter(% Mean range by study)	100.0 - 100.0			
Affected foetuses/litter (% Mean range by study)	49.8 - 58.2			

The overall assessment of the occurred foetal variations after application of morpholine hydrochloride to pregnant rats during GD 6 -19 revealed no dose-response relationship observable if all the different types of variations are summarised and the overall incidences were comparable to the historical control data. Therefore, these findings were considered to be of spontaneous origin and not induced by the treatment.

III. CONCLUSIONS

No treatment-related effects on the development parameters were observed in this study up to the highest dose tested. Therefore, the developmental NOAEL was determined to be at least 750 mg/kg bw/d.

Based on the liver effects of the dams received 250 and 750 mg/kg bw/d, evident by increased organ weights with corresponding clinic-chemistry findings (only at 750 mg/kg bw/d) and haematology findings (mild anaemia) as well as transient reduction of food consumption and body weight gain at 750 mg/kg bw/d, the maternal NOAEL was considered to be 75 mg/kg bw/d.

Additional information from the literature search:

Durotolu P.E. *et al.* Evaluation of precursors of N-nitrosamine in Wistar rat fed high fat diet. Biochem. 76 (2014) 28237-28240.

The objective of this work was to investigate the possible formation of the heterocycyclic Nnitrosomorpholine upon administration of nitrite and morpholine and also the significance of high fat diet on the metabolism of the nitrosamine, using spectrophotometric, cell fractionation, centrifugation, thin layer chromatography, UV irradiation and *in vitro* and *in vivo* methods. The possible endogenous formation of NMOR was detected in urine collected 24h after a combined oral administration of 20 mg nitrite and 40 mg of morpholine/kg through the process of thin layer chromatography. The retention factor (Rf) 0.664 and the purple chromatogram observed in the test urine using a detector NEDSA spray correlated with that of the standard thereby indicating the *in vivo* formation of nitrosamine. Also an *in vitro* study confirmed the formation of nitrosomorpholine at pH 7.4 in an appropriate phosphate buffer. The increase nitrite level after incubation of the liver microsomal fraction for 30min, arising from exposure to ultra violet light irradiation, confirmed the formation of nitrosomorpholine. The histopathological result of rat liver treated with concurrent administration of morpholine and NaNO2 revealed heavy haemorrhage, inflammatory cells, fat deposition and general cytolysis which confirm the toxicity of nitrosomorpholine on the liver. It is concluded that the combined administration of nitrite and morpholine produced a nitrosamine whose metabolism to toxic species in the liver tissue is attributed to the biochemistry and histopathological lesions enhanced by a high fat diet.

Engemann A. et al. Intestinal formation of N-nitroso compounds in the pig cecum model. J. Agric..Food Chem. 2013, 61, 998-1005.

N-Nitroso compounds (NOC) are a group of compounds including N-nitrosamines and N-nitrosamides, which are well-known for their carcinogenic, mutagenic, and teratogenic properties. Humans can be exposed to NOC through the diet and environmentally, or NOC can be formed endogenously in the stomach and intestine. In the intestine, the formation of NOC is supposed to be afforded by the gut microbiota. In this study, the formation of the N-nitrosamines, N-nitrosomorpholine (NMOR) and N-nitrosopyrrolidine (NPYR), and the N-nitrosamides, N-nitrosomethylurea (NMU) and N-nitrosoethylurea (NEU), was investigated in the pig cecum model after the incubation of the corresponding precursor amine or amide with nitrite or nitrate. Following the incubation with nitrate, the formation of NMOR, NPYR, NMU, and NEU was detectable with the microbiota being responsible for the reduction of nitrate to nitrite. After the incubation of nitrite a chemical formation of NOC was shown.

b. M550F003

Metabolite M550F003 is also named Z7.

Report: CA 5.8.1/24
[redacted] 1987 e
Acute oral toxicity with Z7 in rats
DK-470-009
Guidelines: OECD 401, EEC 84/449 B 1
GLP: no

Executive Summary

A single dose of 5000 mg/kg bw of Z7 (Metabolite of BAS 550 F (M550F003); batch: Y 200; purity: >99%) preparations in PEG 400 were administered to groups of 5 fasted male and 5 female animals by gavage. Animals were observed for 14 days. No mortality occurred in the administration groups. Accordingly, the oral LD_{50} was found to be greater than 5000 mg/kg bw:

Rat, oral: LD_{50} > 5000 mg/kg bw

Slight to moderate sedation, ruffled fur and dyspnea were observed after 1, 2, 3, and 5 h in male and female animals. In addition stiff gait and hunched posture were observed. All rats had recovered within 2 days after test article application. In each one male and female animal partly dark-red foci (1 mm) were observed in the lung. No abnormalities were observed in the remaining animals.
Under the conditions of this study the median lethal dose of Z7 after oral administration was found to be greater than 5000 mg/kg bw in rats.

DocID (DK-470-009)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Z7 (Metabolite of BAS 550 F)

Description:	Solid; powder/colorless
Lot/Batch #:	Y 200
Purity/content:	> 99%
Stability of test compound:	Stable for at least 6 months.

2. Vehicle: PEG 400

3. Test animals:

Species:	Rat
Strain:	Wistar / KFM-Han
Sex:	male/female
Age:	approximately 8 - 9 weeks
Weight at dosing (mean):	183 - 199 g (males), 158 – 180 g (females)
Source:	[redacted]
Acclimation period:	At least 7 days
Diet:	Pelleted standard Kliba 343, Batch 73/87 rat maintenance diet, Klingentalmuehle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Group housing (5 per cage) in Makrolon type III cages

Environmental conditions:

Temperature:	19 - 25 °C
Humidity:	40 - 70 %
Air changes:	10 - 15
Photo period:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Dates of work: 18-Aug-1987 - 01-Sep-1987

2. Test article preparation:

The test article was weighed and the vehicle was added. A weight by volume dilution was prepared using a homogenizer. Homogeneity was maintained during treatment using a magnetic stirrer. The preparation was made immediately prior to dosing.

3. Animal assignment and treatment:

The animals received the test article on a mg/kg bw base by oral gavage after being fasted for 12 to 18 hours (access to water was not interrupted). Food was again presented approximately one hour after dosing. A limit test was performed dosing 5 male and 5 female animals with a dose of 5000 mg/kg bw with an application volume of 20 mL/kg bw. Each animal was examined for changes in appearance and behavior four times during day 1, and daily during days 2-15. All abnormalities were recorded. Necropsy of all animals was performed at the end of the observation period. The animals were killed by intraperitoneal injection of sodium pentobarbitone.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the administration groups.

B. CLINICAL OBSERVATIONS

Slight to moderate sedation, ruffled fur and dyspnea were observed after 1, 2, 3, and 5 h in male and female animals. In addition stiff gait and hunched posture were observed. All rats had recovered within 2 days after test article application.

C. BODY WEIGHT

The mean body weights of the administration groups increased throughout the study period.

D. NECROPSY

In each one male and female animal partly dark-red foci (1 mm) were observed in the lung. No abnormalities were observed in the remaining animals.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats for Z7 (Metabolite of BAS 550 F) was determined to be greater than 5000 mg/kg bw.

Report: CA 5.8.1/25
[redacted] 1989 c
4-chloro-3,4-dimethoxybenzophenone: Acute oral and dermal toxicity, skin and eye irritancy and skin sensitising potential
DK-470-011
Guidelines: EEC 67/548, EEC 84/449 B 1, EEC 84/449 B 3, EEC 84/449 B4, EEC 84/449 B 6, EEC 84/449 B5
GLP: yes

Acute oral toxicity

Executive Summary

A single dose of 5000 mg test substance Z7 (in Corn oil) per kg bodyweight was applied to 5 male and 5 female Fischer 344 rats via gavage. Animals were observed for clinical signs various times at the first three days and at least once daily thereafter for a total of 14 days. No animal died during the study period or was found in a moribund state. Accordingly, the oral LD_{50} was found to be greater than 5000 mg/kg bw for males and females, respectively:

Rat, oral: LD_{50} > 5000 mg/kg bw

Except for unkempt appearance at Day 2 no further clinical signs were observed. The mean body weights of the administration groups increased throughout the study period. The available data on acute oral toxicity of the test substance do not meet the criteria for classification according to Regulation EC Directive on dangerous preparations 1999/45/EC and Regulation (EC) No 1272/2008. Classification for acute oral toxicity is therefore not warranted.

(BASF DocID DK-470-011)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - Metabolite of BAS 550 F)

Description:	Solid, buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability	Infra-red spectroscopic fingerprint analysis revealed stability throughout the study period. Test substance formulation in corn oil was checked for stability by means of GC. Stability was confirmed for at least 24 h.

2. Vehicle: Corn oil

3. Test animals:

Species:	Rats
Strain:	Fischer 344
Sex:	male/female
Age:	8-9 weeks
Weight at dosing (mean):	200-212 g (males), 127-141 g (females)
Source:	[redacted].
Acclimation period:	at least 5 days
Diet:	Pelleted diet (PRD, Labsure Animal Foods, Dorset), ad libitum
Water:	Tap water, ad libitum
Housing:	Up to four animals (single sex groups) in cages with stainless-steel wire-mesh walls, floors and tops

Environmental conditions:

Temperature:	19-23°C
Humidity:	30-70%
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 04-Apr-1989 - 18-Apr-1989 (Main study - Experimental period)

2. Animal assignment and treatment:

In a preliminary test groups of one male and one female rat were treated with 560, 1744, and 4248 mg/kg bw test substance by gavage.
In the main test, one group of 5 male and 5 female rats was fasted overnight, weighed and given a single dose of the test substance by gavage. The animals received a single dose of 5000 mg/kg bw test substance in corn oil (10 mL/kg). Three hours after dosing on Day 1 the animals had access to food again.
Clinical signs and symptoms were recorded three times daily for the first three days and once daily for the individual animals up to 14 days post-administration. Individual body weights were determined shortly before administration, weekly thereafter and at the end of the study. Necropsy was performed with all animals on Day 14. The animals were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the range-finding assay when one animal of each sex was treated with 560, 1744, and 4248 mg/kg bw. In the main study no mortality occurred at the limit dose of 5000 mg/kg bw.

B. CLINICAL OBSERVATIONS

The majority of rat appeared unkempt on Day 2. There were no other reactions to treatment and all rats were overtly normal from Day 5.

C. BODY WEIGHT

The mean body weights of the administration groups increased throughout the study period. At Day 1 the body weights were 200-215 g for males and 127-141 g for females. The body weight change for males was 28-35 g (Day 7) and 42-62 g (Day 14). For females the body weight change was 15-23 g (Day 7) and 24-35 g (Day 14).

C. Necropsy

No macroscopic abnormalities were found in the ten rats subjected to necropsy on the fourteenth day of the study.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats was determined to be greater than 5000 mg/kg bw for males and females.

Acute dermal toxicity

Executive Summary

In an acute dermal toxicity study groups of 5 male and 5 female Fischer 344 rats were exposed to a single dose of 2000 mg/kg bw of undiluted 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - batch KSLA No. 9.0574) to the clipped skin under occlusive conditions for 24 hours. The animals were observed for 14 days after administration. Based on the absence of mortality in this study the acute dermal LD_{50} was determined to be greater than 2000 mg/kg bw:

Rat dermal: LD_{50} > 2000 mg/kg bw

The mean body weights of the animals increased as throughout the study period. There were no clinical signs of systemic reaction to treatment. Sites of application of the test material showed slight erythema on Day 2 in one male and one female rat. There were no other dermal changes or irritation reactions. No macroscopic abnormalities were found in the ten rats subjected to necropsy on the fourteenth day of the study.
The available data on acute dermal toxicity of the test substance do not meet the criteria for classification according to Regulation EC Directive on dangerous preparations 1999/45/EC and Regulation (EC) No 1272/2008. Classification for acute dermal toxicity is therefore not warranted.

(BASF DocID DK-470-011)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - Metabolite of BAS 550 F)

Description:	Solid, buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability:	Infra-red spectroscopic fingerprint analysis revealed stability throughout the study period.

2. Vehicle: The test substance was administered undiluted.

3. Test animals:

Species:	Rats
Strain:	Fischer 344
Sex:	male/female
Age:	8-9 weeks
Weight at dosing (mean):	200-212 g (males), 127-141 g (females)
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	Pelleted diet (PRD, Labsure Animal Foods, Dorset), ad libitum
Water:	Tap water, ad libitum
Housing:	Up to four animals (single sex groups) in cages with stainless-steel wire-mesh walls, floors and tops. Single housing during 24 h administration period.

Environmental conditions:

Temperature:	19-23°C
Humidity :	30-70%
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 04-Apr-1989 - 18-Apr-1989 (Main study - Experimental period)

2. Animal assignment and treatment:

In a range-finding study one animal of each sex was treated with 1500 mg test substance per kg bodyweight. In the main study five male and five female rats were treated with 2000 mg test substance per kg bodyweight for 24 h under occlusive conditions. The day before dosing the animals were weighed and the dorsal hair was shorn with fine electric clippers. The test material was moistened with water and held in place with a lint dressing covered with waterproof adhesive tape. At the end of the treatment the dressing was removed and the skin washed with warm dilute detergent solution and then dried. Clinical signs and symptoms were recorded three times daily for the first three days and once daily for the individual animals up to 14 days post-administration. Individual body weights were determined shortly before administration, weekly thereafter and at the end of the study. Necropsy was performed with all animals on Day 14. The animals were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the range-finding assay when one animal of each sex was treated with 1500 mg/kg bw. In the main study no mortality occurred at the limit dose of 2000 mg/kg bw.

B. CLINICAL OBSERVATIONS

There were no clinical signs of systemic reaction to treatment. Sites of application of the test material showed slight erythema on Day 2 in one male and one female rat. There were no other dermal changes or irritation reactions.

C. BODY WEIGHT

All animals had gained weight relative to their initial bodyweights by the end of the 14 day observation period.

C. Necropsy

No macroscopic abnormalities were found in the ten rats subjected to necropsy on the fourteenth day of the study.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats was determined to be greater than 2000 mg/kg bw for males and females, respectively.

Acute dermal irritation

Executive Summary

In an acute dermal irritation study, the skin irritation/corrosion potential of 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - batch KSLA No. 9.0574, purity: 99%) was tested. The intact skin of three male and three female New Zealand White rabbits was exposed to 0.5 g of the unchanged test substance for 4 hours covered with a semi-occlusive dressing. The cutaneous reactions were assessed 30 min, 24, 48 and 72 hours after removal of the patch and then in weekly intervals until day 7 after treatment.
No erythema and no oedema reactions were observed in any animal. Mean scores over 24, 48 and 72 hours for each animal were within 0.0 for erythema and oedema, respectively. In conclusion, 4-Chloro-3',4'-Dimethoxy Benzophenone showed no skin irritation potential to rabbits under the test conditions chosen.

(BASF DocID DK-470-011)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - Metabolite of BAS 550 F)
Description:	Solid, buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability	Infra-red spectroscopic fingerprint analysis revealed stability throughout the study period.
2. Vehicle:	The test substance was administered unchanged.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white
Sex:	male / female
Age:	3-5 months
Weight at dosing:	males: 4.27 – 4.40 kg, females: 3.51 – 3.69 kg
Source:	[redacted].
Acclimation period:	14 days
Diet:	Pelleted diet (Standard Rabbit Diet, S.Q.C., Special Diet Services Ltd.), ad libitum
Water:	Tap water (filtered), ad libitum
Housing:	Single housing in stainless steel wire mesh cages
Environmental conditions:	
Temperature:	15 - 20 °C
Humidity:	30-70%
Air changes:	No data
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 10-Apr-1989 - 18-Apr-1989 (Experimental period)

2. In-vitro pre-test: No *in vitro* pre-test was conducted.

3. Animal assignment and treatment:

The potential of 4-Chloro-3',4'-Dimethoxy Benzophenone to cause acute dermal irritation or corrosion was assessed by a single topical application of 0.5 g of the moistened test substance for 4 hours to the clipped skin of three male and female New Zealand White rabbits using a patch of 2.5 cm x 2.5 cm. The test substance was covered with gauze and held in place by a semi-occlusive elastic adhesive bandage. After 4 hours the dressings were removed, the skin washed with water and dried.
After treatment the animals were examined for erythema, oedema, and other lesions. The mean scores at each time point and group mean scores at 24, 48, and 72 h were calculated.

II. RESULTS AND DISCUSSION

Application of 500 mg 4-Chloro-3',4'-Dimethoxy Benzophenone to the clipped dorsal skin of six rabbits failed to elicit any dermal changes or irritation reactions. Therefore, mean values for erythema and oedema were 0.0 for all animals at any time point.

III. CONCLUSION

Based on the findings of this study, 4-Chloro-3',4'-Dimethoxy Benzophenone showed no skin irritation potential to rabbits under the test conditions chosen.

Eye irritation

Executive Summary

In an eye irritation study, the eye irritation/corrosion potential of 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - batch KSLA No. 9.0574, purity: 99%) was determined by instillation of 100 mg of the unchanged test substance into the conjunctival sac of one eye of six New Zealand White rabbits. No washing was performed. The ocular reactions were assessed approximately 1, 4, 24, 48 and 72 hours and 7 days after the administration of the test substance.
Injection of the conjunctival blood vasculature was apparent in five rabbits one hour and four hours after treatment. No other ocular changes or irritation reactions were observed during the 7-day observation period. The individual mean scores calculated over 24, 48 and 72 hours were 0.0 for corneal opacity, iris lesions, conjunctival redness and chemosis. Based on the findings of this study, 4-Chloro-3',4'-Dimethoxy Benzophenone is not irritating to the eye of rabbits under the test conditions chosen.

(BASF DocID DK-470-011)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - Metabolite of BAS 550 F)
Description:	Solid, buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability	Infra-red spectroscopic fingerprint analysis revealed stability throughout the study period.
2. Vehicle:	The test substance was administered undiluted.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white
Sex:	male / female
Age:	3-5 months
Weight at dosing:	males: 3.90 – 4.46 kg, females: 4.54 – 4.84 kg
Source:	[redacted]
Acclimation period:	14 days
Diet:	Pelleted diet (Standard Rabbit Diet, S.Q.C., Special Diet Services Ltd.), ad libitum
Water:	Tap water (filtered), ad libitum
Housing:	Single housing in stainless steel wire mesh cages
Environmental conditions:	
Temperature:	15 - 20 °C
Humidity:	30-70%
Air changes:	No data
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 17-Apr-1989 - 02-May-1989 (Experimental period)

2. In-vitro pre-test: No *in vitro* pre-test was conducted.

3. Animal assignment and treatment:

The potential of 4-Chloro-3',4'-Dimethoxy Benzophenone to cause acute eye irritation/corrosion was assessed by instillation of 70 mg (0.1 mL bulk volume) of the undiluted test substance into the conjunctival sac of one eye. The lids were held together for a few seconds to prevent loss of material. No washing was performed. The immediate reactions of the rabbits were scored as an initial pain response using a six point scale. The ocular reactions were assessed approximately 1, 4, 24, 48 and 72 hours and 7 and 14 days after dosing. The individual and group mean scores at 24, 48, and 72 h were calculated.

II. RESULTS AND DISCUSSION

The instillation of undiluted 4-Chloro-3',4'-Dimethoxy Benzophenone into the conjunctival sac of one eye of each of six rabbits resulted in no initial pain response. Injection of the conjunctival blood vasculature was apparent in five rabbits one hour and four hours after treatment. No other ocular changes or irritation reactions were observed during the 7-day observation period. The individual mean scores calculated over 24, 48 and 72 hours were 0.0 for corneal opacity, iris lesions, conjunctival redness and chemosis.
For details regarding the individual and mean scores as well as additional findings see Table Table 5.8.1-19.

Table 5.8.1-19: Individual and mean eye irritation scores after ocular application of 4-Chloro-3',4'-Dimethoxy Benzophenone

Readings	Animal	Cornea	Iris	Conjunctiva		Additional findings
		Opacity		Redness	Chemosis	
1 h	01F	0	0	1	0	
	02F	0	0	1	0	
	03F	0	0	1	0	
	04M	0	0	1	0	
	05M	0	0	0	0	Injection of the conjunctival blood vasculature was observed in 5/6 rabbits.
	06M	0	0	1	0	
4 h	01	0	0	1	0	
	02	0	0	1	0	
	03	0	0	1	0	
	04	0	0	1	0	
	05	0	0	0	0	
	06	0	0	1	0	
24 h	01	0	0	0	0	-
	02	0	0	0	0	-
	03	0	0	0	0	-
	04	0	0	0	0	-
	05	0	0	0	0	-
	06	0	0	0	0	-
48 h	01	0	0	0	0	-
	02	0	0	0	0	-
	03	0	0	0	0	-
	04	0	0	0	0	-
	05	0	0	0	0	-
	06	0	0	0	0	-
72 d	01	0	0	0	0	-
	02	0	0	0	0	-
	03	0	0	0	0	-
	04	0	0	0	0	-
	05	0	0	0	0	-
	06	0	0	0	0	-
Mean 24 - 72 h	01	0.0	0.0	0.0	0.0	
	02	0.0	0.0	0.0	0.0	
	03	0.0	0.0	0.0	0.0	
	04	0.0	0.0	0.0	0.0	
	05	0.0	0.0	0.0	0.0	
	06	0.0	0.0	0.0	0.0	
Mean		0.0	0.0	0.0	0.0	

III. CONCLUSION

Based on the findings of this study, 4-Chloro-3',4'-Dimethoxy Benzophenone is not irritating to the eye of rabbits under the test conditions chosen. The available data on acute eye irritation of the test substance do not meet the criteria for classification according to Regulation EC Directive on dangerous preparations 1999/45/EC and Regulation (EC) No 1272/2008. Classification for acute eye irritation is therefore not warranted.

Skin sensitization

Executive Summary

For the determination of potential sensitizing properties of 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - batch: KSLA No. 9.0574, purity: 99%) a maximization test in Dunkin-Hartley guinea pigs was conducted. Based on the results of a pre-test, the intradermal induction was performed with a 0.5% test item preparation in corn oil into the neck region of the animals. The epicutaneous induction (7 days after intradermal induction) and the challenge exposure (14 days after epicutaneous induction) were performed with a 75% test item preparation in vaseline. The study was performed in 5 control and 10 test group animals per sex. Control group animals were treated with the same injection scheme as the test group animals but replacing the test item by the vehicle. Regarding epicutaneous induction, the control group animals were treated with the vehicle. 14 days after the last induction, the challenge was carried out. 0.15 mL of the 75% test substance preparation was applied for 24 hours to the intact skin of the flank under occlusive conditions. 24 and 48 hours after removal of the patch, skin readings were performed. No positive control was included in the study and no information regarding a periodic reliability check of the laboratory was given.
The intradermal induction with 0.5% test substance preparation caused positive reactions in all test group animals of the pre-test. No reactions were observed after epidermal induction with 75% test substance preparation in the animals during the pretest. No data were given for the animals of the main test. The challenge revealed no reactions in any animal of the control or test group, indicating that 4-Chloro-3',4'-Dimethoxy Benzophenone has no skin sensitizing properties in the guinea pig maximization test under the conditions applied.

(BASF DocID DK-470-011)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-Chloro-3',4'-Dimethoxy Benzophenone (Z7 - Metabolite of BAS 550 F)

Description:	Solid, buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability	Infra-red spectroscopic fingerprint analysis revealed stability throughout the study period.

2. Vehicle / Positive control: Vehicles: corn oil (intradermal application), Vaseline (topical application)
Positive control: not available

3. Test animals:

Species:	Guinea Pig
Strain:	Dunkin-Hartley
Sex:	male/female
Age:	5 - 9 weeks (at receipt)
Weight at dosing:	males: 586-693 g, females: 483-577 g
Source:	[redacted]
Acclimation period:	at least 14 days
Diet:	SG1 with vitamin C supplement, Grain Harvesters Ltd., Kent, UK, ad libitum
Water:	Tap water (filtered), ad libitum
Housing:	Groups of 2-3 animals were housed in stainless steel wire mesh cages with a floor area of approximately 1674 cm²

Environmental conditions:

Temperature:	15 - 20 °C
Humidity:	30-70%
Air changes:	No data
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 22-Mar-1989 - 14-Apr-1989 (Main study - Experimental period)

2. Animal assignment and treatment:
The skin sensitizing potential of 4-Chloro-3',4'-Dimethoxy Benzophenone was assessed using the Maximization Test based on the method of Magnusson and Kligman. For this, male and female guinea pigs were randomly allocated to groups. Five animals per sex were used as control group animals and 10 animals per sex in the test group. Based on the results of a pre-test, animals were intradermally induced with 0.5% test substance preparations. Epidermal induction and challenge were conducted with 75% test substance preparations. The animals were closely shorn in the shoulder region using electric clippers followed by an electric razor; two rows of three injections were made, one on each side of the midline.

3. Clinical observation:
The animals were observed for one day during the intradermal range finding test on general toxicity. No information is available from the main test.

4. Body weights:
Individual body weights were determined at the beginning and the end of the study.

5. Pre-test:
100 µL of several dilutions of the test substance (0.05, 0.1, 0.5, and 1.0% in corn oil) were injected intradermally to 2 male and 2 female animals. The animals were examined on the following day to determine the maximum concentration that could be used without causing untoward toxicity. Furthermore, 0.45 mL of several dilutions of the test material (10, 25, 50, and 75%) were absorbed onto 16 cm^2 Whatman No. 3 filter paper patches and applied to the skin of the shaved flanks, covered by occlusive tape, and retained by an elastic adhesive bandage for 24 h. After removal of the patches and bandages the dermal test sites were examined for signs of irritation. A formulation incorporating the maximum possible concentration of test material was selected for topical induction and challenge, as that formulation proved to be non-irritant in the range-finding tests.

6. Main study – intradermal induction:
Based on the results of the pretest, test group animals received intradermal injections of 0.5% test substance preparations. A 0.5% test substance preparation (TS in corn oil) was intradermally injected to ten animals per sex. 6 intradermal injections were applied at the neck region of each animal: front row: 2 injections each of 0.1 mL Freund´s complete adjuvant without test item; middle row: 2 injections each of 0.1 mL of a test item preparation in vehicle at the selected concentration; back row: 2 injections each of 0.1 mL Freund's complete adjuvant / vehicle (1:1) with test item at the selected concentration. Control group animals received the same injections but with the test substance preparation being replaced by the vehicle.

7. Main study – epicutaneous induction:
One week after intradermal induction, 0.45 mL of the 75% test item preparation (in vaseline) was applied to each test group animal under the same conditions as described in the epidermal pretest but for 48 h. The control animals were treated similarly with vehicle only.

8. Main study - challenge:
The challenge was carried out 14 days after the epicutaneous induction. 0.15 mL of the 75% test item preparation (in Vaseline) was applied to the test and control group animal. The animals were exposed under occlusive conditions as described above for 24 hours and skin readings were performed 24 and 48 h after removal of the patch.

9. Evaluation of results
The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitization rate.

10. Positive controls
No information on positive controls was given.

II. RESULTS AND DISCUSSION

A. PRE-TEST

Injections of 0.05 and 0.1% test substance preparations in corn oil caused a positive response in each 2 male and female animals (grade 1). Injections of 0.5% test substance preparations in corn oil also caused a positive response (grade 1-2) in each 2 male and female animals. Injection of 1% substance preparation also caused positive responses in all animals (grade 2). After topical induction no response was observed after treatment with 10, 25, 50, and 75% test substance preparation in vaseline.

B. OBSERVATIONS

No abnormalities were observed during general observation.

C. BODY WEIGHTS

Body weights at start of treatment were within 586-693 g in the male animals and within 483-577 g in the female animals. Body weight gain was observed in any animal throughout the study period.

D. SKIN REACTIONS AFTER INTRADERMAL INDUCTION

According to the results from the pretest, intradermal induction with 0.5% test substance preparation in corn oil leads to a positive response. No details from the main study regarding intradermal induction were available.

E. SKIN REACTIONS AFTER EPICUTANEOUS INDUCTION

According to the results from the pretest, topical application of 75% test substance preparation in petroleum jelly leads to no dermal response. No details from the main study regarding topical induction were available.

F. SKIN REACTIONS AFTER CHALLENGE

The challenge with a 75% test substance preparation in vaseline did not cause any skin reactions in animals of the control group and test group 24 and 48 hours after removal of the patch (see Table 5.8.1-20). Since no borderline results were observed, a 2nd challenge was not performed.

Table 5.8.1-20: Chloro-3',4'-Dimethoxy Benzophenone - Skin reactions after challenge

	Challenge			
Skin findings	**Control group**		**Test group**	
	24 h	**48 h**	**24 h**	**48 h**
Grade 0	10/10#	10/10	20/20	20/20

x/y = number of findings / number of animals tested

G. POSITIVE CONTROL

No information regarding positive control data was given.

III. CONCLUSION

Based on the results of this study it is concluded that 4-Chloro-3',4'-Dimethoxy Benzophenone does not have sensitizing properties in the guinea pig maximization test under the test conditions chosen. 0% of the animals were considered positive after challenge application.

Report: CA 5.8.1/26
[redacted] 1990 c
4-chloro-3,4-dimethoxybenzophenone: A 28 day oral toxicity study in Fischer 344 rats
DK-470-014
Guidelines: EEC 67/548, EEC 84/449 A 5
GLP: yes

Executive Summary

4-chloro-3',4'-dimethoxybenzophenone (Z7 - Batch: KSLA No. 9.0574; Purity: 99%) was administered by gavage to 7 male and female Fischer 344 rats each at concentrations of 0, 15, 60, 250, and 1000 mg/kg bw/day in corn oil for 28 days. Food consumption and body weight were measured weekly and the animals were observed daily for clinical signs of toxicity. After completion of treatment the animals were killed and subjected to gross necropsy, clinical chemistry, haematological and histopathological examinations.
The sole reaction to treatment was staining of the ano-genital zone in high dosed females. Haematology findings for platelet count and plateletcrit were evaluated as incidental. Disturbances of blood chemistry (bilirubin, triglycerides, creatinine, urea nitrogen, cholesterol, protein, ALT, γ-GT, calcium) were considered as mild treatment-related effects. Regarding pathology, treatment-related findings were noted for the liver, kidneys, and caecum including amongst others enlargement of the organs. Only in the kidney histopathological correlates were found including moderate proximal tubular degenerative and regenerative lesions in the renal cortex of rats of both sexes at the high dose level and proximal tubular regenerative foci at 250 (only females) and 1000 mg/kg bw/day. Based on the changes of the liver, kidneys and caecum and the histopathological findings in the kidney that were observed at 250 and 1000 mg/kg bw/day a no observed adverse effect level (NOAEL) was set at 60 mg/kg bw/day for both sexes under the conditions of the present study.

(BASF DocID DK-470-014)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-chloro-3', 4'-dimethoxybenzophenone (Z7 - Metabolite of BAS 550 F)

Description:	Solid / buff colored
Lot/Batch #:	KSLA No. 9.0574
Purity:	99%
Stability of test compound:	The stability of the test substance under storage conditions and the formulations over the test period was demonstrated by analytical measurements.

2. Vehicle and/or positive control: corn oil

3. Test animals:

Species:	Rat
Strain:	Fischer 344
Sex:	Male and female
Age:	6 – 8 weeks at start of administration
Weight at dosing start:	male: 131 – 139 g, female: 112 – 118 g
Source:	[redacted]
Acclimation period:	10 days
Diet:	Powdered diet (LAD 2), ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Single housing in polypropylene cages (floor area: 35x24x16 cm)
Environmental conditions:	
Temperature:	19 - 23 °C
Humidity:	40 - 70 %
Air changes:	NA
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 19-Sep-1989 to 18-Oct-1989

2. Animal assignment and treatment:

4-chloro-3', 4'-dimethoxybenzophenone was administered orally by gavage to groups of 7 male and 7 female rats at doses of 0, 15, 60, 250, and 1000 mg/kg bw/d over a period of 28 days. The dosing volume was 5 mL/kg bw. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation, preparation frequency and analyses of the test-substance preparations:

4-chloro-3', 4'-dimethoxybenzophenone was formulated as a suspension in corn oil. The test item was incorporated in the test formulations at concentrations of 3, 12, 50, and 200 mg/mL. Fresh formulations were prepared daily throughout the study. Before dosing commenced each dose container was inverted (10x) and during dosing the contents of the container were mixed continuously by magnetic stirring device.

The stability of 4-chloro-3', 4'-dimethoxybenzophenone was proven during the study. Homogeneity and concentration control of 4-chloro-3', 4'-dimethoxybenzophenone was verified in samples of all concentrations.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations and pathology

Parameters	**Statistical test**	**Markers in the tables**
All variates except those listed under Wilcoxon´s two sample rank sum test.	A two-way analysis of variance with treatment and block as factor was used for all variates except those analysed by Wilcoxon´s two sample rank sum test. Following analysis of variance, differences between the control and treated group means were assessed for significance using Williams' t-test. On occasions where a monotonic response could not be assumed, Dunnett´s test was used.	* for $p \leq 0.05$ ** for $p \leq 0.01$
Body weight	Body weights were analysed with initial bodyweight as a covariate. Where the covariance relationship was significant ($p<0.05$), the corresponding means were reported as adjusted for initial bodyweight; otherwise means unadjusted for this covariate were reported.	* for $p \leq 0.05$ ** for $p \leq 0.01$
Organ weight	In order to adjust for differences in terminal body weight, organ weights were also considered with terminal bodyweight as a covariate. Means both adjusted and unadjusted for terminal bodyweight were reported.	* for $p \leq 0.05$ ** for $p \leq 0.01$
Non-segmented polymorphic neutrophils, atypical lymphocytes and gamma glutamyl transpeptidase (females only)	Wilcoxon´s two sample rank sum test.	* for $p \leq 0.05$ ** for $p \leq 0.01$
Microscopic abnormalities	Fisher´s exact test for pairwise comparison.	* for $p \leq 0.05$ ** for $p \leq 0.01$

C. METHODS

1. Observations:
A check for moribund and dead animals was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. Each animal was checked for changes of skin, fur, eyes and visible mucous membranes, for abnormal behavior, for disturbances of major body systems and for gross disturbance of food or water consumption.

2. Body weight:
Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on Day 1, 8, 15, 22 and either day 28 (males) or Day 29 (females).

3. Food consumption:
Food consumption was determined weekly over a period of 7 days.

4. Water consumption:
Measurement of water consumption was introduced from Day 21.

5. Ophthalmoscopy:
Not performed in this study.

6. Hematology and clinical chemistry:
Blood samples were taken from each rat at terminal necropsy by cardiac puncture. The blood was held in tubes containing EDTA (Hematology) or lithium heparin (Clinical Chemistry).

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:			
	Red blood cells		*White blood cells*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)
✓	Hemoglobin (Hb)	✓	Differential blood count
✓	Hematocrit (Hct)	✓	Platelet count (PLT)
✓	Mean corp. volume (MCV)	✓	Platelet volume
✓	Mean corp. hemoglobin (MCH)	✓	Plateletcrit ratio
✓	Red cell distribution width	✓	Platelet distribution width

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride			✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Creatinine		
		✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

7. Sacrifice and pathology:
The animals were sacrificed by intraperitoneal injection of a 20% solution of sodium pentobarbitone. All rats were subjected to a detailed post-mortem examination. This included physical examination of the external body surface, all orifices, the cranial cavity, the meningeal surface of the brain, the thoracic, abdominal and pelvic cavities with their associated organs and tissues, the neck and associated organs and tissues and the carcass. All macroscopic abnormalities observed at necropsy were recorded. Terminal body weights and weights of the spleen, heart, kidney, liver, testes, and adrenals were recorded. A bone marrow smear was prepared from each rat, fixed in methanol and stored.
Samples of the adrenals, heart, kidneys, liver, ovaries/testes, spleen and macroscopic abnormalities were taken from all rats at necropsy and fixed in neutral buffered formalin. All tissues retained in the fixative were embedded in paraffin wax, sectioned at 5 µm thickness and stained with haematoxylin and eosin. The histological specimens of macroscopically abnormal tissues found during necropsy and all tissues taken from rats of the control group and those treated at 1000 mg/kg bw/day were assessed by the pathologist. This established the kidneys as a target organ. In order to determine the no effect level, micropathological assessment was extended to include the kidneys of all rats dosed at 60 and 250 mg/kg bw/day.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES
Infra-red spectra taken before and after the study confirmed the stability of the test material. The measured concentrations of the test item in paired samples of administered formulations were found to be in the range 94% to 97% of nominal concentration. The homogeneity of the suspensions and the chemical stability of the test material in the chosen vehicle were demonstrated to be satisfactory.

B. OBSERVATIONS

1. Clinical signs of toxicity
Slight staining of the ano-genital zone was observed on single female rats dosed at 15 and 250 mg/kg bw/day and on all females at the high dose for various periods commencing during the second or third week of the study. All females dosed at 1000 mg/kg bw/day and a single female treated at 250 mg/kg bw/day also showed staining (yellow) of the ano-genital zone on the last day of the study.
Animal technicians visually assessing water consumption considered there to be a treatment-related effect on this parameter. Accordingly, measurement of water consumption during one week confirmed that the water consumption of male and female rats treated with 1000 mg/kg bw/day was slightly greater (20-30%) than that of the control rats or other treated groups. There were no other clinical signs of reaction to oral administration of the test item.

2. Mortality
No mortality was observed throughout the study.

C. BODY WEIGHT AND FOOD CONSUMPTION

Statistically reduced food consumption was observed in the 15 mg/kg bw/day dose group during week 1, but was considered to be too small to be of biological relevance. There were no treatment-related effects upon bodyweight or food consumption [see Table 5.8.1-21]

Table 5.8.1-21: Mean body weight of rats administered 4-chloro-3', 4'-dimethoxybenzophenone for 28 days

	Males					Females				
Dose level [mg/kg bw/d]	0	15	60	250	1000	0	15	60	250	1000
Body weight [g]										
- Day 0	139	131	138	135	138	114	112	115	118	116
- Day 28	217	214	214	228	224	157	153	157	160	154
Overall body weight gain [g]	78	83	76	93	86	43	41	42	42	38

D. BLOOD ANALYSIS

1. Hematological and clinical chemistry findings

A reduction of mean platelet volume in males of all dose groups and of mean corpuscular haemoglobin in all treated female groups and the apparent increase of standard deviation of mean erythrocyte diameter in females of the high dose group caused statistically significant differences from control values, however, each was considered to constitute a minor spontaneous variation of no biological significance. In males a slight reduction of bilirubin at 250 and 1000 mg/kg bw/day and triglycerides at 1000 mg/kg bw/day and an increase of cholesterol in rats at 60 mg/kg bw/day were observed. Due to the absence of changed cholesterol levels in the higher dose groups this effect was considered to be incidental. Females of the high dose group showed statistically significant increases of creatinine, urea nitrogen, gamma glutamyl transpeptidase, calcium, albumin, total protein, cholesterol, alanine aminotransferase, and bilirubin as well as a reduction of chloride levels. These changes were reflected by increases of total protein, albumin and bilirubin in females of the 250 mg/kg bw/day dose group. None of the disturbances of haematological or blood chemical parameters were considered to constitute more than mild treatment-related effects.

Table 5.8.1-22: Selected haematology parameters of rats administered 4-chloro-3', 4'-dimethoxybenzophenone for 28 days

Sex		Males	Females
Parameter	Dose [mg/kg bw/d]		
Platelets [10^9/L]	0	728	793
	15	740	785
	60	760	783
	250	788	803
	1000	831**	800
Mean Platelet Volume [FL]	0	8.0	7.9
	15	7.9	8.0
	60	7.8*	7.8
	250	7.9*	8.0
	1000	7.9*	7.8
Plateletcrit	0	0.586	0.630
	15	0.586	0.627
	60	0.596	0.613
	250	0.623	0.642
	1000	0.656*	0.625

Table 5.8.1-23: Selected clinical chemistry parameters of rats administered 4-chloro-3', 4'-dimethoxybenzophenone for 28 days

Sex		Males	Females
Parameter	Dose [mg/kg bw/d]		
Bilirubin [µnol/L]	0	2.5	2.1
	15	2.3	2.3
	60	2.3	2.2
	250	2.1*	2.9*
	1000	2.0**	2.6*
Protein [g/L]	0	64.2	58.5
	15	62.2	58.3
	60	64.7	58.5
	250	64.7	60.5*
	1000	66.2	63.2**
Urea [mmol/L]	0	5.5	5.2
	15	5.3	5.9
	60	5.0	5.9
	250	5.9	5.5
	1000	5.8	6.5**
ALT [IU/L]	0	102.8	38.5
	15	48.9	38.8
	60	36.4	38.8
	250	44.9	46.3
	1000	60.4	51.5*
γ-GT	0	0.0	0.1
	15	0.0	0.0
	60	0.0	0.1
	250	0.0	0.4
	1000	0.0	0.5**

E. NECROPSY

1. Organ weight

There were no statistically significant differences from control values for group mean absolute weights of the spleen or testes among the groups treated with the test item. The dose-related increase of absolute weight of the liver of rats dosed at 250 or 100 mg/kg bw/day did not exceed 25% of control values but were statistically significant. Analysis of the effect of treatment was similar after adjustment of liver weight for terminal bodyweight. Absolute kidney weights of male rats dosed at 250 or 1000 mg/kg bw/day were slightly but statistically significantly greater than those of male controls. After adjustment for terminal bodyweight, statistically significant differences were apparent between male control values and those of males treated with 60, 250, and 1000 mg/kg bw/day. However, the magnitude of these differences was small and it was considered unlikely that the apparent increase of kidney weight for rats treated at 60 mg/kg bw/day was of any biological significance. The kidneys of female rats showed no treatment-related effect on organ weight. After exclusion of extreme individual values for heart and adrenals, the adrenal weights of male rats dosed at 250 or 1000 mg/kg bw/day were greater than those of corresponding control values and male heart weight, after adjustment for terminal bodyweight, was slightly less among rats treated at the high dose level than for male controls. However after consideration of the natural variation of individual values for these parameters, the differences from control values were considered insufficient to be attributable to an effect of treatment (see Table 5.8.1-23)

Table 5.8.1-24: Selected mean absolute and relative organ weights of rats administered 4-chloro-3', 4'-dimethoxybenzophenone for 28 days

Sex		Males		Females	
Organ weight [g]	Dose [mg/kg bw/d]	Absolute	Relative [%]	Absolute	Relative [%]
Kidney	0	1.35	1.36	1.05	1.04
	15	1.35	1.38	0.99	1.01
	60	1.38	1.41*	1.05	1.03
	250	1.52*	1.45**	1.07	1.05
	1000	1.55**	1.54**	1.06	1.08
Liver	0	6.62	6.73	4.38	4.35
	15	6.65	6.86	4.25	4.36
	60	6.80	6.97	4.49	4.42
	250	7.65*	7.26**	4.69*	4.56*
	1000	8.18**	8.10**	5.03**	5.14**
Adrenals	0	0.039	0.039	0.052	0.052
	15	0.038	0.038	0.048	0.048
	60	0.040	0.040	0.048	0.048
	250	0.041*	0.041	0.047	0.047
	1000	0.045**	0.044**	0.051	0.051

2. Gross and histopathology

Examination of the external appearance of rats killed at completion of treatment revealed yellow staining of the perineal fur in all females of the high dose group, in 2 females dosed at 250 mg/kg bw/day and in a single female dosed at 15 mg/kg bw/day. Slight discoloration and/or slight enlargement of the liver were common in rats dosed at 1000 mg/kg bw/day. A single male of the 250 mg/kg bw/day dose group showed slight hepatic enlargement following treatment. In all males and two females of the high dose group and in two males of the 250 mg/kg bw/day group slight diffuse pallor and/or slight enlargement of the kidneys were observed. In the majority of the animals of the high dose group and in 3 females of the 250 mg/kg bw/day dose group moderate to severe enlargement of the caecum, commonly in association with excessive normal caecal contents, were observed. Other macroscopic abnormalities were considered to be either artefacts or minor variations within the normal range.

Histopathology revealed microscopic abnormalities of the kidney. Moderate proximal tubular degenerative and regenerative lesions were observed in the renal cortex of two male and two female rats treated at the high dose level. The incidence of proximal tubular regenerative foci was increased in males and females of the high dose group and in females of the 250 mg/kg bw/day dose group. No treatment-related histological changes were found in the caecum, the liver or in any other of the histopathological preparations.

Table 5.8.1-25: Incidence of selected macro- and histopathological lesions in rats administered 4-chloro-3', 4'-dimethoxybenzophenone for 28 days

Test group (mg/kg bw/d)	Male animals					Female animals				
	0	15	60	250	1000	0	15	60	250	1000
Kidney										
• Diffuse pallor	-	-	-	1	7	-	-	-	-	2
• Enlargement	-	-	-	1	4	-	-	-	-	-
• Medullary mineralisation	7	-	6	6	6	7	-	7	7	6
• Proximal tubular regeneration										
o Very slight	4	-	4	3	0	3	-	2	2	2
o Slight	0	-	0	2	6**	0	-	0	5*	3
o Moderate	0	-	0	0	1	0	-	0	0	2
• Focal proximal tubular dilatation/epithelial necrosis	0	-	0	0	2	0	-	0	0	2
Caecum										
• Enlargement	-	-	-	-	6	-	-	-	3	7
• Excessive normal caecal contents	-	-	-	-	4	-	-	-	-	7
Liver										
• Enlargement	-	-	-	1	5	-	-	-	-	3
• Dark discoloration	-	-	-	-	4	-	-	-	-	3

* = statistically significant ($p \leq 0.05$); ** = statistically significant ($p \leq 0.01$)

III. CONCLUSIONS

The administration of 4-chloro-3',4'-dimethoxybenzophenone (Z7) by gavage to male and female Fischer 344 rats for 4 weeks caused no signs of general systemic toxicity. The pathological evaluation revealed treatment-related changes of the liver, kidneys and caecum. Only for the kidney histopathological findings were observed at 250 and 1000 mg/kg bw/day. Therefore, the no observed adverse effect level (NOAEL) under the conditions of the present study was 60 mg/kg bw/day for both sexes.

Report:	CA 5.8.1/27 Timm A., 1988 k Salmonella typhimurium and Escherichia coli reverse mutation assay with Z7 DK-470-010
Guidelines:	OECD 471, OECD 472, Toxicity Test Guideline (Japan 1984), EEC 84/449 B 13 Mutagenicity, EEC 84/449 B 14
GLP:	yes

Report:	CA 5.8.1/28 Wollny H.-E., 1995 c Salmonella typhimurium and Escherichia coli reverse mutation assay with Z7 DK-470-024
Guidelines:	OECD 471, OECD 472, Toxicity Test Guideline (Japan 1984), EEC 84/449 B 13 Mutagenicity, EEC 84/449 B 14
GLP:	yes

Comment: **In the first amendment to report DK-470-010, only typing errors were corrected and thus did not influence the outcome of the original study.**

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535 and TA 1537) and E. coli strain WP2 uvrA$^-$ were exposed to Z7 (Batch: Y 200, Purity: > 99%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

In the Ames standard plate test Z7 was tested in concentrations of 1 to 1000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix). Precipitation of the test substance was not observed in the presence or absence of S9 mix. Up to 333.3 µg/plate normal background growth was observed. At higher concentrations the background was slightly reduced. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, the test substance Z7 (Metabolite of BAS 550 F) is not mutagenic in the Ames standard plate test under the experimental conditions of the study.

(BASF DocID DK-470-010)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Z7 (Metabolite of BAS 550 F)

Description: Solid, crystalline, colourless

Lot/Batch #: Y 200

Purity: > 99%

Stability of test compound: Stable for at least 6 months (expiry date 6/88).
The stability of the test substance in the vehicle DMSO has not been determined analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Untreated control: Neither test substance nor solvent were used.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide (NaN_3)	aqua dest.	10 µg/plate
TA 1535	Sodium azide (NaN_3)	aqua dest.	10 µg/plate
TA 1537	4-nitro-o-phenylendiamine (NOPD)	DMSO	50 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	50 µg/plate
WP2 uvrA-	Methyl methane sulfonate (MMS)	aqua dest.	10 µg/plate

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	10 µg/plate
TA 1535	2-aminoanthracene	DMSO	10 µg/plate
TA 1537	2-aminoanthracene	DMSO	10 µg/plate
TA 98	2-aminoanthracene	DMSO	10 µg/plate
WP2 uvrA-	2-aminoanthracene	DMSO	10 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar rats. The rats received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Five days after administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 3 volumes of S9-fraction are mixed with 7 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The rat liver S9-mix was prepared immediately before use and had the following composition (in 100 mM phosphate buffer, pH 7.4):

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	5 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	30 %

The S9 batch was not further characterized (e. g. with benzo(a)pyrene).

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA-

Salmonella typhimurium:

The Salmonella strains (and E. coli if appropriate) are checked for the following characteristics at regular intervals: deep rough character (rfa); ampicillin resistance (R factor plasmid); spontaneous mutation rate.

5. Test concentrations:

Pre-experiment: In the pre-experiment triplicate plates were prepared for each concentration (neg. control; 1, 3, 10, 33, 100, 333, 1000, and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without rat liver S9 mix) for all tester strains indicated above.

Plate incorporation assay I/II: In both experiments triplicate plates were prepared for each concentration (1, 10, 33.3, 100, 333.3, and 1000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without rat liver S9 mix) for all tester strains indicated above.

B. TEST PERFORMANCE:

1. Dates of experimental work: 21-Oct-1987 to 12-Nov-1987

2. Pre-experiment:

The pre-experiment was performed under the same experimental conditions as described for the main experiment.

3. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin and the samples were poured onto minimal agar plates. After incubation at 37°C for 72 hours in the dark, the bacterial colonies (his^+ revertants) are counted.

4. Statistics:

No special statistical tests were performed.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A significant dose-related or a significant and reproducible increase in the number of revertant colonies in at least one tester strain either without S9 mix or after adding a metabolizing system. A significant response is described as a doubling (TA 100) or tripling of the spontaneous mutation rate.

A test substance is generally considered non-mutagenic in this test if:

- No significant dose-related or no significant and reproducible increase in the number of revertant colonies is observed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO and water was not verified analytically.

B. TOXICITY

In the pre-experiment the plates with the test article showed normal background growth up to 333.3 µg/plate in strain TA 98, TA 100, and WP2, respectively. At higher concentrations the background growth was slightly reduced.
According to the dose selection criteria, the test article was tested at the following concentrations in the main experiments: 1, 10, 33.3, 100, 333.3, and 1000 µg/plate.
In the main experiment the background growth was reduced at 1000 µg/plate indicating cytotoxicity. In addition, toxicity was evidenced by a reduction in the number of spontaneous revertants at the highest investigated dose in both experiments.

C. MUTATION ASSAYS

Neither in the first nor in the second experiment with and without metabolic activation a significant dose-dependent increase in number of revertants was observed in any strain tested [Table 5.8.1-26]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

No precipitation of the test article was observed in any experiment.

Table 5.8.1-26: Bacterial gene mutation assay with Z7 - Mean number of revertants

Experiment 1										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control	20±4	22±2	81±5	84±7	14±3	11±1	6±1	5±2	37±6	31±3
Solvent control (DMSO)	16±5	17±4	81±10	82±2	15±4	12±3	8±1	7±2	38±6	33±5
Test item										
1 µg/plate	21±6	16±5	94±4	86±3	15±4	10±2	7±2	6±2	43±5	37±7
10 µg/plate	15±4	16±3	92±8	81±5	16±1	11±4	7±1	7±3	34±1	34±5
33.3 µg/plate	19±7	16±1	94±3	82±3	15±5	12±4	6±4	6±2	30±8	37±6
100 µg/plate	12±2	16±3	94±3	85±4	16±1	11±3	6±1	4±3	40±6	35±4
333.3 µg/plate	14±4	12±7	90±8	84±5	15±1	14±6	5±4	6±2	39±3	38±7
1000 µg/plate	17±4	3±2	94±7	89±7	13±1	3±0	7±1	-	32±5	27±10
Pos. control§	1265±181	1019±90	1295±24	630±56	215±23	504±70	470±47	167±35	458±6	815±83
Experiment 2										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control	20±0	18±1	115±7	104±20	12±4	14±3	18±5	11±1	41±4	30±4
Solvent control (DMSO)	21±6	18±4	90±11	92±15	16±4	12±1	22±1	14±2	34±2	34±2
Test item										
1 µg/plate	21±7	16±1	102±4	100±6	16±4	10±3	16±6	11±1	35±6	31±9
10 µg/plate	18±3	17±3	98±31	81±9	13±4	11±1	17±4	13±5	38±7	34±8
33. 3 µg/plate	19±5	14±5	102±14	75±16	15±3	10±2	13±4	17±1	38±10	36±4
100 µg/plate	18±6	15±6	93±10	88±20	14±1	12±1	14±4	16±3	36±6	40±4
333.3 µg/plate	15±2	16±5	91±9	83±27	9±4	9±4	21±9	15±4	32±0	32±4
1000 µg/plate	17±8	-	-	-	19±3	15±9	7±4	10±4	39±4	27±5
Pos. control§	1503±127	1083±157	1993±162	827±12	188±11	612±520	304±39	221±15	383±57	765±19

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance Z7 (Metabolite of BAS 550 F) is not mutagenic in the Ames standard plate test under the experimental conditions chosen here.

Report: CA 5.8.1/29
Wollny H.-E., 1996 c
2nd amendment to report - Salmonella typhimurium and Escherichia coli reverse mutation assay with Z7
DK-470-026

Guidelines: OECD 471, OECD 472, Toxicity Test Guideline (Japan 1984), EEC 84/449 B 13 Mutagenicity, EEC 84/449 B 14

GLP: yes

Comment: **In the second amendment to report DK-470-010, the results from an additional experiment were provided, where the test substance was tested up to 5000 µ/plate in every strain with and without metabolic activation. This experiment was not included in the original report as only three concentrations were partly evaluable due to cytotoxicity of the test substance. The results of this experiment are provided below. The experiment was performed as described in the original report DK-470-010.**

Table 5.8.1-27: Bacterial gene mutation assay with Z7 - Mean number of revertants (results from amended study)

Experiment 1										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control	31±8	16±7	172±28	140±58	17±8	13±4	12±0	9±3	34±1	31±9
Solvent control (DMSO)	30±6	17±5	76±8	87±15	14±5	11±4	8±1	8±5	42±5	30±3
Test item										
10 µg/plate	30±6	17±1	102±16	83±7	8±4	10±1	8±0	5±2	24±11	30±12
33.3 µg/plate	20±4	18±7	153±67	74±13	18±4	13±5	8±4	8±2	40±11	26±3
100 µg/plate	27±7	18±4	109±3	73±20	17±5	10±1	5±1	7±1	26±10	35±2
333.3 µg/plate	17±5	22±5	83±2	65±8	9±4	0	5±2	10±2	41±11	36±9
1000 µg/plate	0	0	0	0	0	0	0	0	0	0
5000 µg/plate	0	0	0	0	0	0	0	0	0	0
Pos. control§	1430±84	1438±137	1572±25	418±20	378±19	519±76	249±55	221±22	119±80	1265±47

§ = Compound and concentrations see Material and Methods (I.A.2.) of the original report

Report: CA 5.8.1/30
Brooks T.M.,Wiggins D.E., 1989 c
Bacterial mutagenicity studies with 4-chloro-3,4-dimethoxy benzophenone
DK-470-012
Guidelines: EEC 67/548
GLP: yes

Executive Summary
S. typhimurium (strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538) and E. coli strain WP2 uvrA were exposed to 4-chloro-3', 4'-dimethoxy benzophenone (Z7 - Batch: KSLA No. 9.0574, Purity: 99%) using acetone as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the Ames standard plate test 4-chloro-3', 4'-dimethoxy benzophenone was tested at concentrations of 15 to 2000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix). Precipitation of the test substance was observed at concentrations of 2000 µg/plate in the presence or absence of S9 mix. No cytotoxicity was observed up to the highest concentration tested. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study, the test substance 4-chloro-3', 4'-dimethoxy benzophenone (Z7 - Metabolite of BAS 550 F) is not mutagenic in the Ames standard plate test under the experimental conditions of the study.
(BASF DocID DK-470-012)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material 4-chloro-3',4'-dimethoxy benzophenone (Z7 - Metabolite of BAS 550 F)

Description: Solid, buff colored

Lot/Batch #: KSLA No. 9.0574

Purity: 99%

Stability of test compound: Stable during period of use (IR analytical method). The stability of the test substance in the vehicle Acetone was determined analytically (GC/HPLC).

Solvent used: Acetone

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 20 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 98	2-Nitrofluorene	DMSO	5 µg/plate
TA 100	Sodium azide (NaN_3)	Water	2 µg/plate
TA 1535	Sodium azide (NaN_3)	Water	2 µg/plate
TA 1537	9-Aminoacridine	DMSO	25 µg/plate
TA 1538	2-Nitrofluorene	DMSO	5 µg/plate
WP2 uvrA	Potassium dichromate	Water	20 µg/plate

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Benzo(a)pyrene	DMSO	10 µg/plate
TA 98	Benzo(a)pyrene	DMSO	10 µg/plate
TA 1535	2-Aminoanthracene	DMSO	5 µg/plate
TA 1537	Neutral Red	Water	20 µg/plate
TA 1538	Benzo(a)pyrene	DMSO	10 µg/plate
WP2 uvrA-	Benzo(a)pyrene	DMSO	10 µg/plate

3. Activation:

S9 was produced from the livers of induced male Fischer 344 rats pre-treated by intraperitoneal injection of Aroclor 1254 (0.5 g/kg bw). The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The rat liver S9-mix was prepared immediately before use and had the following composition (in 100 mM phosphate buffer, pH 7.4):

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

The S9 batch was not further characterized (e. g. with benzo(a)pyrene).

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538
E. coli: WP2 uvrA

The Salmonella strains (and E. coli if appropriate) are checked regularly for their genotypic characteristics.

5. Test concentrations:

Pre-experiment: no details given

Plate incorporation assay I/II: In both experiments triplicate plates were prepared for each concentration (15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without rat liver S9 mix) for all tester strains indicated above.

B. TEST PERFORMANCE:

1. Dates of experimental work: 07-Apr-1989 to 20-Apr-1989

2. Pre-experiment:

The pre-experiment was performed under the same experimental conditions as described for the main experiment.

3. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.02 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin and the samples were poured onto minimal agar plates. After incubation at 37°C for 48-72 hours in the dark, the bacterial colonies (his^+ revertants) are counted.

4. Statistics:

No special statistical tests were performed.

5. Evaluation criteria:

No details were reported.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle acetone was verified analytically. They were stable for at least one working day. The test item in media had no effect on pH. The test item formed oily smears on the surface of the top agar at 250 µg/plate and above and a visible precipitate was present at 2000 µg/plate showing that the test compound was not totally miscible in the aqueous test system at these amounts.

B. TOXICITY

Based on the results from the pre-experiment the highest concentration of 2000 µg/plate were chosen for the main experiments.
In the main experiments no cytotoxicity was observed when tested up to precipitating concentrations.

C. MUTATION ASSAYS

Neither in the first nor in the second experiment with and without metabolic activation a significant dose-dependent increase in number of revertants was observed in any strain tested [see Table 5.8.1-28]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Table 5.8.1-28: Bacterial gene mutation assay with 4-chloro-3', 4'-dimethoxy benzophenone - Mean number of revertants

Experiment 1*												
Strain	TA 98		TA 100		TA 1535		TA 1537		TA 1538		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Solvent control (Acetone)	22±3	21±2	97±13	85±10	10±3	10±1	16±2	10±1	20±1	17±4	94±8	62±14
Test item												
15.6 µg/plate	23±5	19±5	117±6	90±9	13±5	10±2	11±2	12±2	17±1	16±4	88±12	79±5
31.2 µg/plate	26±4	21±2	105±7	93±12	8±3	10±4	9±2	8±3	20±5	18±4	94±4	81±19
62.5 µg/plate	19±3	21±5	97±15	88±11	11±3	9±3	12±1	10±2	18±7	13±4	80±19	61±7
125 µg/plate	21±3	26±11	91±6	96±9	11±3	9±1	10±2	9±2	20±8	16±4	89±20	69±3
250 µg/plate	20±4	23±7	111±9	96±11	13±4	9±2	14±2	9±3	18±3	15±1	86±13	65±23
500 µg/plate	21±5	18±4	102±13	106±6	8±2	10±3	10±2	8±2	20±2	15±5	84±3	54±12
1000 µg/plate	20±4	16±2	86±6	88±2	8±2	8±1	12±4	8±4	18±6	14±3	88±6	34±5
2000 µg/plate	13±3	10±1	94±12	72±14	8±4	9±2	10±2	8±1	12±0	14±8	89±11	30±7
Pos. control§	375±31	471±11	561±12	502±10	221±53	802±28	289±57	79±7	225±33	517±98	497±12	512±97
Experiment 2*												
Strain	TA 98		TA 100		TA 1535		TA 1537		TA 1538		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Solvent control (Acetone)	18±1	18±5	93±6	87±2	13±4	12±3	12±5	11±1	19±2	16±2	98±4	78±13
Test item												
15.6 µg/plate	20±8	15±3	108±14	90±6	11±4	12±3	13±2	10±1	16±3	15±3	91±10	85±6
31.2 µg/plate	17±2	14±4	100±7	77±9	10±1	13±1	12±5	8±2	23±3	13±1	99±11	75±5
62.5 µg/plate	16±1	19±4	108±7	90±18	12±4	12±1	12±0	11±3	23±3	16±3	89±7	77±8
125 µg/plate	16±4	16±2	100±6	89±10	15±3	12±5	13±2	9±3	20±4	14±3	106±16	87±11
250 µg/plate	21±2	19±3	95±5	85±13	12±2	9±1	9±1	8±2	18±3	14±4	79±14	79±10
500 µg/plate	17±6	12±4	102±3	85±3	10±5	7±3	12±3	7±2	18±2	12±2	84±6	80±4
1000 µg/plate	15±5	14±6	95±26	92±5	10±1	11±4	12±2	6±1	15±2	14±1	91±1	62±10
2000 µg/plate	16±5	14±6	98±17	79±6	10±2	11±2	8±2	11±4	14±5	12±1	90±7	69±12
Pos. control§	378±43	432±50	548±19	386±60	167±8	543±34	250±55	73±6	232±28	465±38	555±74	467±52

§ = Compound and concentrations see Material and Methods (I.A.2.) above; * = the numbers may vary compared to original values due to rounding

III. CONCLUSION

According to the results of the present study, the test substance 4-chloro-3', 4'-dimethoxy benzophenone (Z7 - Metabolite of BAS 550 F) is not mutagenic in the Ames standard plate test under the experimental conditions chosen here.

Report:	CA 5.8.1/31 Brooks T.M.,Wiggins D.E., 1990 c Genotoxicity studies with 4-chloro-3,4-dimethoxy benzophenone: In vitro chromosome studies DK-470-013
Guidelines:	EPA 84-2
GLP:	yes

Report:	CA 5.8.1/32 Brooks T.M., 1993 c Addendum - Genotoxicity studies with 4-chloro-3,4-dimethoxy benzophenone: In vitro chromosome studies DK-470-027
Guidelines:	EPA 84-2
GLP:	yes

Comment: **The report DK-470-027 is an addendum to the study report DK-470-013. In the addendum additional information on materials and method and a summary of the results is given. The information given in the addendum has no influence on the outcome of the original study report DK-470-013.**

Executive Summary

4-Chloro-3',4'-Dimethoxybenzophenone (Z7 - metabolite of BAS 550 F; Batch: KSLA No. 9.0574, Purity: 99%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster Ovary (CHO) cells in the presence and absence of metabolic activation. Based on the results of a pretest for dose selection, 4-Chloro-3',4'-Dimethoxybenzophenone was tested at 45, 225, and 450 µg/mL in the absence of metabolic activation and at 50, 250, and 500 µg/mL in the presence of metabolic activation. The cells were treated for 24 h in the absence of metabolic activation and were sampled directly after exposure (24 h). In the presence of metabolic activation exposure was 3 h with 3 sample times at 8, 12, and 24 h. Vehicle (Acetone) and positive controls (Benzo[a]pyren (B[a]P) and Methylmethanesulfonate (MMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.

Prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, at least 300 well spread metaphases per dose and treatment condition were analyzed for chromosomal aberrations, except for the positive control cultures where less metaphases were scored due to clearly increased aberration rates.

There was no evidence of a substance-related increase in chromosome damage after exposure of cells with up to 450 in the absence or 500 µg/mL in the presence of metabolic activation at any sample time. However, there was a significant increasing trend in the number of isogaps, chromatid gaps or both, with increasing dose, for the 8 h sample time. The positive controls MMS and benzo[a]pyrene induced substantial chromosome damage in any experiment. Thus, under the experimental conditions chosen here, the conclusion is drawn that 4-Chloro-3',4'-Dimethoxybenzophenone (Z7 - Metabolite of BAS 550 F) has no chromosome-damaging (clastogenic) properties under in vitro conditions using CHO cells.

(BASF DocID DK-470-013)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-Chloro-3',4'-Dimethoxybenzophenone (Z7 - Metabolite of BAS 550 F)

Description: Solid, buff colored
Lot/Batch #: KSLA No. 9.0574
Purity: 99%
Stability of test compound: The stability of the test substance under storage conditions was confirmed analytically. The stability of the test substance at room temperature dissolved in the vehicle acetone over a period of 6 days was verified analytically.
Solvent used: Acetone

2. Control Materials:

Negative control: A negative control was not employed in this study.
Solvent control: Acetone
Positive control, -S9: Methylmethanesulphonate (MMS) 6 mg/mL (in water)
Positive control, +S9: 3,4-Benzopyrene 3 mg/mL (in DMSO)

3. Activation: S9 was produced from the livers of F344 rats, pretreated intraperitoneally with 0.5 g/kg Aroclor. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms: Chinese hamster ovary cells (CHO-K1)

5. Culture medium:	Hams F12 medium with glutamine (2 mM) supplemented with 10% (v/v) fetal calf serum (FCS). During exposure to the test substance (3-hour treatment with S9), growth medium with 2% FCS was used.
5. Test concentrations:	
a) Preliminary toxicity assay 1:	1, 10, 50, 100, 250, 500, 750 and 1000 µg/mL (±S9)
Preliminary toxicity assay 2:	25-750 µg/mL (+S9) or 150-750 µg/mL (-S9)
b) Mutation assay:	
Experiment with S9	50, 250, 500 µg/mL (3 hour exposure and 8, 12, and 24 h sampling time)
Experiment without S9:	45, 225, 450 µg/mL without metabolic activation (24 h exposure and 24 h sampling time)

B. TEST PERFORMANCE:

1. Dates of experimental work: 28-Mar-1989 to 16-Nov-1989

2. Preliminary cytotoxicity assay:

In the first cytotoxicity assay CHO cells were treated with 1, 10, 50, 100, 250, 500, 750 and 1000 µg test substance per mL for 3 h (+S9) or 24 h (-S9). After 24 h the cells were fixed and stained, and cell confluence was assessed visually.

In the second cytotoxicity assay CHO cells were treated with 25-750 µg/mL (+S9) or 150-750 µg/mL (-S9) for 3 h (+S9) or 24 h (-S9). After treatment cell counts were performed. The concentration leading to a reduction of 50% of the cell number compared to the control was the maximum concentration used in the main assays.

3. Cytogenicity Assay:

Cell treatment:	Cells were exposed to the test substance, solvent or positive control for 3 hours in the presence of metabolic activation or 24 h in the absence of metabolic activation. The cells were incubated in cell culture bottles at 37°C and 5% CO_2 in triplicates each. Sampling was performed 8, 12, and 24 h after treatment for 3 h (+S9) and at the end of the 24 h treatment (-S9). For determination of cytotoxicity, additional cell cultures were treated in the same way as in the main experiment (24 h sampling ±S9). Growth inhibition was estimated by determination of the mitotic index.
Spindle inhibition:	Colcemide (0.2 µg/mL final concentration) was added to the cultures 2 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment 0.56% KCl solution was added and the cells were fixed by addition of methanol/glacial acetic acid (3:1 v/v).
Slide preparation:	The cells on the slides were stained with Giemsa.
Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. The Vernier co-ordinates were recorded for damaged cells and the number of centromers in each cell analyzed were recorded. A mitotic index based on 500 cells was evaluated from the slides prepared at the 24 h sampling (±S9)

4. Statistics:

The data were analysed using a generalized linear model with a logit link and binomial error structure. Where there were no occurrences of the feature in the solvent control group, Fisher´s exact test was used to compare the untreated group and each treated group with the solvent control group. A test for trend in the dose-response was carried out.

5. Evaluation criteria:

No detailed evaluation criteria were mentioned in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance was confirmed by infra-red spectra and proton magnetic resonance spectrum measurement for the duration of the study period. The stability of the test substance at room temperature in the vehicle acetone over a period of 6 days was verified by HPLC analysis.

B. PRELIMINARY CYTOTOXICITY ASSAY:

Preliminary toxicity assay 1:

The degree of cell confluency observed on test substance exposed cultures was reduced to 50% of that observed on control cultures at 250 µg/mL in the presence and at 750 µg/mL in the absence of S9-mix.

Preliminary toxicity assay 2:

The total cell counts were reduced by an estimated 50% at a concentration of 450 µg/mL in the absence of S9-mix. In the presence of S9-mix little cytotoxicity was observed at concentrations up to 750 µg/mL.

Crystals of test compound were evident in the culture medium at 450 µg/mL and above in the absence of S9-mix and at 250 µg/mL and above in the presence of S9-mix.
The highest concentration selected for the chromosome assay were 450 and 750 µg/mL in the absence and presence of S9-mix, respectively.

C. CYTOGENICITY ASSAYS:

Without S9-mix

Treatment with the test substance at concentrations up to 450 µg/mL for 24 h showed no increase in metaphase chromosome damage compared to the control cultures. The positive control MMS induced a clear increase in chromosomal aberrations.

With S9-mix

Two assays were initially carried out and showed unacceptable levels of damage in the untreated and solvent control groups, respectively. A third experiment was performed using a maximum concentration of 0.25% in the test medium. The highest concentration of the test substance that could be added was 500 µg/mL.

There was no evidence of a substance-related increase in chromosome damage after exposure of cells with up to 500 µg/mL at any sample time (8, 12 or 24 h). However, there was a significant increasing trend in the number of isogaps, chromatid gaps or both, with increasing dose, for the 8 h sample time. The positive control benzo[a]pyrene induced substantial chromosome damage at each of the three sample times.

III. CONCLUSION

The results from this study indicate that 4-chloro-3',4'-dimethoxybenzophenone did not induce chromosome damage in cultured CHO cells either in the presence or in the absence of S9 mix under the experimental conditions applied.

Table 5.8.1-29: Chromosome aberration test with 4-chloro-3',4'-dimethoxybenzophenone without metabolic activation (24 hours treatment, 24 hour sample time)

Compound	Concentration [µg/mL]	Mitotic index	No. of cells examined	Aberrations						
				Numerical	Structural					
					Excluding gaps			Including gaps		
				Polyploid cells [%]	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations
Untreated control	0	0.054	300	0	1	0.003	0.33	7	0.023	2.33
Solvent control	0	0.050	299	0.33	0	0	0	4	0.013	1.34
Test substance	45	0.031	300	0	0	0	0	2	0.007	0.67
	225	0.033	300	0	0	0	0	7	0.023	2.00
	450	0.021	299	0.33	0	0	0	4	0.013	1.34
MMS	20	0.018	299	0.33	131	0.438	28.09	145	0.485	29.77

Table 5.8.1-30: Chromosome aberration test with 4-chloro-3',4'-dimethoxybenzophenone with metabolic activation (3 hours treatment, 8 hour sample time)

Compound	Concentration [µg/mL]	Mitotic index	No. of cells examined	Aberrations						
				Numerical	Structural					
					Excluding gaps			Including gaps		
				Polyploid cells [%]	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations
Untreated control	0	-	299	0.33	0	0	0	7	0.023	2.34
Solvent control	0	-	298	0.67	2	0.007	0.67	8	0.030	3.02
Test substance	50	-	298	0.67	1	0.003	0.34	10	0.034	3.02
	250	-	277	1.42	0	0	0	12	0.043	4.33
	500	-	296	1.33	1	0.003	0.34	16	0.054	5.41
B[a]P	25	-	187	0.53	117	0.525	44.39	149	0.797	51.34

Table 5.8.1-31: Chromosome aberration test with 4-chloro-3',4'-dimethoxybenzophenone with metabolic activation (3 hours treatment, 12 hour sample time)

Compound	Concentration [µg/mL]	Mitotic index	No. of cells examined	Aberrations						
				Numerical	Structural					
					Excluding gaps			Including gaps		
				Polyploid cells [%]	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations
Untreated control	0	-	299	0.33	2	0.007	0.67	3	0.010	1.00
Solvent control	0	-	300	0	2	0.007	0.67	8	0.027	2.33
Test substance	50	-	298	0.67	1	0.003	0.34	7	0.023	2.35
	250	-	299	0.33	0	0	0	7	0.023	2.01
	500	-	299	0.33	0	0	0	6	0.020	2.01
B[a]P	25	-	270	0.74	141	0.522	33.33	152	0.563	34.81

Table 5.8.1-32: Chromosome aberration test with 4-chloro-3',4'-dimethoxybenzophenone with metabolic activation (3 hours treatment, 24 hour sample time)

Compound	Concentration [µg/mL]	Mitotic index	No. of cells examined	Aberrations						
				Numerical	Structural					
					Excluding gaps			Including gaps		
				Polyploid cells [%]	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations	Total aberrations	Mean No. of aberrations per cell	Percentage of cells with aberrations
Untreated control	0	0.036	292	2.67	1	0.003	0.34	7	0.024	2.40
Solvent control	0	0.014	295	1.67	2	0.007	0.68	5	0.017	1.69
Test substance	50	0.004	298	0.67	1	0.003	0.34	5	0.017	1.68
	250	0.014	290	3.33	2	0.007	0.69	5	0.017	1.72
	500	0.002	296	1.33	4	0.014	1.01	8	0.027	2.36
B[a]P	25	0.012	297	1.00	28	0.094	8.08	41	0.138	12.46

Report: CA 5.8.1/33
[redacted] 1993 c
CDMBP - Preliminary oral (gavage) embryotoxicity study in the rat
DK-470-025
Guidelines: OECD 414, EEC 87/302 B, EEC 67/548
GLP: yes

Executive Summary

Three groups of eight mated female rats received CDMBP (Z7 metabolite of DMM) orally by gavage at dosages of 60, 250 and 1000 mg/kg bw/d once daily during post mating days (PMD) 6 to 15 included. A control group of eight animals received the vehicle only (CMC, 1%) over the same period. Animals were sacrificed on PMD20 and the fetuses were removed and examined externally.
There were no treatment-related mortalities, clinical observation or necropsy findings detected in any dose group. In the 1000 mg/kg bw/d group, a slight decrease in body weight gain was observed from PMD6 to PMD9, this finding was considered treatment-related. There were no effects on implantation and postimplantation loss in any dose group. Number, sex and weight of the fetuses were not affected by the treatment. No fetal defects were observed at external examination.
The NOAEL for maternal effects can be set at 250 mg/kg bw/d due to slight decrease body weight gain at 1000 mg/kg bw/d.
The NOAEL for development is considered to be 1000 mg/kg bw/d, the highest tested dose, because no effects were observed in the fetuses.

(DocID DK-470-025)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 4-chloro-3',4'-dimethoxy benzophenone

Description:	solid (powder) / buff-colored
Lot/Batch #:	CBP-A-1050 Ld1
Purity:	97.7%
Stability of test compound:	The test substance was stable as such (expiry date: 17th Sep 1993). The stability in the formulation was verified analytically.

2. Vehicle and/or positive control: Carboxymethylcellulose (1% in aqua dest.)

3. Test animals:

Species:	Rats
Strain:	Crl:CD(SD)BR, Sprague Dawley
Sex:	Female
Age:	8-12 weeks
Weight at dosing:	188-234 g
Source:	[redacted]
Acclimation period:	at least 7 days
Diet:	pelleted diet (Ssniff R10, Ssniff Spezialdiaeten GmbH, Soest, Germany), ad libitum
Water:	water, ad libitum
Housing:	in groups (20-25 animals per cage) prior to and during mating and single housing during the rest of the study period in Makrolon cages type II (E. Becker & Co. GmbH, Castrop-Rauxel, Germany)

Environmental conditions:

Temperature:	19 - 25°C
Humidity:	30 - 70%
Air changes:	NA
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 26/01/1993-24/02/1993

2. Animal assignment and treatment:
The female animals were mated at a ratio of 1:4 in communal cages over night with males of the same strain. The day on which sperm and/or vaginal plug were observed was designated as day 0 of gestation. Immediately after mating the female rats were randomly allocated to treatment groups.

3. Test substance preparation and analysis:
The test substance was daily prepared as a suspension in the vehicle carboxy-methylcellulose (1%). During the first week of treatment samples from each formulation preparation for groups 2 to 4 were taken together with a reference sample from the control group and analysed for determination of concentration, homogeneity, and stability (over 4 and 24 h).

4. Statistics:
Levene´s test for homogeneity of variances was performed followed by a rank transformation (in case of heterogeneity) and the ANOVA for body weight and body weight change. For litter weight the Bartlett´s test for homogeneity of variance was performed followed by the one-way ANOVA. In case of significant results for the ANOVA the Dunnett´s two-tailed t-test was performed to compare treated groups with control group.
For number of corpora lutea, number of implantations, preimplantation loss, total intra-uterine deaths, postimplantation loss, number of live fetuses, and proportion of male fetuses, a rank transformation was performed followed by the Bartlett´s test for homogeneity of variance was performed followed by the one-way ANOVA. In case of significant results for the ANOVA the Dunnett´s two-tailed t-test was performed to compare treated groups with control group. In the event of heterogeneity, the Kruskal-Wallis test ANOVA was performed together with the Wilcoxon rank-sum test to compare each treated group against control.
For mean fetal weight the Bartlett´s test for homogeneity of variance was performed followed by the ANCOVA (covariate: number of fetuses). In case of significant results for ANCOVA, the Dunnett´s two-tailed t-test was performed to compare treated groups with control group.

C. METHODS

1. Observations:

The animals were examined for morbidity and mortality twice daily on working days. All animals were examined at least once daily for signs of ill health or overt signs of toxicity and each finding was recorded.

2. Body weight:

Body weight was determined on days 0, 6, 9, 12, 16, and 20 post-coitum.

3. Terminal procedures:

Necropsy

On day 20 post-coitum, the surviving female rats were sacrificed by carbon dioxide inhalation, dissected, and examined macroscopically for pathological changes. Any abnormalities were recorded.

Uterine/implantation data

The ovaries and uteri were removed and examined and the number of corpora lutea was determined in each ovary. Furthermore, number and position of implantations were subdivided into early and late resorptions and dead and live fetuses. Intrauterine deaths were classified as follows:

- Early resorptions showed decidual or placental tissues only.
- Late resorptions showed embryonic or fetal tissue in addition to placental tissue but excluded fetuses dying in utero within approximately two days prior to the terminal kill.
- Dead fetuses included only the fetuses dying in utero within approximately the last two days.

The uteri of apparently non-pregnant females were immersed in a 10% solution of ammonium sulphide to reveal evidence of implantation.

Fetal data

The fetuses were killed by an intrapleural injection of Eutha 77©. For each live fetus and, if possible, for each dead fetus, the following data were recorded:

- External fetal abnormalities
- Individual fetal weight
- Fetal sex

Dead fetuses were evaluated separately, if applicable. Structural deviations were classified as:

- Malformation: rare and/or probably lethal, e.g. hydrocephaly
- Variation: changes, which regularly occur also in the control groups and which are not of functional significance

All fetuses were initially fixed in ethanol, long-term preservation will be performed in formalin.

The following calculations were performed:

$$\text{Preimplantation loss} = \frac{\text{number of corpora lutea - number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\text{Postimplantation loss} = \frac{\text{number of implantations - number of live fetuses}}{\text{number of implantations}} \times 100$$

$$\text{Sex ratio} = \frac{\text{number of males}}{\text{number of fetuses}} \times 100$$

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical signs of toxicity were reported. In one animal each from the control group and group 2 (60 mg/kg bw) findings in the eyes (i.e. injury, bloody encrusted) were detected.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

A minor effect of treatment with the test item on body weight was observed in group 4 (1000 mg/kg bw) from day 6 to 9 post-coitum, when mean body weight gain was slightly reduced compared to the concurrent control group (see Table 5.8.1-33). In 50 and 250 mg/kg bw dose groups body weight gain was not affected by treatment.

Table 5.8.1-33: Group mean maternal body weight changes during gestation - -grams

HD 121-007

GROUP MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION - grams

	DOSE LEVEL	0 MG/KG	60 MG/KG	250 MG/KG	1000 MG/KG
DAYS 0 TO 6	MEAN	42.23	39.71	42.02	43.67
	S.D.	7.14	3.53	10.54	7.87
	N	6	7	5	6
DAYS 6 TO 9	MEAN	19.32	20.34	21.38	15.35
	S.D.	6.56	5.39	6.14	7.14
	N	6	7	5	6
DAYS 9 TO 12	MEAN	20.25	20.03	24.60	23.33
	S.D.	7.07	3.76	6.08	2.76
	N	6	7	5	6
DAYS 12 TO 16	MEAN	28.15	36.60	29.64	37.42
	S.D.	12.96	7.26	8.70	11.33
	N	6	7	5	6
DAYS 16 TO 20	MEAN	59.70	69.93	65.28	66.22
	S.D.	15.35	9.21	14.14	11.36
	N	6	7	5	6
DAYS 6 TO 16	MEAN	67.72	76.97	75.62	76.10
	S.D.	13.80	10.60	10.78	15.37
	N	6	7	5	6
DAYS 0 TO 20	MEAN	169.65	186.61	182.92	185.98
	S.D.	27.82	17.14	29.77	23.67
	N	6	7	5	6

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05; ** = P≤0.01.
Means calculated excluding dams with no viable embryos/fetuses or with no pups delivered.

D. Terminal procedures

Necropsy

Necropsy did not reveal any findings in the dose groups as well as in the control.

Implantation

Preimplantation loss was normal in all groups including the concurrent control group. Postimplantation loss in the dose groups was similar or lower than in the concurrent control group and within the normal range (see Table 5.8.1-34).

Number, sex, and weight of fetuses

No substance related effect on the mean number of fetuses, the mean fetal weight, and the fetal sex distribution was observed in any of the dose groups (see Table 5.8.1-35).

Fetal defects

At external fetal examination, no malformations or variations were observed in any of the study groups including the control.

Table 5.8.1-34: Implantation data

Implantation Data

(calculated from animals with live fetuses at necropsy)

		Group 1 - 0 mg/kg/day	Group 2 - 60 mg/kg/day	Group 3 - 250 mg/kg/day	Group 4 - 1000 mg/kg/day
Implantations	TOTAL	75	107	65	86
	MEAN	12.5	15.3	13.0	14.3
	SD	5.2	1.1	4.3	3.9
	N	6	7	5	6
Live fetuses	TOTAL	70	101	63	85
	MEAN	11.7	14.4	12.6	14.2
	SD	4.9	1.4	4.4	3.7
	N	6	7	5	6
% of implantations	MEAN	94.3	94.3	96.9	99.1
	SD	5.1	4.6	6.9	2.2
	N	6	7	5	6
Early resorptions	TOTAL	5	6	2	1
	MEAN	0.8	0.9	0.4	0.2
	SD	0.8	0.7	0.9	0.4
	N	6	7	5	6
Late resorptions	TOTAL	0	0	0	0
	MEAN	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	6	7	5	6
Dead fetuses	TOTAL	0	0	0	0
	MEAN	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0
	N	6	7	5	6
Total number of intra-uterine deaths	TOTAL	5	6	2	1
	MEAN	0.8	0.9	0.4	0.2
	SD	0.8	0.7	0.9	0.4
	N	6	7	5	6
% Postimplantation loss	MEAN	5.7	5.7	3.1	0.9
	SD	5.1	4.6	6.9	2.2
	N	6	7	5	6

Significantly different from control: * - 95% confidence level

Statistical analysis was performed by SAS release 6.04

Table 5.8.1-35: Fetal data

Fetal Data

(calculated from animals with live fetuses at necropsy)

		Group 1 - 0 mg/kg/day	Group 2 - 60 mg/kg/day	Group 3 - 250 mg/kg/day	Group 4 - 1000 mg/kg/day
Number of live fetuses	TOTAL	70	101	63	85
	MEAN	11.7	14.4	12.6	14.2
	SD	4.9	1.4	4.4	3.7
	N	6	7	5	6
Litter weight (g)	MEAN	42.8	54.5	47.3	55.9
	SD	16.9	5.2	14.5	12.1
	N	6	7	5	6
Mean fetal weight (g) overall	MEAN	3.8	3.8	3.8	4.0
	SD	0.5	0.1	0.3	0.4
	N	6	7	5	6
Mean fetal weight (g) males	MEAN	3.9	3.9	3.9	4.1
	SD	0.4	0.2	0.4	0.5
	N	6	7	5	6
Mean fetal weight (g) females	MEAN	3.6	3.7	3.7	3.9
	SD	0.1	0.1	0.2	0.4
	N	5	7	5	6
Number of males	TOTAL	35	51	29	40
Number of females	TOTAL	35	50	34	45
Mean proportion of male fetuses	MEAN	56.7	51.4	47.0	46.9
	SD	24.1	21.1	10.7	12.1
	N	6	7	5	6

Significantly different from control: * - 95% confidence level

Statistical analysis was performed by SAS release 6.04

III. CONCLUSIONS

In conclusion, administration of CDMBP (Z7 metabolite of DMM) orally by gavage during the period of organogenesis at dose level of 1000 mg/kg bw/d elicited minimal maternal toxicity (slightly decreased body weight gain during the early treatment period) but no embryotoxicity of external malformations.
Maternal NOAEL was considered to be 250 mg/kg bw/d and developmental NOAEL was the highest tested dose of 1000 mg/kg bw/d.

CA 5.8.2 Supplementary studies on the active substance

Studies submitted in the original Annex I Dossier (2004):

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of are provided under respective chapters.

Pharmacological studies:

Studies on safety pharmacology were performed. Dimethomorph, administered orally at a dose level of 100 mg/kg bw showed no anticonvulsive activity in the mouse. Dimethomorph had no effect on intestinal motility in the male rat but statistically significantly increased motility in the female rat in a dose related manner. Dimethomorph administered orally at a dose level of 100 mg/kg bw had no effect on body temperature in the mouse. Dimethomorph had no effect on blood pressure, respiration, ECG or contractile state of the nictitating membrane. However, a small increase in heart rate above that produced by the vehicle alone was observed at the highest administration dose (intravenous administration of 100 µg/kg bw) of dimethomorph. Dimethomorph administered to the bathing solution at concentrations of 3, 10 and 30 µg/mL had no direct effect on uterine smooth muscle and had no significant effect on cholinerg and adrenerg receptors present in this preparation. Dimethomorph did not directly effect the spontaneous motility of the isolated rabbit ileum neither did it alter the effects of acetycholine and adrenaline on this tissue. Dimethomorph at concentrations of 3, 10 and 30 µg/ml did not directly effect the isolated guinea pig ileum. Dimethomorph potentiated inflammatory reactions at low dose levels but inhibited inflammatory reactions at high dose levels in the male rat. However, since there was no dose-response relationship the relevance of the result obtained with the low dose level is uncertain. Dimethomorph had no effect on inflammatory reactions in the female rat. Dimethomorph, administered intradermally at a concentration of 1 % w/v, had no local anaesthetic activity. Dimethomorph administered orally to male and female mice at doses of 30, 100 and 300 mg/kg demonstrated only small and diverse changes in overt behaviour when analysed in the Irwin test. Dimethomorph, administered orally at a dose level of 100 mg/kg demonstrated no analgesic activity in the mouse. Dimethomorph, administered orally at a dose level of 100 mg/kg had no statistically significant effect on spontaneous locomotor activity in mice. Dimthomorph, administered orally at a dose level of 100 mg/kg, potentiated the pentobarbitone sleeping time. This effect may have been due to the competition with pentobarbitone in pathways of metabolism by liver microsomal enzymes.
The results of these studies did not provide any information which was utilized to establish relevant endpoints in order to calculate appropriate values for the ADI, AOEL or ARfD.

Safety pharmacology - Anticonvulsive activity in mice

[redacted] 1991: Safety pharmacology - anticonvulsive activity in mice. [redacted], unpublished, Reference No. SIP/3/PH

GLP: Yes (certified by the quality assurance of the Toxicol. Laboratories)

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85; purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: Male mice of the CD-1 strain, delivered by [redacted]
A single dose of the vehicle control (water), dimethomorph 100 mg/kg bw, or the standard anticonvulsant Chlordiazepoxide (10 mg/kg bw) were administered orally by gavage to groups of 6 male mice.
Two hours after dosing, each mouse was intravenously injected with a dose of leptazol found by previous experimentation to be convulsive. This dose was 10 mL/kg bw of a 0.8 % solution of leptazol. Animals which survived leptazol administration were killed by cervical dislocation at the end of the test.

Findings:
All animals in the vehicle control group, given sterile water, showed the presence of convulsion when dosed with leptazol.
The standard chlordiazepoxide, protected 60 % of animals from leptazol-induced convulsions.
All animals in the test group, given dimethomorph, showed the presence of convulsions when dosed with leptazol.

Conclusion:
Dimethomorph, administered orally at a dose level of 100 mg/kg bw showed no anticonvulsive activity in the mouse suggesting, that this test article has no substantial depressant action on the central nervous system.

Safety pharmacology - Charcoal meal transit times in the rat small intestines

[redacted]. 1991: Safety pharmacology charcoal meal transit times in the rat small intestines. [redacted] unpublished, Reference No. SIP/9/PH

GLP: Yes (certified by the quality assurance of the Toxicol. Laboratories)

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. ST89/214; purity 94 ± 2%, E-to Z- isomer ratio 45:55
Test animals: Male and female rats of the Crl:SD (CD)BR (VAF plus) strain, delivered by [redacted]

Findings:
The mean distance of charcoal meal transit in the male rat expressed as a percentage of the total length of the intestine was 61.3 %, 58.5 %, 65.0 % and 58.2 % following sterile water 5 mL/kg bw, dimethomorph 30 mg/kg bw, dimethomorph 100 mg/kg bw and dimethomorph 300 mg/kg bw respectively. Statistical analysis showed that dimethomorph had no effect on intestinal motility in the male rat.
The mean distance of charcoal meal transit in the female rat expressed as a percentage of the total length of the intestine was 53.9 %, 60.5 %, 61.8 % and 66.7 % following sterile water 5 mL/kg bw, dimethomorph 30 mg/kg bw, dimethomorph 100 mg/kg bw and dimethomorph 300 mg/kg bw respectively.

Conclusion:
Dimethomorph has no effect on intestinal motility in the male rat but statistically significantly increased motility in the female rat in a dose related manner.

Safey pharmacology - body temperature alteration in mice

[redacted]. 1991: Safety pharmacology - body temperature alteration in mice. [redacted], unpublished, Reference No. SIP/6/PH

GLP: Yes (certified by the quality assurance of the Toxicol. Laboratories)

Guideline: No guideline study

Deviations: Not applicable
Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. ST89/214; purity 94 ± 2 %, E-to Z- isomer ratio 45:55
Test animals: male mice of the CD strain, delivered by [redacted]

Findings:
The mean predose rectal temperature in the vehicle control group (sterile water 10 mL/ kg bw) was 36.0 ^{0}C. The mean rectal temperature at 60, 120 and 240 minutes after dosing was 36.1, 36.6 and 35.2 ^{0}C respectively. The post dose temperatures were not statistically significantly different from the predose temperature.
The mean predose rectal temperature in the group treated with dimethomorph 100 mg/kg bw was 34.8 ^{0}C. The mean rectal temperature at 60, 120 and 240 minutes after dosing was 35.7, 35.4 and 35.0 ^{0}C respectively. The post dose temperatures were not statistically significantly different from the predose temperature.

Conclusion:
Dimethomorph administered orally at a dose level of 100 mg/kg bw had no effect on body temperature in the mouse.

Safety pharmacology - cardiovascular, respiratory and nictitating membrane alterations in cat

[redacted]. 1991: Safety pharmacology - cardiovascular, respiratory and nictitating membrane alterations produced by test compound in the anaesthetized cat. [redacted] unpublished, Reference No. SIP/7/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: three female cats of known lineage, delivered by [redacted]

Findings:
Intravenous administration of noradrenaline produced a rise in blood pressure with very little effect on heart rate or ECG. Effects on respiration and electrical stimulation of the nictitating membrane were negligible. A small relaxation of the nictitating membrane was produced be noradrenaline.
Intravenous administration of noradrenaline produced a fall in blood pressure. The predominant effect of acetylcholine on heart rate was to produce a decrease. No reproducible effect was seen on respiration, EEG or the nictitating membrane response to stimulation. A small relaxation of the nictitating membrane occurred with acetylcholine administration.
Intravenous administration of DMF, the vehicle control, increased blood pressure and caused an increase in heart rate. No consistent effect on respiration ECG or nictitating membrane contraction was observed.
Intravenous administration of dimethomorph increased blood pressure in a manner similar to the vehicle control (DMF). A dose related increase in heart rate was also observed but, only at the highest administration dose (100 µg/kg bw), was the increase greater than that produced by the vehicle alone.
Responses to the standard compounds remained unchanged after dimethomorph on DMF administration.

Conclusion:
Dimethomorph had no effect on blood pressure, respiration, ECG or contractile state of the nictitating membrane. However, a small increase in heart rate above that produced by the vehicle alone was observed at the highest administration dose (100 µg/kg bw) of dimethomorph.

Safety pharmacology - The effect of test compound on rat uterine smooth muscle motility

[redacted] 1991: Safety pharmacology - The effect of test compound on rat uterine smooth muscle motility. [redacted], unpublished, Reference No. SIP/11/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: female rats of Crl:SD (CD) BR strain, delivered by [redacted]

Sub-maximal doses of acetylcholine and adrenaline were given before and after doses of dimethomorph in vehicle (3, 10 and 30 µg/mL) or the equivalent concentrations of vehicle alone. Three preparations received only dimethomorph, two preparations received both dimethomorph and the vehicle and one preparation received only the vehicle.

Findings:
Acetylcholine produced a reproducible contraction of the uterine smooth muscle.
Adrenaline inhibited the acetylcholine-induced contraction.
Dimethomorph had no direct action on the uterine smooth muscle.
Dimethomorph had no statistically significant effect on the response to the acetylcholine or the adrenaline-induced inhibition of acetylcholine.

Conclusion:
Dimethomorph administered to the bathing solution at concentrations of 3, 10 and 30 µg/mL had no direct effect on uterine smooth muscle and had no significant effect on cholinerg and adrenerg receptors present in this preparation.

Safety pharmacology - The effect of test compound on the spontaneous motility of the isolated rabbit ileum

[redacted]. 1991: Safety pharmacology - The effect of test compound on the spontaneous motility of the isolated rabbit ileum. [redacted] unpublished, Reference No. SIP/8/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: New Zealand white rabbit, delivered by [redacted]
Male and female rabbits were killed by an overdose of sodium pentobarbitone given as an intraperitoneal injection. A piece of ileum was removed and suspended in an organ bath. Dose response data for acetylcholine and adrenaline were compared to the effects of 3, 10 and 30 µg/mL dimethomorph on the spontaneous motility of the rabbit ileum in five preparations from five male animals and in five preparations from five female animals. Three preparations from each sex received concentrations of the vehicle, dimethylformamide (DMF), equivalent to that used for the three concentrations of dimethomorph.

Findings:
Acetylcholine produced a dose-related increase in the force of the spontaneous contractions.
Adrenaline inhibited the force of the spontaneous contractions in a dose-related manner.
The predominant action of DMF, the vehicle, was to inhibit the force of spontaneous contractions.
The predominant action of dimethomorph was to inhibit the force of the spontaneous contractions. This inhibition was comparable to that seen with DMF.
DMF appeared to potentiate the effects of acetylcholine and to a certain extent, adrenaline in female tissue.
Dimethomorph had no statistically significant effect on the response to acetylcholine or adrenaline.

Conclusion:
Dimethomorph did not directly effect the spontaneous motility of the isolated rabbit ileum neither did it alter the effects of acetycholine and adrenaline on this tissue.

Safety pharmacology - The effect of the test compound on the isolated guinea-pig ileum and its actions on acetylcholine, histamine, 5-hydroxytryptamine and barium chloride

[redacted] 1991: Safety pharmacology - The effect of test compound on on the isolated guinea-pig ileum and its actions on acetylcholine, histamine, 5-hydroxytryptamine and barium chloride. [redacted] unpublished, Reference No. SIP/13/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: Dunkin Hartley guinea pig, delivered by [redacted]
Male and female guinea pigs were killed by cervical dislocation and a piece of ileum removed and suspended in an organ bath. Sub-maximal doses of acetylcholine, histamine, 5-hydroxytryptamine and barium chloride (BaCl2) were given and then repeated in the presence of hexamethonium, atropine and 1, 10 and 30 µg/mL dimethomorph. A minimum of two preparations for each sex received organ bath concentrations of 3 % of the vehicle, (dimethyl formamide (DMF), the equivalent concentration for 30 µg/mL dimethomorph. One preparation for each sex received the vehicle at all three concentrations (0.3, 1.0 and 3.0 %) used in the administration of dimethomorph.

Findings:
Acetylcholine, histamine, 5-hydroxytryptamine and $BaCl_2$ produced contraction of the guinea pig ileum.
Hexamethonium inhibited the 5-hydroxytryptamine response in female tissue and potentiated the acetylcholine response in male tissue.
Atropine antagonised the responses to all four agonists tested in both male and female tissue.
DMF, the vehicle, produced a concentration dependent inhibition of responses to the four agonists. DMF had no direct effect on the tissue.
Dimethomorph produced a dose-related inhibition of responses to all four agonists, the maximum dose, 30 µg/mL, producing almost complete inhibition of the response. Dimethomorph had no direct effect on the tissue.

Conclusion:
Dimethomorph at concentrations of 3, 10 and 30 µg/mL did not directly effect the isolated guinea pig ileum but appeared to inhibit agonist-induced contractions. However, this inhibition may have been caused by the vehicle used in this study (DMF) which also inhibited contractions caused by the same agonists.

Safety pharmacology - Assessment of potential anti-inflammatory activity using the carrageenan induced rat paw oedema model

[redacted]. 1991: Safety pharmacology - Assessment of potential anti-inflammatory activity using the carrageenan induced rat paw oedema model. [redacted] unpublished, Reference No. SIP/12/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2%, E-to Z-isomer ratio 45:55
Test animals: male and female Crl:SD(CD)BR rats (UK), delivered by [redacted]
Groups of male and of female rats, each group comprising eight male or female animals, were treated with one of five treatments by oral gavage either carboxymethylcellulose (CMC) 2 ml/kg, indomethacin 10 mg/kg, dimethomorph 30 mg/kg, 100 mg/kg oder 300 mg/kg.
One our after oral dosing, oedema was induced in the right hind paw of each animal by a single injection of 0.1 mL 1 % w/v kappa carrageenan. The volume of the right hind paw was then measured at 0, 1, 2.5 and 4 hours after induction of oedema.

Findings:
The vehicle treated animals showed a progressive increase in the volume of the right hind paw of 13, 18 and 23 % in male animals over the period of the experiment and of 5, 25 and 23 % female animals.
Administration of indomethacin to both male and female animals, inhibited the development of oedema.
Administration of dimethomorph to male animals caused a potentiation of the rate of development of oedema at a dose level of 30 mg/kg (although the maximum oedema caused was consistent with that observed in vehicle treated animals), had no effect at a dose level of 100 mg/kg but at 300 mg/kg inhibited oedema formation.
Administration of dimethomorph to female animals had no effect on the rate of development of oedema.

Conclusion:
Dimethomorph potentiated inflammatory reactions at low dose levels but inhibited inflammatory reactions at high dose levels in the male rat. However, since there was no dose-response relationship the relevance of the result obtained with the low dose level is uncertain. Dimethomorph had no effect on inflammatory reactions in the female rat.

Safety pharmacology - Surface anaesthetic activity in the guinea-pig

[redacted]. 1991: Safety pharmacology - Surface anaesthetic activity in the guinea-pig. [redacted], unpublished, Reference No. SIP/10/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: male Dunkin Hartley guinea pigs, delivered by [redacted]
Three groups of male guinea pigs were given 0.1 mL by intradermal injection of either vehicle (50 % sterile water and 50 % dimethylformamide (DMF), 1% lignocaine hydrochloride, of 1 % dimethomorph. Five minutes after intradermal injection, each guinea-pig was tested for presence of anaesthesia by pricking the area of injection and subsequently determining the response on a 0 - 5 scale.

Findings:
The mean score for animals that received 0.1 mL of the vehicle was 1.50.
The mean score for animals given the standard, 1 % lignocaine hydrochloride was 0.00. This was statistically significantly different to the vehicle treated group.
The mean score for the animals treated with 1 % dimethomorph was 2.17. This was not statistically different to the vehicle treated group.

Conclusion:
Dimethomorph, administered intradermally at a concentration of 1 % w/v, had no local anaesthetic activity.

Safety pharmacology - The effect of test compound on the Irwin test in mice

[redacted]. 1991: The effect of test compound on the Irwin test in mice. [redacted] unpublished, Reference No. SIP/1/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: male and female mice of the CD-1 strain, delivered by [redacted]
Eight groups of mice, each group comprising five animals, were treated with one of four treatments, by oral gavage, either vehicle (sterile water) 10 mL/kg, dimethomorph 30 mg/kg, dimethomorph 100 mg/kg, dimethomorph 300 mg/kg. One group of male mice and one group of female mice were administered each treatment. A dose volume of 10 mL/kg was used.
At 45 and 105 minutes after dosing, each mouse was observed, using a simple scoring system, to assess changes in the central, autonomic and somatic nervous systems and dependent, or independent, changes in muscle tone, reflexes, respiration, circulation, renal and gastrointestinal function.

Findings:
In animals administered sterile water, 10 mL/kg, small decreases in grip strength and pain responses were observed in both male and female animals. A small decrease in startle responses was observed in female animals.
Animals treated with dimethomorph showed decreased grip strength and pain responses, similar to vehicle treated animals. In addition, piloerection and increased cutaneous blood flow were observed at all dose levels tested. At the higher dose levels of 100 and 300 mg/kg increased cage dispersal, apathetic behaviour and a change in respiration (a panting effect) were observed. In general, the effects on behaviour seemed most prominent at the 105 minute observation point.

Conclusion:
Dimethomorph administered orally to male and female mice at doses of 30, 100 and 300 mg/kg demonstrated only small and diverse changes in overt behaviour when analysed in the Irwin test.

Safety pharmacology - Tail flick test for analgesia

[redacted] 1991: Tail flick test for analgesia. [redacted] unpublished, Reference No. SIP/5/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: male mice of the CD-1 strain, delivered by [redacted]
Three groups of six male mice were administered either vehicle (sterile water), 10 ml/kg, acetysalicyclic acid, 30 mg/kg, or dimethomorph, 100 mg/kg orally by gavage. A dose volume of 10 mL/kg was used. One hour after dosing the pain response of each mouse was tested by applying a small clip to the base of the tail and in the time taken for the animals to react was measured.

Findings:
The mean time taken for the tails to be flicked after administration of the vehicle control, sterile water, was 0.17 seconds.
The mean time taken for the tails to be flicked after administration of the standard, acetylsalicylic acide, was 1.5 seconds.
The mean time taken for the tails to be flicked after administration of the test compound, dimethomorph, was 0.17 seconds.

Conclusion:
Dimethomorph, administered orally at a dose level of 100 mg/kg demonstrated no analgesic activity in the mouse.

Safety pharmacology - Spontaneous motor activity in mice

[redacted] 1991: Spontaneous motor activity in mice. [redacted] unpublished, Reference No. SIP/2/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: male mice of the CD-1 strain, delivered by [redacted]
Three groups of six male mice were administered either vehicle (sterile water) 10 mL/kg, chlordiazepoxide, 10 mg/kg or dimethomorph, 100 mg/kg orally by gavage. A dose volume of 10 mL/kg was used. One hour after dosing, each mouse was placed individually into a cage divided into two equal areas by means of a line drawn across the middle. The spontaneous motor acticity of each mouse was assessed by counting the number of times it crossed the line in a 3 minute period.

Findings:
The mean number of line crossings made by the mice given the vehicle control, sterile water was 13.67.
The mean number of line crossings made by the mice given the standard, chlordiazepoxide was 16.50.
The mean number of line crossings made by the mice given dimethomorph, was 10.00. This lower value may possibly arise from a higher grooming activity observed in these mice.

Conclusion:

Dimethomorph, administered orally at a dose level of 100 mg/kg had no statistically significant effect on spontaneous locomotor activity in mice. The small reduction in locomotor activity and increase in grooming activity probably reflected individual animal variation.

Safety pharmacology - Potentiation of hexobarbitone sleeping time in mice

[redacted] 1991: Potentiation of hexobarbitone sleeping time in mice. [redacted], Reference No. SIP/4/PH

GLP: Yes (certified by the quality assurance of the [redacted])

Guideline: No guideline study

Deviations: Not applicable

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: Dimethomorph (SAG 151; CME 151); Batch No. T3/85, purity 94 ± 2 %, E-to Z-isomer ratio 45:55
Test animals: male mice of the CD-1 strain, delivered by [redacted]

Three groups of six male mice were administered either vehicle (sterile water) 10 mL/kg, chlordiazepoxide 10 mg/kg or dimethomorph 100 mg/kg orally by gavage. A dose volume of 10 mL/kg was used. Two hours after dosing each mouse was administered sodium pentobarbitone, 60 mg/kg, by intraperitoneal injection. The animals were then placed on their back of side and observed until the righting reflex was regained. The time elapsing between the injection of sodium pentobarbitone and the regaining of the righting reflex, for each animal, was the sleeping time.

Findings:
The mean sleeping time for the vehicle control group, treated with sterile water, was 103.83 minutes.
The mean sleeping time for the standard group, treated with chlordiazepoxide, was 187.17 minutes.
The mean sleeping time for the test group, treated with dimethomorph, was 257.00 minutes.

Conclusion:
Dimthomorph, administered orally at a dose level of 100 mg/kg, potentiated the pentobarbitone sleeping time. This effect may have been due to competition with pentobarbitone in pathways of metabolism by liver microsomal enzymes.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed)

Immunotoxicity studies:

Report: CA 5.8.2/1
[redacted]., 2010 b
BAS 550 F (Dimethomorph) - Immunotoxicity study in male Wistar rats - Administration via the diet for 4 weeks
2010/1043718
Guidelines: EPA 870.7800
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of BAS 550 F (Batch: AC9978-131; Purity: 97.5%) in female Wistar rats was analyzed using dietary dose levels of 0, 300, 800 and 2400 ppm (corresponding to mean intake levels of 23, 61 and 184 mg/kg bw/day, respectively) for 28 days. The parameters used for detection of potential test substance related alterations in the morphology of the immune system included a) the determination of lymphoid organ weights (spleen and thymus) and b) the analysis of the primary humoral (IgM response) immune response to sheep red blood cells (SRBC).
Treatment with BAS 550 F did not result in clinical signs or mortality in any test group. However, in the high dose group (2400 ppm) a significantly lower body weight gain (about 12-19% less as compared to the control) was observed from day 7 day until day 14 of treatment. None of the immunotoxicologically relevant parameters mentioned above were affected by treatment with BAS 550 F up to the highest dose level tested.
Concurrent treatment with positive control substance, cyclophosphamide monohydrate (CPA, 4.5 mg/kg bw/day) induced clear signs of immunotoxicity, demonstrating the reliability of the test system under the study conditions employed.

Based on the obtained results it can be concluded that BAS 550 F does not bear an immunomodulatory/immunotoxic potential under the conditions of this study. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 2400 ppm corresponding to 184 mg/kg bw/day. The NOAEL for systemic toxicity was 800 ppm corresponding to 61 mg/kg bw/day in male Wistar rats.

(DocID 2010/1043718)

I. MATERIAL AND METHODS

1. Test Material:	BAS 550 F (Dimethomorph)
Description:	solid/ white
Lot/Batch #:	AC9978-131
Purity:	97.5%
Stability of test compound:	The test substance was stable over the study period (Expiry date Mar 01, 2015).
2. Vehicle control:	Rodent diet
3. Positive control:	Cyclophosphamide monohydrate (CPA)
Description:	Solid / white
Lot/Batch #:	1362353
Purity:	100% (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date Oct. 2010).
Vehicle for CPA:	Drinking water

4. Test animals:

Species:	Rat
Strain:	Crl:WI(HAN)
Sex:	Male (more sensitive gender based on previous studies)
Age:	34 ± 1 days at delivery; approx. 42 ± 1 days at start of administration
Reason for the selection:	The rat is a frequently used laboratory animal, and there is comprehensive experience with this animal species. Moreover, the rat has been proposed as a suitable animal species by the OECD and the EPA for this type of study.
Weight at dosing:	155-183 g
Source:	[redacted]
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	4 animals per cage in H-Temp (PSU, floor area about 2065 cm^2) cages (TECNIPLAST, Hohenpeißenberg, Germany)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	10/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 01-Sep-2009 - 24-Mar-2010
(In life dates: 09-Sep-2009 (start of administration) to 08-Oct-2009 (necropsy))

2. Animal assignment and treatment:
BAS 550 F was administered to groups of 8 male rats at dietary concentrations of 0, 300, 800 and 2400 ppm for 28 days.
Additionally, 8 male rats were treated orally (gavage) with 4.5 mg/kg bw/day Cyclophosphamide monohydrate (CPA; positive control substance). CPA was administered as a solution in drinking water at a volume of 10 mL/kg. The administered volume was determined based on the most recently determined body weights.
The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.
On day 23 of the study all animals received a single intraperitoneal injection (0.5 mL) of a sheep red blood cell (SRBC)-suspension containing 4 x 10^8 cells/mL for immunization.

3. Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Test substance preparations were mixed once before the start of administration.

The stability of the test substance dimethomorph in the diet over a period of up to 49 days was proven before the start of the study. Homogeneity and concentration analyses of the diet preparations were performed at the beginning of the administration period for all concentrations.

Table 5.8.2-1: Results of homogeneity and concentration control analysis of dimethomorph in rodent diet

Nominal Dose level [ppm]	Sampling	Mean of nominal concentration [%]	Relative standard deviation [%]
300	Sep. 08, 2009	94.7	1.1
800	"	99.1	2.9
2400	"	100.8	1.3

Relative standard deviations of the homogeneity of the dimethomorph samples were quite low, which indicates the homogenous distribution of dimethomorph in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 94.7 to 100.8% of the nominal concentrations confirming the correctness of the concentrations.

The positive control substance preparation (CPA in drinking water) was prepared once at the beginning of the study, split in daily aliquots and deep frozen at -18°C. The mixtures were applied when reaching room temperature. The concentration control of the CPA solution was performed at the beginning of the study. Since the CPA formulation in drinking water was a solution a homogeneity analysis was redundant. The stability analysis conducted revealed the stability of the CPA solution for 66 days when stored frozen.
The actual CPA concentrations were 108.2 and 108.7% of the nominal concentration confirming the correctness of the concentration.

Table 5.8.2-2: Results of concentration control analysis of CPA in drinking water

Nominal Concentration [g/100 mL]	Sampling / Analysis	Analytical concentration [g/100 mL]	Mean of nominal concentration [%]
0.045	Sep. 08, 2009 / Nov. 09, 2009	0.0432	96.0
0.045	Sep. 08, 2009 / Nov. 09, 2009	0.0430	95.6

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table 5.8.2-3: Statistics of clinical examinations

Parameter	Statistical test	Markers in the tables	References
body weight and body weight change	<u>For the test substance and the control groups:</u> A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means <u>For the vehicle and positive control groups:</u> A comparison of the dose group with the control group was performed using the t-test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491 WINER, B.J. (1971): Statistical principles in experimental design. McGraw-Hill, New York, 2nd edition
Clinical pathology parameters	<u>For the test substance and the control groups:</u> Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each test group with the control group was performed using WILCOXON test for the equal medians <u>For the vehicle and positive control groups:</u> Pair-wise comparison of the dose group with the control group was performed using Wilcoxon test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Nonparametric statistics for the behavioural sciences. McGraw-Hill New York SIEGEL, S. (1956): Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York
Pathology weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each test group with the control group was performed using the WILCOXON test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference, Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily. Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. The animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable.

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Food consumption and compound intake:

Food consumption was determined weekly for each cage. The average food consumption per cage was used to estimate the mean food consumption in grams per animal per day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g) on day x, C as the concentration in the food on day x (in mg/kg) and BW_x as body weight on day x of the study (in g).

3. Water consumption:

Drinking water consumption was observed by daily visual inspection of the water bottles for any overt changes in volume.

4. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of the administration period) and thereafter at weekly intervals. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

5. Analysis of the primary immune response:

In the morning blood was taken from the retro-orbital venous plexus from not-fasted animals. The animals were anaesthetized using isoflurane (Isoba®, Essex GmbH Munich, Germany). The blood sampling procedure and the subsequent analysis of the blood and plasma samples were carried out in a randomized sequence.
The following examinations were carried out in 8 male animals per test group.

Primary T-cell dependent antibody response (anti-SRBC IgM ELISA)

Plasma samples from all SRBC immunized animals were analyzed for their specific anti SRBC-IgM titer in an ELISA. Each serum sample was diluted to 1:64 and 1:128. SRBC-IgM concentrations outside the standard curve range were measured in a second test run with an appropriate dilution. Generally, two in-house controls were used for a standard curve. The ELISA was measured with a Sunrise MTP-reader (Tecan AG, Maennedorf, Switzerland), and evaluated with the Magellan-Software of the instrument producer.

6. Necropsy and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following weights were determined for all animals sacrificed at scheduled dates:

1. Anesthetized animals
2. Spleen
3. Thymus

No further histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance BAS 550 F (Dimethomorph) in the diet was demonstrated over a period of up to 49 days at room temperature. As the mixtures were stored no longer than this time period, the stability was guaranteed. The stability of Cyclophosphamide monohydrate (positive control substance) in drinking water was demonstrated for 66 days under study conditions.

Considering the low standard deviation in the homogeneity analysis, it can be concluded that BAS 550 F (Dimethomorph) was distributed homogeneously in feed. Due to the fact that Cyclophosphamide monohydrate (positive control substance) was a solution in drinking water no homogeneity analysis was performed.

The concentration control analyses revealed that the values of the test substance BAS 550 F (Dimethomorph) in feed and the positive control substance (Cyclophosphamide monohydrate) in drinking water were in the expected range of the target concentrations, i.e. were always in a range of 93.8 - 102.4% for the test-substance preparations and 95.6% and 96.0% for the positive control substance, of the nominal concentrations.

B. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs were observed in all animals treated with BAS 550 F (Dimethomorph) and in animals which received Cyclophosphamide monohydrate as positive control.

2. Mortality

No mortality was observed in this study.

B. FOOD CONSUMPTION, DRINKING WATER CONSUMPTION, AND COMPOUND INTAKE

Food consumption was slightly reduced in all animals treated with BAS 550 F (Dimethomorph) during the study. However, a clear dose-response was not observed and these changes were assessed as being incidental. In contrast, a more pronounced effect with regard to food consumption was observed for the positive control animals (Cyclophosphamide monohydrate) with a maximum of -12.9% on days 7 and 14. This change was assessed as treatment-related.

No test substance-related findings were observed regarding drinking water consumption.

Treatment	BAS 550 F				CPA
Dose level	0 ppm	300 ppm	800 ppm	2400 ppm	4.5 mg/kg
Food consumption per cage [g]					
- Day 6 to 7	18.56	17.35	17.15	17.08	16.16
- Day 13 to 14	20.95	20.05	20.71	19.68	18.25
- Day 20 to 21	20.00	18.38	19.09	18.99	17.70
- Day 27 to 28	22.06	21.04	22.52	21.96	20.06

The mean daily test substance intake in mg/kg body weight/day over the entire study period was calculated and is shown in the following table:

Table 5.8.2-4: Calculated intake of BAS 550 F

Test group	Concentration in the diet (ppm)	Mean daily test-substance intake (mg/kg bw/d)
		Males
1	300	23
2	800	61
3	2400	184

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weights in animals of test group 3 (2400 ppm; BAS 550 F) and 1 (300 ppm; BAS 550 F) were slightly lower from day 7 until day 28 but not significantly different when compared to the control animals. Body weights of rats treated with Cyclophosphamide monohydrate were significantly impaired on days 21 and 28, with a maximum of -7.6% on day 28. This finding was considered as being related to treatment with the positive control substance. Body weight changes in animals of test group 3 (2400 ppm; BAS 550 F) were significantly lower from day 7 until day 28 with a maximum of 19.3% less on day 7 when compared to the controls. As the body weight change was nearly 12% less on study day 28 a relation to treatment was indicated. Body weight change of male rats treated with Cyclophosphamide monohydrate was impaired throughout the whole administration period, with a maximum of 21.7% less on day 7. This finding was assessed as treatment-related.

Figure 5.8.2-1: Body weight development of rats administered BAS 550 F for 28 days

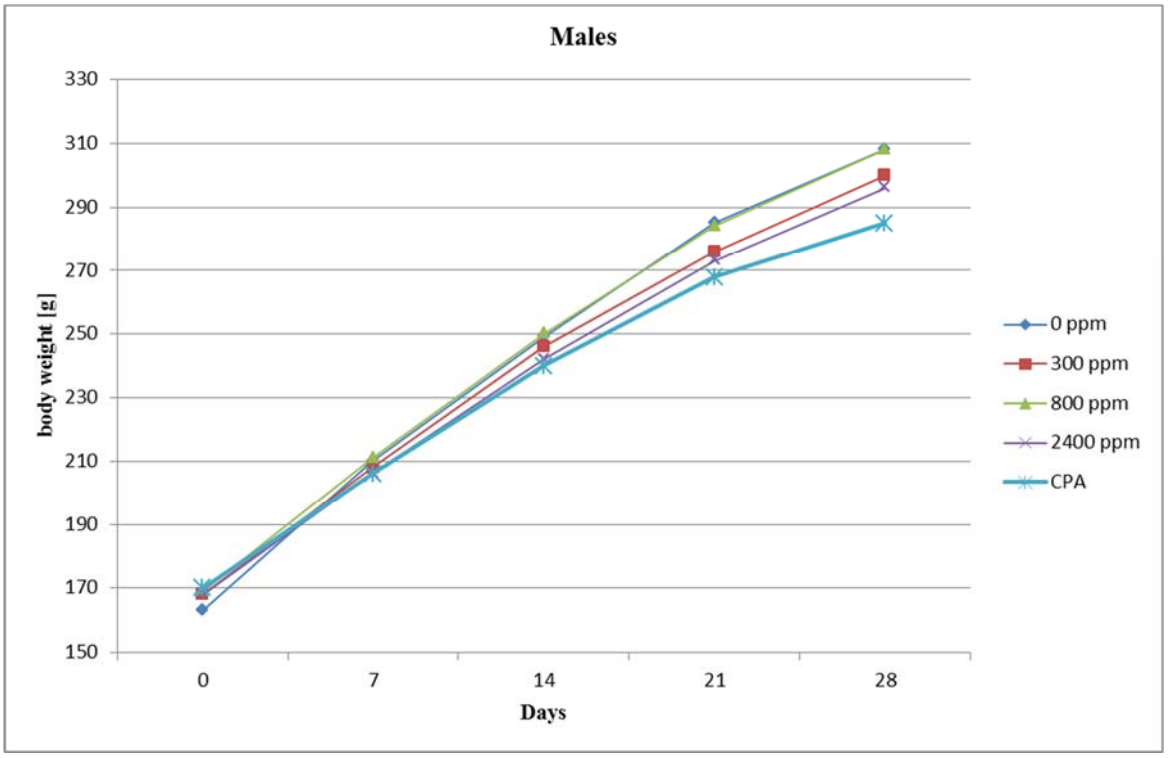


Table 5.8.2-5: Mean body weights of rats administered BAS 550 F or Cyclophosphamide (CPA) for 28 days

Treatment	BAS 550 F				CPA
Dose level	0 ppm	300 ppm	800 ppm	2400 ppm	4.5 mg/kg
Body weight [g]					
- Day 0	163	168	169	168	170
- Day 7	210	208	211	206	206
- Day 14	249	246	250	242	240
- Day 21	285	276	284	273	**268***
- Day 28	308	300	308	296	**285***
Day 28 Δ% (compared to control)	-	-2.77	-0.01	-3.98	-7.64

Table 5.8.2-6: Mean body weight changes of rats administered BAS 550 F or Cyclophosphamide (CPA) for 28 days

Treatment	BAS 550 F				CPA
Dose level	0 ppm	300 ppm	800 ppm	2400 ppm	4.5 mg/kg
Body weight [g]					
- Day 0-7	46.88	40.58	41.95	**37.81****	**36.70****
- Day 0-14	85.53	77.74	81.55	**74.20***	**70.96****
- Day 0-21	121.29	107.86	115.15	**105.34***	**98.44****
- Day 0-28	144.60	131.75	139.21	**127.81***	**114.99****

*: p≤0.05, **: p≤0.01

D. IMMUNOLOGICAL ANALYSES

1. Analysis of the primary T-cell dependent immune response

Six days after immunization, no changes in the SRBC IgM titres were found in male rats dosed with the test substance, whereas the SRBC titres were significantly lower in rats of test group 4 (Cyclophosphamide Monohydrate, positive control group) [see Table 5.8.2-7].

Table 5.8.2-7: Analysis of the specific primary (IgM) immune response to SRBC in rats treated with BAS 550 F or Cyclophosphamide (CPA) for 28 days

Treatment	BAS 550 F				CPA
Dose [ppm]	0	300	800	2400	
[mg/kg bw/day]		23	61	184	4.5
Specific IgM Titer (U/mL)					
- Mean ± SD	2653 ± 2147	5946 ± 7542	2018 ± 777	3728 ± 3253	**740** ** ± **266**
- Median	1477	2711	1873	2303	**820**

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test. two sided)

G. NECROPSY

1. Terminal body weight and organ weights

The absolute mean weights of animals in test groups 1-3 (300, 800 and 2400 ppm, BAS 550 F) did not show relevant differences compared to the control group. The positive control group (test group 4, Cyclophosphamide monohydrate) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result. In addition, the terminal body weights were significantly reduced.

The relative mean weights of animals in test groups 1-3 (300, 800 and 2400 ppm, BAS 550 F) did not show relevant differences compared to the control group. The positive control group (test group 4, Cyclophosphamide monohydrate) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result.

Table 5.8.2-8: Mean absolute and relative organ weights of male rats treated with BAS 550 F or Cyclophosphamide for at 28 days

Sex		**Metconazole**				**CPA**
Dose [ppm]		**0**	**300**	**800**	**2400**	
[mg/kg bw/day			**23**	**61**	**184**	**4.5**
Terminal bodyweight	[g]	289	278	289	279	**265***
	[% of control]	-	96	100	97	**92**
Spleen, absolute	[g]	0.609	0.55	0.586	0.584	**0.366****
	[% of control]	-	90	96	96	**60**
Spleen, relative	[%]	0.21	0.198	0.202	0.207	**0.138****
	[% of control	-	94	96	99	**66**
Thymus, absolute	[mg]	535	496	554	553	**220****
	[% of control]	-	93	103	103	**41**
Thymus, relative	[%]	0.185	0.178	0.192	0.201	**0.083****
	[% of control]	-	96	103	109	**45**

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross pathology

No gross lesions were observed in any test animal. In the absence of any gross lesions, no histopathological investigation was carried out.

III. CONCLUSIONS

Under the conditions of the study BAS 550 F did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to male Wistar rats. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 2400 ppm (184 mg/kg bw/d; highest dose tested). The NOAEL for systemic toxicity was set to 800 ppm (61 mg/kg bw/d). The oral administration of the positive control substance Cyclophosphamide Monohydrate (4.5 mg/kg bw/d) led to clear findings indicative of immunotoxicity. This was represented by significantly lower SRBC IgM antibody titres as well as reduced spleen and thymus weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in male Wistar rats.

Additional information from the literature search:

Kleinstreuer N.C. et al, 2011. Environmental impact on vascular development predicted by high-throughput screening. EHP, 119 (11), 1596-1603. [see KCA 5.8.2/2 2011/1296591]

Abstract:
Understanding health risks to embryonic development from exposure to environmental chemicals is a significant challenge given the diverse chemical landscape and paucity of data for most of these compounds. High-throughput screening (HTS) in the U.S. Environmental Protection Agency (EPA) ToxCast™ project provides vast data on an expanding chemical library currently consisting of > 1,000 unique compounds across > 500 *in vitro* assays in phase I (complete) and Phase II (under way). This public data set can be used to evaluate concentration-dependent effects on many diverse biological targets and build predictive models of prototypical toxicity pathways that can aid decision making for assessments of human develop mental health and disease. We mined the ToxCast phase I data set to identify signatures for potential chemical disruption of blood vessel formation and remodeling. ToxCast phase I screened 309 chemicals using 467 HTS assays across nine assay technology platforms. The assays measured direct inter actions between chemicals and molecular targets (receptors, enzymes), as well as downstream effects on reporter gene activity or cellular consequences. We ranked the chemicals according to individual vascular bioactivity score and visualized the ranking using ToxPi (Toxicological Priority Index) profiles. Targets in inflammatory chemokine signaling, the vascular endothelial growth factor pathway, and the plasminogen-activating system were strongly perturbed by some chemicals, and we found positive correlations with developmental effects from the U.S. EPA ToxRefDB (Toxicological Reference Database) in vivo database containing prenatal rat and rabbit guideline studies. We observed distinctly different correlative patterns for chemicals with effects in rabbits versus rats, despite derivation of in vitro signatures based on human cells and cell-free biochemical targets, implying conservation but potentially differential contributions of developmental pathways among species. Follow-up analysis with anti angiogenic thalidomide analogs and additional in vitro vascular targets showed in vitro activity consistent with the most active environmental chemicals tested here. We predicted that blood vessel development is a target for environmental chemicals acting as putative vascular disruptor compounds (pVDCs) and identified potential species differences in sensitive vascular develop mental pathways.

Analysis:
Putative Vascular Disruptor Compounds (pVDC) were identified using ToxCast data and some target of interest related to embryonic blood vessel formation: VEGFR2 (down regulation of the receptor thyrosine kinase (RTK)), TIE2 (inhibition of the enzymatic activity), CCL2 (down regulation of the proangiogenic chemokine), PAI-1 (perturbation of the plasminogen-activating system via up- or down-regulation of plasminogen activator inhibitor type 1), CXCL10 (up-regulation of the proinflammatory antiangiogenic chemokine), uPAR (perturbation of the plasminogen-activating system via up- or down-regulation of urokinase-type plasminogen activator receptor).
This publication represent more a methodological work to explore potential embryotoxicity in sceening developments. Dimethomorph is cited as a potential pVDC with some activity on uPAR, CCL2 and PAI-1. No numerical values are reported. As dimethomorph have been tested in both rats and rabbit in developmental guideline studies, this work is considered as supplementary information only and do not give new information.

Shah I. et al, 2011. Using nuclear receptor activity to stratify hepatocarcinogens. PLos ONE 6(2): e14584. [see KCA 5.8.2/3 2011/1295091]

Abtract: Nuclear receptors (NR) are a superfamily of ligand-activated transcription factors that control a range of cellular processes. Persistent stimulation of some NR is a non-genotoxic mechanism of rodent liver cancer with unclear relevance to humans. Here we report on a systematic analysis of new in vitro human NR activity data on 309 environmental chemicals in relationship to their liver cancer-related chronic outcomes in rodents. The effects of 309 environmental chemicals on human constitutive androstane receptors (CAR/NR1I3), pregnane X receptor (PXR/NR1I2), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPAR/NR1C), liver X receptors (LXR/NR1H), retinoic X receptors (RXR/NR2B) and steroid receptors (SR/NR3) were determined using in vitro data. Hepatic histopathology, observed in rodents after two years of chronic treatment for 171 of the 309 chemicals, was summarized by a cancer lesion progression grade. Chemicals that caused proliferative liver lesions in both rat and mouse were generally more active for the human receptors, relative to the compounds that only affected one rodent species, and these changes were significant for PPAR (pv0.001), PXR (pv0.01) and CAR (pv0.05). Though most chemicals exhibited receptor promiscuity, multivariate analysis clustered them into relatively few NR activity combinations. The human NR activity pattern of chemicals weakly associated with the severity of rodent liver cancer lesion progression (pv0.05). The rodent carcinogens had higher in vitro potency for human NR relative to non-carcinogens. Structurally diverse chemicals with similar NR promiscuity patterns weakly associated with the severity of rodent liver cancer progression. While these results do not prove the role of NR activation in human liver cancer, they do have implications for nuclear receptor chemical biology and provide insights into putative toxicity pathways. More importantly, these findings suggest the utility of in vitro assays for stratifying environmental contaminants based on a combination of human bioactivity and rodent toxicity.

Analysis:
In this publication, activities of chemicals assessed in the ToxCast program were listed for their potency to activate nuclear receptor related to hepatic carcinogenicity (RXR, SR, LXR, PPAR, PXP, CAR, AhR) and grouped into clusters related to their potential mode of action to induce such an effect. Dimethomorph is cited in category C VIII which correspond to low activity on receptors and very low lesion progression. This publication represent more a methodological work to explore potential hepatocarcinogenicity in sceening developments. As no liver tumors were observed in rats or mice in guideline studies, this publication is considered as supplementary data.

Al-Eryani L. et al, 2015. Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents. Toxicol. Pathol., 43, 482-497. [see KCA 5.8.2/4 2015/1279907]

Abstract: Toxicant-associated fatty liver disease (TAFLD) is a recently identified form of nonalcoholic fatty liver disease (NAFLD) associated with exposure to industrial chemicals and environmental pollutants. Numerous studies have been conducted to test the association between industrial chemicals/environmental pollutants and fatty liver disease both *in vivo* and in *vitro.* The objective of the article is to report a list of chemicals associated with TAFLD. Two federal databases of rodent toxicology studies: Toxicological Reference Database (ToxRefDB; .Environmental Protection Agency) and Chemical Effects in Biological Systems (CEBS, National Toxicology Program) - were searched for liver endpoints. Combined these 2 databases archive nearly 2000 rodent studies. Toxicant-associated steatohepatitis (TASH) descriptor, including fatty change, fatty necrosis, Oil red O-positive staining, steatosis and lipid deposition were queried. Using these search terms, 123 chemicals associated with fatty liver were identified. Pesticides and solvents were the most frequently identified chemicals, while polychlorinated biphenyls (PCBs)/dioxins were the most potent. About 44% of identified compounds were pesticides or their intermediates, and > 10% of pesticide registration studies in ToxRefDB were associated with fatty liver. Fungicides and herbicides were most frequently associated with fatty liver than insecticides. More research on pesticides, solvents, metals, and PCBs/dioxins in NAFLD/TAPLD is warranted due to their association with liver damage.

Analysis:
In this study, dimethomorph was identified as effects were seen in the 90-day rat study. But according to the results, as NOAEL is > 10 mg/kg bw/day, dimethomorph may be not clinically relevant for human steatohepatitis and was not listed as a chemical of concern.

CA 5.8.3 Endocrine disrupting properties

Findings of potential relevance to endocrine disruption identified in repeated dose, investigative and mechanistic studies performed with dimethomorph are discussed in this section.

1. Overview of effects seen in the repeated dose toxicity studies

A – Effects observed in sub-chronic and chronic studies:

No significant effects on endocrine organs or parameters were observed in 28-day or 90-day studies in rats with dimethomorph or Z and E-isomers.

In the 104-week chronic toxicity study in rats, testicular interstitial cell proliferation in males was observed at 2000 ppm, the highest dose tested. After 2 years of treatment, the incidences of testicular tumors in treatment groups were higher than incidences in control groups. However, as discussed during the last EU peer review the increased incidences were not related to treatment with dimethomorph and it was not considered to be oncogenic (see section 5.5).

No significant effects were seen in mice in mid- or long-term studies.

B – Effects observed in dog studies

In the 90-day study in dogs, absolute prostate weights and prostate-to-body weight ratios were significantly reduced, relative to controls at 1350 ppm. The changes in prostate weights noted in the 1350 ppm group were consistent with an apparent increase in the incidence of prostatic interstitial fibrosis for all animals of this group, as compared to the control group (see table 5.3.2-1 and 5.3.2-2 in section 5.3.2).

In the 1-year dog study, statistically significant increases in testes-to-body weight ratios were observed at 450 and 1350 ppm without any histopathological findings. Absolute testes weights were not affected. Decreases in absolute prostate weights (statistically significant) and prostate-to-body weight ratios (non-statistically significant) were observed at 1350 ppm when compared to controls (See section 5.3). The changes in prostate weights noted in the 1350 ppm group were consistent with a slight increase in the incidence and severity of prostatic interstitial fibrosis, as compared to controls. Thus, the tissue shrinkage resulting from interstitial fibrosis was reflected by a reduction in prostate weights only in the 1350 ppm group (see section 5.3).
.

C – Effects observed in reproductive studies

In the 2-generation study in rats, no adverse effects were observed on fertility or reproductive performances as well as development. One finding was considered non adverse: in the 1000 ppm group, the percentage of pups in the F1, F2a and F2b generations which achieved incisor eruption was reduced on one or more days from postnatal days 9 - 11 when compared to controls and the differences were statistically significant. There were no treatment-related effects observed for any other developmental landmark (i.e., pinna unfolding, hair growth, or eye opening). The mean days of incisor eruption for the F1, F2a and F2b litters in the 1000 ppm group were 10.5, 10.5 and 10.7 days, respectively while the mean days of incisor eruption for control litters were 9.6, 9.6 and 9.7 days, respectively, for the same generations. Thus, incisor eruption in the 1000 ppm group was delayed by approximately one day. Rat pups begin to eat solid food in addition to milk at around postnatal day 15. By this time incisor eruption was complete in all groups. Thus, the delay in incisor eruption did not interfere with the development of feeding ability and is not considered an adverse finding (see section 5.6).

In the extended 1-generation study in rats, no indications of adverse effects on fertility or reproductive performance as well as development were observed. A slight increase of the gestation duration was observed at the highest tested dose but was considered not treatment related. Pup body weight development was affected in the 1600 ppm dose group (-13% after birth) but had no effect on pup survival.
A slight effect on anogenital distance/index in mid and high dose males as well as a statistically significant delay in vaginal opening (female F1 offspring of 1600 ppm group) and preputial separation (male F1 offspring of 800 and 1600 ppm group) were observed and may be partly explained by the decreased body weight leading to slow general development. Nipple retention in males was not affected. Slight effects were seen on some endocrine organ weights (relative or absolute or both) in parents and F1A or F1B generations. The only effect which was considered treatment related was a slight decrease in absolute and relative prostate weight in the F1B cohort from 800 ppm and higher. This effect was not observed in parents or F1A cohort. All effects on development observed after treatment with dimethomorph were of low severity and transient in nature. Hormone measurements (luteinizing hormone and testosterone) in male animals of cohort 1A did not reveal any changes.

Table 5.8.3-1: Potentially Endocrine-related effects in in vivo toxicity studies with dimethomorph

Study	Endpoint	Dose level	LOAEL	NOAEL	Reference
Dog. 90-day	Prostate weight reduction (absolute and relative) - prostatic interstitial fibrosis	1350 ppm (43 mg/kg bw/d)	1350 ppm (43 mg/kg bw/d)	450 ppm (15 mg/kg bw/d)	DK-425-002
Dog. 1-year	Testes weight increase (relative)	450 ppm (15 mg/kg bw/d)	450 ppm (15 mg/kg bw/d)	150 ppm (5 mg/kg bw/d)	DK-425-003
	Prostate weight reduction (absolute and relative) - prostatic interstitial fibrosis	1350 ppm (43 mg/kg bw/d)			
Rat. 2-year	Testicular interstitial cell proliferation	2000 ppm (100 mg/kg bw/d)	750 ppm (36 mg/kg bw/d)	200 ppm (9 mg/kg bw/d)	DK-427-006
Rat. 2G	Delay in incisor eruption in pups	1000 ppm (67 mg/kg bw/d)	1000 ppm (67 mg/kg bw/d)	300 ppm (20 mg/kg bw/d)	DK-430-001
Rat extended 1G	Decreased anogenital distance/index and delay of onset of puberty in males	800 ppm (70 mg/kg bw/d)	800 ppm (70 mg/kg bw/d)	300 ppm (26 mg/kg bw/d)	2014/1181670

2. In vitro mechanistic studies on ED properties

Report: CA 5.8.3/1
Woitkowiak C., 2011 d
BAS 550 F (Dimethomorph) - Testing for potential androgenic and antiandrogenic activity using the YAS-assay (AR) (yeast androgen screening)
2011/1140605
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

BAS 550 F (Dimethomorph; Batch: COD-001244; purity 99.8%) was tested to assess an androgenic and/or antiandrogenic activity by using the Yeast Androgen Screening Assay (YAS-Assay) with the hAR yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for androgenic effects (5α-dihydrotestosterone: 10 pM - 1 µM) and antiandrogenic effects (5α-dihydrotestosterone, 5 nM; hydroxyflutamide, 10 µM) were included into the experiment. BAS 550 F was tested at concentrations from 100 pM up to 100 µM. No precipitation and no cytotoxicity were observed up to the highest concentration tested. An increase in the androgen receptor dependent enzyme expression (color development) was not observed. A reproducible inhibition of the androgen effect compared to 5 nM 5α-dihydrotestosterone was observed at a concentration of 100 µM onward.
Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert androgenic effects while slight antiandrogenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.
(DocID 2011/1140605)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 550 F (Dimethomorph)

Description:	Solid, beige
Lot/Batch #:	COD-001244
Purity:	99.8% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01.03.2015
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	DMSO 1% (v/v)
Positive control compounds:	Androgenic control: 5α-dihydrotestosterone Antiandrogenic control: 5α-dihydrotestosterone combined with hydroxyflutamide

3. Test organisms:

Yeast cells (*Saccharomyces cerevisiae*) have been stably transformed with a gene encoding the human androgen receptor (hAR), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an androgen response element and the *LacZ* gene, which encodes the reporter enzyme β-galactosidase. The hAR yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-May-2011 to 27-May-2011

2. Test substance preparation:

The test substance was weighed and topped up with the chosen vehicle to achieve the required concentration of the stock solution. The test substance was dissolved in DMSO. To achieve a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.
Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

2. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for preculture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the preculture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).
The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

3. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of androgenic and antiandrogenic activity in the yeast cells.

Negative controls / Vehicle controls
The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls
Androgenic control:
5α-dihydrotestosterone (dissolved in ethanol)
Final concentrations: 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} mol/L

Antiandrogenic Control:
5α-dihydrotestosterone combined with hydroxyflutamide (dissolved in DMSO)
Final concentrations:
$5x10^{-9}$ mol/L (5α-dihydrotestosterone)/ $1x10^{-5}$ mol/L (hydroxyflutamide)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

4. Evaluation/Assessment

4.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (color development, androgen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

4.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of antiandrogenic activity only nontoxic test substance concentrations are taken into consideration.

4.2 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $5x10^{-9}$ mol/L 5α-dihydrotestosterone achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (color development) based on the experiment.
- The vehicle control did not show color development at 570 nm.

4.3 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of antiandrogenic activity.

A test substance is generally considered non-androgenic in this assay, if:

- Androgen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as androgenic in this assay, if:

- A concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (color development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (color development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly androgenic.

The test substance is considered as antiandrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 20% was observed compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone.
- If a concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone was observed, the test substance is considered to be slightly antiandrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Androgenicity:

An increase in the androgen receptor dependent enzyme expression (color development) was not observed.

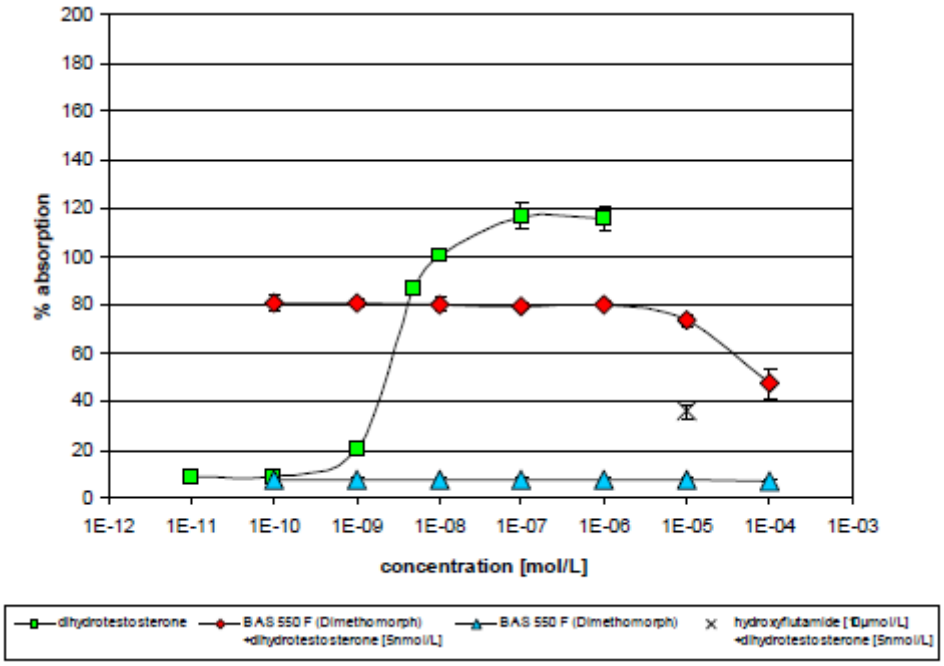
Antiandrogenicity:

A reproducible inhibition of the androgen effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development) was observed at a concentration of 10^{-4} mol/L.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

No cytotoxic effect (decrease of the yeast growth) was observed.

Figure 5.8.3-1: Androgen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10^{-8} M dihydrotestosterone)



III. CONCLUSIONS

Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert androgenic effects, only slight antiandrogenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

Report:	CA 5.8.3/2 Woitkowiak C., 2011 e BAS 550 F (Dimethomorph) - Testing for potential estrogenic and antiestrogenic activity using the YES-assay (ERalpha) (yeast estrogen screening) 2011/1140606
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

BAS 550 F (Dimethomorph; Batch: COD-001244; purity 99.8%) was tested to assess an estrogenic and/or antiestrogenic activity by using the Yeast Estrogen Screening Assay (YES-Assay) with the hERα yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for estrogenic effects (17β-estradiol: 1 pM - 1 µM) and antiestrogenic effects (17β-estradiol, 1 nM; 4-hydroxytamoxifen, 1 µM) were included into the experiment. BAS 550 F was tested at concentrations from 100 pM up to 100 µM. No precipitation and no cytotoxicity were observed up to the highest concentration tested. An increase in the estrogen receptor dependent enzyme expression (color development) was not observed. Furthermore, no inhibition of the estrogen effect compared to 1 nM 17β-estradiol was observed.
Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert estrogenic or antiestrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.
(DocID 2011/1140606)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 550 F (Dimethomorph)

Description:	Solid, beige
Lot/Batch #:	COD-001244
Purity:	99.8% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01.03.2015
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	DMSO 1% (v/v)
Positive control compounds:	Estrogenic control: 17β-estradiol
	Antiestrogenic control: 17β-estradiol combined with 4-hydroxytamoxifen

3. Test organisms: Yeast cells (*Saccharomyces cerevisiae*) have been stably transformed with a gene encoding the human estrogen receptor α (hERα), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an estrogen response element and the *LacZ* gene, which encodes the reporter enzyme β-galactosidase. The hERα yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-May-2011 to 27-May-2011

2. Test substance preparation:

The test substance was weighed and topped up with the chosen vehicle to achieve the required concentration of the stock solution. The test substance was dissolved in DMSO. To achieve a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.

Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

2. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for preculture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the preculture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).
The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

3. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of estrogenic and antiestrogenic activity in the yeast cells.

Negative controls / Vehicle controls
The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls
Estrogenic control:
17β-estradiol (dissolved in ethanol)
Final concentrations: 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/L

Antiestrogenic Control:
17β-estradiol combined with 4-hydroxytamoxifen (dissolved in DMSO)
Final concentrations:
$1x10^{-9}$ mol/L (17β-estradiol)/ $1x10^{-6}$ mol/L (4-hydroxytamoxifen)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

4. Evaluation/Assessment

4.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (color development, estrogen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

4.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of antiestrogenic activity only nontoxic test substance concentrations are taken into consideration.

4.2 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $1x10^{-9}$ mol/L 17β-estradiol achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (color development) based on the experiment.
- The vehicle control did not show color development at 570 nm.

4.3 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of antiestrogenic activity.

A test substance is generally considered non-estrogenic in this assay, if:

- Estrogen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as estrogenic in this assay, if:

- A concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (color development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (color development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly estrogenic.

The test substance is considered as antiestrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 20% was observed compared to $1x10^{-9}$ mol/L 17β-estradiol alone.
- If a concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $1x10^{-9}$ mol/L 17β-estradiol alone was observed, the test substance is considered to be slightly antiestrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Estrogenicity:

An increase in the androgen receptor dependent enzyme expression (color development) was not observed.

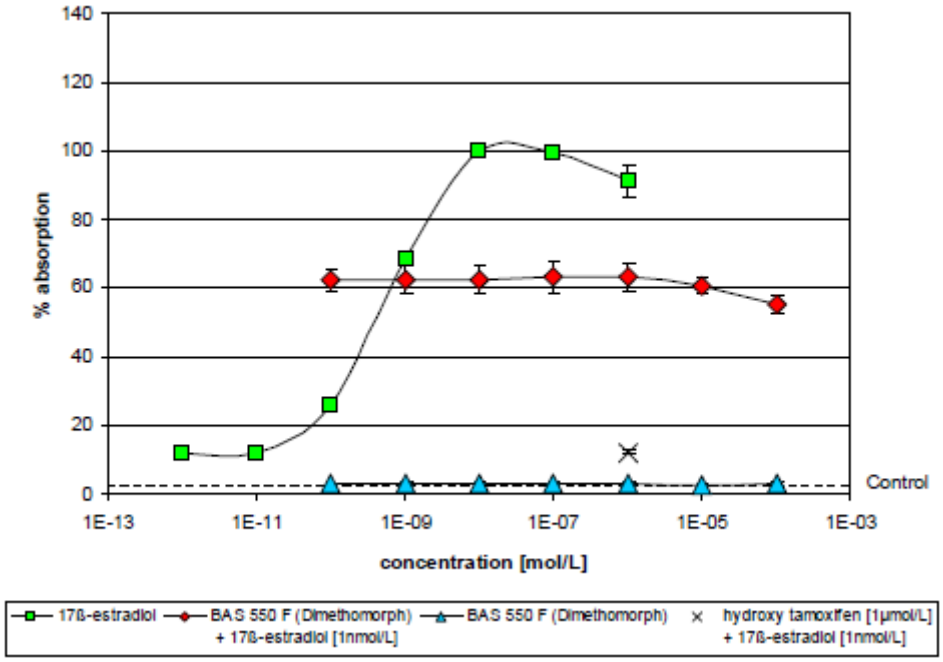
Antiestrogenicity:

A reproducible inhibition of the androgen effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development) was not observed.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

No cytotoxic effect (decrease of the yeast growth) was observed.

Figure 5.8.3-2: Estrogen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10^{-8} M 17β-estradiol)



III. CONCLUSIONS

Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert estrogenic or antiestrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.

Additional information from the literature search:

Bitsch N. et al, 2002. In vitro screening of the estrogenic activity of active components in pesticides (in German). Z. Umweltchem. Okotox. 14 (2) 76-84. [see KCA 5.8.3/3 2002/1028017]

Abstract: Over the last years, the obviously increasing, hormone-dependent impairments observed in human and animals, as well as the increased occurrence of hormone-dependent types of cancers, are sometimes associated with environmental chemicals which ae suspected to imitate or block the effects of natural hormones. For a variety of environmental chemicals an endocrine efficacy could already be demonstrated. Little is known, however, about a possible hormonal activity of plant protection agents which are at present certificated in the Federal Republic of Germany. The aim of the present study was the in vitro testing of at least 57 active ingredients of pesticides and growth regulators certificated in the Federal Republic of Germany, for their possible estrogenic activity. The E-screen-Assay based on the human breast cancer cell line MCF-7 was used as the suitable test system. For at least 8 of the tested substances, an receptor-mediated estrogenic activity could be shown in vitro. With the exception of one substance, 7 active ingredients displayed a very weak affinity for the human estrogen receptor. Their ability to displace 17b-estradiol from the receptor was low. Only the herbicide pendimethalin is to be classified as a full estrogen receptor agonist, it is able to displace the 17b-estradiol almost completely from the receptor. However, the estrogenic potency of pendimethalin, in comparison to 17b-estradiol, was seen to be as small as the estrogenic potency of the other 7 substances tested positively in the E-Screen-Assay. Among these 7 substances, two could not be classified as estrogenically active anymore, because their affinity to the human estrogen receptor was too weak. The relevance of the available results for living organisms should be clarified in further in vivo investigations.

Analysis: in this publication, dimethomorph was devoid of any estrogenic activity. Which confirms the results of the YES assay performed (see 5.8.3/2).

Orton F. et al, 2011. Widely used pesticides with previously unknown endocrine activity revealed as in vitro anti-androgens. Environ. Health Perspect., 109 (6), 794-800. [see KCA 5.8.3/4 2011/1298391]

Abstract: Evidence suggests that there is widespread decline in male reproductive health and anti-androgenic pollutants may play a significant role. There is also a clear disparity between pesticide exposure and endocrine disrupting data, with the majority of the published literature focused on pesticides that are no longer registered for use in developed countries. The aim of this study was to utilise estimated human exposure data to select pesticides to test for anti-androgenic activity, focusing on highest use pesticides. We used European databases to select 134 candidate pesticides based on highest exposure, followed by a filtering step according to known or predicted receptor mediated anti-androgenic potency, based on a previously published quantitative structure-activity relationship (QSAR) model. In total, 37 pesticides were tested for *in vitro* androgen receptor (AR) antagonism. Of these, 14 were previously reported to be AR antagonists ("active"), 4 were predicted AR antagonists using the QSAR, 6 were predicted to not be AR antagonists ("inactive"), and 13 with unknown activity, which were "out of domain" and therefore could not be classified with the QSAR ("unknown"). All 14 pesticides with previous evidence of AR antagonism were confirmed as anti-androgenic in our assay and 9 previously untested pesticides were identified as anti-androgenic (dimethomorph, fenhexamid, quinoxyfen, cyprodinil, λ-cyhalothrin, pyrimethanil, fludioxonil, azinphos-methyl, pirimiphos-methyl). In addition, 7 compounds were classified as androgenic. Due to estimated anti-androgenic potency, current use, estimated exposure, and lack of previous data, we strongly recommend that dimethomorph, fludioxonil, fenhexamid, imazalil, ortho-phenylphenol and pirimiphos-methyl be tested for anti-androgenic effects in vivo. The lack of human biomonitoring data for environmentally relevant pesticides presents a barrier to current risk assessment of pesticides on humans.

Analysis: The authors of this study performed an initial assessment of anti-androgenic properties using a QSAR model, the substances with positive predictions were tested in vitro in an assay in the stability transformed MDA-kb2 cell line. Substances demonstrating a positive response in the MDA-kb2 assay were additionally assessed using the Yeast Androgen Screen (YAS). The following results are presented for dimethomorph:

QSAR prediction	Anti-androgen IC20 (µM)		Cytotoxicty EC20 (µM)		Androgen IC20 (µM)
	MDA-kb2	YAS	MDA-kb2	YAS	MDA-kb2
Anti-androgen	0.263	38.5	> 25	> 50	Negative

The results of the YAS assay are confirmed by the performed by BASF ([see KCA 5.8.3/1 2011/1140605]).

Reif D.M. et al, 2010. Endocrine profiling and prioritization of environmental chemicals using ToxCast data. Environ. Health Perspect., 118 (12), 1714-1720. [see KCA 5.8.3/5 2010/1233632]

Abstract: The prioritization of chemicals for toxicity testing is a primary goal of the U.S. Environmental Protection Agency (EPA) ToxCast program. Phase I of ToxCast used a battery of 467 *in vitro*, high-throughput screening assays to assess 309 environmental chemicals. One important mode of action leading to toxicity is endocrine disruption, and the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) has been charged with screening pesticide chemicals and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife. The goal of this study was to develop a flexible method to facilitate the rational prioritization of chemicals for further evaluation and demonstrate its application as a candidate decision support tool for EDSP. Focusing on estrogen, androgen, and thyroid pathways, we defined putative endocrine profiles and derived a relative rank or score for the entire ToxCast library of 309 unique chemicals. Effects on other nuclear receptors and xenobiotic metabolizing enzymes were also considered, as were pertinent chemical descriptors and pathways relevant to endocrine-mediated signaling. Combining multiple data sources into an overall, weight-of-evidence Toxicological Priority Index (ToxPi) score for prioritizing further chemical testing resulted in more robust conclusions than any single data source taken alone. Incorporating data from *in vitro* assays, chemical descriptors, and biological pathways in this prioritization schema provided a flexible, comprehensive visualization and ranking of each chemical's potential endocrine activity. Importantly, ToxPi profiles provide a transparent visualization of the relative contribution of all information sources to an overall priority ranking. The method developed here is readily adaptable to diverse chemical prioritization tasks.

Analysis: in this publication used for screening of chemicals for prioritization for their potential endocrine effects, dimethomorph was cited in supplementary material. According to this data, no effects on estrogen or thyroid was identified and a potential effect on the androgen pathway reported. This publication is only considered as supplementary material as other studies have been performed to assess this issue.

Orton F. et al, 2014. Mixture effects at very low doses with combination of anti-androgenic pesticides, antioxidants, industrial pollutant and chemicals used in personal care products. Tox. Appl. Pharmacol., 278, 201-208. [see KCA 5.8.3/6 2014/1329032]

Abstract: Many xenobiotics have been identified as in vitro androgen receptor (AR) antagonists, but information about their ability to produce combined effects at low concentration is missing. Such data can reveal whether joint effects at the receptor are induced at low levels and may support the prioritization of in vivo evaluations and provide orientations for the grouping of anti-androgens in cumulative risk assessment. Combinations of 30 AR antagonists from a wide range of sources and exposure routes (pesticides, antioxidants, parabens, UV-filters, synthetic musks, bisphenol-A, benzo(a)pyrene, perfluorooctane sulfonate and pentabromodiphenyl ether) were tested using a reporter gene assay (MDA-kb2). Chemicals were combined at three mixture ratios, equivalent to single components' effect concentrations that inhibit the action of dihydrotesterone by 1%, 10% or 20%. Concentration addition (CA) and independent action were used to calculate additivity expectations. We observed complete suppression of dihydrotestosterone effects when chemicals were combined at individual concentrations eliciting 1%, 10% or 20% AR antagonistic effect. Due to the large number of mixture components, the combined AR antagonistic effects occurred at very low concentrations of individual mixture components. CA slightly underestimated the combined effects at all mixture ratios. In conclusion, large numbers of AR antagonists from a wide variety of sources and exposure routes have the ability of acting together at the receptor to produce joint effects at very low concentrations. Significant mixture effects are observed when chemicals are combined at concentrations that individually do not induce observable AR antagonistic effects. Cumulative risk assessment for AR antagonists should apply grouping criteria based on effects where data are available, rather than on criteria of chem. similarity.

Analysis: this publication is mainly focused on mixture effects. Dimethomorph is listed as a potential anti-androgen with an IC01 (produced 1% Androgen Receptor antagonistic effect) of 6.01 10^{-8} mole/L. These results are in line with assays already performed by the same team and also by BASF.

Archer. E, et al, 2015. The potential anti-androgenic effect of agricultural pesticides used in the Western Cape: In vitro investigation of mixture effects. Water SA, 41 (1), 129-137. [see KCA 5.8.3/7 2015/1278187]

Abstract: Although it is known that environmental chemicals can affect the estrogenic system, far less attention has been paid to chemicals interacting with the androgen receptor (AR). Pesticides, particularly fungicides, have been shown to competitively bind or affect expression of the AR in an inhibiting manner. Few studies have addressed anti-androgenic effects of agrochemicals use in South Africa. The aim of this study was to screen for the ability of commonly-used pesticides (mostly fungicides) in Western Cape agricultural areas to alter the binding of an androgen (DHT) to the human AR (hAR) using a recombinant yeast androgen screen (YAS), and also to test the additivity mixture interaction hypothesis when commonly-used pesticides with similar modes of action (MOAs) are exposed in mixtures Fungicides vinclozolin, folpet, procymidone, dimethomorph, fenarimol, mancozeb, and the insecticide chlorpyrifos, all independently antagonized the binding of the androgen dihydrotestosterone (DHT) to the AR in a dose-dependent manner. The fungicide mancozeb was found to be the most potent anti-androgen in the assay. Binary, equimolar mixtures of the pesticides also antagonized the binding of DHT to the AR, but at lower IC50 concentrations potencies relative to their individual counterparts. The mixtures of the majority of the selected pesticides did not conform to the expected additive mixture interaction. Only the mixture between dimethomorph and mancozeb showed an additive mixture response at IC50 concentration, and, therefore, revealed a more severe AR antagonistic effect compared to their individual counterparts. This study confirmed that pesticides regularly used in agriculture inhibit the binding of androgens to the AR, but when in mixture do not always conform to the predictive addition mixture response model. Also, high relative potencies of individual chemicals in the assay were suppressed when combined with less potent chemicals, showing that the potent chemicals may not be granted access to bind with the AR when exposed in mixture.

Analysis: in this publication, mixture effects of potential AR agonists were assessed. Individual values in the YAS assay were also reported. For dimethomorph, it was concluded that it was a weak AR agonist compared to other substances with an IC50 of 0.38 mM (147.40 mg/L) and with a steep slope in the dose-dependent response indicating a small concentration range of AR agonistic activity. These results are in line with the YAS assay conducted by BASF where AR agonistic activity was seen at high doses with the same profile of the dose-response curve.

Table 5.8.3-2: Summary of in vitro mechanistic studies.

Study	Endpoint	Outcome	Concentration tested		Reference
YES assay	Estrogen activity	Negative	10^{-10} - 10^{-4} mol/L	No effect	Woitkowiak 2011 DocID 2011/1140606
E-screen assay	Estrogen activity	Negative		No effect	Bitsch, 2002 DocID 2002/1028017
YAS assay	Anti-androgenicity	Positive	10^{-10} - 10^{-4} mol/L	IC50: 10^{-4} mol/L	Woitkowiak 2011 DocID 2011/1140605
MDA-kb2 assay	Anti-androgenicity	Positive		IC20: 0.263 µM	Orton, 2011 DocID 2011/1298391
YAS assay	Anti-androgenicity	Positive		IC20: 38.5 µM	Orton, 2011 DocID 2011/1298391
MDA-kb2 assay	Anti-androgenicity	Positive		IC01: 6.01 10^{-8} mole/L	Orton, 2014 DocID 2014/1329032
YAS assay	Anti-androgenicity	Positive	10^{-11} - 10^{-3} mol/L	IC50: 0.38 mM	Archer, 2015 DocID 2015/1278187

3. Discussion on ED properties of dimethomorph

The most widely used definition of an endocrine disruptor is based on the WHO/IPCS (2002): 'An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations'. This definition is purely based on hazard identification, looking at whether the effects reported are regarded to be ED-related and supported by mechanistic information.

According to Regulation (EC) No 1107/2009, Annex II, Point 3.6.5 'an active substance shall only be approved if, (…), it is not considered to have endocrine disrupting properties that may cause adverse effect in humans.'
Nevertheless, there is no regulatory guidance available yet on how to address endocrine disruption (ED) and no final criteria are established.
Pending the adoption of the final scientific criteria for the determination of ED properties, currently the so called Interim criteria are applied. There were two Interim criteria defined within Regulation (EC) No 1107/2009, Annex II, Point 3.6.5:

1) '(…) substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.'

2) 'Substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.'

For dimethomorph there is not sufficient evidence of having endocrine properties that may cause adverse effectes in humans based on the available data.

In *in vitro* studies, some effects related to anti-androgenicity have been reported for dimethomorph. Positive results in YAS and MDA-kb2 assays indicates a potential of dimethomorph for binding to the Androgen Receptors (AR) and subsequently a possible agonism with natural hormones. This occurred at relatively high concentrations *in vitro* and with a steep slope in the dose-dependent response indicating a small concentration range of AR agonistic activity.

To address the relevance of these in vitro findings, *in vivo* studies are available with dimethomorph. According to OECD Conceptual framework for testing and assessment of endocrine disrupters, level 4 studies and a level 5 study (2-generation study) were available in the previous submission and new level 5 study (extended 1-generation study) has been performed to complete the data package in order to provide additional endocrine endpoints which were not included in the 2-generation study.

In these studies, weak effects were observed on endocrine organs:

- In dogs, slight effects were observed on the prostate in the 90-day and the one-year study. These effects were limited to the highest tested dose of 1350 ppm and consisted of prostate weight reduction (absolute and relative) associated with increased severity of prostatic interstitial fibrosis. It should be noted that prostatic interstitial fibrosis is a common finding in dogs, seen in all groups including controls in the two studies and that a clear NOAEL is identified at 450 ppm.
  In the 1-year study, increase of relative testes weight was observed from 450 ppm together with a decrease of body weight gain. As no histopathological findings were observed, no change in absolute testes weight were noted and based on the fact that testes weight was not affected in the 90-day study, it can be assumed that this finding is related to the changes on body weight gain and not related to an endocrine mode of action.

- In rats, a borderline increased incidence of testicular tumors was observed in the 2-year study but this finding was considered not treatment related. This was mainly due to an increased longevity of the animals, the lack of statistical significance in oncogenic effects and the difference of interpretation criterias for the old studies (see section 5.5 for full argumentation). Thus an endocrine mode of action can be ruled out.

- In developmental studies, embryotoxicity was observed in rat and rabbits but only in association with severe maternal toxicity. Such effects were not seen in the 2-generation study and in the extended 1-generation study. Embryotoxicity can be explained by severe maternal toxicity associated with bolus effects of gavage. This effect is not considered to be due to an endocrine mode of action.

- In the reprotoxicity studies, a slight effect was seen in the 2-generation study on pup development (delay of incisor eruption). This was the only effect seen in developmental landmarks, very slight and reversible. As this effects is observed in isolation it is not considered to be due to an endocrine mode of action. In the extended 1-generation study, some effects were observed on developmental lankmarks: decreased anogenital distance/index and delay in onset of puberty in males at doses of about 70 mg/kg bw/day and higher. These effects were mostly concurrent with decreases in body weight and body weight gains. However, all changes seen affected only very sensitive endpoints related to endocrine regulation. Although a mechanism of action involving anti-androgen cannot be completely ruled out this is very unlikely since no other effects were seen on other sensitive endpoints related to an anti-androgenic mode of action: no effects on nipple retention, no effects on sperm, no histological effects on sexual organs, no effects on hormonal levels (LH, testosterone). No effects on reproductive performance were seen in this extended one generation study and at similar doses, no effects on fertility or reproduction were seen in the 2-generation study. Thus, all effects seen with a possible relation to the endocrine system are of low severity and completely reversible.

Conclusion: Some slight anti-androgenic properties have been observed *in vitro*, but with a low potency (high doses and small concentration range for androgen receptor agonism). In a OECD level 5 study, only very slight effects on some very sensitive parameters occurred. No severe effects were observed, all changes were fully reversible and there was no effect on reproductive performance. Moreover, clear NOAELs were determined for all these effects. It can therefore be concluded that dimethomorph should not be considered as an endocrine disruptor.

In addition, based on the legal classification of dimethomorph as Aquatic Chronic 2 - H411, dimethomorph is not considered to be an endocrine disruptor according to the interim criteria of EC 1107/2009.

CA 5.9 Medical Data

A search in the databases listed below - restricted to “pps=human” and “ct d human” - has been performed on October 5th, 2015 via DIMDI-host for the following terms:

- **Dimethomorph**
- **CAS 110488-70-5**
-

ME66	MEDLINE	NLM
ME0A	MEDLINE Alert	NLM
EM74	EMBASE	2005 Elsevier B.V.
EA08	EMBASE Alert	2005 Elsevier B.V.
CL63	CancerLit	NCI
CCTR93	Cochrane Library - Central	Cochrane

2. Crosscheck via ChemIDplus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)

3. Crosscheck via PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez)

4. GUA-internal literature database “FAUST”

5. Regarding the databases HSDB (NLM) and GESTIS (BGIA)

6. Register of the internal medical ward

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Dimethomorph. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to Dimethomorph exposure have not been observed.

Additionally, a health monitoring study has been performed in India on ten vineyard sprayers using dimethomorph 50% WP (ACROBAT), 6 hours/day during 3 days. Clinical examination, haematology, blood chemistry and urinalysis parameters were not affected by the use of dimethomorph. It was concluded that all individuals participated in the experiment well tolerated dimethomorph 50 % WP under the experimental conditions used.

Report: CA 5.9.1/1
Anonymous, 2003 a
Health monitoring study of spraymen exposed to Dimethomorph
2003/1028739
Guidelines: none
GLP: no

Executive Summary:
In this health monitoring study 10 healthy male individuals, aged between 24 and 40 years, were monitored for clinical signs, blood and urine parameters during the study period. The subjects sprayed Dimethomorph 50% WP after mixing with water at 500 g a.i./ha in the grapevine for 6 h/day for three consecutive days with a knapsack sprayer wearing appropriate protective clothing. Clinical examinations, blood and urine parameters were determined before, during and after exposure.
The individuals exposed to Dimethomorph 50% WP did not show any changes in haematology, blood chemistry and urine parameters, which could be attributed to the toxicity of the test substance. None of the individuals had any complaints of any sort. Clinical examination done on the individuals did not reveal any abnormality. In conclusion, Dimethomorph 50% WP was well tolerated in the individuals participating in the study under the experimental conditions applied.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 550 F (Dimethomorph)
   Description: WP preparation containing 500 g/kg of the active substance Dimethomorph; solid / pale white powder
   Batch/purity #: NA

2. **Vehicle and diluent:** Water

3. **Crop:** Grapes
   Application rate: Three applications (6 h per day, 7 am to 1 pm) on 3 consecutive days of Dimethomorph 50% WP after mixing with water at 500 g a.i./ha.
   Study subjects: 10 healthy males aged between 24 and 40 years

B. STUDY DESIGN AND METHODS

1. Dates of work: 25-Mar-2003 – 27-Mar-2003 (field experimental work)

2. Study design
The objective of this study was to monitor the occupational effect on health of individuals exposed to Dimethomorph 50% WP. The subjects sprayed the formulation after mixing with water at 500 g a.i./ha in the grapevine (soon after pruning) for 6 h/day (7 am tot 1 pm) for 3 consecutive days. The spraying was done with knapsack sprayers fitted with hollow cone nozzle. The study participants wore protective clothing while spraying of the test substance.

Meteorological information gathered from the Agronomy Department of the Institute on temperature, relative humidity and rain fall were documented.

3. Clinical examination
All subjects were examined on days -1 (pre-exposure day), days 1, 2 and 3 (exposure days) and on days +5 (post-exposure day). On all days a detailed clinical examination was performed. The examinations included observations for general signs, gastrointestinal effects, neuromuscular signs and cardiorespiratory signs. Ophthalmoscopic examination was performed and the psychological status and vital signs of the subjects were checked. Furthermore, skin reactions and other abnormal reactions were recorded.

3. Laboratory investigations
Blood chemistry, haematology and urinalysis were performed on all study participants on day -1, 1, 3, and +5. About 4 mL of peripheral blood from each subject was collected in EDTA and heparinized vials for haematology and clinical chemistry. On exposure days, blood was collected 2 h after the exposure and on pre-exposure and post-exposure days blood was collected between 1 pm and 2 pm.
Parameters in blood that were determined analytically included red blood cells, haemoglobin, haematocrit, differential blood count, albumin, creatinine, ALT, AST, ALP, glucose and BUN.

II. RESULTS AND DISCUSSION

1. Clinical examination

None of the individuals that sprayed Dimethomorph 50% WP had complaints of any sort during the exposure or after the exposure as evidenced in the case history. Physical examination did not reveal any abnormal signs. Vital signs of all study participants were normal and comparable with that of their pre-exposure data.

2. Laboratory investigations

Haematology, blood chemistry and urine parameters analysed during the exposure days (1 and 3) or post-exposure (day +5) did not differ significantly from the respective values recorded for each individual on pre-exposure (day -1). At both time points of determinations the values were within the normal range. However, one participant showed marginal increase in blood glucose (130 mg/dL), when compared with normal data (historical control) on day -1 and +5. The historical data reach from 63 – 125 mg/mL for glucose. This increase is considered incidental as during the exposure days the blood glucose levels were within the normal range. This subject had generally higher glucose levels throughout the study period (103, 125, 130 and 130 mg/dL) close to the higher limit of the historical data.

Table 5.9.1-1: Range of haematology parameters observed in the subjects during the study period.

Exposure days	WBC (10^3/µL)	RBC (10^6/µL)	Hb (g/dL)	HCT (%)
-1	5.9-12.2	4.32-5.63	14.0-17.3	37.1-45.9
1	5.9-12.6	4.07-5.74	14.0-17.0	35.0-48.0
3	5.9-12.6	4.11-5.37	13.6-17.0	35.9-46.6
+5	6.5-12.1	4.28-5.70	13.2-16.9	36.2-46.6
Historical data	5.0-12.0	4.00-5.50	10.0-18.0	35.0-45.0

WBC: white blood cells; RBC: red blood cells; Hb: haemoglobin; HCT: haematocrit

Table 5.9.1-2: Range of clinical chemistry parameters observed in the subjects during the study period.

Exposure days	Albumin (g/dL)	Glucose (mg/dL)	ALP (IU/L)	BUN (mg/dL)	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dL)
-1	4.0-4.3	82.9-130	26.7-58.1	9.5-15.7	25.5-50.2	15.6-29.4	0.85-1.4
1	3.9-4.5	86.6-125	30.6-61.2	9.7-18.4	22.7-58.0	16.5-28.6	0.85-1.3
3	3.9-4.1	80.5-118	27.1-59.6	9.5-19.0	20.2-61.3	17.5-26.4	0.80-1.4
+5	3.9-4.2	80.5-130	25.5-64.3	9.1-17.0	23.2-63.1	14.4-27.4	0.85-1.2
Historical data	3.2-5.0	70-110	15-112	6.0-20.0	5.0-34	0-40	0.7-1.4

III. CONCLUSION

In a health monitoring study, Dimethomorph 50% WP was well tolerated in ten male individuals, wearing protective clothing, after a knapsack spraying application in grapevine for three consecutive days.

CA 5.9.2 Data collected on humans

From four persons exposed to dust of a Dimethomorph-containing formulation during production, formulation and packaging one of them showed slight irritation of the skin and eyes, another airway irritation, the others showed no health effects.

CA 5.9.3 Direct observations

Dimethomorph is a widely used fungicide. So some cases of slight irritation of the eyes and skin have been reported to BASF in persons exposed to Dimethomorph in combination with other products. These reports could not be verified, and it is not clear whether Dimethomorph was the cause for these irritations.

CA 5.9.4 Epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

As such, no observations regarding health effects after exposure of the general public are known to us.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Analytical methods in biological matrices are not established. Clinical tests are not known. No specific symptoms of poisoning are seen.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

CA 5.9.7 Expected effects of poisoning

Expected effects were derived for acute and subacute studies in animals.

Dimethomorph

Document M-CA. Section 6

RESIDUES IN OR ON TREATED PRODUCTS. FOOD AND FEED AND PLANT METABOLISM

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
31/03/2016	MCA Section 6, Residues CA 6.1/2 and CA 6.1/3: Storage stability data for dimethomorph in strawberry and navy beans: interim report replaced by final report covering a storage interval of up to 24 months. CA 6.1/5: Information included about the delay of storage stability data for morpholin CA 6.3.2/2: Residue data for metabolites in lettuce CA 6.3.3/3: Residue data for metabolites in grapes CA 6.3.4: Information included about the delay of residue data for morpholin CA 6.6.2/2: Field rotcrop residue data for fruiting, legume vegetables and potatoes, including residue data for metabolites CA 6.7.2: Section on MRL calculation updated with the new residue data from grapes and the field rotcrop study CA 6.9: Information on the effect of conversion factors from field residue data on the IEDI calculation was added.	MCA Section6, Version 1, BASF DocID 2016/1000210
30/09/2016	CA 6.1/5: Storage stability data for morpholine provided CA 6.1/6: Due to the inclusion of the study summary under 6.1/5, the table numbering is changed **CA 6.2: OECD summaries for previously evaluated metabolism studies provided** CA 6.2.1/1 14C-Dimethomorph (CME 151) - Metabolism and translocation in vines DK-640-005 CA 6.2.1/2 14C-Dimethomorph (CME 151): Metabolism and translocation in vines - Supplemental data DK-640-010 CA 6.2.1/3 Due to the submission of the old study report, renumbering of the new study and the tables CA 6.2.1/4 Dimethomorph (Chlorophenyl ring - 14C): Metabolism in field grown lettuce - Amended final report DK-640-021 CA 6.2.1/5 Due to the submission of the old study report, renumbering of the new study and the tables CA 6.2.1/6 14C-Dimethomorph (CME 151) (Chlorophenyl ring label) - Metabolism and translocation in potato plants DK-640-004 CA 6.2.1/7 14C-Dimethomorph (CME 151) (Chlorophenyl ring label) - Metabolism and translocation in potato plants - Supplemental data DK-640-009 CA 6.2.1/8 14C-Dimethomorph (CME 151) (Morpholine ring label) - Metabolism and translocation in potato plants DK-640-006	MCA Section6, Version 2, BASF DocID 2016/1103873

	CA 6.2.1/9 14C-Dimethomorph (CME 151) (Morpholine ring label) - Metabolism and translocation in potato plants - Supplemental data DK-640-011 CA 6.2.1/10 Dimethomorph (CME 151) (Chlorophenyl ring-14C) metabolism: The nature of the residue in potato tubers - Supplemental report to report DK-640-014 CA 6.2.1/11 14C-Dimethomorph: Metabolism in tomato plants after uptake through the roots DK-640-020 CA 6.2.2/1 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens DK-440-003 CA 6.2.2/2 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens DK-440-007 CA 6.2.2/3 and 6.2.2/4 Due to the submission of the old study reports, renumbering of the new study and the tables CA 6.2.3/1 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats DK-440-005 CA 6.2.3/2 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats DK-440-008 CA 6.2.3/3, 6.2.3/4 and 6.2.3/5 Due to the submission of the old study reports, renumbering of the new study and the tables CA 6.3.4: Residue data for morpholine provided in strawberry, lettuce, grapes and rotational crop samples CA 6.3.4/1 Determination of the Dimethomorph metabolite Morpholin (Reg.No. 21322) in plant matrices 2015/1243750 CA 6.3.4/2 Study on the residue behavior of Dimethomorph metabolite Morpholine (M550F021) on rotational crops after one application of BAS 550 01 F to bare soil, on wine grapes and table grapes after treatment with BAS 550 02 F, field conditions N and S Europe, 2015 2015/1243751 CA 6.5.1/1: OECD summary of previously evaluated nature of residue at processing study for parent included - BAS 550 F (Dimethomorph): Effects of processing on the nature of the residues due to hydrolysis DK-790-062 CA 6.5.1/2 Nature of the residues at processing study included for metabolite M550F002 - [14C]	

	Reg.No. 4581886: Simulated processing - Hydrolysis at 90°C, 100 °C and 120°C 2016/1209107 CA 6.6.1: OECD summaries of previously evaluated studies provided CA 6.6.1/1 14C-Dimethomorph (CME 151) - Confined accumulation study on rotational crops DK-640-008 CA 6.6.1/2 Dimethomorph (AC 336379): Metabolism of carbon-14 labeled AC 336379 using lettuce, radishes, soybeans, and wheat as rotational crops DK-790-028 CA 6.6.1/3 renumbering of the study CA 6.6.2/2: Study header of rotational crop study (DocID 2015/1241720) corrected CA 6.9: Statement added regarding the dietary exposure for morpholine CA6.9/1 Dimethomorph (BAS 550 F): Evaluation of the relevance of Dimethomorph metabolites in dietary risk assessment 2015/1253634 – Update Chronic RA	
May 2017	CA 6.1/5: Second interim report for storage stability of M550F002 and M550F007 CA 6.1/7: Second interim report for storage stability of morpholine CA 6.1/8: Storage stability data for tomato as third high water crop CA 6.1/9: Additional storage stability data for oilseed rape CA 6.3: Reference to the additional document explaining the strawberry use pattern CA 6.3.2: Additional residue data in lettuce provided CA 6.3.3: Reference added to the ongoing additional residue trials in grapes analyzing for metabolites M550F002 and M550F007 and morpholine CA 6.5.3: Reference added to the ongoing processing study in grapes including analysis for metabolites M550F002 and M550F007 and morpholine CA 6.7.2: MRL calculation for lettuce updated with the additional residue data	MCA Section6, Version 3, BASF DocID 2016/35200

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CA 6 RESIDUES IN OR ON TREATED PRODUCTS. FOOD AND FEED AND PLANT METABOLISM

Dimethomorph (BAS 550 F), a fungicide for use in many vegetables and fruits, is widely registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2007/25/EC. Inclusion entered into force on October 1st, 2007. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until July 31st, 2018 by Implementing Regulation (EU) No 2015/404.

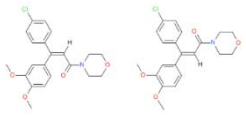
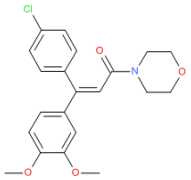
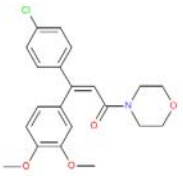
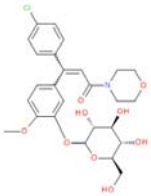
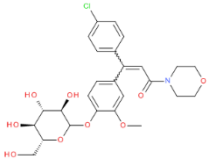
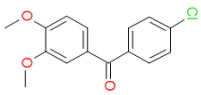
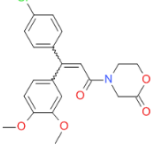
All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the monograph of dimethomorph, in the EFSA conclusion on dimethomorph (2006) and in SANCO/10040/06 -Final document (EU Review Report of November 2006).

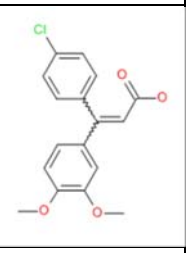
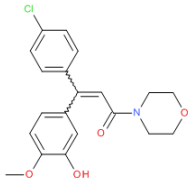
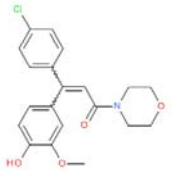
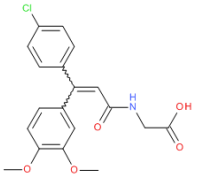
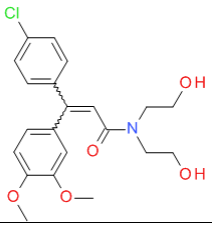
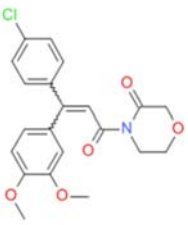
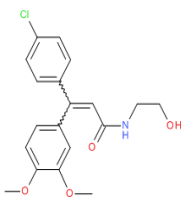
For the current renewal of approval under Regulation (EC) 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section.

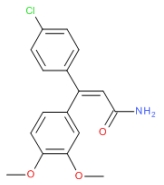
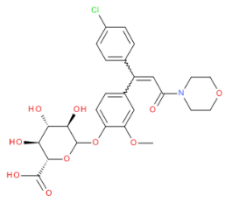
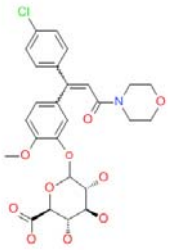
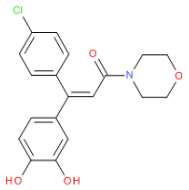
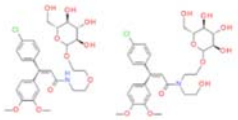
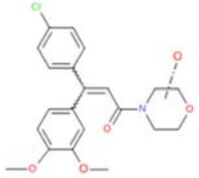
Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective consumer safety chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for consumer safety, no summaries of public literature data on dimethomorph were included in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

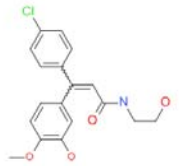
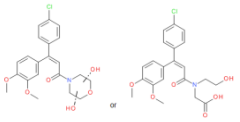
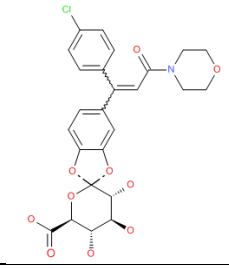
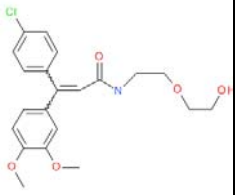
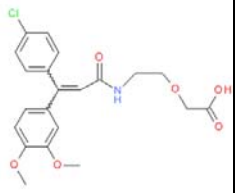
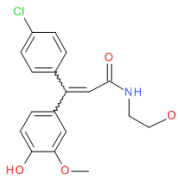
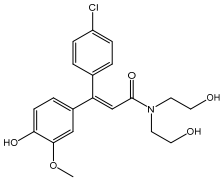
An overview of metabolites identified during consumer safety studies is given below. The list of metabolites occurring in rats is not complete; the complete list can be found in M-CA 5.1 and also in Document N3. The information in the table allows a comparison between the pathways in different test systems.

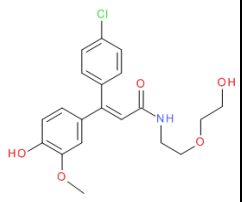
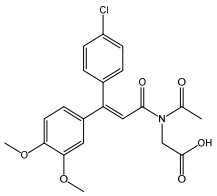
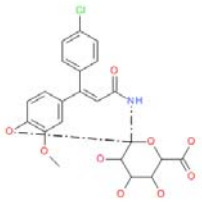
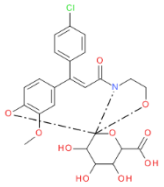
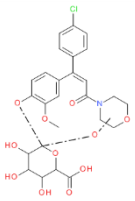
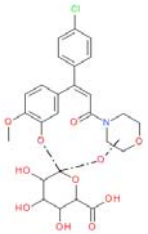
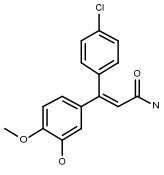
The metabolite overview below is including the different code numbers that are available for each metabolite. Due to historic reasons (e.g. use of different metabolite codes in different study reports), it is unfortunately not possible to use always exclusively one metabolite code for a certain metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Code Numbers			CAS-No Description	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M550F000 BAS 550 F	247723	CL 336379 CME 151	110488-70-5	Crop (grape, lettuce, potato, tomato, rotated crops) Livestock (hen, goat) Rat	
M550F000E	4110868	M550F00E	110488-70-5 (E)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine	Crop (grape, lettuce, potato, tomato, rotated crops) Livestock (hen, goat) Rat	
M550F000Z	4110869	M550F00Z	110488-70-5 (Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine	Crop (grape, lettuce, potato, tomato, rotated crops) Livestock (hen, goat) Rat	
M550F001	4388253	CL199322	5-[E,Z)-1-(4-chlorophenyl)-3-(morpholin-4-yl)-3-oxoprop-1-en-1-yl]-2-methoxyphenyl D-glucopyranoside	Rotational crops (traces)	
M550F002	4581886	CL 411266	4-[(1E,Z)-1-(4-chlorophenyl)-3-(morpholin-4-yl)-3-oxoprop-1-en-1-yl]-2-methoxyphenyl D-glucopyranoside	Grape, rotational crops	
M550F003	247679	Z 7 CL336305	(4-chlorophenyl)(3,4-dimethoxyphenyl)methanone	Lettuce, grapes, potato aerial parts (leaves)	
M550F004	4350503	Z37 CL 153868	4-[(2E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enoyl]morpholin-2-one	Wrongly stated as lettuce metabolite in the previous dossier	

Code Numbers			CAS-No Description	Compound found in	Structure
Substance Code	**Reg. No.**	**Synonyms**			
M550F005	5967179 (E: 4363759) (Z: 4350463)	Z 43	(E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enoic acid	Livestock (poultry)	
M550F006	4060806	Z67 CL 900987	(E,Z)-3-(4-chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one	Lettuce, potato peel Livestock (goat, hen) Rat	
M550F007	4060805	Z69 CL 900986	(E,Z)-3-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one	Grapes, lettuce, potato peel, tomato leaves Livestock (goat, hen) Rat	
M550F008	4108146 E: 5965956 Z: 5965956	Z89. CUR 7117	N-[(E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enoyl]glycine	Livestock (goat, hen) Rat	
M550F009	4626712	Z93 WL 376084 CL 901423	(2E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-N,N-bis(2-hydroxyethyl)prop-2-enamide	Livestock (goat, hen) Rat	
M550F010	4350490	Z94 CUR 7586	4-[(2E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enoyl]morpholin-3-one	Rat (already peer reviewed data only)	
M550F011	4350473	Z95 CUR 7216 CL 153827	(2E,Z)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-N-(2-hydroxyethyl)prop-2-enamide	Livestock (goat, hen) Rat	

Code Numbers			CAS-No Description	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M550F012	E: 4737606 Z: 4350466	Z98 CL 153815	(2)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enamide	Livestock (goat, hen) Rat	
M550F013	4714013		4-[(1E,Z)-1-(4-chlorophenyl)-3-(morpholin-4-yl)-3-oxoprop-1-en-1-yl]-2-methoxyphenyl D-glucopyranosiduronic acid	Livestock (goat, hen) Rat	
M550F015			(2S,3S,4S,5R)-6-[5-[(Z)-1-(4-chlorophenyl)-3-morpholino-3-oxo-prop-1-enyl]-2-methoxy-phenoxy]-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid	Livestock (goat, hen) Rat	
M550F016	E: 4179697 Z: 4708127		3-(4-chlorophenyl)-3-(3,4-dihydroxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one	Livestock (goat), Rat	
M550F017				Grape, rotational crops	
M550F018				Livestock (goat), Rat	
M550F021	21322	morpholine	110-91-8	Grape, lettuce, rotational crops, Livestock (goat, hen), Rat	

Code Numbers			CAS-No Description	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M550F022			(Z)-3-(4-chlorophenyl)-N-(2-hydroxyethyl)-3-(3-hydroxy-4-methoxy-phenyl)prop-2-enamide	Livestock (hen), Rat	
M550F028				Livestock (goat, hen), Rat	
M550F029				Livestock (goat) Rat	
M550F030			(E)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-N-[2-(2-hydroxyethoxy)ethyl] prop-2-enamide	Livestock (goat) Rat	
M550F031			2-[2-[[(E)-3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-enoyl]amino]ethoxy]acetic acid	Livestock (goat) Rat	
M550F033			(E)-3-(4-chlorophenyl)-N-(2-hydroxyethyl)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enamide	Livestock (hen) Rat	
M550F035			(E)-3-(4-chlorophenyl)-N,N-bis(2-hydroxyethyl)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enamide	Livestock (hen)	

Code Numbers			CAS-No Description	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M550F049			(E)-3-(4-chlorophenyl)-N-[2-(2-hydroxyethoxy)ethyl]-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enamide	Livestock (hen) Rat	
M550F053				Livestock (goat)	
M550F062				Livestock (hen) Rat	
M550F069				Livestock (hen) Rat	
M550F074				Livestock (hen) Rat	
M550F076				Livestock (hen) Rat	
M550F091				Livestock (hen)	

CA 6.1 Storage stability of residues

During the last EU peer review data on the storage stability of dimethomorph was evaluated in the categories high acid (grapes and processed products), high starch (potatoes) and high oil content (hops and processed commodities). Dimethomorph was found to be stable under deep-freeze conditions for a period of at least 24 months in grapes (14 months for raisins and 16 months for grape juice), for at least 24 months in potatoes, and for at least 18 months in hops and its processed commodities. Further storage stability studies are available for the categories high water (broccoli, spinach, tomato), high oil (oilseed rape/canola) and high protein content (dry bean). Storage stability was also investigated for animal matrices and is shown in the present update of the active substance dossier.

Plants

The following study is included to provide data for the category high water content, to which the representative crop lettuce belongs, and for the category high oil content.

Report:	CA 6.1/1 Jones J.E. III, 2004 a BAS 550 F: Freezer storage stability of residues of BAS 550 F in broccoli, canola, and spinach 2003/5000425
Guidelines:	EPA 860.1380, EEC 7032/VI/95 rev. 5
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Dimethomorph (BAS 550 F)
	Description:	
	Lot/Batch #:	AC 9978-68A
	Purity:	97.6%
	CAS#:	110488-70-5
	Development code:	CL 336379
	Spiking levels:	0.5 mg/kg
3.	**Test Commodity:**	
	Crop:	Broccoli, spinach, oilseed rape (canola)
	Type:	Brassica vegetables, leafy vegetables (high water), oilseeds (high oil)
	Variety:	Not reported
	Botanical name:	*Brassica oleracea* var. *italic, Spinacia oleracea, Brassica napus*
	Crop parts(s) or processed commodity:	Broccoli, (flower head, stems) spinach (leaves), oilseed rape (seed)
	Sample size:	10 g

B. STUDY DESIGN AND METHODS

1. Test procedure

The freezer stability of BAS 550 F was determined in broccoli, spinach, and canola. Control samples of each matrix were fortified at a level of 0.5 mg/kg with dimethomorph. Samples were placed in glass jars at frozen storage (≤-10°C). All samples were analyzed for recoveries initially after fortification (zero time) and after 4, 8, 15, 18 and 24 months. Additionally, the stability of dimethomorph in extracts stored under refrigerated conditions in the dark was investigated after 0, 1 and 5 days.

2. Description of analytical procedures

The samples were analyzed by BASF method No M 3502 (broccoli and spinach) and M 3463 (canola). The efficiency of the methods was determined at each analysis time period by concurrent recoveries.

For method M 3502 residues of dimethomorph were extracted from spinach and broccoli matrices with acetone and purified by SPE followed by C_{18} SPE.
In method M 3463 residues of dimethomorph were extracted from oilseed matrices by acetone followed by purification by liquid/liquid portioning. Thereafter the extracts were subjected to clean-up by C_{18} SPE followed by SPE. The final analysis of both methods was performed by LC-MS/MS with a limit of quantitation of 0.05 mg/kg.

II. RESULTS AND DISCUSSION

The results obtained from the stored fortified samples indicate that BAS 550 F residues are stable in frozen broccoli (flower head and stems), spinach (leaves), and canola (seed) for at least 24 months. The table below presents a summary of the recoveries from the stored fortified samples. The samples were not corrected for procedural recoveries. The average procedural recoveries are in the range of 70-120%, except for the oilseed rape seed samples after 15, 18 and 24 months. However, corrected for procedural recoveries, the recoveries are acceptable and dimethomorph is considered stable.

Table 6.1-1: Storage stability of dimethomorph in plant matrices

Mean recovery (%)						
A: in stored samples (% of nominal) / B: procedural, in freshly spiked samples						
Months	**A**	B	**A**	B	**A**	B
Methods M 3502 / M 3463						
	Broccoli		**Spinach**		**Oilseed rape**	
0	**93**	84	**93**	103	**82**	78
4	**97**	93	**101**	93	**70**	82
8	**75**	76	**81**	81	**102**	113
15	**75**	90	**89**	97	**54 (73)**	74
18	**71**	88	**84**	85	**54 (71)**	76
24	**75**	84	**88**	92	**60 (72)**	83

() Values in parentheses were corrected for procedural recovery

Table 6.1-2: Extract stability of dimethomorph in plant matrices after storage at 4°C

Matrix	Mean recovery (%)			RSD (%)		
	0 day	**1 day**	**5 days**	**0 day**	**1 day**	**5 days**
Broccoli	88	93	92	8.1	8.4	5.1

III. CONCLUSION

Dimethomorph is considered stable in high water content and high oil content matrices for at least 24 months of frozen storage. Additionally, extracts of dimethomorph have been proven stable for at least 5 days of refrigerated storage in the dark.

Report: CA 6.1/2
Andrews R.S., 2015 a
Freezer storage stability of geometric isomers of BAS 550 F (Reg.No. 4110868, 4110869) in navy bean and strawberry samples
2014/7000495
Guidelines: EPA 860.1380, EPA 860.1500, OECD 506
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:
Description: *E*- and *Z*-dimethomorph (BAS 550 F)
Lot/Batch #: AC11187-92
Purity: 98.9 %
CAS#: 110488-70-5
Spiking levels: 0.05 mg/kg

2. Test Commodity:
Crop: Navy bean (dried seed) and strawberry (fruit)
Sample size: 5 g (stored sample)

B. STUDY DESIGN

1. Test procedure
Plant matrices (navy bean dried seed and strawberry fruit) were fortified separately with *E*-dimethomorph and *Z*-dimethomorph at a concentration level of 0.05 mg/kg (10x LOQ). The spiked samples were stored under frozen storage conditions of about -20°C and analyzed after about 0, 28/29, 82, 182, 274, 365, and 547/558 days (about 0, 1, 3, 6, 9, 12 and 18 months). The exact sampling days are given in the table below.

2. Description of analytical procedures
The residues of dimethomorph were analyzed using BASF method No L0013/02. Dimethomorph was extracted from plant material with a mixture of methanol and water. An aliquot of the extract was centrifuged and diluted. Final determination was performed by LC-MS/MS. The limit of quantitation of the method was 0.005 mg/kg for each isomer. Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen.

II. RESULTS AND DISCUSSION

The table below shows a summary of the stability data for up to 18 months of storage. The study is still ongoing and the results for 24 months storage will be provided in the update to this dossier (submission due in March 2016). The results are expressed as average percentage of the nominal fortification. In order to account for possible variation over the time investigated, the mean corrected recovery results are given in addition in parentheses.

The table below presents a summary of the recoveries for dimethomorph *E*- and *Z*-isomers from the stored fortified samples. The samples were not corrected for procedural recoveries. The average procedural recoveries are in the range of 70-120% after 18 months of frozen storage, the latest sampling interval.

The data indicate that residues of *E*- and *Z*-dimethomorph are stable when stored frozen for at least 18 months in strawberry and dried bean samples.

Table 6.1-3: Storage stability of *E*-dimethomorph and *Z*-dimethomorph in plant matrices

Storage time (days)	Mean recovery (%)			
	A		B	
	E-dimethomorph	*Z*-dimethomorph	*E*-dimethomorph	*Z*-dimethomorph
Navy bean (dried seed)				
0	96, 98 (97)	94, 95 (102)	96, 98 (97)	94, 95 (95)
28	96, 98 (95)	94, 95 (100)	96, 98 (97)	94, 95 (95)
82	104, 94 (94)	109, 105 (95)	104, 94 (99)	109, 105 (107)
182	97, 93 (116)	100, 94 (116)	97, 93 (95)	100, 94(97)
274	90, 90 (109)	89, 91 (109)	90, 90 (90)	89, 91 (90)
365	98, 98 (107)	98, 99 (92)	98, 98 (98)	98, 99 (98)
547	101, 89 (116)	114, 98 (119)	101, 89 (95)	114, 98 (106)
Strawberry (fruit)				
0	91,90 (95)	92, 88 (103)	91, 90 (90)	92, 88 (103)
29	91,90 (92)	92, 88 (99)	91, 90 (90)	92, 88 (99)
82	96, 90 (96)	91, 94 (104)	96, 90 (93)	91, 94 (104)
182	93,92 (108)	96, 95 (99)	93 ,92 (92)	96, 95 (99)
274	85, 87 (115)	88, 89 (108)	85, 87 (86)	88, 89 (108)
365	95, 80 (110)	97, 89 (108)	95, 80 (88)	97, 89 (108)
558	104, 97 (120)	98, 109 (126)	104, 97 (101)	98, 109 (126)

A = mean % recovery in spiked and stored control samples
B = mean % procedural recovery for freshly spiked control samples
() Mean corrected recovery results are given in parentheses

III. CONCLUSION

The results obtained in this storage stability study demonstrate that *E*-dimethomorph and *Z*-dimethomorph are stable in plant matrices with high water and high protein content when stored frozen for up to 18 months.

Report:	CA 6.1/3 Andrews R.S., 2016 a Freezer storage stability of geometric isomers of BAS 550 F (Reg.No. 4110868, 4110869) in navy bean and strawberry samples 2015/7006333
Guidelines:	EPA 860.1380, EPA 860.1500, OECD 506, EEC 7032/VI/97 rev. 5
GLP:	yes (certified by United States Environmental Protection Agency)

This is the final report of the storage stability study of dimethomorph in high protein and high acid matrices, for which the interim report is summarized under CA 6.1/2. This final report ~~will~~ contains the 24-months storage interval. ~~Since the study is currently still ongoing, it will be provided in the dossier update in March 2016.~~

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

Description:	*E*- and *Z*-dimethomorph (BAS 550 F)
Lot/Batch #:	AC11187-92
Purity:	98.9 %
CAS#:	110488-70-5
Spiking levels:	0.05 mg/kg

2. Test commodity:

Crop:	Navy bean (dried seed) and strawberry (fruit)
Sample size:	5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

Plant matrices (navy bean dried seed and strawberry fruit) were fortified separately with *E*-dimethomorph and *Z*-dimethomorph at a concentration level of 0.05 mg/kg (10x LOQ). The spiked samples were stored under frozen storage conditions of about -20°C and analyzed after about 0, 28/29, 82, 182, 274, 365, 547/558 and 726 days (about 0, 1, 3, 6, 9, 12, 18 months and 24 months). The exact sampling days are given in the table below.

3. Description of analytical procedures

The residues of dimethomorph were analyzed using BASF method No L0013/02. Dimethomorph was extracted from plant material with a mixture of methanol and water. An aliquot of the extract was centrifuged and diluted. Final determination was performed by LC-MS/MS. The limit of quantitation of the method was 0.005 mg/kg for each isomer. Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen.

II. RESULTS AND DISCUSSION

The table below presents a summary of the recoveries for dimethomorph *E*- and *Z*-isomers from the stored fortified samples. The samples were not corrected for procedural recoveries. The average procedural recoveries are in the range of 70-120% after 24 months of frozen storage, the latest sampling interval. Exceptions were observed for the *E*-isomer detected in strawberry samples after storage for 558 days and for the *Z*-isomer detected in navy bean and strawberry samples after storage for 547 days and 558 days, respectively, were recovery values were in a range from 121-130%. However, determined recovery values obtained after storage for a longer period (726 days) ranged from 95-112% indicating that both isomers are stable.

Table 6.1-4: Storage stability of dimethomorph in plant matrices

Mean recovery (%)								
A: in stored samples (% of nominal) / B: procedural, in freshly spiked samples								
	A	B	**A**	B	**A**	B	**A**	B
Method L0013/02								
Interval [days]	***E*-isomer**				***Z*-isomer**			
	Navy bean		**Strawberry**		**Navy bean**		**Strawberry**	
0	**94 (97)**	97	**86 (95)**	90	**97 (102)**	95	**93 (103)**	90
28	**93 (95)**	97	-	-	**95 (100)**	95	-	-
29	-	-	**83 (92)**	90	-	-	**89 (99)**	90
82	**93 (94)**	99	**90 (96)**	93	**102 (95)**	107	**96 (104)**	93
182	**110 (116)**	95	**100 (108)**	92	**113 (116)**	97	**95 (99)**	96
274	**98 (109)**	90	**99 (115)**	86	**98 (109)**	90	**96 (108)**	88
365	**105 (107)**	98	**96 (110)**	88	**90 (92)**	98	**100 (108)**	93
547	**110 (116)**	95	-	-	**126 (119)**	106	-	-
558	-	-	**121 (120)**	101	-	-	**130 (126)**	104
726	**112 (111)**	102	**103 (112)**	92	**97 (133)**	73	**95 (134)**	71

() Mean corrected recovery results are given in parentheses

III. CONCLUSION

The results obtained in this storage stability study demonstrate that *E*-dimethomorph and *Z*-dimethomorph are stable in plant matrices with high water and high protein content when stored frozen for up to 24 months.

The following study will provide storage stability data on the dimethomorph metabolite M550F002, which was the most abundant metabolite in the new plant metabolism studies, and on its aglycone, M550F007. ~~The report represents an interim report including data for a storage interval of up to 3 months.~~ A seond interim report is provided in this dossier update covering a storage interval of 18 months (548 days). The final report covering a storage interval of up to 24 months will be available in November 2017.

Report:	CA 6.1/4 Wiltshire K., 2016 a Interim report: Storage stability of the two Dimethomorph metabolites (M550F002 and M550F007) in plant matrices 2014/1186697
Guidelines:	EEC 7032/VI/95 rev. 5, OECD 506, EPA 860.1380
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.1/5 Pugh K., 2017 a Interim Report No. 2 - Frozen storage stability of the two Dimethomorph metabolites (M550F002 and M550F007) in plant matrices 2017/1072429
Guidelines:	EEC 7032/VI/95 rev. 5 Appendix H Storage Stability of Residue Samples (22.07.1997), OECD 506 (Oct. 2007), EPA 860.1380
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	Metabolites of BAS 550 F
	Description:	M550F002, M550F007
	Lot/batch #:	L87-4 (M550F002), L83-12 (M550F007)
	Purity:	93.1% (M550F002), 90.3% (M550F007)
	CAS#:	Not applicable
	Spiking level:	0.01, 0.1 mg/kg
2.	**Test commodity:**	
	Crop:	Lettuce (leaves), oilseed rape (seeds), strawberry (fruits), dried bean (seeds) and potato (tubers)
	Sample size:	5 g (stored sample)

B. STUDY DESIGN AND METHODS

1. Test procedure

Samples of the different plant materials (lettuce leaves, oilseed rape seeds, strawberry fruits, dried bean seeds and potato tubers) were fortified separately 0.1 mg/kg (10x LOQ) of metabolites M550F002 or M550F007. The spiked samples were stored in a freezer at <-18°C. Samples are scheduled to be analysed after storage for approximately 0, 30, 90, 180, 365, 545 and 730 days. The exact storage time is given in Table 6.1-4. Up to date the samples of the storage time points 0, 1, 3, 6, 12 and 18 months could be analysed and are reported in the following. The remaining storage stability data will be reported in due course.

2. Description of analytical procedures

Metabolites M550F002 and M550F007 were analyzed using BASF method No L0013/03. Metabolites were extracted from plant material with a mixture of methanol, water and hydrochloric acid (70/25/5, v/v/v). An aliquot of the extract was centrifuged and partitioned against dichloromethane. The final determination was performed by LC-MS/MS. The limit of quantitation (LOQ) was 0.01 mg/kg for each metabolite in all matrices.

II. RESULTS AND DISCUSSION

A summary of the average recovery rates for metabolites M550F002 and M550F007 after storage times of up to 18~~3~~ months and freshly fortified samples (procedural recoveries) is presented in Table 6.1-4. Mean nominal recoveries after storage are shown as well as corrected recovery rates in order to account for possible analytical variability over the time of analysis.
The corrected recoveries demonstrate that dimethomorph metabolites M550F002 and M550F007 were stable in the investigated plant matrices for a storage period up to 548~~90~~ days.
When looking at the uncorrected nominal recoveries for M550F002, there are values below 70% in high water, high oil, high protein and high starch commodities. However, that has to be seen in the light of the consistent low procedural recoveries for the metabolite, which seems immanent to the method as the method validation data show. When comparing the nominal recoveries at the various storage time points to the 0 day storage time point, it can be seen that there is no significant degradation.
The only exception was for metabolite M550F007 in lettuce after 90, 180, 370 and 547 days of frozen storage, where recoveries lower than 70% were observed. This finding is however inconsistent as in strawberry, which is also high in water (and acid) content, M550F007 was shown to be stable and also M550F002 as a glucoside metabolite is stable in those matrices.
The reasons for this apparent instability (real degradation or binding effects) in lettuce will be further investigated. ~~Recoveries measurements at the additional storage times will also give an indication of whether the effect observed is due to real degradation.~~ The recoveries at the additional timepoints unfortunately confirmed the trend that M550F007 is not stable in the high water matrix lettuce.
The study is still ongoing and the results for storage up to 24 months will be provided in an update to this dossier (results available in November 2017).

Table 6.1-5: Storage stability of metabolites M550F002 and M550F007 in plant matrices

Storage time (days)	Mean recovery (%)			
	A		B	
	M550F002	M550F007	M550F002	M550F007
Lettuce leaves				
0	73.3 (98.1)	87.6 (105)	74.7	83.4
32	66.4 (90.1)	63.2 (76.5)	73.7	82.6
90	66.9 (94.8)	42.5 (49.7)	70.6	85.4
96	-	41.4 (51.1)	-	80.9
180	64.0 (83.4)	29.9 (34.4)	76.8	86.9
370	60.4 (76.6)	22.1 (21.9)	78.9	101
547	57.5 (83.2)	15.6 (16.4)	69.1	95.4
Oilseed rape seeds				
0	74.4 (104)	99.9 (97.1)	71.5	103
32	75.6 (102)	90.9 (102)	74.4	88.8
90	73.1 (99.7)	83.9 (95.4)	73.3	87.9
180	68.1 (98.6)	80.8 (92.5)	69.1	87.4
370	73.5 (93.5)	85.6 (92.6)	78.5	92.5
547	74.4 (102)	104 (103)	72.8	101
Strawberry fruits				
0	76.1 (97.3)	93.7 (97.4)	78.2	96.2
30	75.0 (98.9)	84.6 (95.5)	75.9	88.6
90	71.5 (105)	83.9 (94.8)	68.3	88.6
181	75.1 (105)	88.5 (97.9)	71.8	90.4
365	73.8 (93.1)	94.0 (93.0)	79.3	101
548	76.9 (97.0)	101 (101)	79.2	101
Dried bean seeds				
0	81.9 (98.3)	101 (99.8)	83.4	101
28	77.6 (104)	110 (110)	74.9	101
87	81.7 (102)	94.4 (108)	79.8	87.2
178	78.0 (97.8)	85.4 (99.0)	79.8	86.3
371	82.1 (91.9)	99.9 (101)	89.4	98.9
543	66.4 (89.1)	81.9 (87.1)	74.6	94.0
Potato tubers				
0	71.1 (99.7)	88.0 (101)	71.2	87.1
30	67.1 (92.1)	85.1 (93.8)	72.8	90.7
90	68.3 (96.6)	79.5 (96.4)	70.7	82.5
181	66.8 (90.7)	79.1 (93.2)	73.6	84.9
365	71.5 (86.3)	90.5 (87.5)	82.9	103
548	68.7 (90.6)	89.7 (92.6)	75.9	96.9

A = mean % recovery in spiked and stored control samples
B = mean % procedural recovery for freshly spiked control samples with 0.01 mg/kg and 0.1 mg/kg analyte
() Mean corrected procedural recovery results

III. CONCLUSION

~~The results obtained in this study demonstrate that dimethomorph metabolites M550F002 and M550F007 are stable in plant matrices with high water, oil, acid, protein and starch content for a storage period of up to 90 days. M550F007 in lettuce showed recoveries below 70% while in strawberry, which also has a high water content, the metabolite was stable. This inconsistent finding of an apparently lower storage stability will be further investigated at the remaining storage intervals.~~

The results obtained in this study demonstrate that dimethomorph metabolite M550F002 is stable in plant matrices with high water, high oil, high acid, high protein and high starch content for a storage period of up to 548 days.
M550F007 showed also stability in all categories except in the high water matrix lettuce where recoveries were below 70% in all storage time intervals longer than 30 days.

Report:	CA 6.1/6 Richter S., 2016 a Storage Stability of Morpholine (M550F021, Reg. No. 21322) in plant matrices 2015/1243749
Guidelines:	Pesticide Residue Analytical Methods ENV/JM/Mono (2007)17; OECD Guideline 506: Stability of Pesticide Residues in Stored Commodities (16/10/2007); US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data (08/1996); Commission of the European Communities, Storage Stability of residue samples, Appendix H, 7032/VI/95 rev. 5, 22. July 1997.
GLP:	yes

Report:	CA 6.1/7 Richter S., 2017 a 2nd Interim Report - Storage stability study of Morpholine (M550F021, Reg. No. 21322) in plant matrices 2017/1072427
Guidelines:	OECD-ENV/JM/MONO/(2007)17, OECD 506, EPA 860.1380, EEC 7032/VI/95 rev. 5 Appendix H Storage Stability of Residue Samples (22.07.1997)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

The study 2017/1072427~~2015/1243749~~ ~~will~~ provides storage stability data on the dimethomorph metabolite M550F021 (morpholine) in plants.
~~The report will represent an interim report and was scheduled to be provided in this dossier update in March 2016. However, within the study severe analytical problems were faced, mainly problems with blank values and contamination from other sources (via lab equipment/gloves) due to the widespread use of morpholine in industrial products. This has led to a significant delay of the study so that it is not possible to provide reliable data with this dossier update. An interim report covering a storage interval of up to 6 months can be provided in September 2016 to the Rapporteur Member State.~~

This is the second interim report of the storage stability study of dimethomorph metabolite M550F021 (morpholine) in plant matrices covering a storage interval of up to 12~~6~~ months. The final report will contain the ~~12,~~ 18 and 24-months storage intervals. Since the study is currently still ongoing, the final report will be provided after completion.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:**

Description:	Morpholine (M550F021)
Lot/Batch #:	AC10851-132
Purity:	98.2 %
CAS#:	110-91-8
Spiking levels:	0.20 mg/kg

2. **Test commodity:**

Crop:	Oilseed rape seed, lettuce, strawberry, potato and dried pea
Sample size:	5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

Plant matrices (oilseed rape seed, lettuce, strawberry, potato and dried pea) were fortified with morpholine (M550F021) at a level of 0.20 mg/kg (10x LOQ). The spiked samples were stored under frozen storage conditions of ≤-18°C in a temperature controlled freezer and analyzed after about 0, 30, 90, 180 and 360 days (about 0, 1, 3, 6 and 12months). The exact sampling days are given in the table below. The examination of the storage stability for ~~12,~~ 18, and 24 months is still ongoing.

2. Description of analytical procedures

The residues of M550F021 were analyzed using BASF method No L0013/04. The homogenized crop samples were extracted with acidified methanol after addition of water and internal standard. An aliquot of the extract was filtered and diluted. The final determination of morpholine (M550F021) was performed by LC-MS/MS using the mass transition *m/z* 88 → 70 for morpholine and *m/z* 96 → 78 for the internal standard. The limit of quantitation of the method was 0.02 mg/kg. Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen.

II. RESULTS AND DISCUSSION

The table below presents a summary of the recoveries for dimethomorph metabolite morpholine (M550F021) from the stored fortified samples. The samples were not corrected for procedural recoveries. The average procedural recoveries were in the range of 70-110%. Recoveries after 126 months of frozen storage, the latest sampling interval, were between 70% and 120% (102-108%~~101-107%~~) for lettuce, strawberry, potato tuber and dried peas samples, indicating satisfactory storage stability. For oilseed rape seed a degradation ~~of about~~ to 25%~~40%~~ is observed after a storage period of 126 months. However, storage stability could be demonstrated for at least 3 months with a mean recovery of 76%.

Table 6.1-6: Storage stability of morpholine (M550F021) in plant matrices

Mean recovery (%)										
A: in stored samples (% of nominal) / B: procedural, in freshly spiked samples										
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
Method L0013/04										
Interval [days]	**Oilseed rape seed**		**Lettuce**		**Strawberry**		**Potato tuber**		**Dried peas**	
0	**100 (101)**	99.1	**103 (106)**	96.6	**106 (109)**	97.7	**101 (114)**	88.5	**97.1 (112)**	86.7
22-42	**77.5 (77.5)**	100	**100 (116)**	86.0	**102 (104)**	97.5	**101 (112)**	89.9	**98.0 (92.6)**	106
85	**75.8 (71.9)**	105	**102 (97.4)**	104	**93.3 (92.6)**	101	**102 (101)**	101	**97.0 (100)**	96.6
175-176	**45.6 (54.3)**	84.0	**107 (111)**	96.0	**103 (107)**	96.0	**101 (101)**	99.6	**102 (104)**	97.4
357-358	**26.6 (28.5)**	93.0	**102 (97.3)**	105	**102 (101)**	101	**105 (104)**	101	**108 (107)**	102

() Mean corrected recovery results are given in parentheses

III. CONCLUSION

The results obtained in this storage stability study demonstrate that dimethomorph metabolite M550F021 (morpholine) is stable in plant matrices with high water, high acid, high starch and high protein content when stored frozen for up to 12~~6~~ months. In matrices with high oil content stability was demonstrated for at least 3 months.

The following study in tomatoes is included in this dossier update in order to provide storage stability data for parent dimethomorph in a third high water crop to allow extrapolation of the stoage stability of dimethomorph to the entire group of high water commodities. The study was already reviewed within the MRL review of dimethomorph according to Art. 12 of regulation 396/2005 where EFSA confirmed stability of dimethomorph in tomato of up to 24 months (see EFSA Journal 2011;9(8):2348).
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Report:	CA 6.1/8 Babbitt B., 1998 a CL 336379 (Dimethomorph): Freezer stability of CL 336379 residues in tomato fruit, tomato puree, tomato juice, tomato paste and tomato dry pomace DK-723-040
Guidelines:	EPA 860.1380, EPA 171-4, EPA 40 CFR 158.240
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	BAS 550 F (dimethomorph)
	Description:	
	Lot/Batch #:	AC9978-86A
	Purity:	97.6%
	CAS#:	110488-70-5
	Spiking levels:	Puree, juice, paste and dry pomace: 0.25 mg/kg

2.	**Test commodity:**	
	Crop:	Tomato fruit and processed commodities (puree, juice, paste and dry pomace)
	Sample size:	20-50 g

B. STUDY DESIGN

1. Test procedure

The deep freeze stability of dimethomorph is being investigated in tomato fruits over a period of 24 months and processed tomato commodities over a period of 21 months. Tomato fruits and processed commodities with incurred residues of dimethomorph were used. Untreated tomato fruits and processed commodities samples were spiked with 0.25 mg/kg of dimethomorph and sampled at storage for 6, 12, 18 and 24 months (tomato fruit), or after 6 and 21 months (processed commodities). The samples were stored at -10°C to -20°C in glass bottles and were analyzed at different time intervals.

2. Description of analytical procedures

The method of analysis is described in American Cyanamid Method M 2577. The methodology consists of extracting residues of dimethomorph from the matrix with acetone followed by solid phase extraction cleanup. Measurement of dimethomorph was accomplished by GC-NPD.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1-6. Average dimethomorph recoveries in treated samples ranged from 91-117% in tomato fruits, from 91-96% in tomato paste, from 105-115% in tomato juice, from 97-104% in tomato puree and from 95-105% in tomato dry pomace. No significant change of residue level was observed during the storage period (6-24 month) for any of these matrices.

Table 6.1-7: Storage stability of dimethomorph in tomato fruits and processed matrices

Sample matrix	Average recovery (%)				
	6 months	12 months	18 months	21 months	24 months
Tomato fruit	117	115	91	-	109
Tomato paste	96	-	-	91	-
Tomato juice	115	-	-	105	-
Tomato puree	97	-	-	104	-
Tomato dry pomace	105	-	-	95	-

III. CONCLUSION

Residues of dimethomorph are stable in tomato fruits and processed tomato commodities for at least 21 and 24 months, respectively, when stored under deep frozen conditions.

The following study in oilseed rape is included in this dossier update in order to provide data for parent dimethomorph showing a storage stability of up to 12 months, which is longer than the storage stability derived by the RMS from the already submitted study (see CA 6.1./1, DocID 2003/5000425). This study was already reviewed within the MRL review of dimethomorph according to Art. 12 of regulation 396/2005 where EFSA confirmed stability of dimethomorph in high oil matrix of up to 12 months (see EFSA Journal 2011;9(8):2348).

Report:	CA 6.1/9 Class T., 2000 a Dimethomorph (CL 336379) - Storage stability of Dimethomorph (CL 336379) - Residues in oilseed rape seed at <-18°C DK-326-032
Guidelines:	EEC 91/414 (7032/VI/95), EEC 96/68, EPA 860.1000, EPA 860.1380, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	BAS 550 F (Dimethomorph)
	Description:	
	Lot/Batch #:	CL 336379
	Purity:	97.6%
	CAS#:	110488-70-5
	Spiking levels:	0.02 mg/kg

2.	**Test commodity:**	
	Crop:	Oilseed rape seed
	Sample size:	25 g

B. STUDY DESIGN

1. Test procedure

A storage stability study was conducted with dimethomorph in oilseed rape seed at ≤-18°C for a period of 12 months. Treated and untreated oilseed rape seed were used for determination of residue data at time points 0, 4, 8 and 12 months. All samples were kept frozen at -18°C. After time intervals of 0, 4, 8 and 12 months, triplicate aliquots of treated samples as well as two aliquots of untreated control samples were removed from the storage and analyzed for dimethomorph using gas chromatography with mass selective detection (GC/MSD). Two untreated samples were used for recovery and thus fortified at 0.020 mg/kg dimethomorph at each time point of determination.

2. Description of analytical procedures

Analytical method FAMS 098-02 was used for the extraction and determination of dimethomorph residues in oilseed rape seed. The analyses were carried out using the active ingredient with an E/Z isomer ratio of approximately 44:56.
The residues are extracted from the oilseed rape with acetonitrile. This is followed by liquid-liquid partitioning with n-hexane to remove fatty impurities. Then the extract is subjected to further cleanup by gel permeation chromatography followed by silica gel column chromatography. The content of dimethomorph is determined by gas chromatography using a mass selective detector (GC/MSD).

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1-7. Residue levels remained essentially unchanged over the whole storage period clearly demonstrating the stability of dimethomorph residues during frozen storage.
Procedural recoveries obtained ranged from 75% (4 months) to 118% (8 months) (dimethomorph, arithmetic mean m = 98%, relative standard deviation RSD = 16%). The rate of recoveries of the Z-isomer of dimethomorph varied between 55 % and 59% (arithmetic mean m 57% ± 2%), demonstrating the stability of the isomer ration during storage. The retention times of the isomers of dimethomorph remained essentially unchanged during the 12 months of storage.

Table 6.1-8: Storage stability of dimethomorph in oilseed rape seed

Sample matrix	Average recovery (%)		
	4 months	8 months	12 months
Oilseed rape seed	75	118	97

III. CONCLUSION

Residue levels remained essentially unchanged over the whole storage period clearly demonstrating the stability of dimethomorph residues during frozen storage in oilseed rape seed.

Animals

The following study was not evaluated during the previous peer review of dimethomorph. It is included to prove storage stability of dimethomorph and relevant metabolites in animal tissues and milk.

Report:	CA 6.1/10 Weitzel R., 1994 b Dimethomorph: Storage stability of active ingredient and relevant metabolites in bovine milk and tissues at minus 18°C (Germany, 1992) DK-326-008
Guidelines:	BBA IV 3-3, IVA Guideline Residue Chemistry Part II Storage Stability 1992
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 550 F (Dimethomorph, DMM)
	Description:	
	Lot/Batch #:	CME 151 (parent) Pr 187, Z 67 (dimethomorph/M550F006), CL 900987 (metabolite) Pr 209/3, Z 69 (dimethomorph/M550F007), CL 900986 (metabolite) H 9706, CUR 7117 dimethomorph/M550F008), WL 315582 (metabolite)
	Purity:	99.1% (Dimethomorph) 97.1% (Pr 187) 96.1% (Pr 209/3) 99.9% (H 9706)
	CAS#:	110488-70-5
	Spiking levels:	Dimethomorph: 0.1 mg/kg (milk and tissue) M550F006 (Z 67) and M550F007 (Z69): 0.2 mg/kg (milk) and 0.1 mg/kg (tissue) M550F008 (CUR 7117): 0.1 mg/kg (milk)
2.	**Test Commodity:**	
	Crop:	cow milk, muscle, kidney, liver
	Sample size:	30 g tissues, 20 g milk

B. STUDY DESIGN

1. Test procedure

A storage stability study at ≤-18°C was carried out with dimethomorph (BAS 550 F) and relevant metabolites in bovine milk and tissues. The parent compound was added to untreated milk, muscle, liver and kidney samples at a level of 0.1 mg/kg. Metabolites M550F006 (Z 67) and M550F007 (Z 69) were added to milk at 0.2 mg/kg and to tissues at 0.1 mg/kg. Metabolite M550F008 (CUR 7117) was only added to milk at a level of 0.1 mg/kg. The samples were kept in brown screw-topped jars at ≤-18°C for up to 16 months. At time intervals of four months, samples were removed from storage and have been analyzed for all substances using HPLC with UV detection for milk and GC with P/N or MS detection for tissue analysis.

2. Description of analytical procedures

Milk samples were analyzed for residues of dimethomorph, M550F006 (Z 67), M550F007 (Z 69) and M550F008 (CUR 7117) using method FAMS 024-01 (Shell Forschung GmbH) of 8th February 1991. Muscle, liver and kidney samples were analyzed for residues of BAS 550 F, M550F006 (Z 67) and M550F007 (Z 69) using method FAMS 023-01 (Shell Forschung GmbH).

Principle of method FAMS 024-01

Samples of milk were extracted with (1:3 V/V) acetone using a homogenizer. After centrifugation the liquid phase was decanted from the solid pellet and the acetone was removed by rotary evaporation. The aqueous phase remaining after evaporation of the acetone was partitioned three times with ethyl acetate. The organic solutions were drained through sodium sulfate and then reduced by rotary evaporation. The concentrated residue was dissolved in methanol and injected into a gel permeation chromatography system. The sample was eluted with methanol + 0.01 mol acetic acid/l, concentrated and dissolved in methanol for final HPLC determination.

Principle of method FAMS 023-01

Tissue s were extracted with acetonitrile in a homogenizer. After centrifugation the liquid phase was decanted from the solid pellet. The liquid organic phase was partitioned once with n-hexane. After addition of saturated sodium chloride solution and water, the acetonitrile phase was partitioned twice with dichloromethane. Starting with the third period of analysis (after 8 months), H3P04 (0.1 mol/L) was added to the dichloromethane distribution to improve phase distribution. The combined dichloromethane phases were reduced to dryness. The concentrated residue of the partitions was dissolved in methanol and injected into a gel permeation chromatography system and the sample was eluted with methanol (containing 0.01 mol/L acetic acid from the third phase of analysis on). The concentrated eluate was then dissolved in cyclo-hexanone for final GC determination. A re-validation of method FAMS 023-01 was performed and additional recovery data have been generated (see chapter 4.1, [see KCA 4.1.2/18 2015/1000643].

II. RESULTS AND DISCUSSION

The recoveries of dimethomorph from cow milk and tissues after the various storage periods are summarized Table 6.1-7.

All compounds remained stable over the whole storage period of 16 months in every matrix examined. The analytical methods were validated at the levels used for fortification with every series of analysis (except starting date). The average of all recoveries for dimethomorph, M550F006 (Z 67), M550F006 (Z 69) and M550F008 (CUR 7117) was between 70 and 120% for all samples. M550F008 (CUR 7117) was not tested in tissues.

Table 6.1-9: Storage stability of dimethomorph active ingredient and relevant metabolites in bovine milk and tissues

Sample matrix	Average Recovery (%)				
	0 months	4 months	8 months	12 month	16 month
Milk					
BAS 550 F	84	86	85	92	84
CUR7117	85	78	80	88	94
Z 67	84	70	72	89	85
Z 69	80	83	75	80	86
Muscle					
BAS 550 F	86	88	82	94	89
Z 67	94	92	81	101	87
Z 69	100	88	76	82	85
Liver					
BAS 550 F	89	88	82	86	92
Z 67	106	114	84	95	97
Z 69	116	98	76	78	90
Kidney					
BAS 550 F	84	94	94	89	92
Z 67	89	104	92	96	86
Z 69	86	87	76	98	83

Conclusion:

It can be concluded that dimethomorph and its metabolites M550F006 (Z 67), M550F007 (Z 69) are stable in tissues and milk for at least 16 months when stored under deep frozen conditions. M550F008 (CUR 7117) is stable milk for 16 months of storage time, respectively.

CA 6.2 Metabolism, distribution and expression of residues

During the previous EU Review of the active substance dimethomorph, the metabolism of dimethomorph has been studied in grapes, lettuce, potato, tomato, lactating goat and laying hens. All of these studies have been part of the previous evaluation, are still scientifically valid ~~and are therefore not submitted again in this supplementary dossier~~. ~~For reasons of convenience, a short overview of the main conclusions is given below and short study summaries are included in the following.~~ But with the agreement of Ctgb, a full OECD summary of each old metabolism study was added in this update dossier.

Table 6.2-1: Summary of peer reviewed dimethomorph metabolism studies in plants and livestock

Crop/Species	Label/Dose	Major components	Reference (BASF DocID)
Grape	^{14}C-dimethomorph, chlorophenyl label 4 x 0.9 kg/ha	Dimethomorph (grapes: 86% TRR; leaves 83% TRR) M550F003 (Z7) 1.6% TRR (only in leaves) Metabolite fraction M1 (6-9% TRR) Metabolite fraction M2 (1-5% TRR) M1 and M2 are polar fractions (composed of several minor unknown components)	DK-640-005 DK-640-010 (supplemental data)
Lettuce	^{14}C-dimethomorph, chlorophenyl label 4 x 1.14 kg/ha	Dimethomorph (91.5% TRR) M550F006/M550F007 (Z67/Z69) (0.5% TRR) M550F003 (Z7) (0.5% TRR)	DK-640-021
Potato	^{14}C-dimethomorph, chlorophenyl label 4 x 0.6 kg/ha	Dimethomorph (68% TRR) M550F003 (Z7) (<0.5% TRR); Tubers not investigated due to low residues (<0.019 mg/kg)	DK-640-004 DK-640-009
Potato	^{14}C-dimethomorph, morpholine label 4 x 0.6 kg/ha	Dimethomorph (76% TRR) Tubers not investigated due to low residues (<0.018 mg/kg)	DK-640-006 DK-640-011
Potato	^{14}C-dimethomorph, chlorophenyl label 3 x 0.3 kg/ha	Dimethomorph (peel: 46%TRR, tuber 12% TRR) M550F006 (Z67) 11.6% TRR in peel M550F007 (Z69) 5% TRR in peel Absolute levels in whole tuber were 0.014 mg/kg parent and Z67 <0.003 mg/kg and Z69 <0.002 mg/kg Several minor polar compounds, in sum 8.6% TRR in peel (0.01 mg/kg) and 24% TRR in tuber (0.006 mg/kg)	DK-640-014
Tomato	^{14}C-dimethomorph, chlorophenyl label 8 mg/L (hydroponic system)	Only green parts (leaves) were investigated. Dimethomorph (16-66% TRR) M550F007 (Z69) (13-34%) M550F009 (Z93) (8-17%) M550F011 (Z95) (4-8%) M550F012 (Z98) (1-7%)	DK-640-020

Table 6.2-1: Summary of peer reviewed dimethomorph metabolism studies in plants and livestock

Crop/Species	Label/Dose	Major components	Reference (BASF DocID)
Poultry	^{14}C-dimethomorph, chlorophenyl label, nominal target dose 2 mg/kg bw/d or 40 mg/kg feed Dosing duration 7 days	Dimethomorph and/or its metabolites did not accumulate and were excreted fast and efficiently. Major identified components: Dimethomorph (fat, skin and excreta) M550F006/M550F007 (Z67/Z69) (eggs, liver, kidney, muscle,) M550F009 (WL 376084) (eggs, muscle) M550F011 (CUR 7216) (eggs) M550F005 (Z43) (eggs, kidney).	DK-640-003 DK-640-007
Goat	^{14}C-dimethomorph, chlorophenyl label, nominal target dose 1 mg/kg bw/d or 25 mg/kg feed Dosing duration 7 days	Dimethomorph and/or its metabolites did not accumulate and were excreted fast and efficiently. Major identified components: Dimethomorph (kidney, liver, muscle, fat) M550F006/M550F007 (Z67/Z69) (liver, kidney) M550F009 (WL 376084) (kidney) M550F008 (CUR 7117) (milk)	DK-640-005 DK-640-008

CA 6.2.1 Metabolism, distribution and expression of residues in plants

Metabolism in plants

In the context of the previous submission of dimethomorph for EU Annex I inclusion, plant metabolism studies addressing foliar spray application were carried out in three crops belonging to three different crop categories:

For fruits: grapevines
For root and tuber vegetables: potatoes
For leafy vegetables: lettuce

In addition, investigations on the metabolism of dimethomorph in tomato (green plant parts) have been performed in a hydroponic system in order to investigate the translocation and metabolism after uptake via roots, which should support the drip/drench use pattern for strawberries.

The metabolic pathway of dimethomorph in plants showed a common pattern with regard to the nature of residue. The unchanged parent compound was the only significant component of the total residue found in grapevines (fruit group), potatoes (tuber group) and lettuce (leafy group). The metabolism of dimethomorph was found to proceed along two pathways of which the first one is the major one and the second is rather occurring to a smaller extent in quantitative terms:

1. Demethylation of the two methoxy groups of the dimethoxyphenyl ring to produce metabolites M550F006 (Z67) and M550F007 (Z69). These two metabolites most likely undergo further conjugation with glucose.

2. Hydrolysis of dimethomorph (complete loss of the morpholine moiety) to form metabolite M550F003 (Z7).

In the study with tomato plants treated with dimethomorph by uptake through the roots (hydroponic system) besides the main pathway of demethylation of the methoxy groups an additional pathway has been observed, in which the opening and stepwise degradation of the morpholine ring occurred (leading to M550F009, M550F011 and M550F012).

Based on the results of these metabolism studies, the **parent molecule dimethomorph** (BAS 550 F) has been regarded as the **relevant residue** in **plant matrices** for both **monitoring and risk assessment purposes.**

"The residue definition proposed for monitoring and risk assessment is dimethomorph. No metabolite is included in the residue definition given that the residue pattern is largely dominated by the parent compound." (see *EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph)*

This assessment was also confirmed in 2011 by EFSA during the re-evaluation of the established MRLs according to Regulation (EC) No 396/2005, Art. 12 (see: EFSA Journal 2011;9(8):2348).

Most of the evaluated studies were conducted with the molecule labelled in the chlorophenyl ring, only in one crop group (potato; category root and tuber) investigations with the morpholine label were available. In order to provide further information on the metabolic fate of the morpholine moiety, two new metabolism studies were performed for this submission, one in grapes and one in lettuce. The new metabolism study in grapes was conducted with both labels (chlorophenyl and morpholine ring label) in order to allow a direct comparison between the two labels and also to have a better link to the previous data in grapes with the chlorophenyl label. Since this study confirmed the main metabolic steps already known from the previous data, BASF considered it sufficient to conduct the new lettuce study with the morpholine ring label only.

In the following, ~~short summaries~~ full OECD summaries of the already evaluated studies are shown along with the summaries of the new studies, for reasons of convenience.

Grapes

Schlueter H., 1990: ^{14}C-Dimethomorph (CME 151) - Metabolism and translocation in vines ((DocID DK-640-005)
Schlueter H., 1991: ^{14}C-Dimethomorph (CME 151): Metabolism and translocation in vines - Supplemental data (DocID DK-640-010)

Selected grapes and leaves of grapevines were separately treated four times with ^{14}C-dimethomorph (chlorophenyl label) prepared as an emulsifiable concentrate (CME 151-03, 10% w/v). The treatment was applied on individual grapes or leaves by syringe. The concentration of the active ingredient in the application mixture equaled ca. 900 g dimethomorph/ha, thus resulting in a total application rate of 3.6 kg ai/ha (this means a 2.8N overdosing compared to the actual cGAP in grapes of 5 x 250g ai/ha). Treatment intervals were nine to ten days and the pre-harvest interval was 35 days. Grapes and leaves were sampled after each individual treatment to determine recovery rate of the applied treated solution. After part of the leaves were treated, untreated fruit/leaves of grapevine were sampled to assess translocation of dimethomorph within the plant.Radioactivity was determined by liquid scintillation and combustion analysis. Samples were extracted subsequently with acetone and methanol. Identification was achieved by thin layer chromatography and HPLC-UV. HPLC-MS was used as confirmatory method for identification of the parent compound.

Findings
The Total Radioactivity Residue (TRR) 35 days after last application was 14.6 mg/kg in grapes and 90.1 mg/kg in leaves. From the untreated grapevine, some selected leaves were treated. Untreated grapes and leaves of this grapevine showed maximum TRR values of 0.025 mg/kg and 0.035 mg/kg, demonstrating only a very low translocation of residues from treated to untreated leaves. The Extractable Radioactivity Residue (ERR) for treated grapes and leaves was 98.2% and 96.9% of the TRR, respectively.
The majority of the extractable residue was identified as unchanged parent dimethomorph, which accounted for 86.3% (equivalent to12.6 mg/kg) and 82.9% (equivalent to 74.8 mg/kg) of the TRR in grapes and leaves, respectively. In treated leaves, a trace amount of a degradation product showing similar retention characteristics upon HPLC analysis to the reference compound Z7 (M550F003) was characterized. This compound accounted for only 1.6% (equivalent to 1.44 mg/kg) of the TRR. The remainder of the extractable residues were assigned to the two metabolite fractions M1 and M2, which were found to be composed of several minor unknown polar components. These accounted for 5.8% and 4.7% TRR in grapes and for 8.6% and 1.2% in leaves. Given the high overdosing, the amount of these unknown metabolites in grapes treated according to the actual cGAP would even be lower.

Table 6.2.1-1: Total Radioactive Residues after treatment with ^{14}C-dimethomorph

	Treated grapes	Untreated grapes	Treated leaves
TRR (mg/kg)	14.6	0.026	90.1
Extractable Radioactivity Residue (ERR) (%)	98.2	95.2	96.9
TRR non extractable (%)	1.8	4.2	3.1

Table 6.2.1-2: Identification and quantification of metabolites in treated/untreated grape and treated leave samples at harvest after treatment with ^{14}C-chlorophenyl dimethomorph (extractable fraction)

Metabolite code (Reg.-No of reference substance)	Chlorophenyl label		
	Treated grapes	Untreated grapes	Treated leaves
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Dimethomorph (336379)	12.6 (86.3)	0.0127 (48.8)	74.7 (82.9)
M550F003 (Z7)	Not detected*	Not detected*	1.44 (1.6)
Metabolite Fraction M1	0.84 (5.8)	0.004 (16.7)	7.75 (8.6)
Metabolite Fraction M2	0.70 (4.7)	0.005 (20.8)	1.08 (1.2)
Acetone extract (leaves)	-	-	1.8 (2.0)
Others	0.2 (1.4)	0.002 (9.5)	0.54 (0.6)
Total	*98.2*	*95.8*	*96.9*

Conclusions

The only relevant residue in grapes consists of the parent compound, dimethomorph, which is mainly located on the surface of grapes or leaves. Based on the low level of radioactivity found in untreated leaves and grapes, it was concluded that there is no significant movement from treated to untreated plant parts of vinegrapes.

Report:	CA 6.2.1/1 Schlueter H., 1990 a 14C-Dimethomorph (CME 151) - Metabolism and translocation in vines DK-640-005
Guidelines:	EPA 171-4
GLP:	no

Report:	CA 6.2.1/2 Schlueter H., 1991 a 14C-Dimethomorph (CME 151): Metabolism and translocation in vines - Supplemental data DK-640-010
Guidelines:	EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

Description:	[chlorophenyl-U-^{14}C]-BAS 550 F (chlorophenyl label; E:Z, 47:53) Unlabeled BAS 550 F (E:Z 50:50)	
Lot/batch #:	2271-040 (chlorophenyl label) H 7879 (unlabeled)	
Purity:	99.2% (unlabeled) Radiochemical purity: Specific activity:	98.5% (chlorophenyl label) 1669 MBq/g (chlorophenyl label) 82.16 MBq/g (application mixture)
CAS#:	110488-70-5	
Stability of test compound:	The test item was stable over the test period.	

2. Test commodity:

Crop:	Grapevine
Type:	Berries and small fruits
Variety:	Müller-Thurgau
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed commodity:	Fruits and leaves
Sample size:	Not relevant

B. STUDY DESIGN

The study was carried out at the Biological Experimental Station of Shell Forschung GmbH in Schwabenheim, Germany. The cultivation of the crop took place under natural climatic conditions, whereby the test field was covered with a roofing of plastic foil to avoid a washing off of the radioactivity by rain.

1. Test procedure

Selected grapes and leaves of grapevines were separately treated four times with a mixture of ^{14}C-dimethomorph (chlorophenyl label) and unlabeled dimethomorph prepared as an emulsifiable concentrate (CME 151 03, 10% w/v). The treatment was applied on individual grapes or leaves by syringe. The concentration of the active ingredient in the application mixture equaled ca. 900 g a.i./ha, thus resulting in a total application rate of 3.6 kg a.i./ha (this means a 2.8N overdosing compared to the actual cGAP in grapes of 5 x 250g a.i./ha). Treatment intervals were nine to ten days and the pre-harvest interval was 35 days. Immediately after each treatment, grapes and leaves were taken for recovery measurements. Prior to each further treatment, two grapes were sampled to investigate the potential degradation/biotransformation of the parent compound and the potential formation of metabolites. Mature grapes, all remaining treated fruits and treated leaves were sampled 63 days after the first application. In order to investigate the potential translocation of radioactive residues from the treated leaves, leaves lying above (acropetal to) the treated leaves were sampled. Additionally, (untreated) grapes originating from the untreated twigs were harvested.
At harvest all sample material was weighed and stored at approximately -18°C or below until analysis. The storage conditions stayed the same until analysis started and during the whole period of the metabolism study.

2. Description of analytical procedures

Radioanalysis (TRR): For the determination of the TRR and the measurement of solid residues following solvent extraction or solubilization procedures (RRR), homogenized subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly. In order to determine the background radioactivity untreated sample material were combusted under the same conditions.
Extraction (ERR): Each treated grape was preserved in acetone for approximately 1 hour (acetone wash). The filtered supernatant was subjected to LSC measurements and grapes were homogenized in acetone. The supernatant was separated from the solid residue by centrifugation and the residue was extracted exhaustively one further time with acetone and two times with methanol each extraction step supported by sonication. The extracts from each sample and step were combined according to solvent and aliquots were radioassayed (LSC).
Grapes used for determination of recovery, untreated grapes and all leaf samples (treated and untreated) were processed in the same way as described above, except that no methanol extraction was performed. Extracts containing sufficient amounts of radioactive residues were subjected to radio thin-layer chromatography (TLC).
Treatment of residual radioactive residues (RRR) after solvent extraction: The residues after methanol and water extraction were dried and radioassayed. The residues of selected grapes were subjected to

a) 24 hours soxhlet extraction using methanol.
b) Enzyme treatment (β-glucisidase) followed by treatment with acid (2 N HCl)

3. Identification of metabolites

Identification was achieved by TLC and HPLC-UV. HPLC-MS was used as confirmatory method for identification of the parent compound.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The TRR (combusted) of untreated leaves and grapes were in the same magnitude, ranging from 0.017 to 0.035 mg/kg (Table 6.2.1-1). The TRR in treated leaves (calculated as sum of the ERR and RRR) was very high with 90.1 mg/kg. The high TRR values in treated grapes (14.6 mg/kg) in comparison to the residues obtained in agricultural practice (≤1 mg/kg) can be explained by thorough treatment of each grape, whereas under practical conditions the grapes are protected by the leaves during treatment. Additionally, the threefold higher concentration of the spray mixture and the lack of rainfall has to be considered.

Table 6.2.1-1: Total radioactive residues (TRR) in treated/untreated grapevine matrices

Matrix	TRR calculated [mg/kg]	TRR combusted [mg/kg]
Grape (treated)	14.6	-
Grape (untreated)	-	0.017 – 0.025
Leaf (treated)	90.1	-
Leaf (untreated)	-	0.016 – 0.035

1 TRR was calculated as the sum of ERR + RRR

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in grapes

The results obtained from wasing the surface of the grapevine matrices with acetone and additional extraction with acetone and methanol are summarized in Table 6.2.1-2.
The main portion of radioactive residues was recovered in the acetone wash for both grapes and leaves accounting for 73% and 95% TRR, respectively, indicating that main portions remain on the surface of the skin.
The remaining radioactive residues were mainly extracted by acetone (18% TRR for grapes and 2% TRR for leaves) and methanol (7.4% TRR only applied on grapes). The ERR including the washing fraction accounted for 98% TRR for grapes and for 97% for leaves.
Although the RRR were low (up to 3% TRR), the solid residue after solvent extraction of grapes was further investigated.

Table 6.2.1-2: Extractability of radioactive residues in grapevine samples

Matrix	Acetone wash		Acetone extract		Methanol extract		ERR[1]		RRR [2]		TRR calculated
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Grapes	10.6	72.5	2.7	18.3	1.1	7.4	14.3	98.2	0.3	1.8	14.6
Leaves	85.6	95.0	1.8	2.0	-	-	87.4	97.0	2.7	3.0	90.1

[1] Extractable Radioactive Residue (sum of acetone wash and extracts)
[2] Residual Radioactive Residue (after washing with acetone and after solvent extraction)

2. Identification and characterization of extractable residues in grapevines

TLC investigations of the ERR from treated grapes revealed that the greatest portion (86% TRR) showed the same retention characteristics as the parent compound dimethomorph (Table 6.2.1-3). This radioactive residue possessing identical retention characteristics as the parent compound was isolated and further investigated by HPLC-MS confirming the identity of unchanged dimethomorph. The remainder of the ERR (11.7% TRR) was composed of several unknown mainly polar compounds.
TLC investigation of the acetone surface extract of treated leaves revealed that this residue mainly comprised the unchanged parent compound (83% TRR). These findings were also confirmed by HPLC retention behaviour and by mass spectroscopy of the corresponding radioactive TLC fraction. A further trace amount of radioactive residues detected on the TLC plates showed similar retention characteristics as the reference compound M550F003 (Z7). The identity of this product (1.6% TRR) was confirmed by HPLC.
It was not possible to obtain TLC from the extracts of untreated grapes due to the huge surplus of coextracted plant constituents. In the case of untreated leaves, however, TLC revealed that most of the radioactive residue was made up of the unchanged parent compound.

Table 6.2.1-3: Identification and quantification of metabolites in treated grape and treated / untreated leaf samples at harvest after treatment with ^{14}C-chlorophenyl dimethomorph (extractable fraction)

Designation metabolite code	Grapevine Matrix and Harvesting Timepoint					
	Treated grapes		Treated leaves		Untreated leaves	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Dimethomorph	12.60	86.3	74.70	82.9	0.013	48.8
M550F003 (Z7)	Not detected	Not detected	1.44	1.6	Not detected	Not detected
Metabolite fraction M1	0.84	5.8	7.75	8.6	0.004	16.7
Metabolite fraction M2	0.69	4.7	1.08	1.2	0.005	20.8
Acetone extract (leaves)	-	-	1.80	2.0	-	-
Others	0.20	1.4	0.54	0.6	0.002	9.5
Total	*14.34*	*98.2*	*87.31*	*96.9*	*0.025*	*95.8*

3. Characterization of non-extractable residues

The residual radioactive residues after solvent extraction (RRR) were between 1.8% TRR and 3.0% TRR for treated grapes and leaves, respectively (Table 6.2.1-2). The residue after solvent extraction of grapevine grapes was further investigated and supernatants obtained after Soxhelet extraction, enzyme and acidic treatment were subjected to TLC analysis (Table 6.2.1-4), where the unchanged parent compound was identified and quantified.

Table 6.2.1-4: Characterization of the residual radioactive residues (RRR) after solvent extraction

Fraction / Supernatant	RRR		Soxhlet extraction		Enzyme treatment		HCl treatment	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Dimethomorph	N/A	N/A	0.035	0.24	0.112	0.76	0.006	0.04 [1]
Metabolite fraction M1	N/A	N/A	0.012	0.08	0.007	0.05	0.003	0.02 [1]
Metabolite fraction M2	N/A	N/A	0.007	0.05	0.007	0.05		
Others	N/A	N/A	0.013	0.09	0.010	0.07	0.001	0.01 [1]
Total	*0.261*	*1.8*	*0.068*	*0.47*	*0.136*	*0.93*	*0.026*	*0.18*

1 Determined in the organic phase (0.010 mg/kg or 0.07% TRR) after partition of the supernatant

4. Storage stability

The study was carried out from August 04, 1987, to November 30, 1989, and exceeded 6 months. Information on the storage stability in grapes is sufficiently addressed within the AIR 3 Dossier by investigations in the more recent grape study [see KCA 6.2.1/3 2014/1093386].

Table 6.2.1-5: Summary of identified metabolites in grapevine matrices at 63 days harvest

Component	Grapes						Leaves	
	ERR		RRR		Sum		ERR	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Dimethomorph	12.60	86.3	0.15	1.0	12.75	87.3	74.70	82.9
M550F003 (Z7)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	1.44	1.6
Metabolite Fraction M1	0.84	5.8	0.02 [1]	0.1 [1]	0.86 [1]	5.9 [1]	7.75	8.6
Metabolite Fraction M2	0.69	4.7	0.01 [1]	0.1 [1]	0.70 [1]	4.8 [1]	1.08	1.2

1 0.003 mg/kg or 0.02% TRR were not considered in this sum

III. CONCLUSION

Upon selective treatment of grapes and leaves of a grapevine, the TRR 35 days after last application was 14.6 mg/kg in treated grapes and 90.1 mg/kg in treated leaves. Untreated grapes and leaves of this grapevine showed maximum TRR values of 0.025 mg/kg and 0.035 mg/kg, demonstrating only a very low translocation of residues from treated to untreated leaves. The ERR for treated grapes and leaves was 98.2% and 96.9% of the TRR, respectively.
The majority of the extractable residue was identified as unchanged parent dimethomorph, which accounted for 86.3% TRR (equivalent to12.6 mg/kg) and 82.9% TRR (equivalent to 74.8 mg/kg) in grapes and leaves, respectively. In treated leaves, a trace amount of a degradation product showing similar retention characteristics upon HPLC analysis to the reference compound Z7 (M550F003) was characterized. This compound accounted for only 1.6% (equivalent to 1.44 mg/kg) of the TRR. The remainder of the extractable residues were assigned to the two metabolite fractions M1 and M2, which were found to be composed of several minor unknown polar components. These accounted for 5.8% and 4.7% TRR in grapes and for 8.6% and 1.2% in leaves. Given the high overdosing, the amount of these unknown metabolites in grapes treated according to the actual cGAP would even be lower.
The only relevant residue in grapes consists of the parent compound, dimethomorph, which is mainly located on the surface of grapes or leaves. Based on the low level of radioactive residues found in untreated leaves and grapes, it was concluded that there is no significant movement from treated to untreated plant parts of grapevines.

Report: ~~CA 6.2.1/1~~ CA 6.2.1/3
Rabe U. et al., 2015 a
Metabolism of 14C- Dimethomorph in grapes
2014/1093386
Guidelines: EPA 860.1000, EPA 860.1300: Nature of the Residue in Plants Livestock, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants, JMAFF 59 NohSan No 4200, OECD 501
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

2. Test Material:

Description: [Morpholine-2,3-^{14}C]-BAS 550 F (morpholine label)
[p-chlorophenyl-U-^{14}C]-BAS 550 F (chlorophenyl label)
[acrolyl-^{13}C]-BAS 550 F (acrolyl label)
Unlabeled BAS 550 F
Formulation BAS 550 AB F (=blank formulation of BAS 550 02F; DC formulation)

Lot/Batch #: 219505_CHEM880_P8_1 (morpholine label)
1068-0101 (chlorophenyl label)
1070-0101 (acrolyl label)
AC9978-68A (without label)

Purity: 97.6% (unlabeled)
Radiochemical purity: 98.5% (morpholine label)
96.4% (^{14}C-phenyl label)
Specific activity: 5.79 MBq/mg (morpholine label)
7.7 MBq/mg (chlorophenyl label)

CAS#: 110488-70-5

Stability of test compound: The test item was stable over the test period.

2. Test Commodity:

Crop: Grapes (wine)
Type: Berries and small fruit
Variety: Müller-Thurgau
Botanical name: *Vitis vinifera*
Crop parts(s) or processed commodity: Fruits, leaves, branches
Sample size: Not relevant

B. STUDY DESIGN

The study was carried out at the Agricultural Center of BASF SE in Limburgerhof, Germany.
The cultivation of the crop took place under natural climatic conditions.

1. Test procedure

The crop was treated three times with the radiolabelled test item. The treatment was performed either with a mixture of morpholine-2,3-^{14}C-labelled and unlabelled dimethomorph (morpholine label) or with a mixture of p-chlorophenyl-U-^{14}C-labelled, acrolyl-^{13}C- and unlabelled dimethomorph (chlorophenyl label). The targeted application rate of the test item was 1500 g/ha to adress the critical GAP of 5 x 300 g a.s./ha. In order to reduce the number of necessary radioactive applications, the total rate was allocated in 300 g/ha (approximately 0.268 lb/A) for the first application and 600 g/ha (approximately 0.536 lb/A) for the two subsequent applications. At growth stage BBCH 89, the mature grapes (28 DALA, morpholine label; 14 DALA and 28 DALA chlorophenyl label) were harvested manually. Fruits were separated from branches and leaves, weighed and stored separately. At harvest all sample material was weighed and stored at approximately -18°C or below until analysis. The storage conditions stayed the same until analysis started and during the whole period of the metabolism study. Extracts were stored in a refrigerator or, for longer periods, in a freezer.

2. Description of analytical procedures

Radioanalysis (TRR): For the determination of the TRR, and the measurement of solid residues following solvent extraction or solubilization procedures (RRR), homogenized subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly. In order to determine the background radioactivity untreated sample material were combusted under the same conditions. For the leaves, no background activity was detected.
Extraction (ERR): The homogenized subsamples of grapes and leaves were extracted three times with methanol and two times with water using a blender. The samples were centrifuged and the extracts were decanted. The remaining plant material was subjected to further solubilization steps. The methanol extracts were generally combined and LSC measured. The water extracts were treated likewise. Sample cleanup was performed using an SPE column, fractions were subsequently eluted with water/acetonitrile (40:60).
Extraction efficiency:
To investigate the extraction efficiency, grapes and leaves from the morpholine label (28 DALA) were extracted with the extraction protocols of the main data generation method and of the most important multi-residue methods (BASF method No 575/0, QuEChERS and DFG S19, see also section 4, chapter 4.1 and 4.2).
Treatment of non-extracted radioactivity (RRR): The residues after methanol and water extraction were dried and radioassayed. After ammonia extraction samples were dried, ground and subsequently solubilized with different enzymes (successively with macerozyme/cellulase, amylase/amyloglucosidase, tyrosinase/laccase and glucosidase/hesperidinase). Purified extracts with sufficient radioactivity were analyzed by HPLC.

3. Identification of metabolites

Generally, extracts containing significant radioactive residues (≥0.010 mg/kg) were analyzed using reversed phase HPLC and mass spectrometry (metabolite characterization and identification). A specific HPLC system was used for the investigation of the presence of morpholine.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

For the morpholine label (28 DALA) the calculated TRR of grapes was 0.605 mg/kg. In leaves, the TRR was significantly higher with 4.684 mg/kg. The values for the chlorophenyl label (28 DALA) accounted for 0.654 mg/kg and 7.720 mg/kg in grapes and leaves, respectively. For the chlorophenyl label (14 DALA) the content of radioactivity was 0.889 mg/kg in grapes and 1.348 mg/kg in leaves. Additionally, the TRR was measured by direct combustion analysis followed by LSC. The combusted TRR showed no major differences to the calculated TRR for all the matrices, labels and harvesting times (Table 6.2.1-6).

Table 6.2.1-6: Total radioactive residues (TRR) in grape following foliar treatments

TRRs in treated grape samples					
Matrix	DALA[1]	TRR combusted [mg/kg]		TRR calculated* [mg/kg]	
		Morpholine label	Chlorophenyl label	Morpholine label	Chlorophenyl label
Grape (fruits)	14 DALA	N/A	0.914	N/A	0.889
Grape (leaves)	14 DALA	N/A	1.238	N/A	1.348
Grape (fruits)	28 DALA	0.544	0.623	0.605	0.654
Grape (leaves)	28 DALA	4.712	8.592	4.684	7.720

1 Days after last application
* TRR was calculated as the sum of ERR + RRR
N/A Not applicable

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in grapes

The extractabilities of the grape matrices with methanol and water are summarized in Table 6.2.1-7. The extractability (ERR) of grapes and leaves of all harvest times (14 and 28 DALA) was high for both labels (morpholine or chlorophenyl) and accounted for 82.6 to 89.8% TRR. Thereby, the major part of radioactive residue was extracted with methanol (78.7 to 87.8% TRR) and smaller amounts were extracted with water (0.5 to 6.1% TRR).

Table 6.2.1-7: Extractability of radioactive residues in grape samples

Matrix	Days after last application	Methanol extract		Water extract		ERR[1]		RRR [2]		TRR calculated[3]
		[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Morpholine label										
Grape fruits	28 DALA	0.530	87.7	0.004	0.7	0.535	88.4	0.070	11.6	0.605
Leaves	28 DALA	3.919	83.7	0.285	6.1	4.204	89.8	0.480	10.2	4.684
Chlorophenyl label										
Grape fruits	14 DALA	0.780	87.8	0.005	0.5	0.785	88.3	0.104	11.7	0.889
Leaves	14 DALA	1.100	81.6	0.013	0.9	1.113	82.6	0.235	17.4	1.348
Grape fruits	28 DALA	0.542	82.8	0.007	1.0	0.548	83.8	0.106	16.2	0.654
Leaves	28 DALA	6.075	78.7	0.395	5.1	6.470	83.8	1.250	16.2	7.720

[1] ERR = Extractable Radioactive Residue (extraction with methanol and water)
[2] RRR = Residual Radioactive Residue (after solvent extraction with methanol and water)
[3] TRR was calculated as the sum of ERR + RRR

Extraction efficiency

In addition, the extraction efficiency of three alternative extraction procedures (for important residue methods) was investigated in samples of both matrices treated with morpholine labelled test substance. The extractions were performed according to BASF residue analytical method 575/0 (L0013/01), multimethod DFG S19 and multimethod QuEChERS. In order to compare the extraction efficiency, the amount of parent compound released with the different residue methods was set in proportion to the results from the metabolism study (extraction with methanol and water). For grapes, comparable amounts of dimethomorph were extracted with the residue methods (93.2% to 97.5% of the metabolism study). For leaves, the investigated methods showed comparable extractabilities (residue method 575/0: 89.2% of the metabolism study, DFG S19: 99.0% of the metabolism study) with the exception of the multimethod QuEChERS, which resulted in a lower extractability (71.4 % of the metabolism study.

Table 6.2.1-8: Comparison of extraction efficiency of radioactive residues from grape samples using different analytical methods

Matrix	Grape fruits				Leaves			
Extraction Method	Metabolism Investigations	Residue Method 575/0	Multimethod QuEChERS	Multimethod DFG S19	Metabolism Investigations	Residue Method 575/0	Multimethod QuEChERS	Multimethod DFG S19
	[% TRR]							
Dimethomorph *E*-Isomer	22.9[1]	22.6	23.4	20.6	31.1[1]	27.2	22.3	31.3
Dimethomorph *Z*-Isomer	37.4[1]	36.2	34.4	35.6	31.9[1]	28.9	22.6	31.1
Dimethomorph (Sum Isomers)	60.3	58.8	57.9	56.2	63.0	56.2	45.0	62.4
Dimethomorph [%MET][2]	100.0	97.5	96.0	93.2	100.0	89.2	71.4	99.0

1 Sum of respective isomer of all quantitative analyses
2 Extraction efficiency of parent compound compared to extraction method used in the metabolism study (in %)

2. Identification and characterization of extractable residues in grapes

The peak assignment was based on HPLC-MS investigations from extracts of both labels and matrices. Additionally, co-chromatography experiments and retention time comparison of the components with the ^{14}C-signals of the HPLC analyses were used.
In both labels (morpholine and chlorophenyl) the *E/Z*-isomers of the parent compound dimethomorph were identified by accurate mass measurement and an isotope pattern typical for the respective label. In the mopholine label the *E/Z*-isomers of M550F002, up to three isomers of the metabolite M550F017 and up to two isomers of the metabolite M550F007 and M550F018 were identified by accurate mass calculation and MS/MS fragment analysis. Additionally, a mass of 403 u was characterized by MS/MS analysis together with M550F018 and was therefore assigned along with this metabolite. For samples of the chlorophenyl label extracted ion chromatograms (EIC) were used for the metabolite identification. Thereby, the two isomers of M550F002, up to four isomers of M550F017, up to two isomers of M550F006 and M550F007 as well as M550F018 in combination with M = 403 u and the parent compound (*E/Z*-isomers) were identified. Since the MS peak of M550F006 did not correspond to a radio-peak in the HPLC patterns, it was not reported as an identified metabolite in the summary tables as it could not be quantified. For the label specific metabolite morpholine (M550F021), the peak was assigned on the basis of co-chromatography experiments and comparison of retention times with the radiolabelled reference item.

In grapes and leaves, the (*E*)- and (*Z*)-isomers of dimethomorph were the main components identified for both labels at 28 DALA and for the chlorophenyl label likewise at 14 DALA with concentrations between 19.6 and 31.4% TRR for the (*E*)-isomer and between 30.9 and 38.8% TRR for the (*Z*)-isomer. Metabolite M550F007, resulting from demethylation of the parent compound, was detected partially in combination with M=403 and M550F018 (eluting in one peak for the chlorophenyl label) in small amounts in grapes of the morpholine and chlorophenyl label (1.5 and 2.4% TRR, respectively). Hydroxylation of the morpholine ring results in the formation of the metabolite M550F018, which was observed in grapes and leaves harvested 14 DALA (3.3% TRR and 1.0% TRR) and in grapes and leaves at 28 DALA of the chlorophenyl label (4.8% TRR and 2.3% TRR) as well as in leaves of the morpholine label (1.8% TRR). These levels represent the sum of both isomers. Isomers of the metabolite M550F002, the corresponding glycosylated product of M550F007, were detected in both matrices for both labels in concentrations of up to 13.6% TRR for the sum of both isomers. Isomers of the metabolite M550F017, which resulted from an oxidative ring opening of the morpholine ring, were found in leaves of the morpholine label and in grapes of the chlorophenyl label at low concentrations (up to 3.5% TRR for a specific isomer). For the morpholine label, up to 4.6 % TRR were identified as M550F021 (morpholine), which results from cleavage of the parent molecule and was therefore label-specific.

HPLC-characterized radioactivity consisted of up to 4 peaks (below or equal to 0.011 mg/kg and 1.9% TRR) in grapes of the morpholine label and of up to 2 peaks (below or equal to 0.019 mg/kg and 2.9% TRR) in grapes of the chlorophenyl label (28 DALA). In leaves, HPLC-characterized radioactivity amounted to a maximum of 3 peaks (below or equal to 0.085 mg/kg and 1.8% TRR) or 2 peaks (below or equal to 0.172 mg/kg, 2.2% TRR) for the morpholine or chlorophenyl label, respectively.

Table 6.2.1-9: Residues of [p-chlorophenyl-^{14}C]-dimethomorph in grapes

Designation Metabolite Code	Grapevine Matrix and Harvesting Timepoint							
	Grapes 14 DALA		Leaves 14 DALA		Grapes 28 DALA		Leaves 28 DALA	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
M550F021 (Morpholine)	-	-	-	-	-	-	-	-
M550F002 - 31.6 min	-	-	0.023	1.7	0.013	2.0	0.116	1.5
M550F002 - 34.9 min	0.026	2.9	0.052	3.9	0.036	5.4	0.153	2.0
M550F017 - 33.3 min	-	-	-	-	-	-	-	-
M550F017 - 35.9 min	-	-	-	-	-	-	-	-
M550F017 - 38.6 min	-	-	-	-	0.021	3.3	-	-
M = 403 + M550F018 - 44.3 min	0.008	0.9	0.013	1.0	0.022	3.3	0.120	1.6
M550F007 / M = 403 + M550F018 - 45.5 min	0.021	2.4	-	-	0.010	1.5	0.056	0.7
(*E*)-Dimethomorph	0.274	30.9	0.423	31.4	0.128	19.6	2.119	27.5
(*Z*)-Dimethomorph	0.345	38.8	0.430	31.9	0.250	38.1	2.386	30.9

Table 6.2.1-10: Residues of [morpholine-2,3-^{14}C]-dimethomorph in grapes

Designation Metabolite Code	Grapevine Matrix and Harvesting Timepoint			
	Grapes 28 DALA		Leaves 28 DALA	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
M550F021 (Morpholine)	0.012	2.0	0.215	4.6
M550F002 - 31.6 min	0.027	4.4	-	-
M550F002 - 34.9 min	0.056	9.2	0.109	2.3
M550F017 - 33.3 min	-	-	0.090	1.9
M550F017 - 35.9 min	-	-	0.162	3.5
M550F017 - 38.6 min	-	-	0.062	1.3
M = 403 + M550F018 - 44.3 min	-	-	0.064	1.4
M = 403 + M550F018 - 45.5 min	-	-	0.020	0.4
M550F007	0.009	1.5	-	-
(*E*)-Dimethomorph	0.138	22.9	1.454	31.1
(*Z*)-Dimethomorph	0.226	37.4	1.496	31.9

E/Z isomer ratio of parent dimethomorph

While the ratio of the (*E*)- and (*Z*)-isomer of dimethomorph in leaves was approximately 1:1 for both labels, the relative amount of the (*E*)-isomer in grapes was lower in comparison to the (*Z*)-isomer. In grapes, the E/Z isomer ratio was 38 : 62 (morpholine label, 28 DALA), 44 : 56 (chlorophenyl label, 14 DALA), and 34 : 66 (chlorophenyl label, 28 DALA).

3. Characterization of non-extractable residues

The residual radioactive residues after solvent extraction (RRR) were between 11.6 and 16.2 % of the TRR for grapes and 10.2 and 17.4 % of the TRR for leaves. The residues after solvent extraction were further solubilized using a combination of sequential solubilization steps, whereby 1.5 to 5.0 % and 1.7 to 4.2 % TRR were additionally released of the chlorophenyl and morpholine label, respectively (see Table 6.2.1-11 and Table 6.2.1-12).

Table 6.2.1-11: Characterization of the residual radioactive residues (RRR) after solvent extraction (chlorophenyl label)

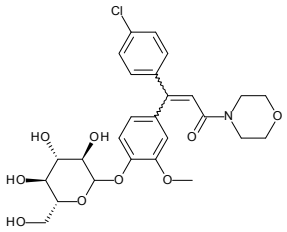
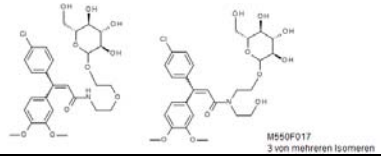
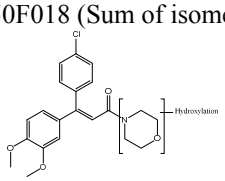
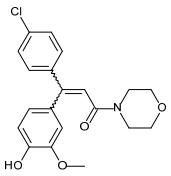
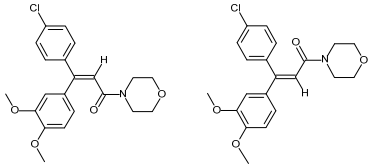
Fraction / Supernatant	Grapes 14 DALA		Leaves 14 DALA		Grapes 28 DALA		Leaves 28 DALA	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
RRR	0.104	11.7	0.235	17.4	0.106	16.2	1.250	16.2
Ammonia Solubilizate	0.009	1.0	0.032	2.3	0.008	1.3	0.165	2.1
Macerozyme / Cellulase Solubilizate	0.001	0.1	0.003	0.3	0.001	0.1	0.078	1.0
α-Amylase / β-Amylase / Amyloglucosidase Solubilizate	0.001	0.1	0.003	0.2	0.001	0.2	0.038	0.5
Tyrosinase / Laccase Solubilizate	0.001	0.1	0.003	0.2	0.005	0.8	0.085	1.1
Glucosidase / Hesperidinase Solubilizate	0.001	0.1	0.002	0.2	0.001	0.2	0.024	0.3
Sum of Solubilized Radioactive Residues	0.013	1.5	0.044	3.2	0.017	2.6	0.390	5.0
Final Residue	0.086	9.7	0.160	11.9	0.085	13.0	0.839	10.9
Sum of Solubilized Radioactive Residues + Final Residue	0.099	11.2	0.204	15.1	0.102	15.6	1.228	15.9

Table 6.2.1-12: Characterization of the residual radioactive residues (RRR) after solvent extraction (morpholine label)

Fraction / Supernatant	Grapes 28 DALA		Leaves 28 DALA	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
RRR	0.070	11.6	0.480	10.2
Ammonia Solubilizate	0.006	1.0	0.146	3.1
Macerozyme / Cellulase Solubilizate	0.002	0.3	0.018	0.4
α-Amylase / β-Amylase / Amyloglucosidase Solubilizate	0.001	0.1	0.015	0.3
Tyrosinase / Laccase Solubilizate	0.001	0.1	0.010	0.2
Glucosidase / Hesperidinase Solubilizate	0.001	0.1	0.009	0.2
Sum of Solubilized Radioactive Residues	0.010	1.7	0.198	4.2
Final Residue	0.067	11.1	0.255	5.4
Sum of Solubilized Radioactive Residues + Final Residue	0.077	12.8	0.453	9.7

The following table provides a summary of identified metabolites in grape matrices.

Table 6.2.1-13: Summary of identified metabolites in grape matrices at 28 days harvest

Designation Metabolite code	Grape matrix and harvesting time							
	Grapes 28 DALA		Leaves 28 DALA		Grapes 28 DALA		Leaves 28 DALA	
	Chlorophenyl label				Morpholine label			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
M550F021 (Morpholine) 	-	-	-	-	0.012	2.0	0.215	4.6
M550F002 (sum of isomers) 	0.049	7.4	0.269	3.5	0.083	13.2	0.109	2.3
M550F017 (sum of isomers) 	0.021	3.3	-	-	-	-	0.314	6.7
M550F018 (Sum of isomers)* 	0.032	4.8	0.176	2.3	-	-	0.084	1.8
M403*					-	-		
M550F007 * 	0.010	1.5	0.056	0.7	0.009	1.5	-	-
Dimethomorph (sum of isomers) 	0.378	57.7	4.505	58.4	0.364	60.3	2.95	63.0

*for the chlorophenyl label, M550F007 was coeluting with M550F018 and M403; in both labels M550F018 and M403 were coeluting; as a worst case each metabolite was assigned the full peak quantity

4. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph (BAS 550 F) in grapes is shown in Figure 6.2.1-1. The main peaks detected in this study correspond to the two isomers of the parent compound dimethomorph. The metabolic pathway in grapes is characterized by three initial metabolic steps followed by susequent Phase II reactions (conjugation):
Metabolite M550F007 results from demethylation of the parent compound. Subsequently, the metabolite M550F002 is formed by a glycosylation step from M550F007. Furthermore, hydroxylation of the morpholine ring of the parent compound leads to the formation of different isomers of metabolite M550F018. Metabolite M550F017 results from an oxidative ring-opening reaction of the morpholine ring of dimethomorph (either at the nitrogen or oxygen leading to various constitutional isomers) followed by glycosylation. Furthermore, cleavage of the parent molecule was observed to a low extend, leading to the generation of morpholine (M550F021).

5. Storage stability

In all cases, the chromatograms of re-analyzed extracts and of re-extracted matrices showed similar peak patterns as the initial analyses. For the morpholine label, the storage stability was confirmed in the homogenized matrices over a storage interval of at least 361 and 371 days (for grapes and leaves, respectively). In methanol extracts of the same label, the storage stability was shown for a storage interval of at least 882 days for grapes and 273 days for leaves. Similarly, the storage stability was also confirmed for the investigated homogenized matrices of the chlorophenyl label (28 DALA, 115 days for grapes and 125 days for leaves). The storage stability of the corresponding methanol extracts was confirmed for a period of at least 109 days for grapes and 114 days for leaves. Though the initial analysis was performed comparatively late in case of the morpholine label, the highly similar metabolite pattern between both labels strongly suggest the stability of metabolites.
It can be concluded, that the storage stability investigations performed for both matrices (grapes and leavesshowed that metabolites were stable during the time of investigation.

III. CONCLUSION

The concentrations of radioactive residues in grapes at 28 DALA were 0.605 mg/kg and 0.654 mg/kg for the morpholine and chlorophenyl label, respectively. The TRR in grapes at 14 DALA was slightly higher and accounted for 0.889 mg/kg (chlorophenyl label). In leaves, the TRR was significantly higher with 4.684 mg/kg and 7.720 mg/kg at 28 DALA for the morpholine and the chlorophenyl label, respectively. At 14 DALA, the TRR in leaves was lower with 1.348 mg/kg (chlorophenyl label), probably due to a higher water content of the matrix.

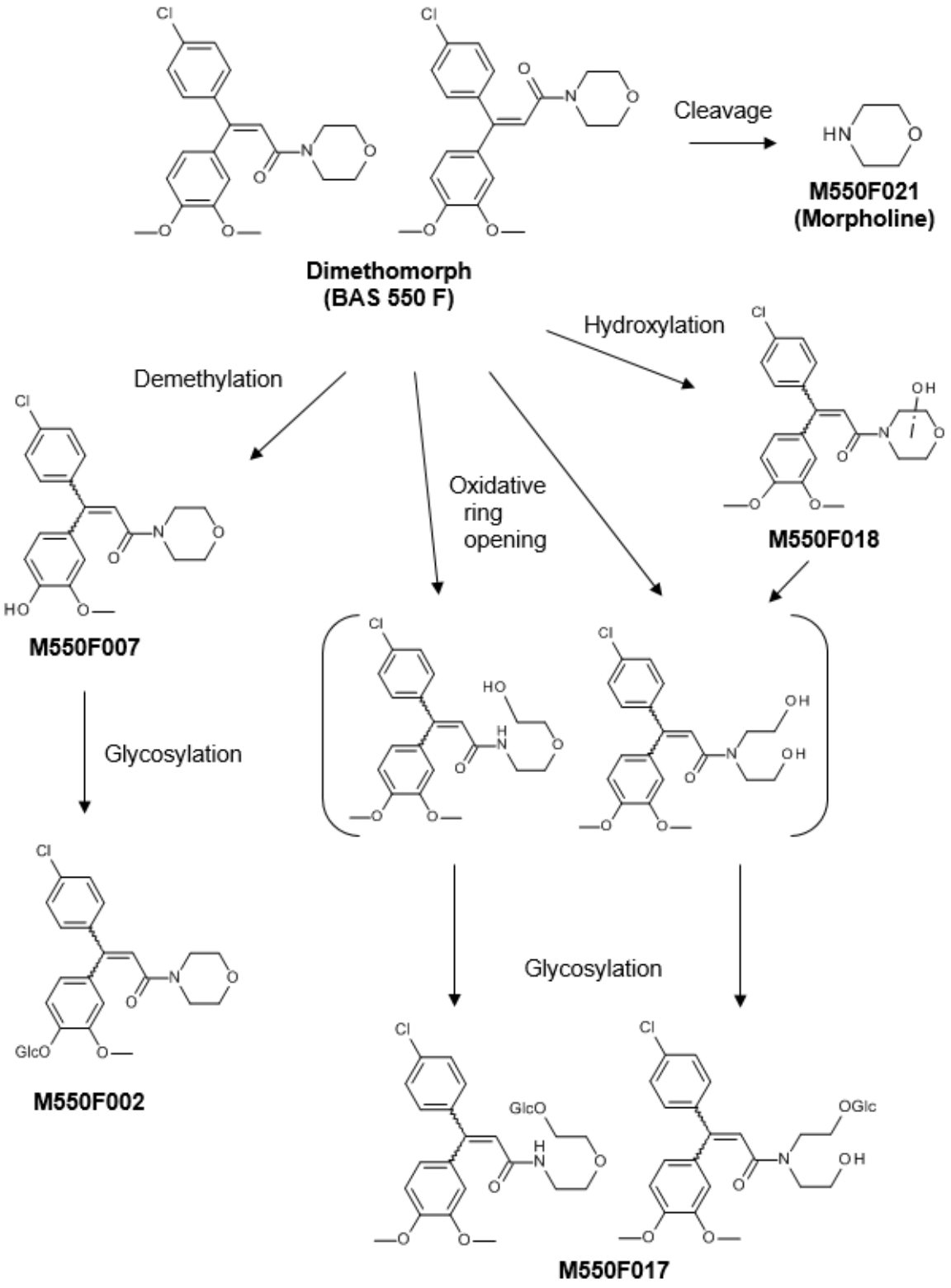
Most of the radioactive residues were extracted with methanol for both matrices and labels (78.7 to 87.8% TRR) and only minor amounts of residues were extracted with water (up to 6.1% TRR). The residual radioactive residues after solvent extraction (RRR) were between 11.6 and 16.2% TRR for grapes and 10.2 and 17.4 % TRR for leaves. Small amounts were solubilized using a combination of sequential solubilization steps (1.5 to 5.0% TRR) were additionally released.

In grapes and leaves, dimethomorph (*E*- and *Z*-isomers) was the main component identified for both labels. Individual isomer concentrations ranged from 19.6 to 31.4% TRR for the (*E*)-isomer and from 30.9 to 38.8% TRR for the (*Z*)-isomer, totaling 58 to 70 % TRR in leaves and grapes over all harvest times. The ratio of the (*E*)- and (*Z*)-isomer of dimethomorph in leaves was approximately 1:1 for both labels, while the *E/Z* isomer ratio in grapes was 38 : 62 (morpholine label, 28 DALA), 44 : 56 (chlorophenyl label, 14 DALA), and 34 : 66 (chlorophenyl label, 28 DALA).

Besides parent compound the most predominant metabolite was M550F002, resulting from demethylation of the *p*-methoxy group and subsequent glucosylation. This was the only metabolite occurring in amounts >10% TRR. Its respective aglycon, M550F007, was detected partially in combination with M=403 and M550F018 (eluting in one peak for the chlorophenyl label) in small amounts in grapes of the morpholine and chlorophenyl label (1.5 and 2.4% TRR, respectively).
Further observed metabolites were M550F018 (hydroxylation of the morpholine ring), M550F017 (ring opening of morpholine ring and glucosylation) and metabolite M550F021 (morpholine). All these metabolites occurred only in subordinate amounts (<5% TRR). M550F021 was the only label-specific metabolite, all other metabolites were observed in both labels.

It can be concluded that metabolism of dimethomorph in grapes is not extensive. The major compound in quantitatve terms is the unchanged parent compound. The most important metabolic step is the demethylation of the dimethoxy ring and subsequent conjugation with glucose. Further observed metabolic steps were the hydroxylation and opening of the morpholine ring and to a low extend cleavage and release of the intact morpholine moiety.
These metabolic steps were already observed in the previous metabolism study evaluated in the last EU peer review. Although the metabolite M550F003 (Z7), which was detected in trace amounts in grape leaves of the previous study, was not found in this new study (despite specifically searching for it via MS), the overall metabolic pathway was found to be the same for both the already peer reviewed and the new study and thus the previous knowledge of the metabolism of dimethomorph in grapes is confirmed.

Figure 6.2.1-1: Proposed metabolic pathway of dimethomorph in grapes



Putative intermediates not found
Glc: Glucose

Lettuce

Goodyear A., 1995: Dimethomorph (Chlorophenyl Ring-^{14}C): Metabolism in Field Grown Lettuce (DocID DK-640-021)

Lettuce seeds were grown in soil containing plastic trays in a glasshouse The metabolism of chlorophenyl ring labelled ^{14}C-dimethomorph was investigated in lettuce plants following four successive foliar applications of dimethomorph in a DC (dispersed concentrate) formulation equivalent to a mean treatment rate of 1.14 kg a.s./ha. Applications were made 30 days after sowing of lettuce seed and then again at intervals of 9, 10, and 11 days. Mature lettuce was sampled 4 days after the last application. Samples were subsequently extracted with acetone and acetone/water (50:50). The extractable radioactivity was characterized and quantified by radio HPLC analysis. Metabolite identification was performed by comparison with reference substances and/or LC/MS.

Findings

The total radioactivity (TRR) in lettuce sampled four days after the final application was 102 mg/kg. 96.7 mg/kg (94.8% TRR) was extracted with acetone, followed by another 3.7 mg/kg (3.6% TRR) by acetone:water (50:50 v/v). The non-extractable residue accounted for 1.6 mg/kg (1.57% TRR) and was not further investigated. The majority of the extractable residue in lettuce acetone extract (91.5%; 93.3 mg/kg) was identified as unchanged parent, dimethomorph. The E/Z isomer ratio was approximately 57:43. Further metabolites in the concentrated acetone extract were identified by MS as M550F003 (synonyms CL 336305 or Z7), accounting for 0.5% TRR (0.5 mg/kg) and M550F006 (synonyms CL 900987 or Z67), accounting for 0.5% TRR (0.5 mg/kg). The presence of M550F007 (synonyms CL 900986 or Z69) was also confirmed since 4 peaks were seen in the HPLC-MS trace analyzing for M550F006. In the previous dossier and EU peer review the presence of M550F004 (synonym Z37) was stated. However, this was a mistake due to the fact that the synthesized reference standard for M550F004 had the same retention time as metabolite M550F006 (Z67). Via LC-MS analysis the presence of M550F006 could be confirmed, but not the presence of M550F004 (as described in the report on pp. 37-39).

The remainder of the extractable residue was composed of several minor unknown polar components (1.5 mg/kg, 1.5% TRR) which were not further identified. This polar fraction (i.e. radioactivity remaining at the origin of the TLC or unretained on HPLC) was isolated and subjected to acidic, basic and enzymatic treatments, followed by HPLC analysis. The acidic treatment resulted in the biggest portion of radioactivity being retained. Analysis of the retained peaks showed similar retention times to M550F006, suggesting that the polar fraction, at least partly, contained conjugates of M550F006 (Z67).

Table 6.2.1-16: Summary of residues found after treatment of lettuce with ^{14}C-dimethomorph (chlorophenyl label)

Analyte	Residue mg/kg (%TRR)		
Dimethomorph	*E*-isomer	*Z*-isomer	Sum *E*+*Z*
	52.9 (51.9%)	40.4 (39.6%)	93.3 (91.5%)
M550F006 (Z67)/ M550F007 (Z69)	0.5 (0.5%)		
M550F003 (Z7)	0.5 (0.5%)		

~~Conclusions~~

~~The relevant residue of ^{14}C-dimethomorph in lettuce consisted primarily of the unchanged parent compound. Other metabolites identified were M550F003 (Z7) and M550F006/M550F007 (Z67/Z69) and were clearly below 5% TRR and thus of minor importance. Analysis of the polar radioactivity following acid treatment showed evidence of small amounts of M550F006 (Z67) and/or M550F007 (Z69) indicating that the primary desmethyl metabolites of dimethomorph are subsequently metabolized via conjugation.~~

Report:	CA 6.2.1/4 Goodyear A., 1995 a Dimethomorph (Chlorophenyl ring - 14C): Metabolism in field grown lettuce - Amended final report DK-640-021
Guidelines:	EEC 91/414, EPA 171-4
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

Description:	^{14}C-dimethomorph (chlorophenyl label; E:Z; 52:48) BAS 550 F (unlabeled)
Lot/batch #:	S1050/1 (labeled) 1513 (unlabel)
Purity:	99.7% (unlabeled) Radiochemical purity: >98.0% (labeled) Specific activity: 23.6 µCi/mg (0.873 MBq/mg, labeled)
Application mixture	0.619 - 0.661 µCi/mg
CAS#:	110488-70-5
Stability of test compound:	The test item was stable over the test period.

2. Test commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Little gem
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Whole plant without roots, roots
Sample size:	Not relevant

B. STUDY DESIGN

The study was carried out at the test facility Hazleton Europe (HE, North Yorkshire, England, name changed to Corning Hazleton Europe, CHE, between issue of the draft and final report).

1. Test procedure

The metabolism of ^{14}C-dimethomorph has been investigated in field grown lettuce plants following four successive foliar applications (DC formulation) equivalent to a mean treatment rate of 1.14 kg a.s./ha. Applications were made 30 days after sowing of lettuce seed and again at intervals of a further 9, 10 and 11 days. Whole plant samples without roots were taken for analysis two hours after the first application and 4 days after the final application.

Additionally collected root and soil samples were not analysed.

Plant material was stored frozen at -20°C until analysis started and during the whole period of the study.

2. Description of analytical procedures

The lettuce shoots (2 hours after application) were homogenized by maceration in the presence of chloroform. The mature lettuce sample (4 days after the final application) was homogenized by maceration in the presence of acetone. The amount remaining unextracted in each sample was determined by combustion analysis. The residue levels in the crop samples were calculated from the fresh weight of plant material and the total amount of radioactivity present.

Extraction (ERR): Homogenized subsamples of immature lettuce (harvested 2 hours after the first application) were extracted three times with chloroform. Homogenized subsamples of mature lettuce were extracted three times with acetone, followed by three times extraction with acetone/water (50:50, v/v). The extracts were concentrated using a rotary evaporator. During concentration, the sample was divided into two phases, which were labeled 'organic' and 'aqueous'. Liquid samples were directly mixed with a scintillation fluid and the radioactivity was determined by LSC.

Treatment of residual radioactive residues (RRR) after solvent extraction: For the measurement of solid residues following solvent extraction (RRR), the residues were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC).

3. Identification of metabolites

All extracts of immature and mature lettuce were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for quantification of the residues. Co-chromatography was performed with unlabeled dimethomorph using HPLC.
To confirm the identity of dimethomorph and the metabolites M550F003 (synonyms CL 336305 or Z7), M550F004 (synonym Z37) and M550F006 (synonyms CL 900987 or Z67) in the lettuce acetone extract, aliquots of the respective reference standards and sample were analysed by GC/MS.

Peak assignment was based on GC-MS and LC-MS with the parent reference standard (*E*- and *Z*-isomers) or metabolite standards of M550F003 (Z7), M550F004 (Z37), M550F006 (Z67) and M550F009 (Z93). The parent compound was confirmed by GC-MS and LC-MS. Metabolites M550F003 (Z7) and M550F006 (Z67) were confirmed by LC-MS.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) was calculated by summing up the extracted radioactivity and the remaining radioactivity in the residue after solvent extraction. The calculated TRR of immature lettuce was 348.4 mg/kg (extraction with chloroform) and the calculated TRR of mature lettuce was 102.0 mg/kg (extraction with acetone and acetone/water, 50:50, v/v).

Table 6.2.1-14: Total radioactive residues (TRR) in lettuce following foliar treatments

TRRs in treated lettuce samples		
Matrix	**Time after application**	**TRR calculated [1] [mg/kg]**
Whole plant without roots (immature)	2 hours after first application	348.4
Whole plant without roots (mature)	4 days after last application	102.0

1 TRR was calculated as the sum of ERR + RRR

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in lettuce

The extractabilities of the lettuce matrices either with chloroform (immature lettuce) or with acetone and acetone/water (50:50, v/v, mature lettuce) are summarized in Table 6.2.1-15.
For immature lettuce, the majority of the residue (97.4% TRR; 339.3 mg/kg) was extractable into chloroform with maceration, the remaining solid residue comprising 2.6% TRR and 9.1 mg/kg.

For mature lettuce, the majority of the residue (94.9% TRR; 96.7 mg/kg) was extractable into acetone with maceration, with a further 3.6% (3.7 mg/kg) extractable into acetone : water (1:1, v/v). The residue after solvent extraction (RRR) accounted for 1.6% TRR; 1.6 mg/kg. During concentration of the acetone extract the sample was divided into two phases, these were labeled 'organic' and 'aqueous' and contained 39.1% TRR (39.9 mg/kg) and 55.8% TRR (56.9 mg/kg) of the total residue respectively (see Table 6.2.1-16).

Table 6.2.1-15: Extractability of radioactive residues in lettuce samples

Matrix	Chloroform extract		Acetone extract		Acetone/water extract		RRR [1]		TRR calculated [2]
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[mg/kg]
Chlorophenyl label									
Whole plant without roots (immature)	97.4	339.3	N.A.	N.A.	N.A.	N.A.	2.6	9.1	348.4
Whole plant without roots (mature)	N.A.	N.A.	94.9	96.7	3.6	3.7	1.6	1.6	102.0

1 RRR = Residual Radioactive Residue (after solvent extraction)
2 TRR was calculated as the sum of ERR + RRR
N.A. Not applicable

2. Extraction efficiency

For the high water matrix lettuce, extractions using either chloroform or acetone and subsequently acetone/water (50:50, v/v) resulted in comparable amounts of extracted radioactive residues (see Table 6.2.1-15). With chloroform, 97.4 % of the TRR were extracted. Using acetone and subsequently acetone/water (50:50, v/v) in sum 98.5 % of the TRR were extracted.

3. Identification and characterization of extractable residues in lettuce

The results of the identification and characterization of extractable residues in lettuce are shown in Table 6.2.1-16.

For immature lettuce, which was harvested two hours after test item application, characterization of the radioactivity extractable into chloroform from the lettuce plants using TLC and HPLC methods showed only unchanged ^{14}C-dimethomorph to be present.

Similar characterization of the mature lettuce plant extracts showed that greater than 93% TRR of the radioactivity present was unchanged ^{14}C-dimethomorph in addition to small amounts of polar compounds and unidentified regions of radioactivity. The polar compounds were detected as origin material on TLC and unretained material on HPLC with maximum levels of 3.0 mg/kg (3.1% TRR) and 2.3 mg/kg (2.4% TRR) obtained respectively.
The unidentified regions of radioactivity were observed by TLC only, with a total of between 0.68 mg/kg (0.7% TRR) and 0.87 mg/kg (0.9% TRR) present. Reference to the chromatography of authentic metabolite standards indicated the unidentified radioactive material may correspond to the metabolites M550F003 (Z7) and either M550F004 (Z37) or M550F006 (Z67).

Subsequent re-analysis of the mature lettuce acetone extract by LC-MS confirmed that the unidentified radioactive material corresponded to the metabolites M550F003 (Z7) and M550F006 (Z67). The data obtained from LC-MS also showed that metabolite M550F007 (synonyms CL 900986 or Z69, a positional isomer of M550F006) may be present in the sample. The metabolites M550F003 (Z7) and M550F006 (Z67) (and/or M550F007, Z69) were each present at levels of 0.5 mg/kg (0.5% of the total radioactive residue present).

Polar radioactivity unretained on HPLC was isolated and subjected to acidic, basic and enzymatic treatment, followed by analysis using HPLC. The basic and enzymatic treated samples showed little change to the original chromatographic profile with the majority of the radioactivity still present as polar unretained material. However, each region accounted for less than 10% TRR. The acid treated sample produced a greater proportion of radioactivity retained under the HPLC conditions used, with two main regions observed. These regions possessed similar chromatographic properties to the isomers of the metabolite M550F006 (Z67) and/or M550F007 (Z69), however insufficient radioactivity was present to permit further structural identification.

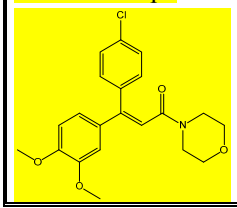
Table 6.2.1-16: Summary of identified and characterized residue of dimethomorph in lettuce using HPLC

Sample	Immature lettuce		Mature lettuce							
	Chloroform extract		Acetone organic phase		Acetone aqueous phase		Acetone:water		TRR calculated [1]	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Residue in extract	339.3	97.4	39.9	39.1	56.9	55.8	3.7	3.6	100.5	98.5
Dimethomorph (*E*-isomer)	151.6	43.5	22.3	21.9	31.6	31.0	1.9	1.9	55.8	54.8
Dimethomorph (*Z*-isomer)	186.7	53.6	16.9	16.6	22.6	22.2	1.5	1.5	41.0	40.3
Unretained	-	-	-	-	2.4	2.4	0.2	0.2	2.6	2.6
Background	0.7	0.2	0.6	0.6	0.4	0.4	-	-	1.0	1.0

1 TRR was calculated as the sum of ERR + RRR

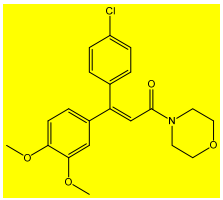
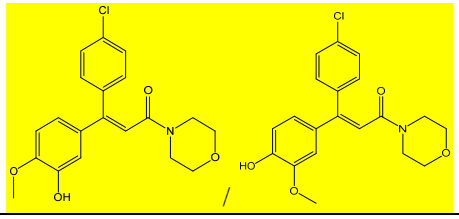
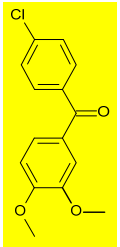
The following table provides a summary of identified metabolites in immature and mature lettuce.

Table 6.2.1-17: Summary of residues found in immature and mature lettuce after treatment with ^{14}C-dimethomorph

Analyte	Residue mg/kg (%TRR)					
	Immature lettuce			Mature lettuce		
Dimethomorph 	*E*-isomer	*Z*-isomer	Sum *E*+*Z*	*E*-isomer	*Z*-isomer	Sum *E*+*Z*
	151.6 (43.5%)	186.7 (53.6%)	338.3 (97.1%)	55.8 (54.8%)	41.0 (40.3%)	96.8 (95.1%)

The following table provides a summary of identified metabolites the concentrated acetone extract in mature lettuce.

Table 6.2.1-18: Summary of residues found in mature lettuce concentrated acetone extract after treatment with ^{14}C-dimethomorph

Analyte		Residue mg/kg (%TRR)
BAS 550 F (*E*)		52.9 (51.9%)
BAS 550 F (*Z*)		40.4 (39.6%)
Sum E +Z		93.3 (91.5%)
M550F006 (Z67) / M550F007 (Z69)		0.5 (0.5%)
M550F003 (Z7)		0.5 (0.5%)

4. Determination of isomer ratio of dimethomorph in lettuce

The isomer ratio for the dimethomorph detected by HPLC in the mature lettuce samples showed an increased amount of the *E*-isomer present (*E*:*Z*, 55:40) compared to the sample taken 2 hours after the initial test article application (*E*:*Z*, 44:54). This change may have been the result of the instability of the *Z*-isomer when exposed to light.

5. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph (BAS 550 F) in lettuce is shown in Figure 6.2.1-2: . The metabolism of dimethomorph is not extensive as the majority (greater than 93 mg/kg; 91% TRR) of the radioactive residue in the mature lettuce plants was shown by TLC and HPLC to be unchanged ^{14}C-dimethomorph. In addition to dimethomorph two unidentified radioactive regions were detected using TLC, these accounting for a maximum of 0.9% of the total residue, which were identified as metabolites M550F003 (Z7) and M550F006 (Z67).

Radioactivity chromatographing at the origin on TLC and unretained on HPLC was also observed, this accounted for a maximum of 3.1% TRR of the radioactive residue and was thought to be polar metabolites. Analysis of this polar radioactivity following acidic and basic treatment showed evidence for small amounts of M550F006 (Z67) and M550F003 (Z7) being released. This result would indicate that primary metabolites of dimethomorph are subsequently further metabolised via conjugation mechanisms.

6. Storage Stability

The study was initiated on 10 August 1993 and completed on 7 August 1995. The practical phase of the study was started on 15 September 1993 and completed on 19 January 1995 and consequently exceeds 6 months of storage interval. Information on the storage stability in lettuce is sufficiently addressed within the AIR 3 Dossier in the more recent study with the morpholine label [see KCA 6.2.1/5 2015/1000601].

III. CONCLUSION

The total radioactivity (TRR) in lettuce sampled four days after the final application was 102 mg/kg. 96.7 mg/kg (94.8% TRR) was extracted with acetone, followed by another 3.7 mg/kg (3.6% TRR) by acetone:water (50:50 v/v). The non-extractable residue accounted for 1.6 mg/kg (1.57% TRR) and was not further investigated. The majority of the extractable residue in lettuce acetone extract (91.5%; 93.3 mg/kg) was identified as unchanged parent, dimethomorph. The E/Z isomer ratio was approximately 57:43. Further metabolites in the concentrated acetone extract were identified by MS as M550F003 (synonyms CL 336305 or Z7), accounting for 0.5% TRR (0.5 mg/kg) and M550F006 (synonyms CL 900987 or Z67), accounting for 0.5% TRR (0.5 mg/kg). The presence of M550F007 (synonyms CL 900986 or Z69) was also confirmed since 4 peaks were seen in the HPLC-MS trace analyzing for M550F006. In the previous dossier and EU peer review the presence of M550F004 (synonym Z37) was stated. However, this was a mistake due to the fact that the synthesized reference standard for M550F004 had the same retention time as metabolite M550F006 (Z67). Via LC-MS analysis the presence of M550F006 could be confirmed, but not the presence of M550F004 (as described in the report on pp. 37-39).

The remainder of the extractable residue was composed of several minor unknown polar components (1.5 mg/kg, 1.5% TRR) which were not further identified. This polar fraction (i.e. radioactivity remaining at the origin of the TLC or unretained on HPLC) was isolated and subjected to acidic, basic and enzymatic treatments, followed by HPLC analysis. The acidic treatment resulted in the biggest portion of radioactivity being retained. Analysis of the retained peaks showed similar retention times to M550F006, suggesting that the polar fraction, at least partly, contained conjugates of M550F006 (Z67).

The relevant residue of ^{14}C-dimethomorph in lettuce consisted primarily of the unchanged parent compound. Other metabolites identified were M550F003 (Z7) and M550F006/M550F007 (Z67/Z69) and were clearly below 5% TRR and thus of minor importance. Analysis of the polar radioactivity following acid treatment showed evidence of small amounts of M550F006 (Z67) and/or M550F007 (Z69) indicating that the primary desmethyl metabolites of dimethomorph are subsequently metabolized via conjugation.

Report:	CA 6.2.1/5 Lewis C.J.,Cooper T., 2015 a [Morpholine-2,3-14C]-BAS 550 F: Metabolism in lettuce 2015/1000601
Guidelines:	UK Health and Safety Good Laboratory Practice Regulations 1999 (No. 3106), OECD 501 - Metabolism in crops (adopted January 8 2007)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

2. Test Material:

Description:	[Morpholine-2,3-^{14}C]-BAS 550 F (morpholine label) Unlabeled BAS 550 F Formulation BAS 550 AB F (=blank formulation of BAS 550 02 F; DC formulation)
Lot/Batch #:	858-0201 (morpholine label) AC9978-68A (without label)
Purity:	97.6% (unlabeled) Radiochemical purity: 99.0% (morpholine label) Specific activity: 5.57 MBq/mg (morpholine label)
CAS#:	110488-70-5
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Not reported
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Immature and mature lettuce
Sample size:	Not relevant

B. STUDY DESIGN

The in-life phase of the study was carried out at the test site, AgroChemex Ltd. The analytical phases of the study were carried out at the test facility, Smithers Viscient (ESG).

1. Test procedure

The lettuces were foliar treated three times with a mixture of ^{14}C-labeled (morpholine ring label) and unlabeled dimethomorph at a nominal rate of 225 g a.s./ha per application. The first application was carried out 7 days before immature harvest and 21 days before mature harvest, the second application was immediately after immature harvest and 14 days before mature harvest, and the last application was 7 days before mature harvest. Immature lettuces were harvested at BBCH 45 (7 days after the first application), immediately before the second application. Mature lettuces and control lettuces were harvested at BBCH 49 (7 days after the last application). Plant material (lettuce leaf) was stored frozen until analysis started and during the whole period of the study. Extracts were stored in a freezer.

2. Description of analytical procedures

Radioanalysis (TRR): For the determination of the TRR, and the measurement of solid residues following solvent extraction (RRR), homogenized subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). Liquid samples were directly mixed with a scintillation fluid and the radioactivity was determined by LSC.

Extraction (ERR): Homogenized subsamples of lettuce were extracted three times with methanol, three times with water and once with acetone. The remaining plant residue was dried, ground, and aliquots were analyzed by LSC, following combustion.

Extraction efficiency:

In addition to the methanol, water and acetone extractions used in the metabolism investigations, samples were extracted with acetone/water (2:1, v/v) (Residue Method A; DFG S19), and with methanol/water/2M HCl (70:25:5, v/v/v) (Residue Method B; data generation method 575/0). The aim was to show that dimethomorph (E and Z isomers) as the only main residue in lettuce was efficiently extracted with the solvents used in these analytical residue methods.

3. Identification of metabolites

All extracts of immature and mature lettuce were analyzed by high performance liquid chromatography (HPLC) for quantification of the residues. Peak assignment was based on co-chromatography with the parent reference standard or radiolabeled morpholine reference standard. The parent compound was confirmed by mass spectrometry.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) was calculated by summing the extracted radioactivity and the remaining radioactivity in the residue after solvent extraction. The calculated TRR of immature lettuce was 5.92 mg/kg and the calculated TRR of mature lettuce was 8.30 mg/kg. The combusted TRR values were 6.84 and 9.78 mg/kg, respectively. The differences between direct combusted values and calculated values are considered to be due to inaccuracies associated with direct combustion of the homogenate. Thus, the quantification of metabolite residues is based on the calculated TRR as the more reliable value.

Table 6.2.1-19: Total radioactive residues (TRR) in lettuce following foliar treatments

TRRs in treated lettuce samples			
Matrix	**DALA[1]**	**TRR combusted [mg/kg]**	**TRR calculated* [mg/kg]**
		Morpholine label	**Morpholine label**
Immature lettuce	7 DAFA[2]	6.841	5.922
Mature lettuce	7	9.776	8.304

1 Days after last application
2 Days after first application / immediately before second application
* TRR was calculated as the sum of ERR + RRR

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in lettuce

The extractabilities of the lettuce matrices with methanol, water and acetone are summarized in Table 6.2.1-23. The extractability was 98.6% TRR for immature lettuce and 97.2% TRR for mature lettuce. For both matrices the major part of the radioactivity was extracted with methanol (immature: 96.3% TRR and mature: 95.1% TRR) and only minor amounts were subsequently released with water and acetone.

Table 6.2.1-20: Extractability of radioactive residues in lettuce samples

Matrix	**Methanol extract**		**Water extract**		**Acetone extract**		**ERR[1]**		**RRR[2]**		**TRR calculated[3]**
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[mg/kg]
Morpholine label											
Immature lettuce	96.3	5.700	2.0	0.118	0.3	0.021	98.6	5.839	1.4	0.083	5.922
Mature lettuce	95.1	7.893	1.8	0.147	0.3	0.026	97.2	8.066	2.9	0.238	8.304

1 ERR = Extractable Radioactive Residue (extraction with methanol, water and acetone)
2 RRR = Residual Radioactive Residue (after solvent extraction with methanol, water and acetone)
3 TRR was calculated as the sum of ERR + RRR

Extraction efficiency

Extractability was assessed for the residue methods DFGS19 as the proposed enforcement method and also for BASF method 575/0, the main data generation method. The results are compiled in Table 6.2.1-21 and show that the extractability of BAS 550 F (sum of parent compound isomers) with the extraction methods DFG S19 and 575/0 is very similar to the extractions used in the metabolism investigations for both mature and immature harvest as they showed an efficiency of 98.3 and 102.7% and in immature lettuce and of 92.4 and 99.1% in lettuce of mature harvest.

Thus both methods proved to be suitable for reliable determination of BAS 550 F in leafy matrices.

Table 6.2.1-21: Total identified radioactive residues extracted from lettuce samples with alternative extraction solvents

	Immature			Mature		
Extraction Method	**Metabolism Investigations**	**Acetone: Water (2:1 v/v) (Multi-Method DFG S19)**	**Methanol: Water: 2M HCl (75:25:5 v/v/v) (BASF Method 575/0)**	**Metabolism Investigations**	**Acetone: Water (2:1 v/v) (Multi-Method DFG S19)**	**Methanol: Water: 2M HCl (75:25:5 v/v/v) (BASF Method 575/0)**
Metabolite	**[mg/kg]**					
BAS 550 F (*E*-isomer)	3.028	2.955	3.074	4.330	3.836	4.115
BAS 550 F (*Z*-isomer)	2.131	2.114	2.222	2.951	2.892	3.097
BAS 550 F (total)	5.159 (100%*)	5.069 (98.3%)	5.296 (102.7%)	7.281 (100%*)	6.728 (92.4%)	7.212 (99.1%)

* The percentage extraction efficiency

2. Identification and characterization of extractable residues in lettuce

In all extracts and at both harvests, the most abundant component was the parent compound dimethomorph, which accounted for a total of 87.1% TRR (immature) or 87.8% TRR (mature) (5.16 or 7.28 mg/kg, respectively), split between two isomers. The observed E/Z ratio was 59:41 in immature and mature samples, respectively.
Apart from the peaks accounting for parent compound, a polar region was observed in the extracts. This fraction was not retained on the column in the original HPLC system but was isolated and chromatographed on a more suitable HPLC system. It was shown to be comprised of several components (up to eight peaks), one of which could be identified as morpholine (M550F021) by co-chromatography with the radiolabeled reference standard. The maximum amount of morpholine present was 1.6% TRR (0.09 mg/kg) for the immature harvest and 3.0% TRR (0.25 mg/kg) for the mature harvest. Other characterized radioactivity accounted for 6.7% TRR (immature) or 3.7% TRR (mature) (0.39 or 0.30 mg/kg, respectively) and were composed of several minor metabolites, up to 7 peaks (max. 0.129 mg/kg, 2.19% TRR) in immature lettuce and up to 8 peaks (max. 0.125 mg/kg, 1.51% TRR) in mature lettuce.

Table 6.2.1-22: Summary of identified and characterized residue of [morpholine-2,3-^{14}C]-dimethomorph in lettuce

Metabolite	Matrix			
	Immature lettuce		Mature lettuce	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residue				
Identified				
(E)-Dimethomorph	3.028	51.1	4.330	52.2
(Z)-Dimethomorph	2.131	36.0	2.951	35.6
Dimethomorph (sum of isomers)	5.159	87.1	7.281	87.8
M550F021 (morpholine)[1]	0.094	1.6	0.251	3.0
Total identified	**5.253**	**88.7**	**7.532**	**90.8**
Characterized				
Total characterized from ERR (up to 7 peaks, max. 0.129 mg/kg, 2.19% TRR)	**0.390**	**6.7**	-	-
Total characterized from ERR (up to 8 peaks, max. 0.125 mg/kg, 1.51% TRR)	-	-	**0.303**	**3.7**
Total identified and characterized from ERR	**5.643**	**95.4**	**7.835**	**94.5**
Final Residue (Residual Radioactive Residue, RRR)	**0.083**	**1.4**	**0.238**	**2.9**
Grand total	**5.726**	**96.8**	**8.073**	**97.4**

3. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph (BAS 550 F) in lettuce is shown in Figure 6.2.1-2. The metabolism of dimethomorph is not extensive as the unchanged parent compound represented 87-88% of the TRR. Dimethomorph is metabolized in lettuce to small amounts of M550F021 (morpholine) and other minor metabolites. Desmethyl metabolites (M550F006/M550F007) were detected by LC-MS, however could not be quantified as they did not correspond to radiopeaks in the HPLC pattern. Thus, they are present only in trace amounts.

4. Storage stability

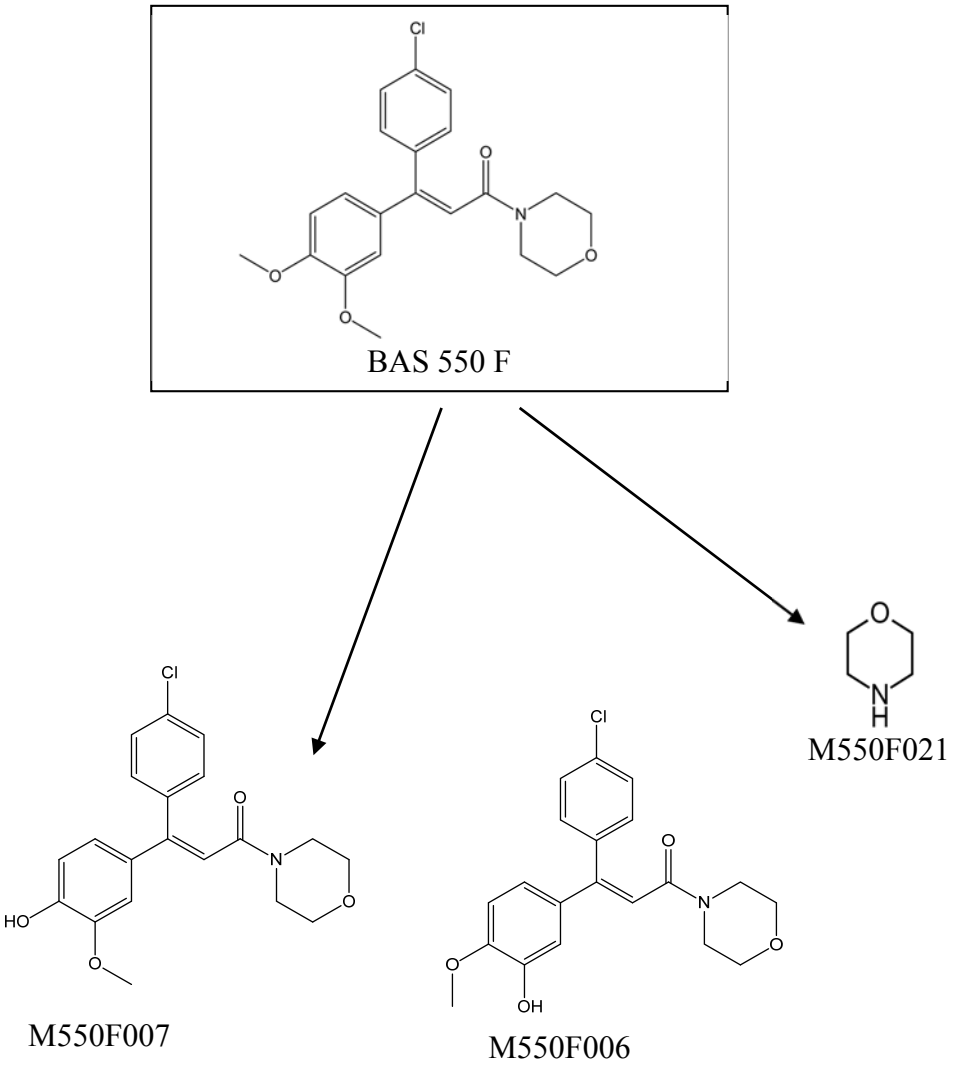
Initially each harvest was analyzed as its individual pots. The extractions were then repeated with a sample of the combined plant material from both pots for each harvest. Immature harvest extracts were initially analyzed 20-21 days after harvest and mature harvest extracts were initially analyzed 36 days after harvest. Analysis of the combined harvest samples took place 197 days after harvest for the immature harvest extracts and 183-194 days after harvest for the mature harvest extracts. There were no significant changes in the metabolite profiles between the individual pot extracts and the combined extracts showing the samples were stable in frozen storage for the relevant period.

III. CONCLUSION

After foliar application of dimethomorph on lettuce (approximately 225 g a.s./ha) on up to three occasions, high amounts of residues were detected (8.30 mg/kg at mature harvest).
For both harvests, radioactive residues were predominantly extracted with methanol (immature harvest: 96.3% TRR and mature harvest: 95.1% TRR) and only minor amounts were subsequently released with water (≤2% TRR) and acetone (≤0.3% TRR).

Dimethomorph is not extensively metabolized in lettuce, parent comprised 87.8% TRR at mature harvest. One minor metabolic step that was observed is the cleavage and release of the intact morpholine ring, leading to the formation of small amounts of M550F021 (morpholine), accounting for a maximum of 3.0% TRR. The presence of desmethylated metabolites M550F006 and M550F007 was confirmed by LC-MS but since they are not connected to the minor peaks in the radiochromatogram they were not quantifiable and thus occur only in trace amounts.

Figure 6.2.1-2: Proposed metabolic pathway of dimethomorph in lettuce



Potatoes

No new metabolism studies were performed in potatoes, representing the third crop group, for the following reasons: for potato already studies with both radiolabels (chlorophenyl and morpholine ring label) are available and were evaluated and accepted during the EU peer review of dimethomorph. Additionally, the two new studies in grape and lettuce largely confirmed the metabolic steps known from the previous studies. Since the previous studies showed a common pathway across all investigated crop groups, it was concluded that a new study in a third crop group would not provide substantial new information on the metabolic pathway of dimethomorph in plants.

In the following, ~~short summaries~~ full OECD summaries of the already evaluated studies are provided.

~~**Thiele J., 1990: Dimethomorph (Chlorophenyl Ring Label): The Metabolism and Translocation in Potato Plants (DocID DK-640-004)**~~
~~**Thiele J., 1990: Dimethomorph (Chlorophenyl Ring Label): The Metabolism and Translocation in Potato Plants (Amendment DK-640-009)**~~
~~**Thiele J., 1990: (Morpholine Ring Label): Metabolism and Translocation in Potato Plants (DocID DK-640-006)**~~
~~**Thiele J., 1991: (Morpholine Ring Label): Metabolism and Translocation in Potato Plants – Supplemental data (DocID DK-640-011)**~~

~~Potatoes were grown in pots in sandy loam soil under greenhouse conditions. Plants were sprayed with chlorophenyl labelled or morpholine labelled ^{14}C-dimethomorph (CME 101-03, 10% w/v) four times at a rate of 600 g a.s./ha with intervals of 10 days. Compared to the registered cGAP in potatoes (8 x 180 g a.s./ha), this represented a 1.6N overdosing. Potato tubers and green plant parts were sampled after each application for recovery of applied dose at harvest (PHI 7days). Radioactivity was determined by liquid scintillation and combustion analysis. Green matter and tuber samples were extracted with acetone and methanol. The extractable radioactivity was characterized and quantified by radio TLC/HPLC. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by mass spectrometry.~~

~~**Table 6.2.1-26: Test conditions - potato (greenhouse studies)**~~

~~**Position of label**~~	~~**Chlorophenyl ring label**~~	~~**Morpholine ring label**~~
~~Nominal application rate [g a.s./ha]~~	~~600~~	~~600~~
~~Number of applications~~	~~4~~	~~4~~
~~Interval between applications [days]~~	~~10~~	~~10~~
~~Comparison to the maximum recommended use rate~~	~~1.6N~~	~~1.6N~~
~~Sampling of plant material [days after final treatment]~~	~~7 (potato plants, tuber)~~	~~7 (potato plants, tuber)~~

Findings

At harvest, total radioactive residues amounted to 23.49 mg/kg in potato tops (leaves and stems) for the chlorophenyl label and 18.18 mg/kg for the morpholine label while the TRR in tubers were 0.008 mg/kg, or 0.012 mg/kg (tubers from lower layer of pots) and 0.019 mg/kg or 0.018 mg/kg (tubers from upper layer of pots) for chlorophenyl or morpholine label, respectively. Because of the low total radioactivity residues found in potato tubers, no characterization of the residues was undertaken. The bulk of the radioactivity, 97.8% or 97.9% from potato tops treated with chlorophenyl or morpholine labelled ^{14}C-dimethomorph was extractable with acetone and methanol. Of the extractable residues, the proportion which was recovered in acetone wash amounted from 61.3-72.3% of the total recovered radioactivity, indicating that the major portion of the radioactive residues were still associated with potato plants as a surface residue. The greatest portion of the extractable radioactivity was associated with unaltered parent accounting for 68% or 75.9% of the TRR for chlorophenyl or morpholine label, respectively. Only a trace amount (<0.5%) of the degradation product showing similar retention time upon TLC analysis as the reference compound M550F003 (Z7) was characterized for the chlorophenyl label.

Table 6.2.1-27: Total radioactivity residues in potato tops and tuber at harvest following application of ^{14}C-Chlorophenyl or ^{14}C-Morpholine dimethomorph grown in Greenhouse.

Label	Total radioactive residues in mg/kg		
	Tuber		Tops
	Lower layer pot	Upper layer pot	
Chlorophenyl label	0.008	0.019	23.49
Morpholine label	0.012	0.018	18.18

Conclusions

The investigation of the metabolism of dimethomorph in potato lead to the conclusion that the relevant residue in potato green matter consists primarily of the parent compound dimethomorph. The low residues in potato tubers suggest that translocation of residues from aerial plant parts to tubers is negligible.

Edwards V. T., 1992: Dimethomorph (CME 151, Chlorophenyl Ring Label): The Nature of the Residue in Potato Tubers (DocID DK-640-014)

Potatoes growing in a lysimeter were treated three times with a 150 g/L Dispersion Concentrate of dimethomorph at rates of 300 g a.s./ha with treatment intervals of 10 days. Compared to the current registered eGAP in potatoes (8 x 180 g ai/ha), this means a slight underdosing (0.63 N). The potato tubers and aerial parts were harvested at maturity, approximately four weeks after the last application. Radioactivity was determined by liquid scintillation and combustion analysis. Characterization was achived by TLC and confirmed by GC-MS. Peel and peeled potatoes were analyzed separately.

Table 6.2.1-28: Test conditions - potato (outdoor lysimeter study)

Position of label	**Chlorophenyl ring label**
Nominal application rate [g a.s./ha]	300
Number of applications	3
Interval between applications [days]	10
Comparison to the maximum recommended use rate	0.63N
Sampling of plant material [days after final treatment]	28 (potato plants, tubers, peel)

Findings

Most of the radioactivity was found in the potato leaves accounting for about 98.5%. Only 0.8% (0.121 mg/kg) of applied radioactivity was found in the peel and 0.7% (0.025 mg/kg) in the peeled potato tubers. The calculated TRR concentration in whole potato tuber was 0.044 mg/kg. Both, peeled tuber and tuber peel were extracted with methanol/water and water, reaching an ERR of 47.7% and 63.3% TRR for tuber and peel, respectively. The extracts from peel and peeled tuber were partitioned with dichloromethane to provide an initial quantification of total organo-soluble and water-soluble components.

The organo-soluble fractions of peel and peeled tuber consisted of parent dimethomorph, accounting for 46.3% TRR (0.056 mg/kg) and 12.0% TRR (0.003 mg/kg) in peel and tuber, respectively. The E/Z ratio was found to be approximately 1:3 in both peel and tubers. In organo-soluble component of the peel the desmethyl derivatives of parent, M550F006 (Z67) and M550F007 (Z69) were identified and accounted for 11.6% (0.014 mg/kg) and 4.96% (0.006 mg/kg) of the TRR, respectively. In the peeled potato, the desmethyl derivatives M550F006 (Z67) and M550F007 (Z69) were not observed. The water-soluble fraction in both peel and peeled tuber was investigated by radio-HPLC and found to consist of several minor polar components accounting for 8.6% (0.01 mg/kg) and 24.0% (0.006 mg/kg) of the TRR, respectively. These compounds were not identified since they were not matching with the retention times of the reference standards of parent or M550F006 or M550F007. The calculated concentration of unchanged parent, M550F006 (Z67) and M550F007 (Z69) in whole potato is equivalent to 0.014, <0.003, and <0.002 mg/kg, respectively.

Table 6.2.1-29: Extraction of radioactivity after treatment with ^{14}C-dimethomorph chlorophenyl ring label (%TRR)

Solvent	Tuber peel	Peeled tuber
Methanol/water	55.3	47.4
Water	2.2	0.0
Repeat methanol/water	5.5	n.a.
Repeat water	0.3	n.a.
ERR	63.3	47.7
Unextractable (RRR)	28.3	45.1
Total recovered	91.6	92.8

n.a. Not analyzed; no repeat extractions were performed because initial extraction procedure did not recover any radioactivity in water extract

Table 6.2.1-30: Summary of identified components in potato samples after treatment with ^{14}C-chlorophenyl ring-labelled dimethomorph (lysimeter study)

	Organo-soluble		Water-soluble	
	Tuber peel	Peeled tuber	Tuber peel	Peeled tuber
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Dimethomorph	0.056 (46.3)	0.003 (12.0)	0.002 (21.0)	0.012 (29.4)
M550F006 (Z67)	0.014 (11.6)	n.d.	n.d.	n.d.
M550F007 (Z69)	0.006 (4.96)	n.d.	n.d.	n.d.

n.d. Not detected; the water soluble fractions was composed of several minor polar components which precluded further characterization

Conclusion

The nature of residues found in peel and peeled potatoes treated with ^{14}C-dimethomorph labelled in the chlorophenyl ring is mainly parent compound dimethomorph. Traces of the desmethyl derivatives of parent, M550F006 (Z67) and M550F007 (Z69), were only found in potato peel.

Report:	CA 6.2.1/6 Thiele J., 1990 a 14C-Dimethomorph (CME 151) (Chlorophenyl ring label) - Metabolism and translocation in potato plants
Guidelines:	DK-640-004 EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Report:	CA 6.2.1/7 Thiele J., 1991 a 14C-Dimethomorph (CME 151) (Chlorophenyl ring label) - Metabolism and translocation in potato plants - Supplemental data
Guidelines:	DK-640-009 EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Report:	CA 6.2.1/8 Thiele J., 1990 b 14C-Dimethomorph (CME 151) (Morpholine ring label) - Metabolism and translocation in potato plants
Guidelines:	DK-640-006 EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Report:	CA 6.2.1/9 Thiele J., 1991 b 14C-Dimethomorph (CME 151) (Morpholine ring label) - Metabolism and translocation in potato plants - Supplemental data
Guidelines:	DK-640-011 EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

3. Test material:

Description:	[p-chlorophenyl-U-^{14}C]-BAS 550 F (chlorophenyl label) *E*:*Z*; 47 :53
	[Morpholine-2,3-^{14}C]-BAS 550 F (morpholine label) *E*:*Z*; 50:50
	BAS 550 F (unlabeled) Formulation CME 151 03 (0.993 kg/L, EC)
Lot/Batch #:	S 1050 (chlorophenyl label) 1040 (*E*-isomer), 1041 (*Z*-isomer) (morpholine label) H 7879 (unlabeled) Ka 16/89 (CME 151 03)
Purity:	99.2% (unlabeled) Radiochemical purity: ≥98.0% Specific activity: 873.2 MBq/g (chlorophenyl label) 691.9 MBq/g (morpholine label)
CAS#:	110488-70-5
Stability of test compound:	The test item was stable over the test period.

2. Test commodity:

Crop:	Potato
Type:	Root and tuber vegetables
Variety:	Bintje
Botanical name:	*Solanum tuberosum*
Crop parts(s) or processed commodity:	Tuber, aerial parts
Sample size:	Not relevant

B. STUDY DESIGN

The study was carried out at the Biological Experimentation Station of Shell Forschung GmbH in Schwabenheim, Germany. The cultivation of the crop took place under natural climatic conditions.

1. Test procedure

Potatoes were grown in pots in sandy loam soil under greenhouse conditions. Plants were sprayed with chlorophenyl labeled or morpholine labeled ^{14}C-dimethomorph (CME 151 03, 10% w/v, EC) four times at a rate of 600 g a.s./ha with intervals of 10 days. Compared to the registered cGAP in potatoes (8 x 180 g a.s./ha), this represented a 1.6N overdosing. Potato tubers (from upper and lower layer of the pots) and green plant parts were sampled after each application for recovery of applied dose and at harvest (PHI 7 days). At harvest, all sample material was weighed and stored at approximately -18°C or below until analysis.

2. Description of analytical procedures

Radioanalysis (TRR): Radioactivity was determined by liquid scintillation and combustion analysis.
Extraction (ERR): Potato tops samples (leaves and stems) were extracted with acetone and methanol. After the methanol extraction, the top samples were allowed to dry and stored in a fume cupboard at room temperature before the combustion analysis.

3. Identification of metabolites

The extractable radioactivity was characterized and quantified by radio TLC/HPLC. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by mass spectrometry. Generally, TLC investigations of methanol and acetone radioactive extracts revealed that 26.5% of the extractable radioactivity was composed of several unknown, mainly polar compounds whose identification was no longer possible. The identity of the radioactive compound recovered from the aerial parts was further confirmed by UV-spectroscopy, HPLC and MS investigation of the radioactive fractions isolated from TLC plates. Only a trace amount (<0.5%) of the degradation product showing similar retention time upon TLC analysis as the reference compound M550F003 (Z7) was characterized for the chlorophenyl label.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

At harvest (7 DALA), total radioactive residues amounted to 23.49 mg/kg in potato tops (leaves and stems) for the chlorophenyl label and 18.18 mg/kg for the morpholine label while the TRR in tubers were 0.008 mg/kg or 0.012 mg/kg (tubers from lower layer of pots) and 0.019 mg/kg or 0.018 mg/kg (tubers from upper layer of pots) for chlorophenyl or morpholine label, respectively. The results of TRR found are presented in Table 6.2.1-23.

Table 6.2.1-23: Total radioactive residues (TRR) in potato tops and tuber at harvest following application of ^{14}C-chlorophenyl dimethomorph grown in greenhouse

Matrix	DALA[1]	TRR combusted [mg/kg]	
		Morpholine label	Chlorophenyl label
Tuber (lower layer pot)	7	0.012	0.008
Tuber (upper layer pot)	7	0.018	0.019
Tops	7	18.18	23.49

[1] Days after last application

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in potatoes

The bulk of the radioactivity, 97.8% or 97.9% from potato tops treated with chlorophenyl or morpholine labeled ^{14}C-dimethomorph was extractable with acetone and methanol. Of the extractable residues, the proportion which was recovered in acetone wash amounted from 61.3-72.3% of the total recovered radioactivity, indicating that the major portion of the radioactive residues were still associated with potato plants as a surface residue.
The extractabilities of the potato matrices with acetone and methanol are summarized in Table 6.2.1-24.

Table 6.2.1-24: Extractability of radioactive residues in potato samples

Matrix	Days after last application	Acetone extract		Methanol extract		ERR[1]		RRR [2]		TRR calculated[3]
		[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Chlorophenyl label										
Potato tops	7 DALA	14.42	61.3	8.54	36.5	22.96	97.8	0.52	2.3	100
Morpholine label										
Potato tops	7 DALA	13.11	72.3	4.69	25.7	17.8	97.9	0.38	2.1	100

[1] ERR = Extractable Radioactive Residue (extraction with acetone and methanol)
[2] RRR = Residual Radioactive Residue (after solvent extraction with acetone and methanol)
[3] TRR was calculated as the sum of ERR + RRR

2. Identification and characterization of extractable residues in potatoes

Because of the low total radioactivity residues found in potato tubers, no characterization of the residues was undertaken. In the aerial parts, the greatest portion of the extractable radioactivity was associated with unaltered parent accounting for 68% or 75.9% of the TRR for chlorophenyl or morpholine label, respectively. Only a trace amount (<0.5%) of the degradation product showing similar retention time upon TLC analysis as the reference compound M550F003 (Z7) was characterized for the chlorophenyl label.

3. Storage stability

For both labels, the mature potato samples were taken 8 June 1989 and the analytical phase of the study was completed on 7 November 1989 and consequently was within 6 months of storage interval.

4. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph (BAS 550 F) in potatoes is shown in Figure 6.2.1-3**:** **Metabolic pathway of dimethomorph in potatoes**. The key step of the metabolism of dimethomorph in potatoes after foliar application were demethylation of the 2-methoxy groups of the dimethoxyphenyl ring to produce metabolites M550F006 (Z67) and M550F007 (Z69), resulting in a hydroxyl group that most likely forms the corresponding glucose conjugate and hydrolysis of dimethomorph to form metabolite M550F003 (Z7).

III. CONCLUSION

The investigation of the metabolism of dimethomorph in potato lead to the conclusion that the relevant residue in potato green matter consists primarily of the parent compound dimethomorph. The low residues in potato tubers suggest that translocation of residues from aerial plant parts to tubers is negligible.

Report:	CA 6.2.1/10 Edwards V.T., 1992 a Dimethomorph (CME 151) (Chlorophenyl ring-14C) metabolism: The nature of the residue in potato tubers - Supplemental report to report SHGR.89.071 DK-640-014
Guidelines:	EPA 171-4
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

Description:	[p-chlorophenyl-U-^{14}C]-BAS 550 F (chlorophenyl label) *E:Z*; 47 :53 BAS 550 F (unlabeled, formulation SF 07460, 150 g/L, DC)
Lot/batch #:	S 1050/1 (chlorophenyl label)
Purity:	Radiochemical purity: ≥97.0% (Formulation DC) Specific activity: 873.2 MBq/g (chlorophenyl label)
CAS#:	110488-70-5
Stability of test compound:	The test item was stable over the test period.

2. Test commodity:

Crop:	Potato
Type:	Root and tuber vegetables
Variety:	Quarta
Botanical name:	*Solanum tuberosum*
Crop parts(s) or processed commodity:	Tuber
Sample size:	Not relevant

B. STUDY DESIGN

The study was carried out at the Biological Experimentation Station of Shell Forschung GmbH in Schwabenheim, Germany. The cultivation of the crop took place under natural climatic conditions.

1. Test procedure

Potatoes growing in a lysimeter were treated three times with a formulation (DC, 150 g/L) containing chlorophenyl labeled dimethomorph at rates of 300 g a.s./ha with treatment intervals of 10 days. Compared to the current registered cGAP in potatoes (8 x 180 g as/ha), this means a slight underdosing (0.63N). The potato tubers and aerial parts were harvested at maturity, approximately four weeks after the last application.
At harvest, all sample material was weighed and stored at approximately -18°C or below until analysis.

2. Description of analytical procedures

Radioanalysis (TRR): Radioactivity was determined by liquid scintillation and combustion analysis.
Extraction (ERR):
Aerial parts (leaves and stem) were rinsed with acetone for 15 seconds (acetone wash) and then dried and subjected to analysis of the remaining radioactivity by combustion analysis.
Potato tubers were carefully peeled and both tuber peel and peeled tuber were extracted with methanol/water and water. The radioactivity remaining unextracted from both peel and peeled tubers was measured by combustion giving a total recovery through the extraction process. The methanol/water and water extracts from potato peel and peeled tuber were partitioned with dichloromethane to provide an initial quantification of total organo-soluble and water-soluble components.
Sub-samples of dichlormethane and aqueous phase from both peel and peeled tuber were analyzed by TLC and HPLC, respectively.

3. Identification of metabolites

Characterization was achieved by TLC and confirmed by GC-MS. Peel and peeled potatoes were analyzed separately. The leaves sampled at the final harvest were analyzed only by combustion for total radioactive residues.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Most of the radioactivity was found in the leaves (leaves + acetone wash) accounting for about 98.5%. The ^{14}C-TRR in tuber peel and peeled tuber at harvest were 0.121 mg equivalent dimethomorph/kg fresh weight (0.8% of applied radioactivity) and 0.025 mg equivalent dimethomorph/kg fresh weight (0.7% of applied radioactivity), respectively. The calculated TRR concentration in whole potato tuber was 0.044 mg/kg.
Because of the low total radioactivity residues found in potato tubers, no characterization of the residues was undertaken. The results of TRR found are presented in Table 6.2.1-25.

Table 6.2.1-25: Total radioactive residues (TRR) in potato tops and tuber at harvest following application of ^{14}C-chlorophenyl dimethomorph (outdoor lysimeter study)

TRRs in treated potato samples [mg/kg]			
Matrix	DALA[1]	TRR combusted [mg/kg]	Recovered radioactivity [%]
		Chlorophenyl label	
Peel	28 DALA	0.121	0.8
Peeled tuber	28 DALA	0.025	0.7
Leaves Acetone wash	28 DALA	1.359	5.6
Leaves After wash	28 DALA	22.41	92.4
Whole potato	28 DALA	0.044	1.5

1 Days after last application

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in potatoes

Both peeled tuber and tuber peel were extracted with methanol/water and water, reaching an ERR of 47.7% and 63.3% TRR for tuber and peel, respectively. Most of the residue was extractable with methanol/water. The recovery through the extraction procedure was calculated by summing the radioactivity in the extracts and that measured by combustion.
The extractabilities of the potato matrices with methanol/water and water are summarized in Table 6.2.1-26.

Table 6.2.1-26: Extractability of radioactive residues in potato samples

Matrix	Days after last application	Methanol/water		Water extract		ERR[1]		RRR [2]		TRR calculated[3]
		[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Chlorophenyl label										
Tuber peel	28 DALA	0.074	60.8	0.003	2.5	0.077	63.3	0.034	28.3	91.6
Peeled tuber	28 DALA	0.012	47.7	n.a.	n.a.	0.012	47.7	0.011	45.1	92.8

[1] ERR = Extractable radioactive residue (extraction with methanol/water and water)
[2] RRR = Residual radioactive residue (after solvent extraction with methanol/water and water)
[3] TRR was calculated as the sum of ERR + RRR
n.a. Not analyzed; no repeat extractions were performed because initial extraction procedure did not recover any radioactivity in water extract

2. Identification and characterization of extractable residues in potatoes

The organo-soluble fractions of peel and peeled tuber consisted of parent dimethomorph, accounting for 46.3% TRR (0.056 mg/kg) and 12.0% TRR (0.003 mg/kg) in peel and tuber, respectively. The *E/Z* ratio was found to be approximately 1:3 in both peel and tubers. In organo-soluble component of the peel the desmethyl derivatives of parent, M550F006 (Z67) and M550F007 (Z69) were identified and accounted for 11.6% (0.014 mg/kg) and 4.96% (0.006 mg/kg) of the TRR, respectively.
In the peeled potato, the desmethyl derivatives M550F006 (Z67) and M550F007 (Z69) were not observed. The water-soluble fraction in both peel and peeled tuber was investigated by radio-HPLC and found to consist of several minor polar components accounting for 8.6% (0.01 mg/kg) and 24.0% (0.006 mg/kg) of the TRR, respectively. These compounds were not identified since they were not matching with the retention times of the reference standards of parent or M550F006 or M550F007. The calculated concentration of unchanged parent, M550F006 (Z67) and M550F007 (Z69) in whole potato is equivalent to 0.014, <0.003, and <0.002 mg/kg, respectively. The presence of dimethomorph in the dichlormethane phases from both peel and peeled tuber was confirmed by GC-MS.

Table 6.2.1-27: Summary of identified components in potato samples after treatment with ^{14}C-chlorophenyl ring-labeled dimethomorph (lysimeter study)

Metabolite	Matrix							
	Tuber peel				Peeled tuber			
	[mg/kg]		[% TRR]		[mg/kg]		[% TRR]	
Total radioactive residue								
Identified								
	Organo-soluble		Water-soluble		Organo-soluble		Water-soluble	
Dimethomorph	0.056	46.3	0.002	21.0	0.003	12.0	0.012	29.4
M550F006 (Z67)	0.014	11.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M550F007 (Z69)	0.006	4.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. Not detected; the water soluble fractions were composed of several minor polar components which precluded further characterization

4. Storage stability

All samples were stored in a freezer at approximately -18°C until used. The maximal storage period was between 4 to 6 months.

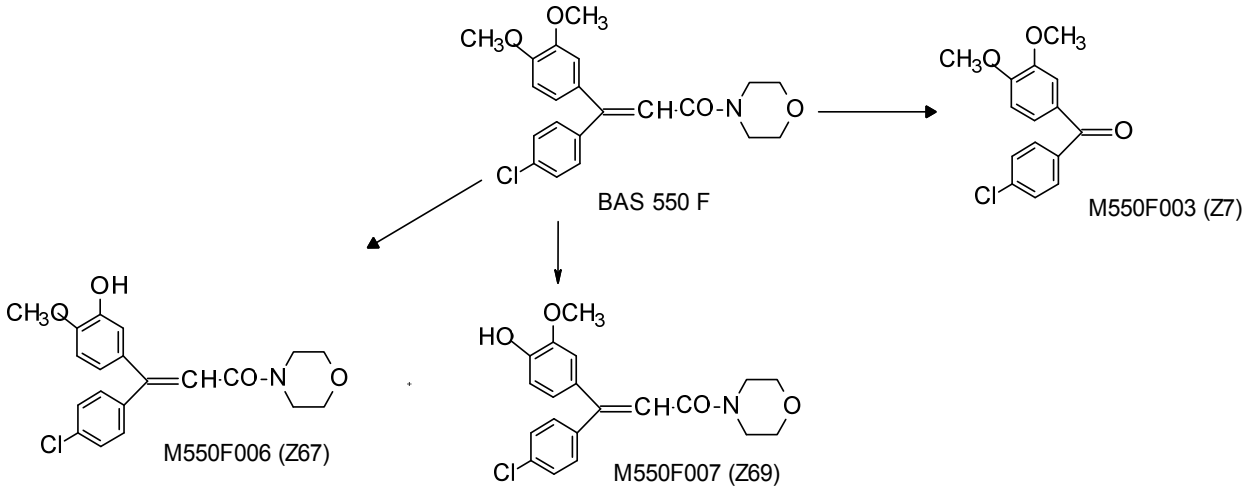
5. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph (BAS 550 F) in potatoes is shown in Figure 6.2.1-3. The key step of the metabolism of dimethomorph in potatoes after foliar application was demethylation of the 2-methoxy groups of the dimethoxyphenyl ring to produce metabolites M550F006 (Z67) and M550F007 (Z69), resulting in a hydroxyl group that most likely forms the corresponding glucose conjugate and hydrolysis of dimethomorph to form metabolite M550F003 (Z7).

III. CONCLUSION

The investigation of the metabolism of dimethomorph in potato lead to the conclusion that the nature of residues found in peel and peeled potatoes treated with ^{14}C-dimethomorph labeled in the chlorophenyl ring is mainly parent compound dimethomorph. Traces of the desmethyl derivatives of parent, M550F006 (Z67) and M550F007 (Z69) were only found in potato peel.

Figure 6.2.1-3: Metabolic pathway of dimethomorph in potatoes



Tomatoes

The following study was considered as supplemental information in the last peer review since it investigated the fate of dimethomorph after uptake via the roots, which was no representative use pattern in the last peer review. Since the drip/drench use in strawberries combines both foliar exposure as well as uptake from residues in soil via roots, the study is included again into the supplementary dossier. It can provide -together with the metabolism studies for foliar spray and the rotational crop studies- information about the metabolism of dimethomorph for the drip/drench use pattern.

~~Schlueter H., Varga J. 1995, ^{14}C-Dimethomorph: Metabolism in tomato plants after uptake through the roots (DocID DK-640-020)~~

~~Tomato plants were treated with ^{14}C-labelled dimethomorph (chlorophenyl label) by adding Dimethomorph to the nutrient media (8 mg/L) of a hydroponic system for uptake through the roots for a 7-day application period. Samples of the above ground tomato plant parts were taken immediately at the end of the 7-day application period and, 14 and 28 days thereafter. Since the aim of the study was the characterization of metabolites after root uptake and since the largest amount of residues was expected to be found in the green plant parts, neither roots nor tomato fruits were investigated (fruits were also not yet present at sampling). The sampled material was analysed by LSC (radioactivity levels) and TLC (characterization of nature of the residues). For characterization of the nature of residues, plant samples were extracted with successively with acetone, methanol, and methanol/water 4:1. For confirming metabolite identities, HLPC-MS was used.~~

~~Findings~~

~~The distribution of residues between extractable residue (ERR) and non-extractable residues as well as the total radioactive residues in the samples from the different time points is shown in the table below.~~

~~Table 6.2.1-36: Extractability of residues in tomato plants without roots~~

~~Days after 7-day application period~~	~~Residue mg/kg parent equivalent (% of TRR)~~		
	~~0~~	~~14~~	~~28~~
~~Extractable radioactive residues (ERR)*~~	~~23.84 (97.3%)~~	~~11.37 (90.7%)~~	~~5.83 (83.3%)~~
~~Residual radioactive residues (RRR)~~	~~0.67 (2.7%)~~	~~1.16 (9.3%)~~	~~1.17 (16.7%)~~
~~Total radioactive residues (TRR)~~	~~24.52~~	~~12.52~~	~~7.00~~

~~* Sum of all extracts (Acetone, Methanol and Methanol/Water 4:1)~~

The nature and magnitude of residues is summarized in the following table:

Table 6.2.1-37: Nature of the residue at each sampling time

Days after 7-day application period	Residue mg/kg (% of TRR)		
	0	14	28
Dimethomorph	16.2 (66%)	3.5 (28%)	1.12 (16%)
M550F007 (Z69), mainly as its glucoside M550F002	≤3.2 (≤13%)	2.25-4.13 (18-33%)	0.91-2.38 (13-34%)
M550F009 (Z93)	1.96 (8%)	2.13 (17%)	0.63 (9%)
M550F011 (Z95)	≤1.47 (≤6)	0.5-1.0 (4-8%)	≤0.49 (7%)
M550F012 (Z98)	≤1.47 (≤6)	0.13-0.63 (1-5%)	≤0.49 (7%)

Dimethomorph was the main compound found at each sampling event with 16-66% of TRR. For all three sampling times, estimation of metabolite content indicated that the demethylated product M550F007 (Z69) was the main metabolite for each sampling time with ca. 13-34% of TRR. This metabolite was identified by TLC and MS after acidic hydrolysis, showing that it is present in a conjugated form, probably as its glucoside M550F002. The contents of metabolites M550F009 (Z93), M550F011 (Z95) and M550F012 (Z98) were lower with 8-17%, 4-8% and 1-7% of the TRR.

Conclusion

This study showed that dimethomorph is readily taken up by the tomato plants through the roots when applied in hydroponic solution. Dimethomorph is translocated and metabolized in tomato green plant parts via two pathways: (1) Demethylation of the p-methoxy group of the dimethoxyphenyl ring followed by conjugate formation; (2) Opening and stepwise degradation of the morpholine ring.

These metabolic steps are similarly observed in the other plant metabolism studies after foliar spray application, especially the new grape metabolism study. Thus, the metabolic pathways of dimethomorph in plants show a common pattern with regard to the nature of residue, regardless of whether the uptake route is via roots or above ground plant parts. It can be concluded that the metabolic pathway is qualitatively similar for foliar spray as well as drip/drench use patterns.

Report:	CA 6.2.1/11 Schlueter H.,Varga J., 1995 a 14C-Dimethomorph: Metabolism in tomato plants after uptake through the roots
Guidelines:	DK-640-020 EPA 171-4
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

3. Test material:

Description:	^{14}C-BAS 550 F (chlorophenyl label; E:Z, 54:46) Unlabeled BAS 550 F
Lot/batch #:	S 1050/1 (chlorophenyl label) Re 8/5 (unlabeled)
Purity:	99.1% (unlabeled) Radiochemical purity: 98% (chlorophenyl label) Specific activity: 0.873 MBq/mg (chlorophenyl label) 199.46 MBq/g (application mixture)
CAS#:	110488-70-5
Stability of test compound:	The test item was stable over the test period.

2. Test commodity:

Crop:	Tomato
Type:	Fruiting vegetable
Variety:	Professor Rudloff
Botanical name:	*Solanum lycopersicum* L.
Crop parts(s) or processed commodity:	Whole plant without roots
Sample size:	Not relevant

B. STUDY DESIGN

The in-life phase of the study was carried out at Cyanamid Forschung GmbH, Department of Chemical Research.

1. Test procedure

Tomato plants were treated with ^{14}C-labeled dimethomorph (chlorophenyl label) by adding dimethomorph to the nutrient media (8 mg/L) of a hydroponic system for uptake through the roots for a 7-day application period. Samples of the above ground tomato plant parts were taken immediately at the end of the 7-day application period and 14 and 28 days thereafter. Since the aim of the study was the characterization of metabolites after root uptake and since the largest amount of residues was expected to be found in the green plant parts, neither roots nor tomato fruits were investigated (fruits were also not yet present at sampling). The sampled material was analyzed by liquid scintillation counting (LSC) and thin layer chromatography (TLC). For characterization of the nature of residues, plant samples were extracted successively with acetone, methanol and methanol/water (4/1, v/v). For confirming metabolite identities, HLPC-MS was used.

2. Description of analytical procedures

Radioanalysis of radioactive residues: For the determination of the total radioactive residues (TRR) and the measurement of residual radioactive residues after solvent extraction (RRR), homogenized subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by LSC. Liquid samples were directly mixed with a scintillation fluid and the radioactivity was determined by LSC.
Extraction (ERR): Homogenized subsamples of tomato leaves were extracted two times with acetone, methanol and methanol/water (4/1, v/v). Aliquots of extracts were dried (rotary evaporator), re-suspended in water and partitioned three times against dichloromethane, whereby the obtained dichloromethane phases were combined. Further, another aliquots of extracts were dried, re-suspended in 2 N HCl and incubated for approximately 8 hours at room temperature and/or for 3 hours at 80°C. Obtained solutions were diluted with water and partitioned as described above.

3. Identification of metabolites

Extracts were analyzed by TLC. For confirming metabolite identities, HLPC-MS was used.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) was calculated by summing the extracted radioactivity and the remaining radioactive residues after solvent extraction. The calculated TRR of in tomato plants (without roots) accounted for 24.52 mg/kg directly after termination of the treatment and declined to 12.52 mg/kg after 14 days and to 7.00 mg/kg after 28 days after termination of the application.

Table 6.2.1-28: TRR in tomato plants

DALA[1]	TRR calculated [mg/kg]
0	24.52
14	12.52
28	7.00

1 Days after last application

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in tomato

The extractabilities of the tomato plant sampled at the three different time-points are summarized in Table 6.2.1-29. The extractability was high ranging from 83.2% to 97.3% TRR for. For tomato plants sampled at 0 DALA, similar portions of radioactive residues were extracted with acetone and methanol (54% and 42% TRR, respectively), while only low amount were subsequently extracted with methanol/water (2% TRR).
For tomato plants sampled at 14 DALA and 28 DALA, major part of radioactive residues was extracted with methanol (55% TRR and 46% TRR, respectively), lower amounts were obtained by applying acetone extraction (31% TRR and 21% TRR). For samples of 14 DALA minor amounts (5% TRR) and for 28 DALA similar amounts (17% TRR) compared to acetone extraction were released by methanol/water extraction.

Table 6.2.1-29: Extractability of radioactive residues in tomato plant samples

DALA	Acetone extract		Methanol extract		Methanol/water extract		ERR [1]		RRR[2]		TRR
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
0	13.11	53.5	10.28	41.9	0.45	1.9	23.84	97.3	0.67	2.7	24.52
14	3.88	30.9	6.93	55.2	0.55	4.6	11.37	90.7	1.16	9.3	12.52
28	1.46	20.8	3.22	45.9	1.15	16.5	5.83	83.2	1.17	16.8	7.00

1 Extractable radioactive residue (extraction with acetone, methanol and methanol/water)
2 Residual radioactive residue after solvent extraction

2. Identification and characterization of extractable residues in lettuce

Dimethomorph was the main compound found at each sampling event with 16-66% TRR. For all three sampling time points, determination of metabolite content indicated that the demethylated product M550F007 (Z69) was the main metabolite accounting for 13-34% TRR. This metabolite was identified by TLC and MS after acidic hydrolysis, showing that it is present in a conjugated form, probably as its glucoside M550F002. The contents of metabolites M550F009 (Z93), M550F011 (Z95) and M550F012 (Z98) were lower with 8-17%, 4-8% and 1-7% TRR.

Table 6.2.1-30: Summary of identified components in tomato

Days after 7-day application period	0		14		28	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Dimethomorph	16.2	66	3.5	28	1.12	16
M550F007 (Z69), mainly as its glucoside M550F002	≤3.2	≤13	2.25-4.13	18-33	0.91-2.38	13-34
M550F009 (Z93)	1.96	8	2.13	17	0.63	9
M550F011 (Z95)	≤1.47	≤6	0.5-1.0	4-8	≤0.49	≤7
M550F012 (Z98)	≤1.47	≤6	0.13-0.63	1-5	≤0.49	≤7

3. Storage stability

The study was carried out from October 29, 1993, to February 15, 1995, thus the analytical phase probably exceeded 6 months. Information on the storage stability in high water matrices is sufficiently addressed within the AIR3 Dossier by the investigations in the new lettuce study. This should also cover the investigations in tomato plant parts of this study.

4. Proposed metabolic pathway

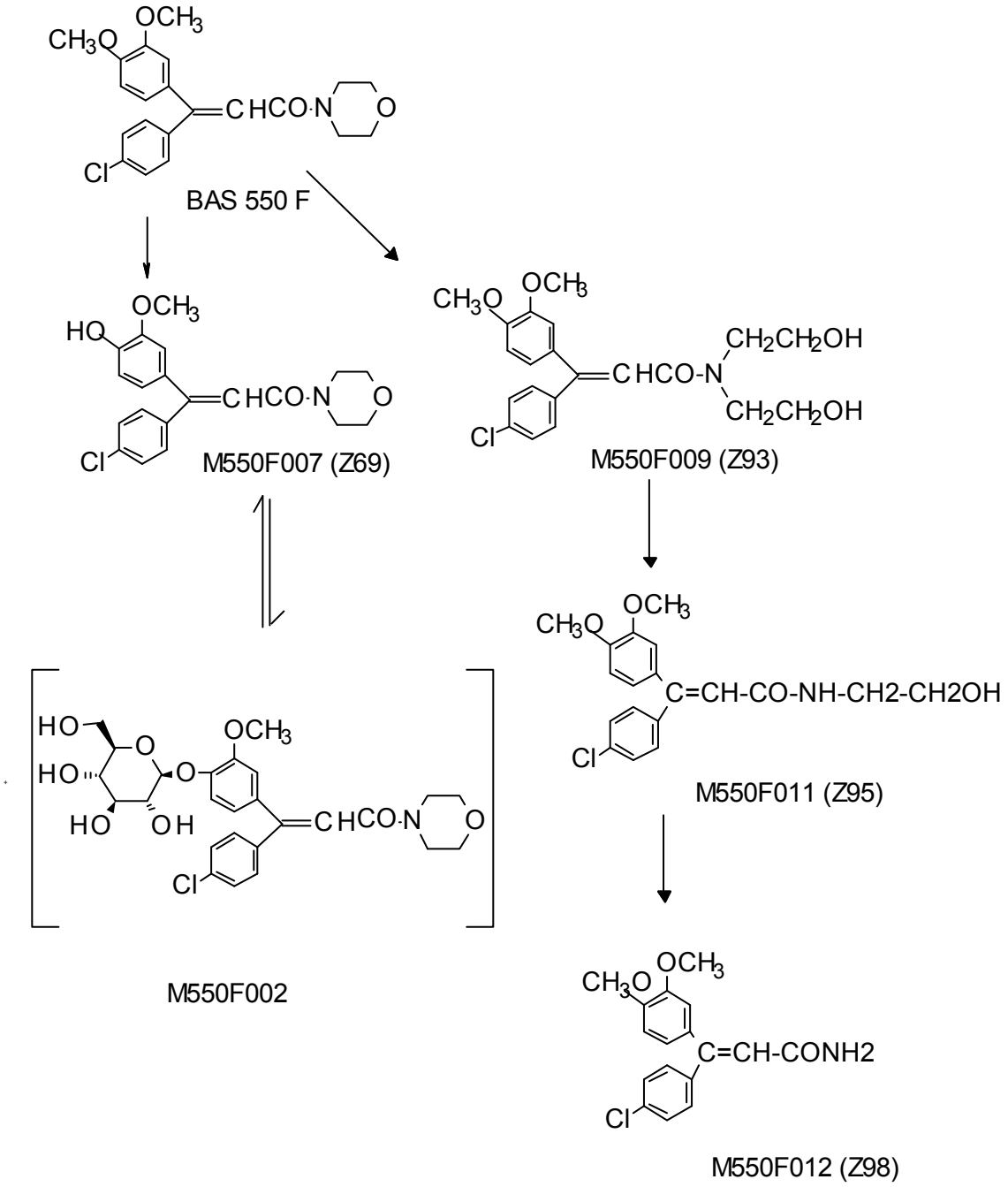
The proposed metabolic pathway of dimethomorph (BAS 550 F) in tomato is shown in Figure 6.2.1-4**: Metabolic pathway of dimethomorph tomato plant (uptake via roots)**. The metabolism of dimethomorph follows two main metabolic steps:

- Demethylation of the p-methoxy group
- Opening and stepwise degradation of the morpholine ring

III. CONCLUSION

This study showed that dimethomorph is readily taken up by the tomato plants through the roots when applied in hydroponic solution. Dimethomorph is translocated and metabolized in tomato green plant parts via two pathways: (1) Demethylation of the p-methoxy group; (2) Opening and stepwise degradation of the morpholine ring.
These metabolic steps are similarly observed in the other plant metabolism studies after foliar spray application, especially the new grape metabolism study. Thus, the metabolic pathways of dimethomorph in plants show a common pattern with regard to the nature of residue, regardless of whether the uptake route is via roots or above ground plant parts. It can be concluded that the metabolic pathway is qualitatively similar for foliar spray as well as drip/drench use patterns.

Figure 6.2.1-4: Metabolic pathway of dimethomorph tomato plant (uptake via roots)



Overall conclusions plant metabolism

The metabolic pathways of dimethomorph in plants of three different crop groups (fruits, leafy vegetables, root & tuber vegetables) show a common pattern with regard to the nature of residue. Metabolism of dimethomorph in plants is not extensive. The unchanged parent is the quantitatively most predominant component of the total residue. Besides that the following main metabolic steps were observed:

1) Demethylation at the dimethoxyphenyl ring, resulting in a hydroxyl group, yielding metabolites M550F006 (Z67) and M550F007 (Z69). These metabolites undergo subsequent Phase II-reactions by being conjugated with glucose (M550F002).

A summary of the old and new metabolism data show that the demethylation at the *p*-position is more predominant (yielding M550F007), whereas the respective *m*-demethylated product M550F006 was only observed in trace amounts in some of the previous studies. While previous studies only suggested the glycosylation step, this was now confirmed in the new grape study as being a major metabolic step, yielding substantial amounts of M550F002, which was the only metabolite present in amounts >10% TRR.

2) Hydroxylation of the morpholine ring (M550F018)

Previous studies have not identified M550F018, probably due to the reason that it had not been available as reference standard. With the better technology for identification (MS/MS) used in the new studies, this metabolite could be identified. It is possible that this metabolite serves as a precursor (i.e. the initial step) of the morpholine ring opening and subsequent degradation, which was an observed metabolic step also in the previous metabolism studies.

3) Opening of the morpholine ring, followed by glucose conjugation (M550F017), and its step-wise degradation (M550F009, M550F011, M550F012)

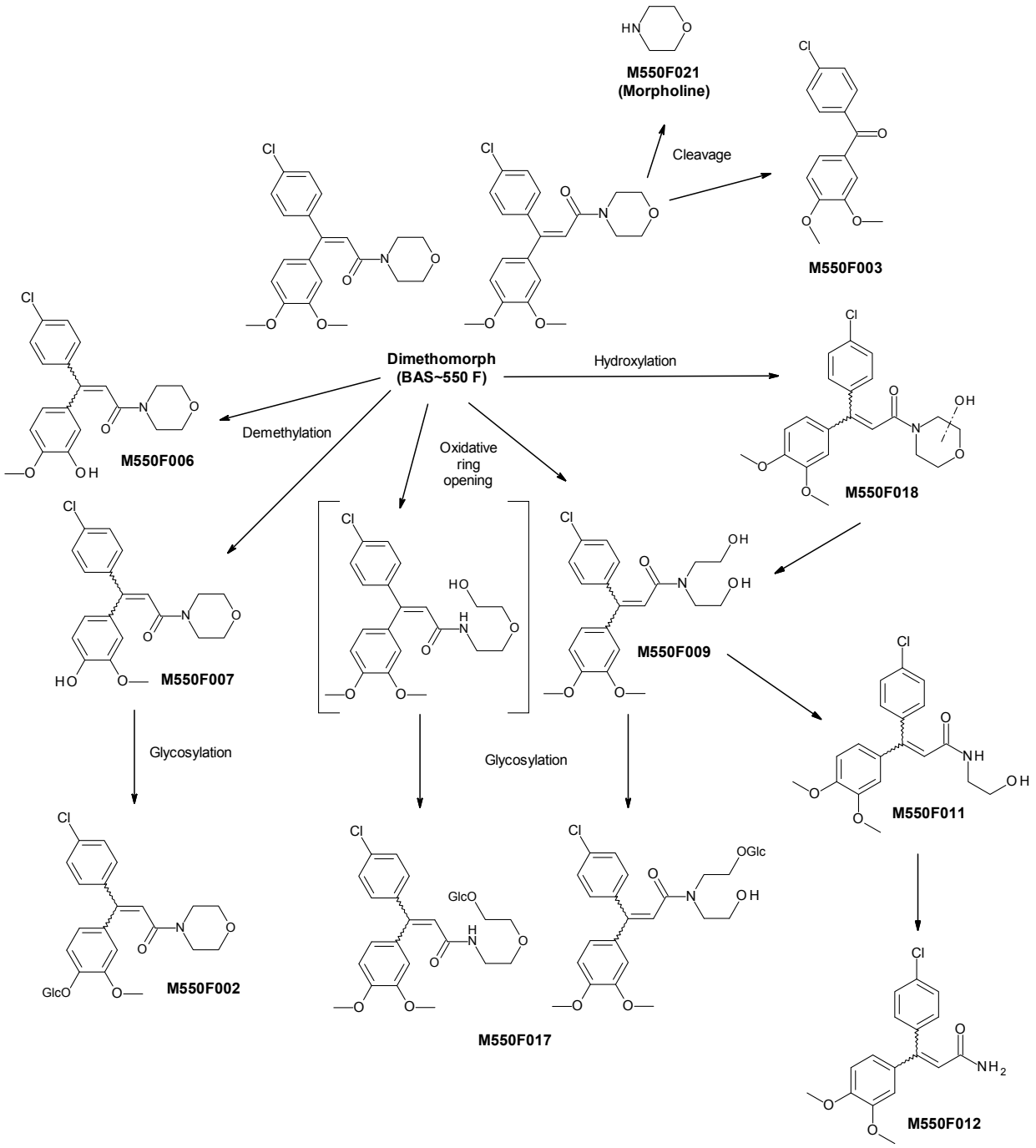
Again, metabolite M550F017 was not identified in the previous studies probably due to limitations of the technology. Step-wise degradation was an observed pathway in tomato leaves upon uptake of dimethomorph via the roots (hydroponic study).

4) Cleavage/hydrolysis (M550F003) and release of the intact morpholine ring (M550F021).

This pathway was shown to be of subordinate significance for both metabolites. Metabolite M550F003 was only observed in previous studies (not in the new grape study although specifically searching for it by MS) and also only in trace amounts. It probably results from cleavage or hydrolysis of the parent molecule. Metabolite M550F021 (morpholine) was not observed in the previous studies due to the position of the radiolabel in the chlorophenyl ring and also due to the limited technology used at that time. It was now identified as one metabolite among other substances in the polar fraction accounting for 4.6% TRR at most.

It can be concluded that the new metabolism studies confirm the previous understanding of the metabolism of dimethomorph and enlarge it due to the state-of-the-art technology used for metabolite identification. The new studies have also shown that besides M550F021 (morpholine), which occurred only in minor amounts, no other label specific metabolite was observed. This corroborates the conclusion that the metabolic fate of dimethomorph is sufficiently described by studies with the chlorophenyl label. The new studies also demonstrate that metabolism after spray application and root uptake (drip/drench use pattern) is going along similar pathways, with the most predominant metabolic step being the demethylation and further conjugation. The opening of the morpholine ring was an observed step also after foliar spray application and the step-wise degradation of the morpholine moiety (observed only after root uptake) is quantitatively of subordinate importance.
A summary of the metabolic steps observed in plants is shown in Figure 6.2.1-5.

Figure 6.2.1-5: Metabolic pathway of dimethomorph in plants (summary of peer reviewed and new data)



Putative intermediates not found
Glc: Glucose

Metabolism in livestock

During the previous EU Review of the active substance dimethomorph, for investigation of the metabolism of dimethomorph in animals, metabolism studies were carried out in two livestock species and in the rat:

For poultry:	Laying hens
For ruminants:	Lactating goats

The metabolism study in poultry showed that besides the parent compound (found in gizzard, fat, skin and excreta), the metabolites M550F006/M550F007 (Z67/Z69) were determined (in yolks, plasma, liver, kidney, muscle, heart, and excreta), indicating that the primary metabolic fate of dimethomorph in poultry is - similar to plants- the demethylation of one of the methoxy groups. In a second pathway, dimethomorph was found to be metabolized via oxidative stepwise degradation of the morpholine ring, resulting in the occurrence of metabolites M550F009 (synonyms Z93 or WL 376084), M550F011 (synonyms Z95 or CUR 7216), M550F008 (CUR 7117) and M550F005 (Z43).

The metabolism study in lactating goats showed that the major component of the extractable residue in plasma, liver, kidney, heart, muscle, and fat was the unchanged parent compound. In addition, mainly M550F006/M550F007 (Z67 and Z69) were detected, indicating that also in goats dimethomorph was metabolized via demethylation of the two methoxy groups. As an alternative pathway, again the stepwise degradation of the morpholine ring was observed, leading to the occurrence of metabolites M550F009 (WL 376084), M550F005 (Z43), and M550F008 (CUR 7117, in milk).

From these results it can be concluded that the metabolic pathways in goats and hens were similar and proceeds along two routes:

1. Demethylation of the two methoxy groups of the dimethoxyphenyl ring to produce M550F006 (Z67)and M550F007 (Z69). These two metabolites most likely undergo further transformation by forming conjugates with their free hydroxyl moiety.

2. Ring-opening and stepwise degradation of the morpholine ring producing metabolites M550F009 (WL 376084), M550F011 (CUR 7216), M550F008 (CUR 7117), and M550F005 (Z43).

Based on the results of these metabolism studies, the **parent molecule dimethomorph** (BAS 550 F) was defined as **relevant residue** in **animal matrices** for both **monitoring and risk assessment** (for poultry and milk to be considered as default residue definition).

"The expert meeting proposed dimethomorph as default residue definition for monitoring and risk assessment. It must however be kept in mind that this definition should be revised for milk and poultry product in case of animal exposure exceeding the trigger value of 0.1 mg/kg feed." (*EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph)*

All the available studies were carried out with the radiolabel in the chlorophenyl ring. Also, due to the technology applied (only TLC), metabolite identification and quantification is not satisfying current standards and also no information on the E/Z isomer ratio was generated. Moreover, for some tissues bound residues were not further investigated. In order to generate information on the metabolic fate of the morpholine moiety, and to address all other mentioned points, new metabolism studies were conducted in both livestock species. Both studies were performed with the chlorophenyl and morpholine ring label in parallel to have a better comparability of the two labels. These new studies supplement the available and already peer-reviewed data.

In the following, ~~short summaries~~ full OECD summaries of the already evaluated studies are shown for reasons of convenience, along with the summaries of the new studies.

CA 6.2.2 Poultry

[redacted] 1990: ^{14}C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens (DocID DK-440-003)
[redacted] 1991: ^{14}C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens-First amendment to report (DocID DK-440-007)

Three groups of laying hens were orally dosed twice daily (except for last day, one dose) with a target dose of 2.0 mg ^{14}C-dimethomorph (chlorophenyl label)/kg of body weight for 7 consecutive days. Each hen received 15 oral doses. Assuming a feed consumption of 0.1 kg/day and a mean body weight of 2 kg, the daily target dose of 2.0 mg/kg bw/d would correspond to a nominal dietary burden of 40 mg/kg feed dry matter. Hens of the various groups were sacrificed at different time points according to the type of samples and their purpose. Animals of group 1 were sacrificed after 3, 5, and 8 hours post last dose to determine blood peak levels. Animals of group 2 were sacrificed after 4, 5, 6 and 7 days in order to monitor depuation of blood levels. Animals of group 3 were sacrificed at 3 hours post last dose (= blood peak level) to collect excreta, tissues and eggs. Finally, a fourth dose group was present to monitor depuration of residues in eggs for up to 12 days after last dose. Extraction of egg yolk, white and plasma was done after acetone precipitation with methanol/water and subsequent acetone/acidic water extraction.
Fat with skin was extracted with Dichloromethane. The other tissues were first incubated with phosphate buffer at 37.5°C for 18 hours, then extracted multiple times with methanol and methanol/water and then submitted to a Soxhlet extraction.
Metabolite analysis and identification was done by TLC and comparison with reference standards.

Findings
Excretion of radioactivity was very effective with 84.8% of the administered dose recovered in excreta. Less than 0.1% of the dose was found in eggs, 0.4% was allocated to organs and tissues. Absorption of ^{14}C-dimethomorph was rapid as blood peak levels were 0.129 and 0.292 mg/kg three hours after the first and 15th administration.

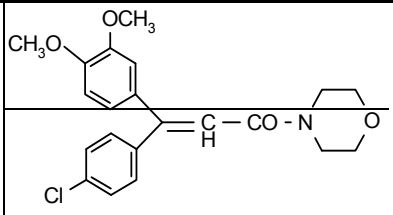
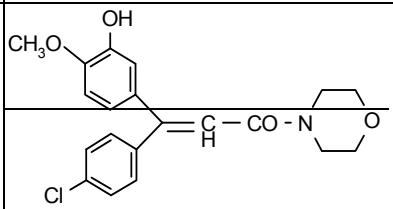
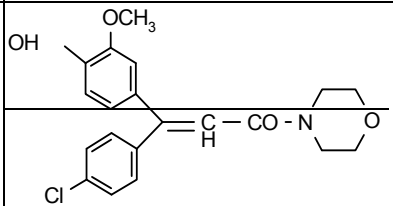
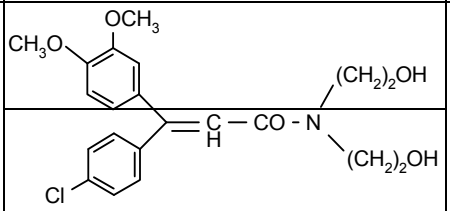
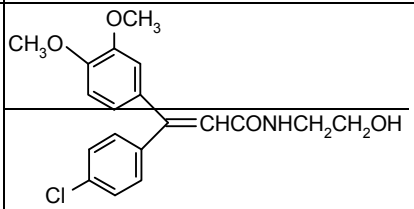
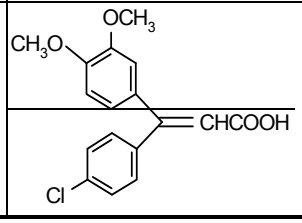
Table 6.2.2-1: Total radioactive residues (Group 3) in laying hens after dosing with ^{14}C-dimethomorph (nominal dose: 40 ppm based on feed intake), sacrifice 3 hours post last dose

Matrix	Chlorophenyl label TRR [mg/kg]*
Eggs	0.011-0.490**
Muscle	0.016
Fat with skin	0.037
Kidney	0.301
Heart	0.049
Gizzard	0.038
Blood	0.172
Plasma	0.158
Liver	1.051

* TRR values were corrected for background
** Represents residue levels in egg white, shells and yolk.

In heart, and gizzard, low concentrations of residual radioactivity (0.038-0.049 mg/kg) were found, also in muscle and fat with skin (0.016 and 0.037 mg/kg, respectively). The highest TRR was observed in liver and kidney with 1.051 and 0.301 mg/kg, respectively. In whites of eggs, radioactivity levels were very low (0.011 mg/kg) and a plateau level was reached after the third or fourth administration. In yolks, the level of radioactive residues was higher (0.381 mg/kg). At the end of the 12 day depuration period, the level approached close to background level (0.023 mg/kg). From eggs white, plasma and edible organs/tissues 73.9, 81.7% and 78-105.4% of radioactivity was extractable. This was achieved by multiple steps as described above. The identified metabolites and their quantities are summarized in the following table:

Table 6.2.2-2: Summary of metabolite identities and quantities in egg and edible matrices of laying hen tissues after dosing with ^{14}C-dimethomorph/chlorophenyl label

Metabolite code	Metabolite Identity	Egg yolks mg/kg (% TRR)	Liver mg/kg (% TRR)	Kidney mg/kg (% TRR)	Muscle mg/kg (% TRR)	Fat mg/kg (% TRR)
Dimethomorph BAS 550 F		ND	ND	ND	ND	0.017 (45.9)
M550F006 (Z67)		0.070 (18.4)	0.132 (12.6)	0.032 (10.6)	0.003 (18.8)	ND
M550F007 (Z69)						
M550F009 (WL 376084)		0.018 (4.72)	ND	ND	0.002 (12.5)	ND
M550F011 (CUR 7216)		0.038 (9.97)	ND	ND	ND	ND
M550F005 (Z43)		0.045 (11.8)	ND	0.015 (4.98)	ND	ND

In egg yolks, the major metabolite fraction M550F006/M55F007 (Z67/Z69) accounted for 0.07 mg/kg/18.3%TRR. Metabolite fractions M550F005 (Z43) (0.045 mg/kg), M550F011 (CUR 7216) (0.038 mg/kg) and M550F009 (WL 376084) (0.018 mg/kg) were also detected. In liver, the major metabolite fractions were M550F006/M55F007 (Z67/69) (0.132 mg/kg) and an unknown metabolite fraction L4 (0.105 mg/kg). In kidney, the major metabolite fractions were M550F006/M55F007 (Z67/Z69) and M550F005 (Z43) with 0.032 and 0.015 mg/kg. In muscle, heart, gizzard, fat and skin, all metabolite fractions were detected in minor amounts accounting together for 0.009-0.031 mg/kg. In muscle and heart, M550F006/M550F007 (Z67/Z69) (0.003 and 0.008 mg/kg), and M550F009 (WL 376084) (0.002 and 0.009 mg/kg) were detected, respectively. In gizzard and fat with skin, the parent compound ranged from 0.005-0.017 mg/kg.

Conclusion

The study demonstrated that dimethomorph and/or its metabolites are neither accumulated nor retained in laying hens after repeated oral administration. Besides the parent compound (fat, skin and excreta), metabolites M550F006/M550F007 (Z67/Z69) (eggs, liver, kidney, muscle,) was determined indicating that dimethomorph in laying hen was primarily metabolized via demethylation of one of the phenolic methoxy groups. Additionally, dimethomorph was metabolized via opening and degradation of the morpholine ring resulting in the occurrence of M550F009 (WL 376084) (eggs, muscle), M550F011 (CUR 7216) (eggs) as well as M550F005 (Z43) (eggs, kidney).

Report:	CA 6.2.2/1 [redacted] 1990 a 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens DK-440-003
Guidelines:	EPA 171-4, EPA 540/9-82-023
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Report:	CA 6.2.2/2 [redacted] 1991 a 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to laying hens DK-440-007
Guidelines:	EPA 171-4, EPA 540/9-82-023
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Description:	BAS 550 F (chlorophenyl label, *E:Z* 73:27) BAS 550 F (unlabeled, *E:Z* 49.5:50.5)
Lot/batch #:	S 1050 (chlorophenyl label) H 7879 (unlabeled)
Purity:	Radiochemical purity: Chlorophenyl label: 99.7% Chemical purity: Unlabeled: 99.2% Specific activity: Chlorophenyl label: 873 MBq/g Chlorophenyl dose: 373 MBq/g
CAS#:	110488-70-5
Development code:	Not reported
Stability of test compound:	The test item was stable for the test period.

2. Test animals

Species:	Laying hen
Variety:	White leghorn hybrids
Gender:	Female
Age at dosing:	25-38 weeks
Weight at dosing:	1.6-1.9 kg
Number of animals:	30 (4 groups)
Acclimation period:	at least 7 days

Diet:	Powdered hen feed 565 (No 364), *ad libitum* (KLIBA, Klingentalmuehle AG, CH-4303 Kaiseraugust, Switzerland)
Water:	Tap water, *ad libitum*
Housing:	Stainless steel metabolism cage

Environmental conditions	
Temperature:	21-23°C
Humidity:	38-74%
Photoperiod:	16 h light / 8 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral (intubation): Amount of dose:	2.0 mg/kg bw/day, 40 mg/kg feed (chlorophenyl label)
Vehicle:	Solution of cremophor, carboxymethyl-cellulose and NaCl by a Hamilton syringe connected to a stainless steel intubation tube
Timing:	Twice daily (once daily on the last days)
Duration:	8 consecutive days

2. Sample collection

Egg collection:	Twice daily (when available)
Excreta collection:	Daily
Interval from last dose to sacrifice:	3-8 h
Tissues harvested & analyzed:	Liver, kidney, muscle (breast and thigh), fat (omental, subcutaneous and renal), blood, heart, gizzard (without lining and contents), skin, cage wash

3. Test system

The metabolism and distribution of dimethomorph was investigated in 30 laying hens following a repeated oral administration of [p-chlorophenyl-^{14}C]-BAS 550 F at a mean dose level of 1.96-2 mg/kg bw/day for eight consecutive days. The test item was prepared in a solution of cremophor, carboxymethylcellulose and NaCl and administered orally by intubation using a Hamilton syringe connected to a stainless steel intubation tube. Details of the study design are summarized in Table 6.2.2-1.

Table 6.2.2-1: Overview of experimental design of [^{14}C]-BAS 550 F to laying hens

Animal number	Mean daily dose [mg/kg bw]	Treatment days	Sacrifice	Sampling
Hens 1-9	2.00	8	3, 5 and 8 h after last dosing	Blood: 0.5, 1, 2, 3, 5 and 8 h after the first and the last dose Final: Liver, kidney, muscle, fat
Hens 10-18	2.00	8	4, 5, 6 and 7 days after last dosing	Blood: twice daily 24 hours after each administration Final: Liver, kidney, muscle, fat
Hens 19-24	1.96	8	At blood peak level (3 hours post last dose)	Eggs: Twice daily Excreta: At 24 hour intervals Cage wash: After sacrifice Final: Blood/plasma, heart, liver, kidney, gizzard (without lining and contents), muscle (breast and thigh), fat (perirenal and omental), skin and carcass including the content of gizzard
Hens 25-30	1.96	8	Up to 12 days after last dosing	Eggs: Twice daily prior to dosing and during administration; 24 h intervals after last dosing during depuration phase of 12 days

4. Sampling and storage

Excreta and cage wash (using water and/or acetone) samples were collected prior to first dose and for each 24 h period until sacrifice. Eggs were collected during 24 hour prior dose administration and then collected (when available) at least twice daily. At approximately 3-8 hours post final dose the hens were sacrificed and edible tissues (heart, liver, kidney, muscle and fat), gizzard (without lining and contents), skin and the carcass including the content of gizzard were removed *post mortem*. Any whole eggs still in the oviduct at termination were collected. All samples were stored at approximately -20°C.

5. Description of analytical methods and extraction procedures

Radioanalysis: The radioactive residues were measured by liquid scintillation counting (LSC) analysis. Homogenized samples of blood/plasma, edible organs and tissues, egg yolks and whites were mixed with a tissue solubilizer prior to measurements. Solid debris (excreta, cage wash and egg shells) were analyzed by combustion analysis followed by LSC.

Thin-layer chromatography (TLC): TLC was applied for characterization of the metabolite patterns in egg yolks, plasma and extracts of organs, tissues and excreta. HPLC analysis coupled with radioactivity measurement (RAM) was used for the determination of the isomer ratio (E:Z) of the parent compound dimethomorph.

Extraction:

Samples of the different matrices were homogenized and extracted repeatedly with different solvents (see Table 6.2.2-2).

Egg yolk and plasma were extracted once with a mixture of methanol/water (8/2, v/v) and once with a mixture of acetone/water (1/1, v/v; adjusted at pH 2 with HCl) after protein precipitation with acetone. Due to low radioactive residue levels, *egg white* was not extracted after protein precipitation. After incubation with phosphate buffer, *liver and kidney* were extracted two times with methanol/water (8/2, v/v), once with methanol and exhaustively with methanol Soxhlet. *Muscle, heart and gizzard* were extracted as described for liver and kidney after incubation with phosphate buffer and exogenous enzymes obtained from rat liver and kidney. *Skin* was extracted two times with dichloromethane, once with methanol once with methanol/water (8/2, v/v) and exhaustively with methanol Soxhlet. *Fat* was extracted once with dichloromethane and *excreta* three times with methanol/water (8/2, v/v), once with methanol and exhaustively with methanol Soxhlet.

Additionally, to determine whether radioactive residues were acid-labile or conjugated with natural products and to characterize the aglycons, hydrolysis (with HCl) was performed on aqueous samples or protein pellets of egg yolk, plasma, liver, kidney and excreta.

The radioactive residues after solvent extraction were not further investigated.

Table 6.2.2-2: Overview of extractions performed on poultry matrices

Matrix	Solvent			
	Procedure 1	Procedure 2	Procedure 3	Procedure 4
Egg yolk	methanol/water (8/2, v/v)	acetone/water pH 2(1/1, v/v)	-	-
Plasma	methanol/water (8/2, v/v)	acetone/water pH 2 (1/1, v/v)	-	-
Liver	2x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	-
Kidney	2x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	-
Muscle	2x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	
Heart	2x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	
Gizzard	2x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	
Skin	2x dichloromethane	methanol	methanol/water (8/2, v/v)	methanol Soxhlet
Fat	dichloromethane	-	-	-
Excreta	3x methanol/water (8/2, v/v)	methanol	methanol Soxhlet	-

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in all matrices are summarized in Table 6.2.2-4 and the excretion balance of radioactive residues by hens after repeated dosing is given in Table 6.2.2-3.
At sacrifice, the recovered radioactive residues in excreta and cage wash amounted together to 87.8% of the administered dose. Negligible amounts were detected in eggs (<0.1% of the dose) and low amounts were present in edible organs/tissues and blood (0.4% of the dose).
The highest levels of radioactive residues were determined in excretory organs liver and kidney (1.051 mg/kg and 0.301 mg/kg, respectively) and low concentrations were recovered in edible organs/tissues (heart, gizzard, fat and skin) ranging from 0.037 mg/kg to 0.049 mg/kg. The lowest amount of radioactive residue was detected in muscle accounting for 0.016 mg/kg.
Similar levels of radioactive residues were determined for blood and plasma, indicating that no significant association of radioactive residues with blood cells occurred.
Albeit elevated radioactive residues were observed for egg yolk, these levels of radioactive residues decrease to background levels following a zero-order kinetics within 12 days after termination of repeated treatment. For egg white, overall very low levels of radioactive residues were recovered.

Table 6.2.2-3: Balance of radioactive residues and excretion pattern in laying hens after repeated dosing of BAS 550 F

Sample	Time after first administration [h]	Dose recovered in sample [% administered radioactivity]
Excreta	0-24	10.5
	24-48	10.4
	48-72	12.2
	72-96	13.0
	96-120	12.9
	120-144	12.4
	144-168 [1]	12.3
	168-171	1.1
Subtotal		84.8
Cage wash		2.9
Total excreted		87.8
Total in eggs	0-171	<0.1
Total in edible tissues/organs and blood	171 [2]	0.4
Total		**88.2**

1 Selected for further analysis
2 At sacrifice

Table 6.2.2-4: Total radioactive residues in hen matrices after repeated dosing of BAS 550 F

Matrix	TRR [mg/kg] [1]
Liver	1.051
Kidney	0.301
Muscle	0.016
Heart	0.049
Gizzard	0.038
Fat	0.037
Skin	0.037
Blood	0.188
Plasma	0.158
Egg yolk (pooled)	0.381
Egg white (pooled)	0.011

1 Corrected for background, except for blood

B. EXTRACTION OF RESIDUES

Table 6.2.2-5 contains the extractability of radioactive residues from hen matrices sampled after repeated dosing of BAS 550 F.
In eggs and plasma, the major amount of radioactive residues (73.9% - 84.5% TRR) was not bound to proteins after exhaustive extraction. In the edible organs/tissues, radioactive residues were almost completely extracted (78.0% - 105.4% TRR), indicating that in the laying hen BAS 550 F and / or its metabolites were not bound.
Radioactive residues in egg yolks, plasma, liver, kidney, muscle, heart, gizzard, fat, skin and excreta were further analyzed by TLC and characterized by their partition characteristics (Table 6.2.2-6).
The residual radioactive residue after solvent extraction ranged from 7.0% to 36.3% and was not further analyzed, except for egg yolk and plasma, where HCl hydrolysis was applied on RRR (Table 6.2.2-7).

Table 6.2.2-5: Extractability of hen matrices after [^{14}C]-BAS 550 F treatment

Matrix	Solvent extraction		Soxhlet		ERR[1]		RRR[2]		Total [3]	
	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR
Egg yolk	0.177	46.5	N/A	N/A	0.177 [4]	46.5 [4]	0.204	53.5	0.381	100.0
Egg white	0.009	81.7	N/A	N/A	0.009 [4]	81.7 [4]	0.002	18.3	0.011	100.0
Plasma	0.117	73.9	N/A	N/A	0.117 [4]	73.9 [4]	0.041	26.1	0.158	100.0
Liver	0.589	64.1	0.128	13.9	0.717 [5]	78.0 [5]	0.334	36.3	1.051	114.3
Kidney	0.200	75.2	0.035	13.2	0.235 [5]	88.4 [5]	0.066	24.6	0.301	113.0
Muscle	0.013	96.5	0.001	8.9	0.014 [5]	105.4 [5]	0.002	15.6	0.016	121.0
Heart	0.031	67.6	0.005	12.4	0.036 [5]	79.9 [5]	0.013	29.8	0.049	109.7
Gizzard	0.032	90.0	0.002	6.0	0.034 [5]	96.0 [5]	0.004	12.7	0.038	108.7
Fat	0.035	97.8	N/A	N/A	0.035	97.8	0.002	7.0	0.037	104.8
Skin	0.029	84.1	0.003	7.5	0.032	91.6	0.005	14.3	0.037	105.9
Excreta [6]	10.3	86.8	0.9	7.7	11.2	94.5	1.1	9.1	12.3	103.6

1 Extractable radioactive residue
2 Residual radioactive residues after solvent extraction
3 Sum of ERR + RRR
4 Including supernatant after protein precipitation
5 Including buffer incubation prior to extraction
6 Instead of [mg/kg] % of the applied dose is given
N/A Not applicable

Table 6.2.2-6: Partition of radioactive residues extracted from hen matrices

Solvent		Egg yolk		Plasma		Fat		Skin		Excreta	
		[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]
Organic extracts	Dichloromethane	37.2	0.142	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Hexane	N/A	N/A	N/A	N/A	11.8	0.004	8.5	0.003	1.0	0.1
	Acetonitrile	N/A	N/A	N/A	N/A	86.0	0.031	N/A	N/A	N/A	N/A
	Hexane/ethyl acetate	N/A	N/A	N/A	N/A	N/A	N/A	78.9	0.028	N/A	N/A
	Ethyl acetate	2.9	0.011	35.5	0.056	N/A	N/A	N/A	N/A	32.8	3.9
	Ethyl acetate, pH 1.0	1.4	0.005	27.7	0.044	N/A	N/A	N/A	N/A	18.0	2.1
	Water	1.9	0.007	10.7	0.017	N/A	N/A	N/A	N/A	N/A	N/A
Aqueous extract	Ethyl acetate	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	24.2	2.9
	Water	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	10.8	1.3

Solvent		Liver		Kidney		Muscle		Heart		Gizzard	
		[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]
Organic extracts	Hexane 1	9.9	0.091	8.1	0.022	1.1	<0.001	8.5	0.004	0.9	<0.001
	Hexane 2	0.9	0.008	1.3	0.003						
	Dichloromethane/ ethyl acetate	35.1	0.323	40.3	0.107	68.6	0.009	54.0	0.024	63.0	0.022
	Ethyl acetate, pH 1.0	4.4	0.040	8.4	0.022	N/A	N/A	N/A	N/A	N/A	N/A
Aqueous extract	Dichloromethane/ ethyl acetate	10.0	0.092	8.0	0.021	N/A	N/A	N/A	N/A	N/A	N/A
	Ethyl acetate, pH 7.0	0.9	0.008	0.7	0.002	N/A	N/A	N/A	N/A	N/A	N/A
	Water	9.4	0.087	6.8	0.018	11.6	0.002	10.0	0.004	14.5	0.005
	Precipitate	7.4	0.068	14.8	0.040	9.0	0.001	5.8	0.003	7.3	0.003

1 % of recovered radioactivity = % TRR
N/A Not applicable

Table 6.2.2-7: HCl hydrolysis of the RRR and subsequent partition of radioactive residues

Solvent	Egg yolk		Plasma	
	[%] [1]	[mg/kg]	[%] [1]	[mg/kg]
Ethyl acetate	35.7	0.136	11.4	0.018
Ethyl acetate, pH 7.0	2.3	0.009	2.3	0.003
Aqueous phase and final residue	15.5	0.059	12.4	0.020

1 % of recovered radioactivity = %TRR

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

Summaries of identified and characterized residues are shown in Table 6.2.2-8 to Table 6.2.2-10. A summary of all identified metabolites and their distribution in edible matrices is given in Table 6.2.2-11: . Identification was accomplished by comparison of obtained Rf-values with those of reference compounds. For quantification of labeled components, concentrated extracts and processed subsamples of egg yolk, plasma, liver, kidney, muscle, heart, gizzard, fat, skin and excreta were analyzed TLC.

In egg yolk, the major portion of radioactive residues was identified as M550F006/M550F007 (Z67/69) and accounted for 0.070 mg/kg. Metabolites M550F005 (Z43) and M550F011 (CUR 7216) amounted to 0.045 mg/kg and 0.038 mg/kg, respectively. Besides, metabolites M550F009 (WL 376084), M550F003 (Z7) and Z41 were detected at levels ranging from 0.009 mg/kg to 0.018 mg/kg.

In plasma, besides five unknown minor components (below or equal to 0.018 mg/kg), metabolites M550F006/M550F007 (Z67/69) and M550F005 (Z43) were detected and accounted for 0.022 mg/kg and 0.025 mg/kg, respectively.

In liver, the main portion amounted to 0.132 mg/kg and was identified as metabolites M550F006/M550F007 (Z67/69). The remaining not identified portions were below or equal to 0.080 mg/kg.

In kidney, metabolites M550F006/M550F007 (Z67/69) were identified accounting for the major portion (0.032 mg/kg). Additionally, metabolite M550F005 (Z43) was identified at a concentration of 0.018 mg/kg. The ten unknown components were detected being below or equal to 0.020 mg/kg.

In muscle, heart, gizzard, fat and skin, all metabolites were present at minor levels (below or equal to 0.017 mg/kg).

In muscle and heart, besides two minor components, M550F006/M550F007 (Z67/69; 0.003 mg/kg and 0.008 mg/kg) and M550F009 (WL 376084) (0.002 mg/kg and 0.009 mg/kg) were found. In gizzard, fat and skin, the parent compound BAS 550 F ranged from 0.005 mg/kg to 0.017 mg/kg. Three unknown components were present in a range from 0.001 mg/kg to 0.010 mg/kg. Only in gizzard, metabolite M550F009 (WL 376084) was additionally recovered amounting to 0.004 mg/kg.

In excreta, extracted radioactive residues amounted to 94.5% TRR (11.2% of the dose). The main portion yielded 1.7% of the dose and compromised metabolites M550F006/M550F007 (Z67/69). Besides ten unknown components, the parent compound BAS 550 F (0.2% of the dose) and metabolite M550F009 (WL 376084; 0.5% of the dose) were detected.

Table 6.2.2-8: Summary of all identified and characterized residues in eggs and plasma of laying hens after repeated dosing with BAS 550 F

Components	Egg yolk		Egg white		Plasma	
	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]
Identified						
BAS 550 F	-	-	N/A	N/A	-	-
M550F006/M550F007 (Z67/Z69)	18.3	0.070	N/A	N/A	14.0	0.022
M550F005 (Z43)	11.9	0.045	N/A	N/A	15.5	0.025
M550F009 (WL 376084)	4.6	0.018	N/A	N/A	-	-
M550F003 (Z7)	2.3	0.009	N/A	N/A	-	-
Z41	4.3	0.016	N/A	N/A	-	-
M550F011 (CUR 7216)	10.0	0.038	N/A	N/A	-	-
Total identified	**51.4**	**0.196**	**N/A**	**N/A**	**29.5**	**0.047**
Characterized						
Number of components	1		-		5	
Component with highest radioactive residue	3.2	0.012	-	-	11.6	0.018
Total Characterized	**3.2**	**0.012**	**81.7**	**0.009**	**45.1**	**0.071**
Total identified and/or characterized	**54.6**	**0.208**	**81.7**	**0.009**	**74.6**	**0.118**

N/A Not applicable

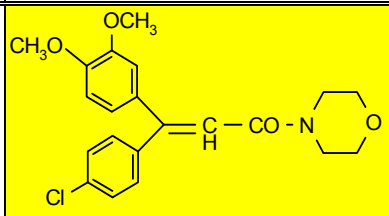
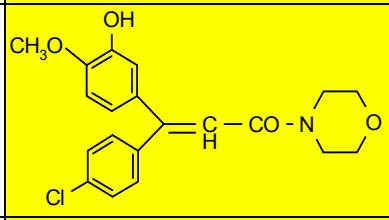
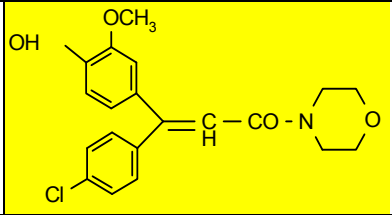
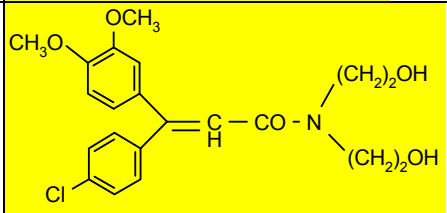
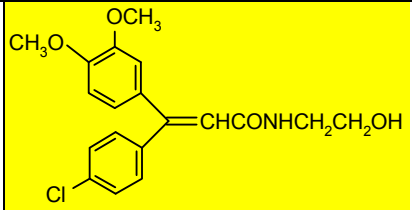
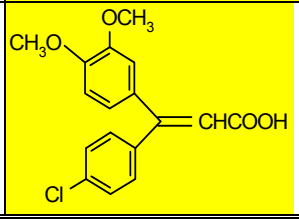
Table 6.2.2-9: Summary of all identified and characterized residues in tissues of laying hens after repeated dosing with [BAS 550 F

Components	Liver		Kidney		Muscle		Heart		Gizzard	
	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]
Identified										
BAS 550 F	-	-	-	-	-	-	-	-	13.6	0.005
M550F006/M550F007 (Z67/Z69)	14.4	0.132	12.0	0.032	24.6	0.003	18.9	0.008	-	-
M550F005 (Z43)	-	-	6.8	0.018	-	-	-	-	-	-
M550F009 (WL 376084)	-	-	-	-	13.5	0.002	19.9	0.009	10.8	0.004
Total identified	**14.4**	**0.132**	**18.8**	**0.050**	**38.1**	**0.005**	**38.8**	**0.017**	**24.4**	**0.009**
Characterized										
Number of components	11		10		2		2		3	
Component with highest radioactive residue	8.7	0.080	7.5	0.020	22.7	0.003	17.0	0.008	19.3	0.007
Total Characterized	**45.0**	**0.414**	**37.9**	**0.100**	**30.5**	**0.004**	**23.7**	**0.011**	**38.6**	**0.013**
Total identified and/or characterized	**59.4**	**0.546**	**56.7**	**0.150**	**68.6**	**0.009**	**62.5**	**0.028**	**63.0**	**0.022**

Table 6.2.2-10: Summary of all identified and characterized residues in fat, skin and excreta of laying hens after repeated dosing with BAS 550 F

Components	Fat		Skin		Excreta	
	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]
Identified						
BAS 550 F	47.3	0.017	24.2	0.009	1.7	0.2
M550F006/M550F007 (Z67/Z69)	-	-	-	-	14.0	1.7
M550F009 (WL 376084)	-	-	-	-	3.8	0.5
Total identified	**47.3**	**0.017**	**24.2**	**0.009**	**19.5**	**2.4**
Characterized						
Number of components	3		3		10	
Component with highest radioactive residue	27.7	0.010	26.7	0.009	16.9	2.0
Total Characterized	38.7	0.014	54.7	0.019	55.5	6.5
Total identified and/or characterized	**86.0**	**0.031**	**78.9**	**0.028**	**75.0**	**8.9**

Table 6.2.2-11: Summary of metabolite identities and quantities in egg and edible matrices of laying hen tissues after repeated dosing with BAS 550 F

Metabolite code	Metabolite Identity	Egg yolks mg/kg (%) TRR	Liver mg/kg (%) TRR	Kidney mg/kg (%) TRR	Muscle mg/kg (%) TRR	Fat mg/kg (%) TRR
Dimethomorph BAS 550 F		-	-	-	-	0.017 (47.3)
M550F006 (Z67)		0.070 (18.3)	0.132 (14.4)	0.032 (12.0)	0.003 (24.6)	-
M550F007 (Z69)						
M550F009 (WL 376084)		0.018 (4.6)	-	-	0.002 (13.5)	-
M550F011 (CUR 7216)		0.038 (10.0)	-	-	-	-
M550F005 (Z43)		0.045 (11.9)	-	0.018 (6.8)	-	-

D. STORAGE STABILITY

The storage stability of radioactive residues in matrix and extracts was investigated for egg yolk, liver, kidney and fat. Storage at -20 °C for up to 6 months did neither affect extractability, partition behaviour nor the metabolite pattern.

E. METABOLIC PATHWAY

BAS 550 F was extensively metabolized and efficiently excreted (87.8% of the dose) in the hens. An overview on quantified metabolites is provided in Table 6.2.2-11.
Metabolites M550F006/M550007 (Z67/69) were detected as the major metabolites of BAS 550 F, indicating that biotransformation in hens primarily occurs via demathylation of one of the phenolic methoxy groups. Additionally, biotransformation of BAS 550 F follows a step-wise ring cleavage of the morpholine mojety, whereby metabolites M550F009 (WL 376084), M550F005 (Z43) and M550F011 (CUR 7216) are formed.

III. CONCLUSION

The metabolism and distribution of dimethomorph was investigated in 30 laying hen following a repeated oral administration of [p-chlorophenyl-^{14}C]-BAS 550 F at a mean dose level of 1.96-2 mg/kg bw/day for eight consecutive days.
The study demonstrated that dimethomorph and/or its metabolites are neither accumulated nor retained in laying hens after repeated oral administration. Besides the parent compound (fat, skin and excreta), metabolites M550F006/M550F007 (Z67/Z69) (eggs, liver, kidney, muscle,) was determined indicating that dimethomorph in laying hen was primarily metabolized via demethylation of one of the phenolic methoxy groups. Additionally, dimethomorph was metabolized via opening and degradation of the morpholine ring resulting in the occurrence of M550F009 (WL 376084) (eggs, muscle), M550F011 (CUR 7216) (eggs) as well as M550F005 (Z43) (eggs, kidney).

Report: CA 6.2.2/3
[redacted] 2014 a
BAS 550 F: Metabolism in the laying hen
2015/1000604
Guidelines: 2004/10/EC of 11 February 2004, EPA 860.1300: Nature of the Residue in Plants Livestock, OECD Test Guideline 503 - Metabolism in livestock, EEC 91/414 (7030(VI/95 Rev. 3)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report: CA 6.2.2/4
[redacted] 2016 a
Report Amendment - BAS 550 F: Metabolism in the laying hen
2016/1002113
Guidelines: 2004/10/EC of 11 February 2004, EPA 860.1300: Nature of the Residue in Plants Livestock, OECD Test Guideline 503 - Metabolism in livestock, EEC 91/414 (7030(VI/95 Rev. 3)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Description: [p-chlorophenyl-^{14}C]-BAS 550 F (chlorophenyl label, *E:Z* 45:55)
[Morpholine-2,3-^{14}C]-BAS 550 F (morpholine label, *E*:*Z* 46:54)
[Acrolyl-^{13}C]-BAS 550 F (carbon-13 label; E:Z 38:62)
[Morpholine-^{15}N]-BAS 550 F (nitrogen-15 label, *E:Z* 44:56)
BAS 550 F (unlabeled, *E:Z* 44:56)

Lot/batch #: 1068-0101 (chlorpophenyl label)
858-0101 (morpholine label)
1070-0101 (carbon-13 label)
1071-0105 (nitrogen-15 label)
AC9978-68A (unlabeled)

Purity: Radiochemical purity:
Chlorophenyl label: 96.4%
Morpholine label: 98.6%
Chemical purity:
Carbon-13 label: 91.3%
Nitrogen-15 label: 97.3%
Unlabeled BAS 550 F: 97.6%
Specific activity:
Chlorophenyl label: 7.70 MBq/mg
Morpholine label: 5.74 MBq/mg
Chlorophenyl dose: 1.959 MBq/mg
Morpholine dose: 1.931 MBq/mg

CAS#: 110488-70-5
Development code: Not reported
Stability of test compound: The test item was stable for the test period for both labels.

2. Test Animals

Species: Laying hen
Variety: "Lohmann Brown"
Gender: Female
Age: Adult
Weight at dosing: 1.85 kg (chlorpophenyl label), 1,73 kg (morpholine label)
Number of animals: 22 (2 control, 10 per label)
Acclimation period: at least 21 days

Diet: 200 g concentrate, either Saracen RS Layer Pellets supplied by Saracen Horse Feed, (Lillico), Country Layer Pellets supplied by Heygates and Sons Country Feeds or OYTA Shells supplied by Van der Endt Louwerse
Water: Tap water, *ad libitum*
Housing: Metabolic cage with a mesh floor suitable with a mesh floor suitable for the separate collection of excreta

Environmental conditions
Temperature: 15-26°C
Humidity: 20-68%
Photoperiod: 12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:
Amount of dose: 1.06 mg/kg bw/day, 14.61 mg/kg feed (chlorophenyl label)
1.12 mg/kg bw/day, 15.83 mg/kg feed (morpholine label)
Food consumption: 0.136 kg DM/day (chlorophenyl label)
0.124 kg DM/day (morpholine label)
Vehicle: Gelatin capsule administered by forceps
Timing: Once daily
Duration: 14 consecutive days

2. Sample collection

Egg collection: Twice daily (when available)
Excreta collection: Daily
Interval from last dose to sacrifice: 3-6 h
Tissues harvested & analyzed: Liver, kidney, muscle (breast and thigh), fat (omental, subcutaneous and renal), blood, bile, GI tract and contents, carcass

Table 6.2.2-12: Overview of experimental design of [^{14}C]-BAS 550 F to laying hens

Animal number	Radio-label	Mean daily dose		Treatment days	Sacrifice	Sampling
		[mg/kg feed]	[mg/kg bw]			
Hens 01-10F	U-^{14}C-Chlorophenyl label	1.06	14.61	14	3-6 h after last dosing	Eggs: twice daily Excreta: at 24 hour intervals Cage wash: at 24 hour intervals Final: Liver, kidney, muscle (breast and thigh), fat (omental, subcutaneous and renal), blood, bile, GI tract and contents, carcass
Hens 11-20F	2,3-^{14}C-morpholine label	1.12	15.83	14	3-6 h after last dosing	

3. Test system

The metabolism and distribution of dimethomorph was investigated in 10 laying hen following a repeated oral administration of [p-chlorophenyl-^{14}C]-BAS 550 F or [morpholine-2,3-^{14}C]-BAS 550 F at respective mean dose levels of 1.06 mg/kg bw/day (14.61 mg/kg feed) or 1.12 mg/kg bw/day (15.83 mg/kg feed) for fourteen consecutive days. The test item was prepared in gelatin capsules and administered orally using a forceps. Details of the study outline are summarized in Table 6.2.2-12.

4. Sampling and storage

Excreta and cage wash (using methanol:water 1:1, v:v) samples were collected prior to first dose and for each 24 h period until sacrifice. Eggs were collected during 24 hour prior dose administration and then collected (when available) at least twice daily. At approximately 3-6 post final dose the hens were sacrificed and edible tissues (liver, kidney, muscle and fat), bile, blood, the GI tract and parts of the carcass were removed *post mortem*. Any whole eggs still in the oviduct at termination were collected. All other partially formed eggs present in the carcass were retained separately and were analyzed for determination of the total radioactive residue. All samples were stored at ca. –20°C.

5. Description of analytical methods

The radioactivity of homogenized extracts of the liquid samples (egg white, egg yolk, cage wash, blood, bile) was determined by LSC (liquid scintillation counting) analysis. Solid debris (excreta, GI tract content, tissues) were analyzed for radioactivity content by combustion analysis followed by LSC. Following the separate combustion for TRR analysis of all muscle types and all fat types the entire samples were combined, thoroughly mixed and analyzed as composite muscle and composite fat since the TRRs of the individual muscle types and fat depots were all in a similar range. Edible matrices were homogenized and extracted repeatedly with different solvents (see Table 6.2.2-13).

Liver, kidney and composite muscle were homogenized and extracted repeatedly with acetonitrile, acetonitrile:water and in case of the chlorophenyl label with methanol (all), water (liver and kidney) and ethanol (liver). In case of the morpholine label liver, kidney and composite were extracted with acetonitrile, acetonitrile:water and water, while liver was additional extracted with methanol and ethanol. For composite fat and yolk samples were extracted with dichloromethane and in case of fat (chlorophenyl label) additionally with ethyl acetate and in case of yolk (morpholine label) additional with ethyl acetate and acetone. Additionally to the first extraction, two more extraction regimes were performed to compare dichloromethane-, acetone- and acetonitrile based extractions (see Table 6.2.2-13).

Further investigation of non-solvent extractable residues in egg yolk, egg white, liver, kidney and fat (RRR) was conducted using protease and lipase enzymes.

Table 6.2.2-13: Overview of extractions performed in poultry

Matrix	Extraction solvent (repetitions)					
	Procedure 1	Procedure 2	Procedure 3	Procedure 4	Procedure 5	Procedure 6
Chlorophenyl label						
Egg yolk (Dichloromethane)	Dichloromethane (x2)	-	-	-	-	-
Egg yolk (Acetone)	Acetone (x2)	Acetone (x4)	-	-	-	-
Egg yolk (Acetonitrile)	Acetonitrile (x3)	-	-	-	-	-
Egg white	Acetonitrile (x2)	-	-	-	-	-
Liver	Acetonitrile (x3)	Acetonitrile:Water (4:1 v:v) (x4)	Methanol (x4)	Ethanol (x2)	Water (x2)	-
Kidney	Acetonitrile (x3)	Acetonitrile:Water (4:1 v:v) (x4)	Methanol (x3)	Water (x3)	-	-
Muscle	Acetonitrile (x3)	Acetonitrile:Water (4:1 v:v) (x3)	Methanol (x2)	-	-	-
Fat (Dichloromethane)	Dichloromethane (x3)	Ethyl Acetate (x1)	-	-	-	-
Fat (Acetone)	Acetone (x2)	Acetone (x2)	-	-	-	-
Fat (Acetonitrile)	Acetonitrile (x7)	-	-	-	-	-
Morpholine label						
Egg yolk (Dichloromethane)	Dichloromethane (x6)	Ethyl Acetate (x2)	Acetone (x2)	-	-	-
Egg yolk (Acetone)	Acetone (x7)	-	-	-	-	-
Egg yolk (Acetonitrile)	Acetonitrile (x7)	-	-	-	-	-
Egg white	Acetonitrile (x2)	Acetonitrile (x1)	Acetonitrile:Water (4:1 v:v) (x2)	Methanol (x1)	Water (x4)	Water (pH 2.5) (x2)
Liver	Acetonitrile (x3)	Acetonitrile:Water (4:1 v:v) (x4)	Methanol (x4)	Ethanol (x2)	Water (x2)	Water (pH 2.5) (x3)
Kidney	Acetonitrile (x3)	Acetonitrile:Water (4:1 v:v) (x4)	Water (x3)	Water (pH 2.5) (x2)	-	-
Muscle	Acetonitrile (x2)	Acetonitrile:Water (4:1 v:v) (x3)	Water (x4)	-	-	-
Fat (Dichloromethane)	Dichloromethane (x2)	-	-	-	-	-
Fat (Acetone)	Acetone (x3)	Acetone (x2)	-	-	-	-
Fat (Acetonitrile)	Acetonitrile (x8)	-	-	-	-	-

HPLC analysis was performed on relevant samples and extracts with a significant residue. Residues present in eggs and tissues following solvent extraction were investigated by HPLC and HPLC-MS/MS both using the same HPLC method (HPLC 2). Identification of metabolites was performed by accurate mass HPLC-MS/MS analyses. On-line radio-detection for quantification and fraction collection and TopCount analysis were used to confirm the assignment and quantification. Moreover the identity of some radiolabeled components was tested also by co-chromatography with the authentic reference items. The unretained polar fraction observed in the morpholine label samples was isolated and analysed by a second HPLC method, HPLC 3.

Metabolites were defined as identified, where a positive identification of a particular component was made using at least one chromatographic system in conjunction with mass spectrometric detection (accurate mass positive ion full scan and product ion evaluation including direct comparison with the fraction collected [^{14}C]-radio-chromatograms). Where the residue was unable to be identified or the residue was not large enough to warrant identification analysis, then the residue was classified as characterized.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in all matrices are summarized in Table 6.2.2-15. The radioactivity recovered in all matrices accounted for 95.12% (chlorophenyl label) and 90.60% (morpholine label) of the total dose (see Table 6.2.2-14). Until sacrifice, the radioactive residues excreted and cage wash amounted to 87.53% and 3.88%, respectively for the chlorophenyl label and 82.86% and 2.88%, respectively for the morpholine label. Furthermore in the gastro intestinal tract, its contents and in bile a total of 3.39 % (chlorophenyl label) and 3.25% (morpholine label) of the administered radioactivity was found. Radioactivity associated with kidney, muscle, fat and whole blood accounted in the chlorophenyl label for <0.01% and was in the range <0.01-0.05% of the administered dose in the morpholine label. Radioactive residues in egg yolk accounted for 0.14% (chlorophenyl label) or 0.64% (morpholine label) of the dosed radioactivity and reached a plateau concentration in the range of approximately 0.095 mg/kg (chlorophenyl label) or 0.537 mg/kg (morpholine label) within 11 or 10 days, respectively. Radioactive residues in egg white accounted for 0.01% (chlorophenyl label) or 0.27% (morpholine label) and in partially formed eggs for 0.05% (chlorophenyl label) or 0.31% (morpholine label). The radioactive residues in liver were 0.12% (chlorophenyl label) and 0.25% (morpholine label) of the dosed radioactivity.

Table 6.2.2-14: Recovery of radioactivity in laying hens following 14 consecutive administrations of [^{14}C]-BAS 550 F at a target dose level of 12 mg /kg dry weight diet/day

Sample	Dose recovered in sample [% administered radioactivity]	
	Chlorophenyl label	Morpholine label
Excreta	87.53	82.86
Cage wash	3.88	2.88
Egg yolk	0.14	0.64
Egg white	0.01	0.27
Liver	0.12	0.25
Kidney	<0.01	0.02
Breast muscle	<0.01	0.05
Thigh muscle	<0.01	0.04
Omental fat	<0.01	0.02
Subcutaneous fat	<0.01	0.01
Renal fat	<0.01	<0.01
G. I. Tract	1.42	1.72
G. I. Tract contents	1.87	1.51
Partially formed egg	0.05	0.31
Bile	0.10	0.02
Whole blood	<0.01	<0.01
Total	**95.12**	**90.60**

Table 6.2.2-15: Total radioactive residues in edible hen matrices

Matrix	Timing	Mean TRR [mg/kg]			
		Measured	Calculated	Measured	Calculated
		Chlorophenyl label		Morpholine label	
Liver	Terminal	0.770	0.710	1.186	1.143
Kidney	Terminal	0.196	0.197	0.747	0.652
Breast muscle	Terminal	0.011	-	0.128	-
Thigh muscle	Terminal	0.029	-	0.151	-
Composite muscle	Terminal	0.012	0.013	0.132	0.133
Omental fat	Terminal	0.018	-	0.290	-
Subcutaneous fat	Terminal	0.015	-	0.245	-
Renal fat	Terminal	0.033	-	0.406	-
Composite fat	Terminal	0.029 / 0.020*#	0.026 / 0.023*	0.359 / 0.318*#	0.353 / 0.323*
Egg yolk pool	168-312 hours	0.256	0.276 / 0.290*	1.294	1.397 / 1.380*
Egg white pool	168-312 hours	0.006	0.006	0.147	0.157

Calculated TRR= sum of ERR + RRR

* First value for DCM based extraction, second value for acetone based extraction

\# Generated a fresh pool of fat; as the renal fat was exhausted, the pool consisted of omental and subcutaneous fat only, combined in the ratio 2:1 (w:w)

B. EXTRACTION OF RESIDUES

The extractability of the edible tissues was ranging from 30.6% (egg white, morpholine label) to 95.5% (fat, chlorophenyl label) of the combusted TRR (see Table 6.2.2-16). In liver, where radioactivity accounted for 0.770 or 1.186 mg/kg (chlorophenyl label or morpholine label), 54.5% or 58.7% of the combusted TRR were extracted. An additional 35.9% and 37.4% (chlorophenyl label or morpholine label) of the TRR was released from liver by enzymatic digestion (see Table 6.2.2-8). In kidney radioactivity accounted for 0.196 or 0.747 mg/kg (chlorophenyl label or morpholine label) and 65.2% or 48.5% of the combusted TRR were extracted. Additional 49.0% and 40.2% (chlorophenyl label or morpholine label) of the TRR was released from kidney by enzymatic digestion. In egg yolk (2.56 mg/kg and 1.294 mg/kg) extracted radioactivity accounted for 40.4% TRR for the chlorophenyl label and ranged from 90.5% to 55.3% for the morpholine label depending on the extraction eluent. Additional 82.8% and 16.9% to 47.3% (chlorophenyl label or morpholine label) of the TRR was released from egg yolk by enzymatic digestion. In fat (0.020-0.029 mg/kg and 0.318-0.359 mg/kg; chlorophenyl label or morpholine label) extracted radioactivity ranged from 76% to 95.5% TRR for the chlorophenyl label and from 88.0% to 92.8% TRR for the morpholine label depending on the extraction eluent. Additional 10.2% (morpholine label) of the TRR was released from fat (dichlormethane based extraction referring to the 88%) by enzymatic digestion (see Table 6.2.2-17).
Residues in composite muscle were 0.012 mg/kg and 0.132 mg/kg (chlorophenyl label or morpholine label) and 81.7% and 40.5% were extracted. Further extraction by enzymatic digestion was attempted, but no useful data could be obtained. In egg white residues of 0.006 mg/kg and 0.147 mg/kg (chlorophenyl label or morpholine label) were determined and 91.0% and 30.6% were extracted. Further 73.8% of the TRR could be released by enzymatic digestion from egg white of the morpholine label (referring to 30.6%).
The residual radioactive residue after solvent extraction ranged from 8.8% to 75.5%. After successfully conducted enzymatic digestion final residues accounted for 0.4% to 3.4% of the respective TRR (see Table 6.2.2-17).
In conclusion, it can be stated that solvent extractability was generally rather modest for most matrices despite using many extraction steps. However, for all matrices enzyme treatment with protease and lipase liberated most of the bound radioactivity.

Table 6.2.2-16: Extractability of hen matrices after [^{14}C]-BAS 550 F treatment

Matrix	TRR combusted		ERR[1]		RRR[2]		Recovery
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Chlorophenyl label							
Liver	0.770	100.0	0.419	54.5	0.291	37.7	92.2
Kidney	0.196	100.0	0.127	65.2	0.070	35.9	101.1
Composite muscle	0.012	100.0	0.009	81.7	0.004	35.0	116.7[4]
Composite fat (dichlormethane extr.)	0.029	100.0	0.023	79.6	0.003	9.4	89.0
Composite fat (acetone extr)#	0.020	100.0	0.019	95.5	0.004	20.3	115.8[4]
Composite fat (acetonitrile extr.)*	0.020	100.0	0.016	81.3	-	-	-
Egg yolk pool (dichlormethane extr.)	0.256	100.0	0.104	40.6	0.176	68.6	109.2
Egg yolk pool (acetone extr.)	0.256	100.0	0.104	40.6	0.186	72.5	113.1
Egg yolk pool (acetonitrile extr.)*	0.256	100.0	0.058	22.7	-	-	
Egg white pool	0.006	100.0	0.005	91.0	0.001	22.0	113.0
Morpholine label							
Liver	1.186	100.0	0.695	58.7	0.448	37.8	96.5
Kidney	0.747	100.0	0.362	48.5	0.290	38.8	87.3
Composite muscle	0.132	100.0	0.054	40.5	0.079	60.0	100.5
Composite fat (dichlormethane extr.)	0.359	100.0	0.316	88.0	0.037	10.3	98.3
Composite fat (acetone extr)#	0.318	100.0	0.295	92.8	0.028	8.8	101.6
Composite fat (acetonitrile extr.)*	0.318	100.0	0.065	20.5	-	-	-
Egg yolk pool (dichlormethane extr.)	1.294	100.0	1.171	90.5	0.226	17.5	108.0
Egg yolk pool (acetone extr.)	1.294	100.0	0.716	55.3	0.664	51.3	106.6
Egg yolk pool (acetonitrile extr.)*	1.294	100.0	0.150	11.6	-	-	-
Egg white pool	0.147	100.0	0.046	30.6	0.111	75.5	106.1

1 ERR = Extractable radioactive residue
2 RRR = Residual radioactive residues (alternatively post extraction solid: PES)
3 Sum of ERR + RRR
4 Recovery based on low residues and multiple extraction steps
* The extraction was performed in order to assess the extractability of the radioactive residue in fatty matrices by acetonitrile. Analysis of the solid debris was not performed.
# Includes radioactivity in oil layer

Table 6.2.2-17: Summary of released non extractable radioactive residues in hen matrices

Matrix	RRR[1]		Protease digestion		Lipase digestion		Final residue		Recovery
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Chlorophenyl label									
Liver	0.291	37.7	0.136	17.6	0.139	18.1	0.004	0.5	96
Kidney	0.070	35.9	0.049	25.2	0.047	23.8	0.002	0.9	139[2]
Egg yolk pool (dichlormethane based extraction)	0.176	68.6	0.114	44.4	0.098	38.4	0.003	1.3	123[2]
Morpholine label									
Liver	0.448	37.8	0.262	22.1	0.181	15.3	0.005	0.4	100
Kidney	0.290	38.8	0.221	29.6	0.079	10.6	0.004	0.6	105
Composite muscle	0.079	60.0	-	-	-	-	-	-	-
Composite fat (dichlormethane based extraction)	0.037	10.3	0.025	7.1	0.011	3.1	0.001	0.4	103
Egg yolk pool (dichlormethane based extraction)	0.226	17.5	0.176	13.6	0.043	3.3	0.014	1.1	103
Egg yolk pool (acetone based extraction)	0.664	51.3	0.362	28.0	0.250	19.3	0.035	2.7	97
Egg White	0.111	75.5	0.108	73.8	NA	NA	0.005	3.4	102

1 Residual radioactive residues (alternatively post extraction solid: PES)
2 High recovery due to multiple extraction steps
3 Muscle RRR sample further investigated using enzymatic techniques, but no useful data was obtained

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

Summaries of identified and characterized residues are shown in Table 6.2.2-18 to Table 6.2.2-19 for the chlorophenyl label and in Table 6.2.2-20 to Table 6.2.2-21 for the morpholine label. A summary of all identified metabolites and their distribution in edible matrices is given in Table 6.2.2-22 (chlorophenyl label) and Table 6.2.2-23 (morpholine label). Identification was accomplished by HPLC in conjunction with mass spectrometric detection. For quantification of labeled components, concentrated extracts of eggs, liver, kidney, composite fat and composite muscle were analyzed directly by HPLC (using the same HPLC method as for LC-MS analysis).
In those cases where metabolites were co-eluting, each metabolite was assigned the entire peak amount as a worst case assumption.

Chlorophenyl label
In the dichloromethane extract of egg yolk (ERR: 0.100 mg/kg; see Table 6.2.2-20) one peak was identified, representing 0.017 mg/kg or 6.6% TRR, as a co-elution of the metabolites M550F006 (*Z*) and M550F012. The rest of the radioactivity was distributed among 12 peaks, of which no one was greater than 0.006 mg/kg (2.3% TRR). The acetone extraction (ERR: 0.104 mg/kg) provided a much higher degree of identification, with a total residue of 0.071 mg/kg (28.0% TRR) being identified. This residue was identified as unchanged BAS 550 F (*E*) (0.001 mg/kg; 0.5% TRR) and the following metabolites M550F012 (0.030 mg/kg; 11.8% TRR); M550F006 (*Z*) (0.024 mg/kg; 9.3% TRR); M550F028 (0.024 mg/kg; 9.4% TRR); M550F011 (0.016 mg/kg; 6.3% TRR) and M550F007 (*E*) (0.008 mg/kg; 3.1% TRR).
In addition, the following metabolites were also observed: M550F022 (0.008 mg/kg; 3.1% TRR); M550F038 (0.008 mg/kg; 3.1% TRR); M550F018 (0.004 mg/kg; 1.5% TRR); M550F091 (0.004 mg/kg; 1.5% TRR) and M550F033 (0.004 mg/kg; 1.7% TRR). Here, the residual radioactivity was distributed among 4 peaks, accounting for max. 0.003 mg/kg or 1.2% TRR.

Analysis of the liver extract (ERR: 0.419 mg/kg) identified a total residue of 0.170 mg/kg (22.3% TRR). This residue was identified as M550F013 (0.047 mg/kg; 6.3% TRR), M550F012 (0.042 mg/kg; 5.4% TRR), M550F028 (0.030 mg/kg; 3.8% TRR) and M550F006 (*Z*) and M550F008 (*Z*), each representing 0.029 mg/kg (3.7% TRR), as they co-eluted.
Other observed metabolites included M550F069 (0.025 mg/kg; 3.3% TRR); either M550F049 or M550F035 (0.022 mg/kg; 3.0% TRR); M550F011 (0.018 mg/kg; 2.3% TRR); M550F007 (*E*) (0.016 mg/kg; 2.0% TRR); M550F033 (0.015 mg/kg; 2.0% TRR) and M550F062 and M550F074 or M550F076 each representing 0.012 mg/kg (1.6% TRR), as they co-eluted. M550F038 (0.012 mg/kg; 1.5% TRR) and M550F022 (0.009 mg/kg; 1.2% TRR) were also observed. The residual extracted radioactivity was distributed among numerous components (43 to 44 peaks), of which the biggest accounted for 0.012 mg/kg or 1.6% TRR.

Analysis of the kidney extract (ERR: 0.127 mg/kg) identified a total residue of 0.024 mg/kg (12.3% TRR). In this extract, the following metabolites were identified: M550F022 (0.012 mg/kg; 6.1% TRR), M550F012 (0.012 mg/kg; 6.2% TRR), M550F008 (*Z*) (0.009 mg/kg; 4.8% TRR) and M550F006 (*Z)* (0.003 mg/kg; 1.4% TRR). Also in this matrix the residual extracted radioactivity was distributed among numerous components, with (13 peaks, no one greater than 0.008 mg/kg, 4.1% TRR).

Analysis of the fat extract was performed twice, firstly following a dichloromethane extraction (ERR: 0.023 mg/kg), and then with an acetone extraction (ERR = 0.019 mg/kg).
In the dichloromethane extract, two late eluting components were observed (with similar retention times as the parent compound), which could not be unambiguously identified, however, each peak was <0.010 mg/kg. The acetone extract was unfortunately unsuitable for chromatographic analysis.

The egg white extract (ERR: 0.005 mg/kg) and the muscle extracts (ERR = 0.009 mg/kg; distributed between three extracts, all <0.010 mg/kg) were not further analyzed due to their low levels of radioactivity.

The non-extractable residues after solvent extraction of egg yolk, liver and kidney were 68.6, 37.7 and 35.9% TRR, respectively and were therefore further investigated with protease and lipase enzymes, whereby the bound radioactivity was nearly completely released (see Table 6.2.2-19). This demonstrates that the majority of the non-extractable radioactivity was bound, or incorporated, into the endogenous protein and lipid fraction. Solubilized radioactivity was characterized by HPLC, but warranted no further identification information.

Morpholine label
The dichloromethane extract of the egg yolk (ERR: 1.171 mg/kg) was unable to provide any identification of the extracted radioactivity. 21 peaks were observed, of which the most predominant one was a polar fraction, unretained on the first chromatographic system (accounting for 0.041 mg/kg or 3.1% TRR). Chromatography on HPLC 3 showed that this polar region contained at least two components, one of which had the same retention time as morpholine. This component represented a residue of 0.023 mg/kg (1.7% TRR).
The acetone extraction of egg yolk (ERR: 0.716 mg/kg) was able to provide a much higher degree of identification, with a total residue of 0.200 mg/kg (15.4% TRR) being identified. This residue was identified as the two isomeric forms of parent compound BAS 550 F. The residual extracted radioactivity was distributed among 13 peaks, no single peak accounting for more than 0.008 mg/kg or 0.6% TRR.

Analysis of the egg white extract (ERR: 0.046 mg/kg) revealed a multitude of components, the largest of which represented an unretained, polar fraction accounting for 0.012 mg/kg (8.2% TRR). Analysis with HPLC3 showed that this fraction contains a single peak, which did not co-chromatograph with morpholine. All other characterized peaks represented no more than 0.002 mg/kg or 1.3% TRR each.

Analysis of the liver extract (ERR: 0.695 mg/kg) provided identification for components representing a residue of 0.051 mg/kg (4.3% TRR). The largest component represented a residue of 0.013 mg/kg (1.1% TRR) and was identified as a co-elution of M550F033 and M550F013. Other observed metabolites were largely the same as seen in the liver of the chlorophenyl label (M550F028, M550F011, M550F007, M550F069, M550F049/M550F035), each accounting for <0.010 mg/kg and <0.1% TRR.
The characterized residue consisted of at least 63 components, of which none represented more than 0.008 mg/kg and 0.7% TRR, except one unretained, polar fraction (0.265 mg/kg; 22.4% TRR). Upon chromatography on HPLC 3, at least 10 separate radioactive components were observed, the largest of which accounted for 0.052 mg/kg (4.5% TRR). One component had the same retention time as morpholine, representing a residue of 0.003 mg/kg (0.3% TRR).

Analysis of the kidney extract (ERR: 0.362 mg/kg) showed eight peaks, the largest single component representing 0.008 mg/kg (1.0% TRR) except one unretained, polar fraction (0.258 mg/kg; 34.6% TRR). This fraction was collected and re-analyzed on HPLC 3, whereby six separate radioactive components were observed, with the largest residue associated to a single component being 0.173 mg/kg (23.2% TRR). One component had the same retention time as morpholine, representing a residue of 0.011 mg/kg (1.5% TRR).

Analysis of the muscle extract (ERR: 0.054 mg/kg) showed three components, the largest of which was an unretained, polar fraction (0.038 mg/kg; 28.7% TRR). This fraction was collected and re-analyzed with HPLC 3, showing that it consist of at least 4 components, the largest of which accounted for 0.015 mg/kg (11.6% TRR).

In the acetone extract of fat (ERR: 0.295 mg/kg) the only component identified was BAS 550 F, occurring as the two isomeric forms (*E* and *Z*) and accounting for 0.038 mg/kg (12.1% TRR). Also in the dichloromethane extract of fat two peaks were observed with the same retention time as the E and Z isomer of dimethomorph, accounting in sum for 0.029 mg/kg and 8.1% TRR.

The non-extractable residues after solvent extraction of liver, kidney, fat, egg yolk (dichloromethane extraction), egg yolk (acetone extraction) and egg white were 37.8, 38.8, 10.3, 17.5, 51.3 and 75.5% TRR, respectively and were therefore further investigated with protease and lipase enzymes, where applicable, whereby the bound radioactivity was nearly completely released (see Table 6.2.2-19). This demonstrates that the majority of the non-extractable radioactivity was bound, or incorporated, into the endogenous protein and lipid fraction. The solubilized radioactivity was further characterized by HPLC. In all cases, the enzyme treatment released a polar fraction, which was then shown (by HPLC 3) to contain in some cases free morpholine as a minor component.

Table 6.2.2-18: Summary of identified and characterized residues in eggs of laying hens after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Components	Egg yolk (dichlormethane based)		Egg yolk (acetone based)		Egg white	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified						
BAS 550 F (*E*)	-	-	0.5	0.001	-	-
M550F012 [42.1 min]	-	-	2.5	0.006	-	-
M550F033	-	-	1.7	0.004	-	-
Co-elutions of: M550F006 (*Z*) [44.7 min] M550F012 [44.6 min]	6.6	0.017	9.3	0.024	-	-
M550F011 [38.6 min] M550F028 [38.5 min]	-	-	3.2	0.008	-	-
M550F022 [37.1 min] M550F028 [37.0 min]	-	-	3.1	0.008	-	-
M550F038 [39.4 min] M550F028 [39.4 min]	-	-	3.1	0.008	-	-
M550F011 [40.9 min] M550F007 (*E*) [41.3 min]	-	-	3.1	0.008	-	-
M550F091 [40.2 min] M550F018 [40.1 min]	-	-	1.5	0.004	-	-
Total identified	**6.6**	**0.017**	**28.0**	**0.071**	NA	NA
Characterized						
Number of peaks	12		4		-	
Peak with highest radioactive residue	2.3	0.006	1.3	0.003	-	-
Characterized by extraction	-	-	8.4	0.022	91.0	0.005
Total characterized	11.7	0.031	12.7	0.33	91.0	0.005
Total identified and/or characterized	**18.5**	**0.047**	**40.6**	**0.104**	**91.0**	**0.005**
Extracted by enzyme digestion	82.8	0.212	-	-	-	-
Final residue	1.3	0.003	72.5	0.186	22.0	0.001
Grand total*	**109.2**	**0.276**	**113.1**	**0.290**	**113.0**	**0.006**

* Including radioactive losses during extraction procedure (data not shown)
NA Not analyzed by HPLC; residue in extract <0.010 mg/kg

Table 6.2.2-19: Summary of identified and characterized residues in tissues of laying hens after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Components	Liver		Kidney		Composite muscle		Composite fat (dichlormethane based)		Composite fat (acetone based)	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified										
M550F007 (*E*)	2.0	0.016	-	-	-	-	-	-	-	-
M550F012 [42.1 min]	1.7	0.013	-	-	-	-	-	-	-	-
M550F022	1.2	0.009	6.4	0.012	-	-	-	-	-	-
M550F033	2.0	0.016	-	-	-	-	-	-	-	-
Co-elutions of: M550F012 [44.6 min] M550F006 (*Z*) [44.7 min] M550F008 (*Z*) [44.5 min][a]	3.7	0.029	-	-	-	-	-	-	-	-
M550F012 [44.6 min] [b] M550F008 (*Z*) [44.5 min	-	-	4.8	0.009	-	-	-	-	-	-
M550F012 [44.6 min] [b] M550F006 (*Z*) [44.7 min]	-	-	1.4	0.003	-	-	-	-	-	-
M550F011 [38.6 min] M550F028 [38.5 min]	2.3	0.018	-	-	-	-	-	-	-	-
M550F074 **or** M550F076 [30.7 min] M550F062 [30.2 min]	1.6	0.012	-	-	-	-	-	-	-	-
M550F069 [28.6 min] M550F013 [29.7 min]	3.3	0.025	-	-	-	-	-	-	-	-
M550F013 [32.8 min] M550F049 **or** M550F035 [33.3 min]	3.0	0.022	-	-	-	-	-	-	-	-
M550F038 [39.4 min] M550F028 [39.4 min]	1.5	0.012	-	-	-	-	-	-	-	-
Total identified	**22.3**	**0.170**	**12.3**	**0.024**	**NA**	**NA**	**<0.1**	**<0.001**	**NA**	**NA**
Characterized										
Number of peaks	43-44		13		-		2		-	
Peak with highest radioactive residue	1.6	0.012	4.1	0.008	-	-	28.7	0.008	-	-
Characterized by extraction	3.5	0.027	7.6	0.016	81.7	0.009	3.1	0.001	20.1	0.004
Total identified and/or characterized	**48.3**	**0.372**	**53.7**	**0.106**	81.7	0.009	**55.8**	**0.016**	**20.1**	**0.004**
Extracted by enzyme digestion	35.7	0.275	49.0	0.096	-	-	-	-	-	-
Final residue	0.5	0.004	0.9	0.002	35.0	0.004	9.4	0.003	20.3	0.004
Grand total*	**92.2**	**0.710**	**101.1**	**0.197**	**116.7**	**0.013**	**89.0**	**0.026**	**109.3**	**0.023**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)
a Component not observed in extract 3
b For M550F012, this is the same isomer

Table 6.2.2-20: Summary of identified and characterized residues in eggs of laying hens after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Components	Egg yolk (dichlormethane based)		Egg yolk (acetone based)		Egg white	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified						
BAS 550 F (*E*)	-	-	8.8	0.114	-	-
BAS 550 F (*Z*)	-	-	6.6	0.086	-	-
Total identified	**NA**	**NA**	**15.4**	**0.200**	**NA**	**NA**
Characterized						
Number of peaks-HPLC 2	21		13		23	
Peak with highest radio-active residue	1.3	0.017	0.6	0.008	1.3	0.002
Number of peaks-HPLC 3	2		-		1	
Peak with highest radioactive residue	1.7	0.023	-	-	8.2	0.012
Characterized by extraction	-	-	-	-	4.0	0.007
Total identified and/or characterized	**7.4**	**0.092**	**20.2**	**0.264**	**24.8**	**0.037**
Extracted by enzyme digestion	16.9	0.219	47.3	0.612	73.8	0.108
Final residue	1.1	0.014	2.7	0.035	3.4	0.005
Grand total*	**108.0**	**1.397**	**106.6**	**1.380**	**106.1**	**0.157**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)
Number of peaks - HPLC 2 do not contain peaks, which were included in Number of peaks - HPLC3

Table 6.2.2-21: Summary of identified and characterized residues in tissues of laying hens after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Components	Liver		Kidney		Composite muscle		Composite fat (dichlormethane based)		Composite fat (acetone based)	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified										
BAS 550 F (*E*)	-	-	-	-	-	-	-	-	7.5	0.024
BAS 550 F (*Z*)	-	-	-	-	-	-	-	-	4.6	0.014
M550F033	0.6	0.007	-	-	-	-	-	-	-	-
M550F069	0.7	0.008	-	-	-	-	-	-	-	-
Co-elutions of: M550F013 [29.7 min] M550F049 or M550F035 [30.2 min]	0.6	0.008	-	-	-	-	-	-	-	-
M550F033 [32.7 min] M550F013 [32.8 min]	1.1	0.013	-	-	-	-	-	-	-	-
M550F028 [38.5 min] M550F011 [38.6 min]	0.6	0.007	-	-	-	-	-	-	-	-
M550F007 (*E*) [41.3 min] M550F011 [40.9 min]	0.7	0.008	-	-	-	-	-	-	-	-
Total identified	**4.3**	**0.051**	**<0.1**	**<0.001**	**NA**	**NA**	**NA**	**NA**	**12.1**	**0.038**
Characterized										
Number of peaks-HPLC 2	63+unassigned peaks		7		2		2[b]		-	
Peak with the highest residue	0.7	0.008	1.0	0.008	0.4	0.001	4.9	0.017	-	-
Number of peaks-HPLC 3	10		6		4		-		-	
Peak with highest residue	4.5	0.052	23.1	0.173	11.6	0.015	-	-	-	-
Characterized by extraction	-	-	-	-	-	-	-	-	47.2	0.150
Total identified and/or characterized	**37.9**	**0.450**	**37.4**	**0.279**	**29.3**	**0.039**	**8.1**	**0.029**	**59.3**	**0.188**
Extraction by enzyme digestion	37.4	0.443	40.2	0.300	-[a]	-[a]	10.2	0.036	-	-
Final residue	0.4	0.005	0.6	0.004	60.0[a]	0.079[a]	0.4	0.001	8.8	0.028
Grand total*	**96.5**	**1.143**	**87.3**	**0.652**	**100.5**	**0.133**	**98.3**	**0.353**	**101.6**	**0.323**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)
a Enzyme digestion was attempted, but no results were obtained
b Both peaks refer to the two isomer of parent
Number of peaks - HPLC 2 do not contain peaks, which were included in Number of peaks - HPLC3

Table 6.2.2-22: Summary of identified residues in hen after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

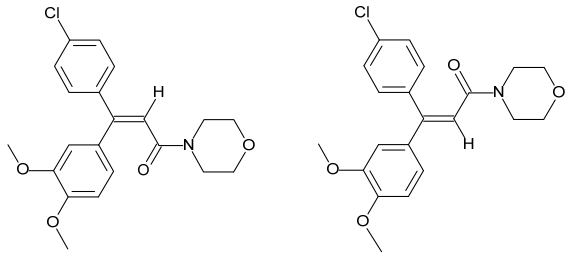
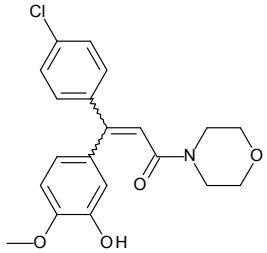
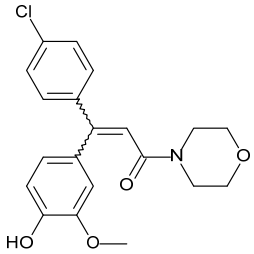
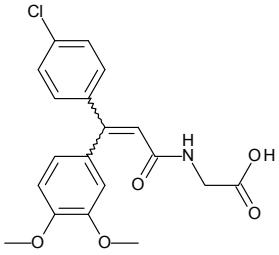
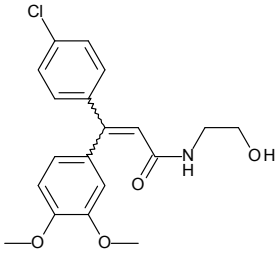
Metabolite	Structure	Egg yolk (dichlor-methane)	Egg yolk (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 550 F (*E*)		ND	0.001 (0.5)	ND	ND
BAS 550 F (*Z*)		ND	ND	ND	ND
Sum E +Z		*ND*	*0.001 (0.5)*	*ND*	*ND*
M550F006 (*E*)		ND	ND	ND	ND
M550F006 (*Z*)		0.017[A] (6.6)	0.024[A] (9.3)	0.029[A] (3.7)	0.003[A] (1.4)
M550F007 (*E*)		ND	0.008[A] (3.1)	0.016 (2.0)	ND
M550F007 (*Z*)		ND	ND	ND	ND
M550F008 (*E*)		ND	ND	ND	ND
M550F008 (*Z*)		ND	ND	0.029[A] (3.7)	0.009[A] (4.8)
M550F011 (sum of isomers)		ND	0.016[A] (6.3)	0.018[A] (2.3)	ND

Table 6.2.2-22: Summary of identified residues in hen after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

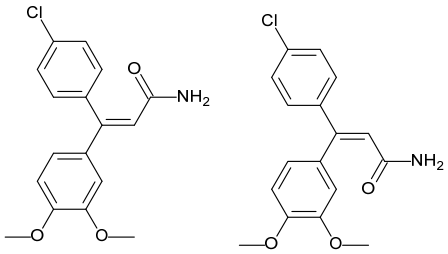
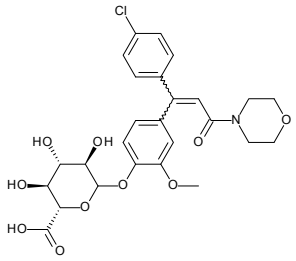
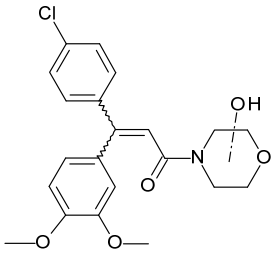
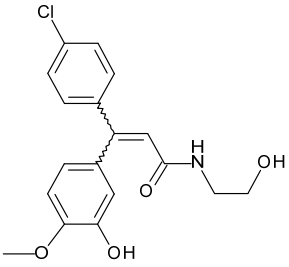
Metabolite	Structure	Egg yolk (dichlor-methane)	Egg yolk (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F012 (sum of isomers)		0.017[A] (6.6)	0.030[A] (11.8)	0.042[A] (5.4)	0.012[A] (6.2)
M550F013 (sum of isomers)		ND	ND	0.047[A] (6.3)	ND
M550F018 (sum of isomers)		ND	0.004[A] (1.5)	ND	ND
M550F022 (sum of isomers)		ND	0.008[A] (3.1)	0.009 (1.2)	0.012 (6.1)

Table 6.2.2-22: Summary of identified residues in hen after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

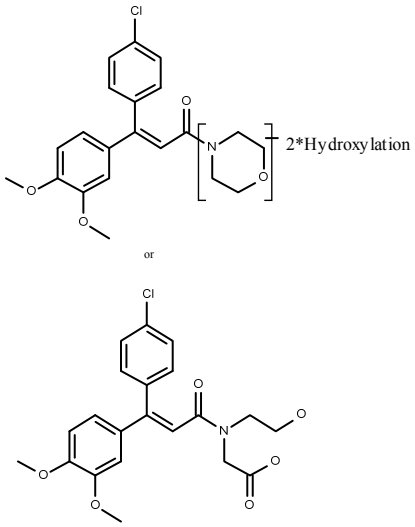
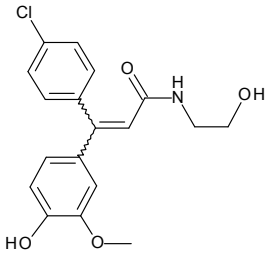
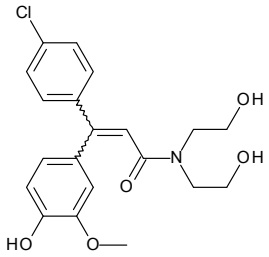
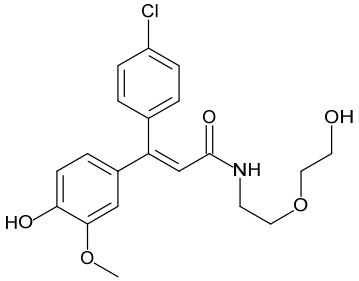
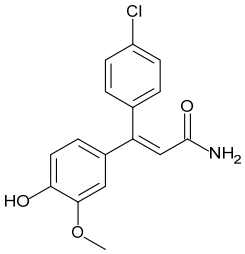
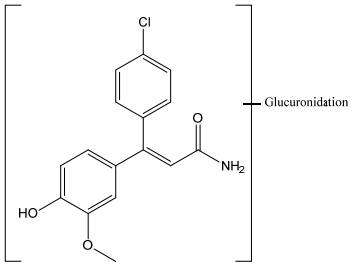
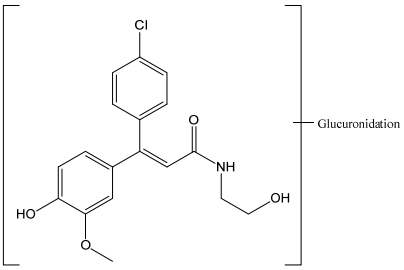
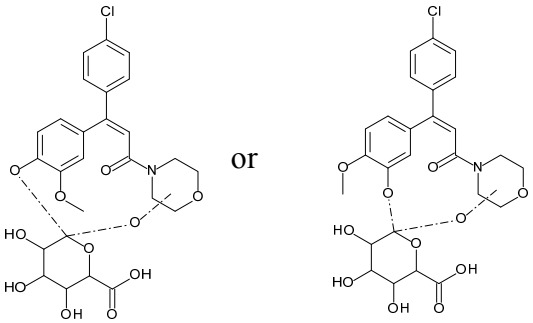
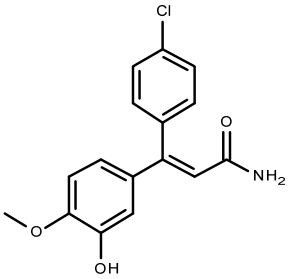
Metabolite	Structure	Egg yolk (dichlor-methane)	Egg yolk (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F028 (sum of isomers)		ND	0.024[A] (9.4)	0.030[A] (3.8)	ND
M550F033 (sum of isomers)		ND	0.004 (1.7)	0.015 (2.0)	ND
M550F035 **OR** M550F049	 **OR** 	ND	ND	0.022[A] (3.0)	ND

Table 6.2.2-22: Summary of identified residues in hen after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Metabolite	Structure	Egg yolk (dichlor-methane)	Egg yolk (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F038 (sum of isomers		ND	0.008[A] (3.1)	0.012[A] (1.5)	ND
M550F062		ND	ND	0.012[A] (1.6)	ND
M550F069		ND	ND	0.025[A] (3.3)	ND
M550F074 **OR** M550F076		ND	ND	0.012[A] (1.6)	ND
M550F091 (sum of isomers)		ND	0.004[A] (1.5)	ND	ND

ND Not Detected
A Co-eluting component

Table 6.2.2-23: Summary of identified residues in hen after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

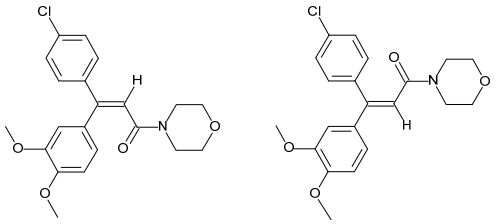
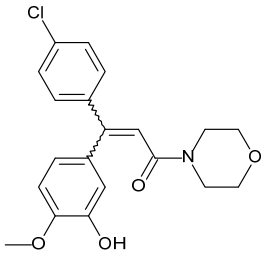
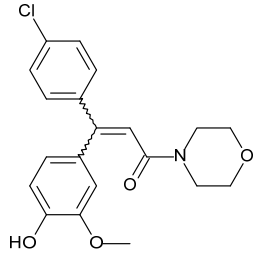
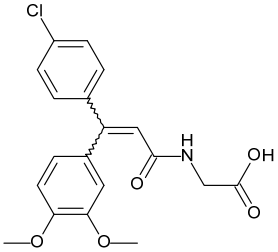
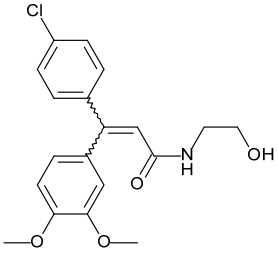
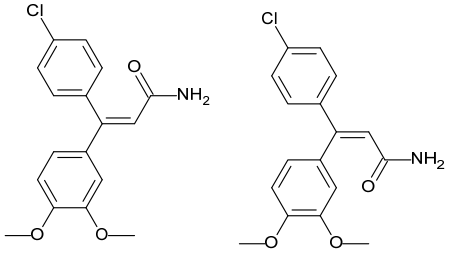
Metabolite	Structure	Egg yolk (acetone)	Liver	Fat (acetone)
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR
BAS 550 F (*E*)		0.114 (8.8)	ND	0.024 (7.5)
BAS 550 F (*Z*)		0.086 (6.6)	ND	0.014 (4.6)
Sum E+Z		*0.200 (15.4)*	*ND*	*0.038 (12.1)*
M550F006 (*E*)		ND	ND	ND
M550F006 (*Z*)		ND	ND	ND
M550F007 (*E*)		ND	0.008[A] (0.7)	ND
M550F007 (*Z*)		ND	ND	ND
M550F008 (*E*)		ND	ND	ND
M550F008 (*Z*)		ND	ND	ND
M550F011 (sum of isomers)		ND	0.015[A] (1.3)	ND
M550F012		ND	ND	ND

Table 6.2.2-23: Summary of identified residues in hen after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

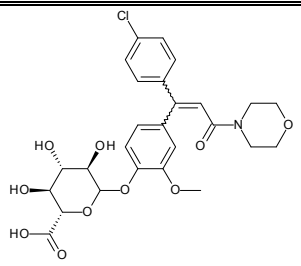
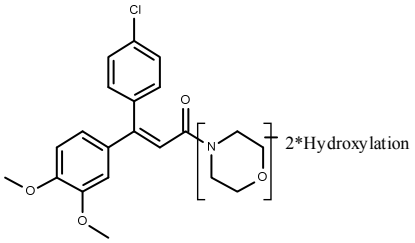
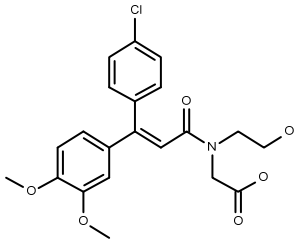
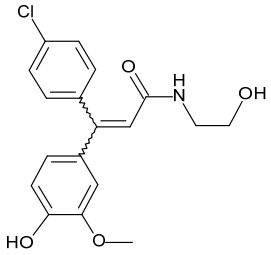
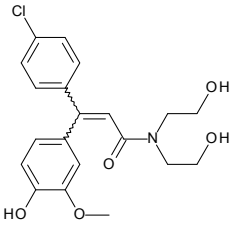
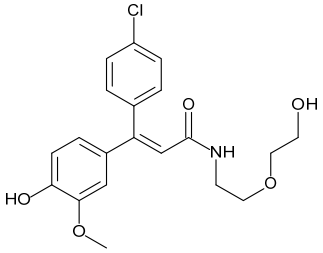
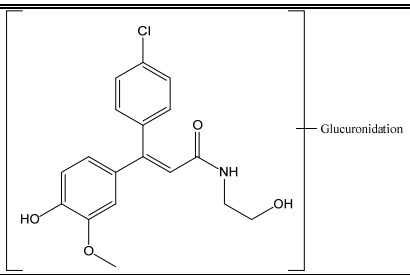
Metabolite	Structure	Egg yolk (acetone)	Liver	Fat (acetone)
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR
M550F013 (sum of isomers)		ND	0.021[A] (1.7)	ND
M550F028 (sum of isomers)	 or 	ND	0.007[A] (0.6)	ND
M550F033 (sum of isomers)		ND	0.020[A] (1.7)	ND
M550F035 **OR** M550F049	 **OR** 	ND	0.008[A] (0.6)	ND

Table 6.2.2-23: Summary of identified residues in hen after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Metabolite	Structure	Egg yolk (acetone)	Liver	Fat (acetone)
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR
M550F069		ND	0.008 (0.7)	ND

ND Not detected
A Co-eluting component

Identification of the polar region

The polar fraction that was observed through all matrices of the morpholine radiolabel (either in extracts or in released radioactivity after enzyme treatment of non-extractable residues, see Table 6.2.2-24) was shown to consist, in most cases, of multiple components, which could not clearly be identified despite significant effort using several methods. Hereby several LC-MS/MS techniques (HILIC), co-chromatography with potential metabolites, TLC methods and enzyme deconjugation techniques (glucoronidase, sulphatase hydrolysis) were used to identify the polar fraction. Since this remained unsuccessful, additionally an in-vitro-study in goat hepatocytes was performed to support the identification of the polar region [see KCA 6.2.3/5 2015/1231130]. However, no sufficient amount of the polar region was produced in the hepatocytes to allow MS identification.

An alternative HPLC method (HPLC 3) was used to analyze the isolated polar fraction. Co-chromatography with the radioactive reference substance morpholine confirmed the assumption that M550F021 (morpholine) might be part of the polar fraction (Table 6.2.2-24). Considering all analytical results from this study it is hypothesized that an incorporation of C2 fragments (stemming from the degradation of the morpholine moiety) into biosynthetic pathways is a relevant decomposition mechanism for BAS 550 F, resulting in small endogenous molecules of polar nature.

Table 6.2.2-24: Summary of quantification of polar region and M550F021 (morpholine)

Matrix	Additional information	Polar region (0-6 min; HPLC 2)		M550F021 (HPLC 3)	
		mg/kg	%TRR	mg/kg	%TRR
Morpholine label					
Egg yolk	Dichloromethane based extraction	0.041	3.1	0.023	1.7
	Dichloromethane based extraction PES-Hydrolysate	0.208	16.0	0.071	5.4
	Acetone based extraction PES-Hydrolysate	0.472	36.5	0.113	8.7
Egg white	Extraction	0.012	8.2	n.d.	n.d.
	PES-Hydrolysate	0.107	72.9	n.d.	n.d.
Liver	Extraction	0.265	22.4	0.003	0.3
	PES-Hydrolysate	0.132	11.2	0.076	6.4
Kidney	Extraction	0.261	34.8	0.011	1.5
	PES-Hydrolysate	0.182	24.4	0.07	7.3
Muscle	Extraction	0.038	28.7	n.d.	n.d.
Fat	PES-Hydrolysate	0.026	7.4	0.024	6.9

ND Not detected
PES Post extraction solid
PES Hydrolysate after enzyme digestion with protease and lipase

Extraction efficiency

In the study several extraction procedures were performed. For the matrices liver, kidney, muscle and egg white usually acetonitrile was used and provided good extractability. Where extractability with acetonitrile was not yet sufficient, sequential solvents were used to increase the extractability, but still the majority of residues was found in the acetonitrile (or aqueous acetonitrile) extract in all cases (see Table 6.2.2-25 and Table 6.2.2-27). The data generation methods for animal tissues, FAMS 023-01, uses acetonitrile as extraction solvent, thus the identical solvent as employed in the metabolism study. For this reason, no further investigations on extraction efficiency in muscle, liver, kidney and egg white were considered necessary.

For the matrices egg yolk and fat initial extraction was based on dichloromethane. For both matrices an acetone and acetonitrile based extraction was additionally performed. The data generation method FAMS 023-01 for fat and method FAMS 054-01 for eggs both use acetonitrile as solvent. Data for both solvents (acetone, acetonitrile) are shown in comparison with dichloromethane (see Table 6.2.2-26 and Table 6.2.2-28). In case of fat of the chlorophenyl label, acetonitrile proved to be an equally effective solvent, with 81.3% TRR extracted compared to 76.5% TRR with dichloromethane. For fat of the morpholine label, acetonitrile proved to be significantly less efficient with 20.5% TRR vs. 88% TRR extracted with dichloromethane. In egg yolk of both labels, acetone proved to be the better extraction solvent as compared to acetonitrile, providing more or less similar extraction efficiency as dichloromethane. The data from these extractions are provided in Table 6.2.2-25 to Table 6.2.2-28.

Table 6.2.2-25: Extraction efficiency of tissues and egg white treated with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Matrix	Acetonitrile		Acetonitrile/Water		Methanol		Ethanol		Water	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Liver	0.172	22.4	0.086	11.1	0.131	17.1	0.010	1.3	0.020	2.6
Kidney	0.077	39.8	0.032	16.3	0.012	6.4	-	-	0.006	2.7
Muscle	0.006	53.4	0.002	17.7	0.001	10.6	-	-	-	-
Egg white	0.005	91.0	-	-	-	-	-	-	-	-

Table 6.2.2-26: Extraction efficiency of fat and egg yolk treated with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Matrix	Dichloromethane		Acetone		Acetonitrile (Solvent of data generation method)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fat*	0.022	76.5	0.017	85.0	0.016	81.3
Egg yolk	0.104	40.6	0.104	40.6	0.058	22.7

* For fat, different underlying TRRs for dichloromethane extraction (0.029 mg/kg) and acetone/acetonitrile extraction (0.020 mg/kg) as a fresh fat pool had to be generated (see also footnote to Table 6.2.2-6)

Table 6.2.2-27: Extraction efficiency of tissues and egg white treated with [morpholine-2,3-^{14}C]-BAS 550 F

Matrix	Acetonitrile		Acetonitrile/ Water		Methanol		Ethanol		Water		Water (pH 2.5)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Liver	0.244	20.6	0.148	12.5	0.191	16.1	0.021	1.8	0.034	2.9	0.057	4.8
Kidney	0.152	20.4	0.097	13.0	-	-	-	-	0.103	13.8	0.010	1.3
Muscle	0.018	13.9	0.015	11.0	-	-	-	-	0.021	15.6	-	-
Egg white	0.018	12.4	0.003	1.8	0.001	0.4	-	-	0.021	14.2	0.003	1.8

Table 6.2.2-28: Extraction efficiency of fat and egg yolk treated with [morpholine-2,3-^{14}C]-BAS 550 F

Matrix	Dichloromethane		Acetone		Acetonitrile (Solvent of data generation method)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fat*	0.316	88.0	0.148	46.7	0.065	20.5
Egg yolk	1.018	78.7	0.716	55.3	0.150	11.6

* For fat, different underlying TRRs for dichloromethane extraction (0.359 mg/kg) and acetone/acetonitrile extraction (0.318 mg/kg) as a fresh fat pool had to be generated (see also footnote to Table 6.2.2-6)

Storage stability

As the extraction and analysis phase of the study exceeded 6 months after sacrifice, a storage stability assessment was performed. Extracts of egg yolk, liver and kidney for the chlorophenyl label and egg yolk, liver, kidney and fat for the morpholine label were re-analyzed at the end of the experimental phase using HPLC method 2, and the initial and stability chromatograms were compared to assess whether the components showed stability during the course of the study. The profiles were comparable showing stability over the course of the study.

Metabolic pathway

The proposed metabolic pathway of BAS 550 F (Reg. No. 247723) in laying hens is provided in Figure 6.2.2-1. BAS 550 F was extensively metabolized in the hens. An overview on quantified metabolites is provided in Table 6.2.2-13 and Table 6.2.2-14. The identified metabolites comprise the following phase I and phase II conversions of the parent compound, BAS 550 F:

The phase I metabolism is characterized by a demethylation of the dimethoxyphenyl ring (M550F006 and M550F007) or hydroxylation of the morpholine-ring (M550F018 and M550F028) including several consecutive and sequential metabolites of oxidative degradation of the morpholine ring (M550F011, M550F008 and M55F012). Both steps (demethylation and morpholine ring degradation) result also in metabolites M550F022, M550F033 (and the corresponding conjugate M550F069), M550F038, M550F091 (and the corresponding conjugate M550F062), M550F035, M550F049 and M550F050.

Data show that a cleavage of the molecule and release of the free morpholine (M550F021) is probable. The phase II metabolism is characterized by conjugation to glucuronides of demethylated metabolites (M550F013, M550F074 and M550F076).

The polar fraction that was observed through all matrices (either in extracts or in released radioactivity after enzyme treatment of non-extractable residues) was shown to consist in most cases of multiple components, which could not clearly be identified despite much effort. One of the polar components seems to be free morpholine (M550F021). Considering all analytical results it is hypothesized that an incorporation of C2 fragments (stemming from the degradation of the morpholine moiety) into biosynthetic pathways is a relevant decomposition mechanism for BAS 550 F, resulting in small endogenous molecules of polar nature. Considering all analytical results it is hypothesized that an incorporation of C2 fragments in biosynthesis pathways is a relevant decomposition procedure of BAS 550 F. This hypothesis is based on the consecutive and sequential degradation of the morpholine ring yielding C2 fragments and the incorporation of radioactivity in polar molecules.

Stereoisomer analysis

The dose formulation of BAS 550 F is a mixture of *E* and *Z*-isomer in a ratio ~45:55. The *E*/*Z*-isomer ratio was investigated in all edible matrices, which contained BAS 550 F (see Table 6.2.2-29).

Fatty matrices like egg yolk and fat contained BAS 550 F. The isomeric ratio in these matrices ranged from 57:43 to 62:38 showing a slight shift in favor of the *E*-isomer.

Table 6.2.2-29: Summary of E/Z ratio of BAS 550 F in edible poultry matrices

Application Formulation / Matrix	Additional information	%TRR		Ratio (%)	
		E-isomer	*Z*-isomer	*E*-isomer	*Z*-isomer
Chlorophenyl label					
Application formulation	CoA	n.a.	n.a.	43	57
	Dose formulation*	42.9**	53.3**	45	55
Egg yolk	Acetone based extraction	0.5	n.d.	n.a.	n.a.
Morpholine label					
Application formulation	CoA	n.a.	n.a.	44	56
	Dose formulation*	42.6**	54.5**	44	56
Egg yolk	Acetone based extraction	8.8	6.6	57	43
Fat	Acetone based extraction	7.5	4.6	62	38

n.d. Not detected
n.a. Not applicable
* Method 2; prior dosing;
** Area (%)

III. CONCLUSION

After oral dosing of BAS 550 F (Reg. No. 247723) to laying hens for 14 consecutive days, residues were rapidly and efficiently excreted. Approximately 91-95% of the total mean dose was recovered, the majority of which was present in the excreta (82.9-87.5%) and GI tract and contents (3.2%). Relatively low amounts were recovered in cage wash (2.9-3.9%). Radioactivity associated with edible portions (eggs and tissues) accounted for approximately <1-1.6% of the administered dose.
Radioactive residues in eggs were mostly associated with egg yolk and reached a plateau in egg yolks after 10-11 days for both radiolabels. Residues in tissues were consistently higher for the morpholine label compared to the chlorophenyl label.

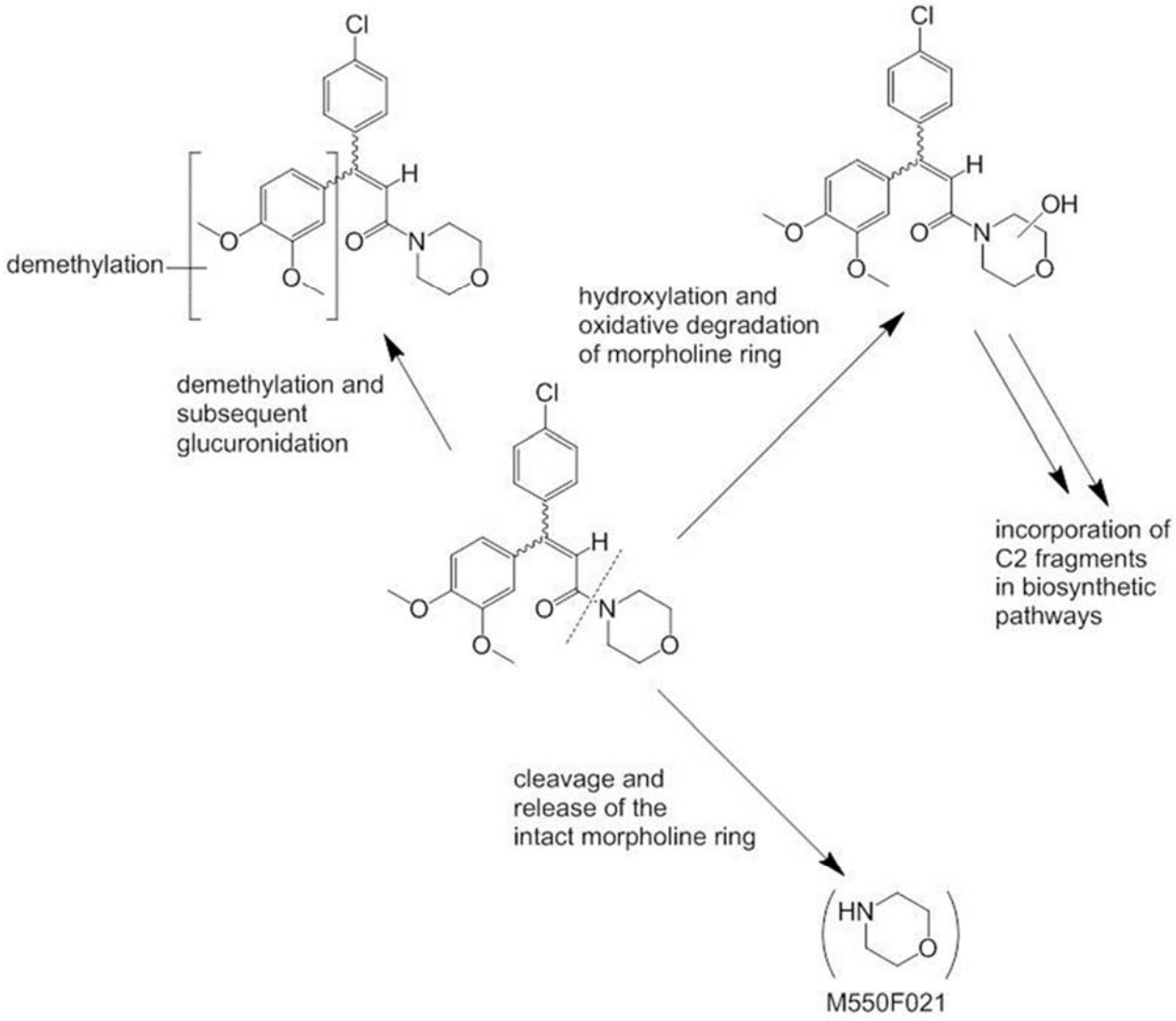
Parent BAS 550 F was extensively metabolized in laying hens. In the group dosed with chlorophenyl labeled BAS 550 F, the unchanged was only detected in egg yolk as the *E*-isomer (0.001 mg/kg; 0.5% TRR).
Metabolites formed through the hydroxylation and/or oxidative degradation of the morpholine moiety of BAS 550 F were observed in the egg yolk, liver and kidney extracts. M550F012 was observed in egg yolk, liver and kidney, accounting for up to 11.8% TRR in the egg yolk, up to 5.4% TRR in the liver and up to 6.2% TRR in the kidney, as a co-eluting species in all matrices. M550F028 accounted for up to 9.4% TRR in egg yolk and up to 3.8% TRR in the liver. M550F011 was observed in the egg yolk (6.3% TRR) and the liver (2.3% TRR).
M550F008 (*Z*) was observed in liver and kidney, accounting for up to 3.7% TRR in the liver and up to 4.8% TRR in the kidney, as a co-eluting species in both matrices. M550F022 was observed in egg yolk, liver and kidney, accounting for 3.1% TRR in the egg yolk, 1.2% TRR in the liver and up to 6.1% TRR in the kidney.
Furthermore, the following metabolites, resulting from demethylation, were observed: M550F006 (*Z*), accounting for 1.4% TRR in kidney, 3.7% TRR in liver and 9.3% TRR in egg yolk; M550F007 (*E*) with 3.1% TRR in egg yolk and 2.0% TRR in liver and M550F013 (the glucuronide of M550F007) with 6.3% TRR in liver.

In hens dosed with morpholine labeled BAS 550 F, the unchanged parent was detected in egg yolk and fat. In the egg yolk, BAS 550 F represented 0.200 mg/kg (15.4% TRR) and in the fat 0.038 mg/kg (12.1% TRR).
Besides that, in the liver most of the metabolites known from the chlorophenyl label were identified, i.e. metabolites M550F013, M550F033, M550F028, M550F011, M550F007, M550F069, and M550F049/M550F035. In samples of the morpholine label, a polar fraction was most predominant, which was shown to consist of several components, one of which was tentatively identified as free morpholine.

It can be concluded, that metabolism of dimethomorph in poultry is dominated by demethylation at the dimethoxy ring and conjugation to glucuronides, and even more by the hydroxylation and opening and step-wise degradation of the morpholine ring, including complete degradation to C2 fragments, which are then subsequently incorporated into natural biosynthetic pathways, rendering small endogenous molecules of polar nature.

Figure 6.2.2-1: Proposed metabolic pathway for dimethomorph in the laying hen (considering peer reviewed and new data)

Main metabolic steps



( ) indicates metabolite is characterised

Figure 6.2.2-1: Proposed metabolic pathway for dimethomorph in the laying hens (considering peer reviewed and new data)

Metabolites from demethylation and glucuronidation of the dimethoxyphenyl ring

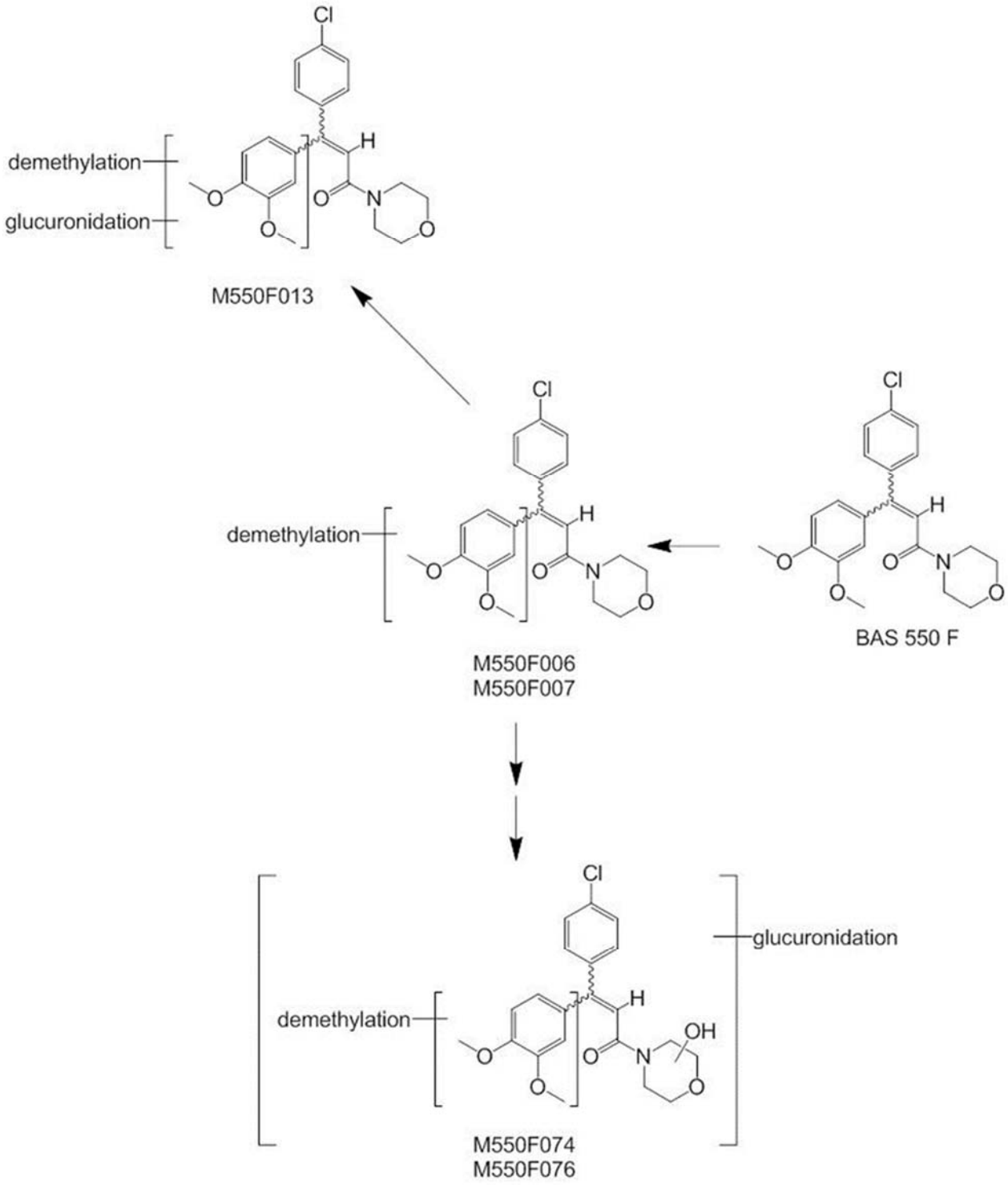


Figure 6.2.2-1: Proposed metabolic pathway for dimethomorph in the laying hens (considering peer reviewed and new data)

Metabolites from hydroxylation and oxidative degradation of the morpholine ring and demethylation of the dimethoxyphenyl ring

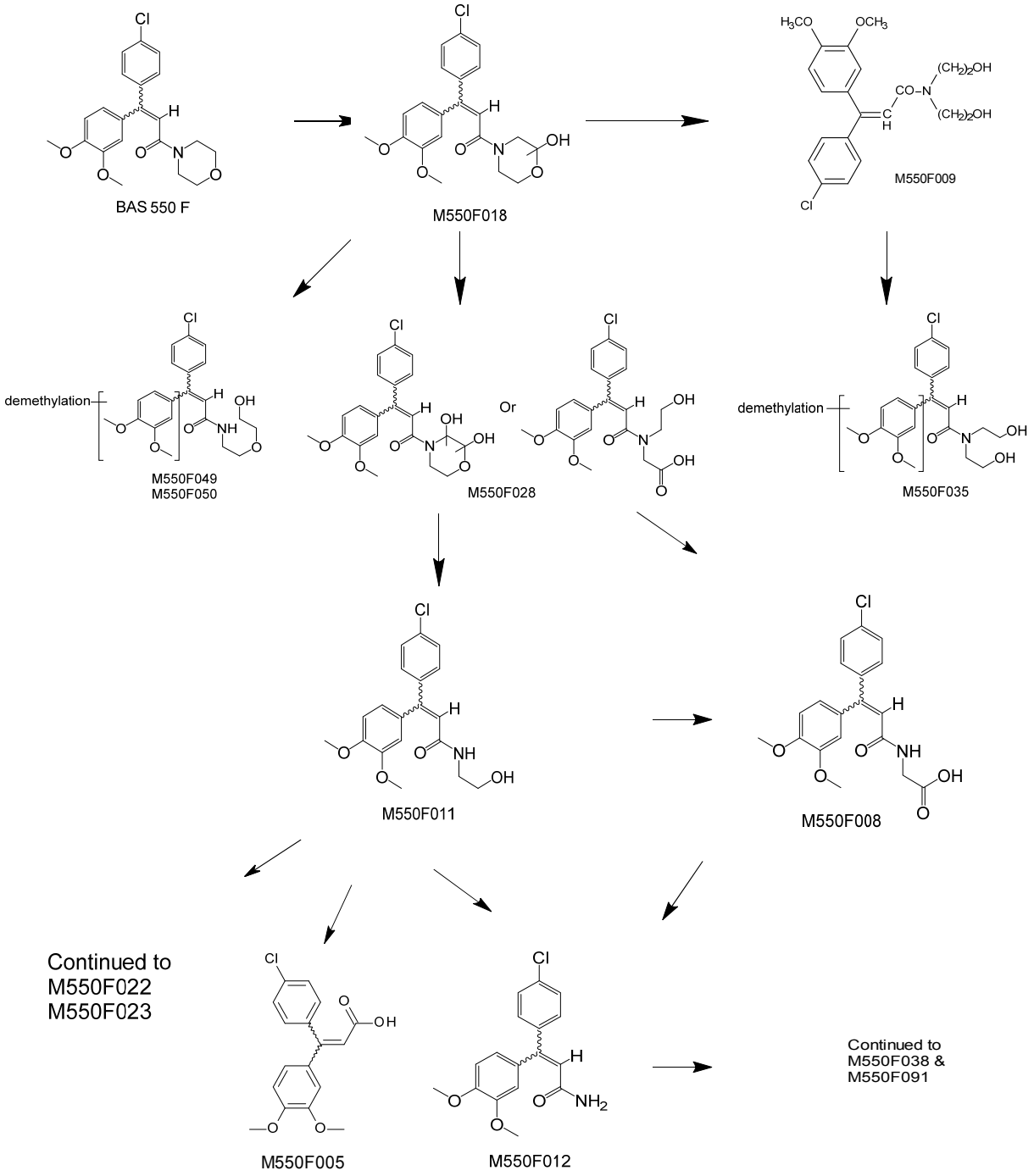
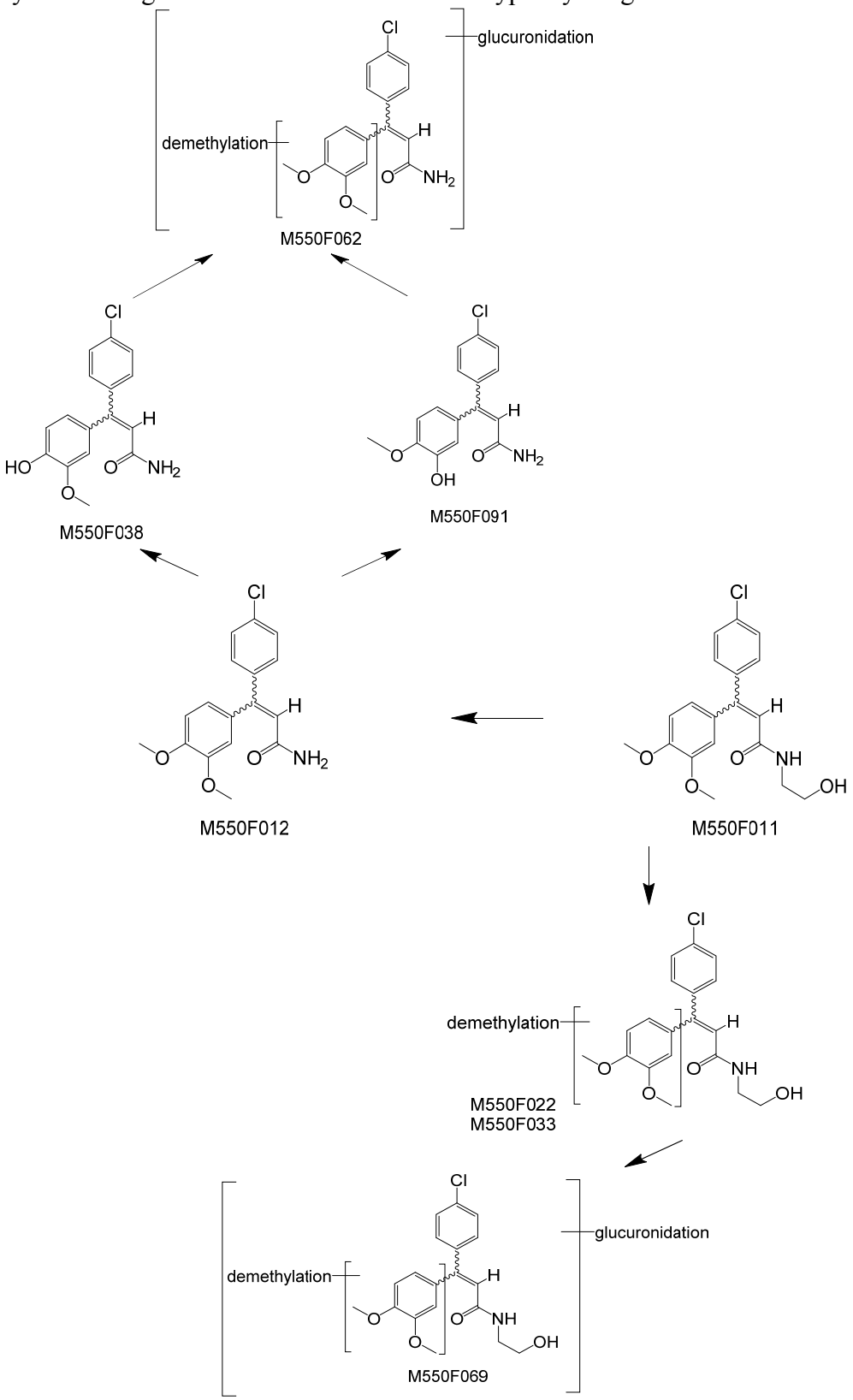


Figure 6.2.2-1: Proposed metabolic pathway for dimethomorph in the laying hens (considering peer reviewed and new data)

Metabolites from hydroxylation and oxidative degradation of the morpholine ring and demethylation and glucuronidation of the dimethoxyphenyl ring



CA 6.2.3 Lactating ruminants

[redacted] 1990: ^{14}C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats (DocID DK-440-005)
[redacted] 1991: ^{14}C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats-First amendment to report (DocID DK-440-008)

Two lactating goats were orally dosed twice daily (except for last day, one dose) with a target dose of 1.0 mg ^{14}C-dimethomorph (chlorophenyl label)/kg of body weight for 7 consecutive days. Each goat received 15 oral doses. Assuming a feed consumption of 2 kg/day and a mean body weight of 50 kg, the daily target dose would correspond to a nominal dietary burden of 25 mg/kg feed. Both animals were sacrificed at the peak blood level determined after the first administration, which was 4 hours post last dose. Tissues were extracted with methanol/water and methanol, followed by Soxhlet extraction, where necessary. Analysis and identification of metabolites was carried out using TLC and comparison with reference standards.

Findings
Excretion of radioactivity from goats was very effective with 85.1-88.2% of the administered dose recovered in excreta. The radioactivity level in milk reached a plateau after the 3rd administration (i.e. after 2 days) and were 0.10/0.11 mg/kg at the end of the dosing period. After fractionation of the milk, only low amounts of radioactivity were detected in milk fat and the protein pellet (0.004-0.015 mg/kg). The highest TRR was observed in liver and kidney with 7.134 (mean) and 0.28 (mean) mg/kg, respectively. TRR in muscle and fat was 0.027 and 0.073 mg/kg, respectively.

Table 6.2.3-1: Total radioactive residues in edible matrices, blood and bile after dosing of lactating goats with ^{14}C-[chlorophenyl]-dimethomorph (values following background subtraction

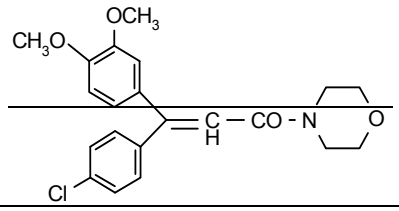
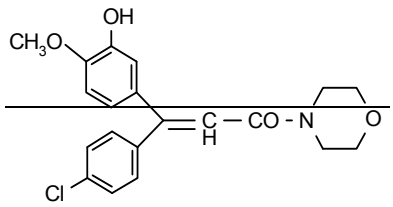
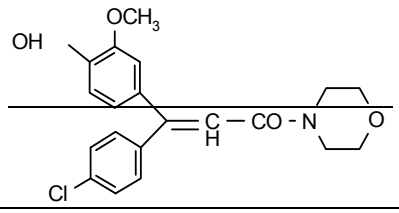
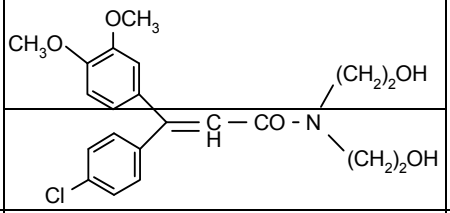
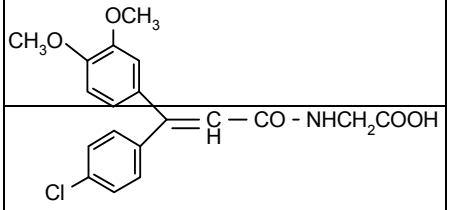
Matrix	TRR [mg/kg]*	
	Goat 1	Goat 2
Kidney	0.289	0.270
Liver	7.718	6.550
Heart	0.073	0.050
Muscle	0.032	0.022
Fat	0.088	0.057
Blood	0.066	0.066
Bile	16.289	9.204

* Values following background subtraction

The extractable radioactive residue in kidney, liver, muscle and fat was 95.4, 90.5, 99.4 and 99.3%, respectively.
In milk, metabolite M550F008 (CUR 7117) was detected as the polar metabolite fraction (0.047-0.052 mg/kg; goat 1 and 2, respectively).

In kidney, besides the parent compound (0.019-0.034 mg/kg) mainly M550F006 (Z67) (0.030-0.035 mg/kg) and M550F009 (WL 376084; 0.016-0.021 mg/kg) were detected. In liver, beside the main component of unchanged parent (4.507-5.767 mg/kg) M550F006 (Z67) (0.137-0.421 mg/kg) and M550F007 (Z69) (0.115-0.328 mg/kg) were detected. In muscle and fat only the parent compound was detected in the range from 0.002-0.006 mg/kg and 0.045-0.065 mg/kg.

Table 6.2.3-2: Summary of metabolite identities and quantities in edible matrices of goat tissues and milk after dosing with ^{14}C-dimethomorph/chlorophenyl label

Metabolite code (Reg. No. of reference substance)	Metabolite Identity	Milk mg/kg (% TRR*)	Muscle mg/kg (% TRR*)	Fat mg/kg (% TRR*)	Liver mg/kg (% TRR*)	Kidney mg/kg (% TRR*)
Dimethomorph		ND	0.002 (7.41)	0.045 to 0.065 (61.6 to 89.0)	4.507 to 5.767 (63.2 to 80.8)	0.019 to 0.034 (6.79 to 12.1)
M550F006 (Z67)		ND	ND	ND	0.137 to 0.421 (1.92 to 5.90)	ND
M550F007 (Z69)		ND	ND	ND	0.115 to 0.328 (1.61 to 4.60)	
M550F009 (WL 376084)		ND	ND	ND	ND	0.016 to 0.021 (5.71 to 7.50)
M550F008 (CUR 7117)		0.047 to 0.052 (45.6 to 50.5)	ND	ND	ND	ND

* The %TRR was calculated based on the average TRR value of goat 1 and 2.
ND Not detected

~~**Conclusion**~~

~~In conclusion, the major component of the extractable residue in kidney, liver, muscle and fat was unchanged parent. In addition, mainly M550F006 (Z67) (kidney and liver) and M550F007 (Z69) (liver) were detected indicating that dimethomorph in the lactating goat was initially metabolized via demethylation of one of the phenolic methoxy-groups. Alternatively, dimethomorph was metabolized via morpholine-ring opening and degradation resulting in M550F009 (WL 376084) (kidney) and M550F008 (CUR 7117) (milk).~~

Report:	CA 6.2.3/1 [redacted], 1990 b 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats DK-440-005
Guidelines:	EPA 171-4, EPA 540/9-82-023
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Report:	CA 6.2.3/2 [redacted], 1991 b 14C-Dimethomorph (CME 151): Absorption, distribution, metabolism and excretion after repeated oral administration to lactating goats DK-440-008
Guidelines:	EPA 171-4, EPA 540/9-82-023
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Description:	[p-chlorophenyl-^{14}C]-BAS 550 F (chlorophenyl label, *E*:*Z* 49:51) BAS 550 F (unlabeled, *E*:*Z* 50:50)
Lot/batch #:	SF 01/01 (chlorophenyl label) H 7879 (unlabeled)
Purity:	Radiochemical purity: Chlorophenyl label: after purification 98.9 % Chemical purity: Unlabeled BAS 550 F: 99.2% Specific activity: Chlorophenyl label: 45.12 mCi/g (l.669 MBq/mg)
CAS#:	110488-70-5
Development code:	CHE 151
Stability of test compound:	The ^{14}C-dimethomorph (CHE 151) proved to be sufficiently stable as shown by TLC- and HPLC analyses of the radioactivity in the formulation after the first (radiochemical purity: 99.0% and *E*:*Z*-ratio 48:52) and the 15th administration (radiochemical purity: 99.2 % and *E*:*Z*-ratio: 50:50).

The aim of the present study were to elucidate the absorption, distribution, metabolism and excretion of dimethomorph in lactating goats.

2. Test animals

Species:	Goat
Variety:	Saanen (white goat)
Gender:	Female
Age:	Mature (lactating)
Weight at dosing:	Four days before administration, 44 kg (goat 1) and 54 kg (goat 2).
Number of animals:	2
Acclimation period:	7 days

Diet:	Hay and ruminant feed *ad libitum* (KLIBA, Klingentalmuehle AG, CH-4303 Kaiseraugst/Switzerland).
Water:	Tap water, *ad libitum*
Housing:	During acclimation and the study, stainless steel goat metabolism cage (W. Ehret Versuchstiertechnik, Emmendingen/FRG).

Environmental conditions	
Temperature:	22 ± 3°C
Humidity:	40-70%
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

The study was conducted at RCC Umweltchemie AG, Itingen, Switzerland and was completed on 11 October 1990.

1. Dosing regime

Oral:	**Amount of dose:**	2 x 0.5 mg/kg bw/day (1.0 mg/kg bw/day, chlorophenyl label), 25 mg/kg feed
	Food consumption:	The slight variations in body weights, the similar daily milk production during acclimation and administration as well as the constant urine and feces production during administration indicated a normal food consumption and hence a normal health status of both goats during the in-life phase of the study
	Vehicle:	Oral intubation
	Timing:	Twice daily at 9.00 and 17.00 hours
	Duration:	7 consecutive days (15 doses)

2. Sample collection

Milk collection:	Twice daily
Urine and feces collection:	Daily
Interval from last dose to sacrifice:	4 h
Tissues harvested & analyzed:	Blood, urine, feces, milk, bile, liver, kidney, composite muscle (round, loin and flank), composite fat (perirenal, omental and subcutaneous), heart

3. Test system

Two lactating goats were orally dosed twice daily (except for last day, one dose) with a target dose of 1 mg ^{14}C-dimethomorph (chlorophenyl label)/kg bw/day, respectively, for 7 consecutive days. Each goat received 15 oral doses. Assuming a feed consumption of 2 kg/day and a mean body weight of 50 kg, the daily target dose would correspond to a nominal dietary burden of 25 mg/kg feed.

The test item was prepared as solution and administered using a Hamilton syringe connected to a stomach tube. Details of the study outline are summarized in Table 6.2.3-1.

Table 6.2.3-1: Dose level following administration of ^{14}C-BAS 550 F to lactating goats at a target dose level of 25 mg/kg dry weight diet/day

Goat number	Radio-label	Nominal daily dose		Treatment days	Sacrifice	Sampling
		[mg/kg feed]	[mg/kg bw]			
Goat 1+2	U-^{14}C-chlorophenyl label	25*	2 x 0.5	7	4 h after last dosing (at blood peak level as determined after the first administration)	Day 1: Blood: pre-dose, 0.5, 1, 2, 3, 4, 6, 8 hours (immediately prior to the second dose administration) and prior to each administration Milk: twice daily prior administration Urine/Feces: at 24 hour intervals Final: Blood, urine, feces, milk, bile, liver, kidney, muscle (round, loin and flank), fat (perirenal, omental and subcutaneous), heart

* Target value

4. Sampling and storage

Blood samples were taken from the jugular vein of the goat prior to dosing and after the first administration at 0.5, 1, 2, 3, 4, 6 and 8 hours, i.e. immediately prior to the second administration. Furthermore, immediately prior to each administration and at sacrifice, blood was collected. Urine and feces were collected at 24 hour intervals. The goat was milked twice daily and immediately prior to sacrifice.
Both animals were sacrificed at the peak blood level determined after the first administration, which was 4 hours post last dose. After sacrifice, edible tissues (liver, kidney, muscle and fat), heart and bile were removed. All samples were stored at ca. –20°C.

5. Description of analytical methods

Radioanalysis
Subsamples of blood and plasma, milk, urine were taken for determination of radioactivity for the respective sampling intervals. The radioactivity of urine, bile and milk (for milk after digest with Soluene 350, tissue solubilizer) were determined by LSC (liquid scintillation counting) analysis.
Feces were lyophilized and homogenized. Organs and tissues were sampled after sacrifice and homogenized. Blood and plasma, feces and homogenates of organs and tissues were analyzed for radioactivity content by combustion analysis followed by LSC.

Extraction
For milk, fat was separated by filtration and proteins were precipitated using acetone. The protein-free fraction (whey) was concentrated to its water phase, which was partitioned three times with ethyl acetate (1:2, v/v). The recovered organic phase was concentrated and analyzed by TLC and HPLC.
For plasma, proteins were precipitated using acetone. The protein pellet was extracted with methanol:water (8:2, v/v).

Tissues were extracted three to four times with methanol/water (8:2, v/v) and methanol, followed by Soxhlet extraction where necessary. Analysis and identification of metabolites was carried out using TLC and comparison with reference standards.

Analysis and identification of metabolites
Thin-layer chromatography (TLC) was used for characterization of metabolite patterns in bile, plasma and extracts of organs and tissues. From defatted milk, a major milk metabolite (Mi4) was isolated, which, based on TLC, high voltage electrophoresis and derivatization, proved to be identical to M550F008 (CUR 7117).

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Absorption of ^{14}C-dimethomorph (CME 151) occurred rapidly: low blood peak levels of 0.0337 mg/kg for goat 1 and 0.0238 mg/kg for goat 2 were reached at 4 hours after the first administration. Plateau values (0.0272-0.0671 mg/kg) were reached after the 4th administration.

The radioactivity level in milk reached a plateau after the 3rd administration (i.e. after 2 days) and were 0.10/0.11 mg/kg at the end of the dosing period. After fractionation of the milk, only low amounts of radioactivity were detected in milk fat and the protein pellet (0.004-0.015 mg/kg).

Excretion of the test article mainly proceeded via the feces (69.5% and 73.9% of the dose). In urine excreted radioactivity accounted for 15.6% and 14.3% of the dose. Very low amounts (below 0.1% of the dose) were excreted in milk. Together with residual radioactivity in cage wash, organs, tissues and blood, total recovery at the blood peak level, i.e. 4 hours after the last administration, amounted to 88.3% and 91.8% of the dose for goats 1 and 2, respectively.

The total radioactive residues in all matrices are summarized in Table 6.2.3-2: to Table 6.2.3-4.
At sacrifice, minor amounts of radioactivity (0.022-0.088 mg/kg) were detected in heart, muscle and fat of both goats (Table 6.2.3-2:). Small but significant levels of radioactivity were only found in the excretory organs kidney (0.270-0.289 mg/kg) and liver (6.550-7.718 mg/kg) as well as in bile (9.204-16.289 mg/kg), indicating that excretion was in full progress.

Table 6.2.3-2: Radioactive residues in edible matrices, blood and bile after dosing of lactating goats with ^{14}C-BAS 550 F

Matrix	Radioactive residues [mg/kg]*	
	Goat 1	Goat 2
Kidney	0.289	0.270
Liver	7.718	6.550
Heart	0.073	0.050
Composite muscle	0.032	0.022
Composite fat	0.088	0.057
Blood	0.066	0.066
Bile	16.289	9.204

* Values following background subtraction

Table 6.2.3-3: Recovery of radioactivity in lactating goats after repeated (15x) oral administration of ^{14}C-BAS 550 F at a target dose level of 1.0 mg/kg/day (values not corrected for background)

Sample	Dose recovered in sample [%]*	
	Goat 1	Goat 2
Faeces	69.5	73.9
Urine	15.6	14.3
Cage wash	0.9	1.6
Milk	<0.1	<0.1
Total excreted	**86.0**	**89.8**
Organs/tissues/blood	2.3	2.0
Total	**88.3**	**91.8**

* Values not corrected for background

Table 6.2.3-4: Total radioactive residues in edible goat matrices, blood and bile after administration of ^{14}C-BAS 550 F (values not corrected for background)

Matrix	Radioactive residues and dose recovered in sample			
	Goat 1		Goat 2	
	% dose	mg/kg	% dose	mg/kg
Liver	1.9	7.725	1.5	6.557
Kidney	<0.1	0.297	<0.1	0.278
Heart	<0.1	0.081	<0.1	0.058
Composite muscle	0.2*	0.040	0.1*	0.030
Composite fat	0.1*	0.096	0.1*	0.065
Blood	0.1*	0.066	0.1*	0.066
Bile	<0.1	16.301	0.2	9.216
Total	**2.3**	**-**	**2.0**	**-**

* Calculated on the assumption of 40%, 12% and 8% of the body weight for muscle, fat and blood, respectively.

B. EXTRACTION OF RESIDUES

The extractable radioactive residues in kidney, liver, muscle and fat accounted for between 90.3-108.6% of the radioactivity (Table 6.2.3-5).
In liver, where the radioactivity accounted for 7.718 or 6.550 mg/kg (goat 1 or goat 2), 90.0% or 90.9% of the TRR were extracted, respectively.

Table 6.2.3-5: Extractability of goat matrices after treatment with ^{14}C-BAS 550 F

Matrix	Extractable (room temperature)		Extractable (Soxhlet)		ERR[1]		RRR[2]		Sum[3]
	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[mg/kg]	[%] TRR	[%] TRR
Goat 1									
Liver	6.613	86.8	0.244	3.2	6.857	90.0	0.861	11.3	101.3
Kidney	0.268	97.6	-	ND	0.268	97.6	0.021	7.8	105.4
Composite muscle	0.028	100.3	-	ND	0.028	100.3	0.004	15.4	115.7
Composite fat	0.078	102.3	0.005	6.3	0.083	108.6	0.005	6.1	114.7
Heart	0.061	82.9	0.007	10.0	0.068	92.9	0.005	6.8	99.7
Milk fat (filtration)	-	-	-	-	0.014	14.3	-	-	96.6
Milk whey (acetone extract)	-	-	-	-	0.080	78.8	-	-	
Milk protein pellet (acetone precipitate)	-	-	-	-	0.004	3.5	-	-	
Plasma (ethyl acetate and aqueous phase)	-	-	-	-	0.049	85.2	0.014	24.7	109.9
Goat 2									
Liver	5.567	87.2	0.236	3.7	5.803	90.9	0.747	11.7	102.6
Kidney	0.252	93.1	-	ND	0.252	93.1	0.018	6.7	99.8
Composite muscle	0.019	98.7	-	ND	0.019	98.7	0.003	13.6	112.3
Composite fat	0.049	82.5	0.005	7.8	0.054	90.3	0.003	4.9	95.2
Heart	0.038	84.5	0.007	15.8	0.045	100.3	0.005	11.2	111.5
Milk fat (filtration)	-	-	-	-	0.015	14.3	-	-	101.8
Milk whey (acetone extract)	-	-	-	-	0.088	83.1	-	-	
Milk protein pellet (acetone precipitate)	-	-	-	-	0.005	4.4	-	-	
Plasma (ethyl acetate and aqueous phase)	-	-	-	-	0.052	84.4	0.015	23.4	107.8

ND Not determined
1 ERR = Extractable radioactive residue
2 RRR = Residual radioactive residues
3 Sum of ERR + RRR

Table 6.2.3-6: Distribution of radioactive residues *via* extraction and partitioning

Matrix	Distribution of radioactive residues											
	Ethyl acetate		Hexane		Dichloro-methane /ethyl acetate		Ethyl acetate*		Aqueous phase		ERR	
	[mg/kg]	%] TRR	[mg/kg]	%] TRR	[mg/kg]	%] TRR	[mg/kg]	%] TRR	[mg/kg]	%] TRR	[mg/kg]	%] TRR
Goat 1												
Milk[1]	0.076	74.4	-	-	-	-	-	-	0.004	4.4	0.080	78.8
Plasma[2]	0.023	40.4	-	-	-	-	0.021	37.0	0.005	7.8	0.049	85.2
Kidney	-	-	0.005	1.9	0.175	63.9	0.065	23.5	0.023	8.3	0.268	97.6
Liver	-	-	0.107	1.4	6.415	84.2	0.228	3.0	0.107	1.4	6.857	90.0
Heart	-	-	0.004	5.6	0.060	81.7	0.002	2.8	0.002	2.8	0.068	92.9
Muscle	-	-	0.001	2.2	0.025	89.4	0.001	4.0	0.001	4.7	0.028	100.3
Fat	-	-	0.006	8.0	0.073	95.8	0.003	3.9	0.001	0.9	0.083	108.6
Bile[3]	14.977	93.6	-	-	-	-	0.256	1.6	1.056	6.6	16.289	101.8
Goat 2												
Milk[1]	0.084	79.3	-	-	-	-	-	-	0.004	3.8	0.088	83.1
Plasma[2]	0.024	39.1	-	-	-	-	0.024	38.2	0.004	7.1	0.052	84.4
Kidney	-	-	0.005	1.8	0.161	59.4	0.054	20.0	0.032	11.9	0.252	93.1
Liver	-	-	0.115	1.8	5.426	85.0	0.147	2.3	0.115	1.8	5.803	90.9
Heart	-	-	0.002	5.3	0.037	82.4	0.003	6.3	0.003	6.3	0.045	100.3
Muscle	-	-	<0.001	1.7	0.017	88.5	0.001	4.0	0.001	4.5	0.019	98.7
Fat	-	-	0.002	2.9	0.052	86.4	<0.001	0.5	<0.001	0.5	0.054	90.3
Bile[3]	4.492	46.9	-	-	-	-	2.892	30.2	1.820	19.0	9.204	96.1
Bile[4]	6.986	67.1	-	-	-	-	-	-	2.218	21.3	9.204	88.4

* Ethyl acetate after acidifying of aqueous phase to pH 1.0
1 Partitioning of whey from milk (0-4 hours after last administration)
2 Protein precipitation and partitioning of plasma at sacrifice
3 Partitioning after HCl treatment
4 Partitioning after glucuronidase digest

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

A summary of identified and characterized residues are shown in Table 6.2.3-7. A summary of identified metabolites in edible matrices and their distribution is given in Table 6.2.3-8.

Radioactivity in milk, plasma, kidney, liver, heart, muscle, fat and bile of both goats was further analyzed. In milk, metabolite M550F008 (CUR 7117) was detected as the polar metabolite fraction (0.047-0.052 mg/kg; goat 1 and 2, respectively). The other five unknown metabolite fractions occurred in amounts below 0.015 mg/kg.

In plasma, the parent compound (0.003-0.004 mg/kg) and M550F006 (Z67, 0.005 mg/kg) occurred in minor amounts. Additionally, at least seven unknown metabolite fractions were found below 0.015 mg/kg.

In kidney, besides the parent compound (0.019-0.034 mg/kg), mainly M550F006 (Z67, 0.030-0.035 mg/kg) and M550F009 (WL 376084, 0.016-0.021 mg/kg) were detected. In liver, beside the main component of unchanged parent (4.507-5.767 mg/kg), M550F006 (Z67, 0.137-0.421 mg/kg) and M550F007 (Z69, 0.115-0.328 mg/kg) were detected. In muscle and fat only the parent compound was detected in the range from 0.002-0.006 mg/kg and 0.045-0.065 mg/kg, respectively.

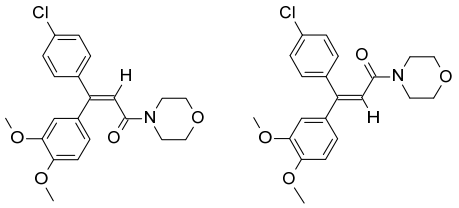
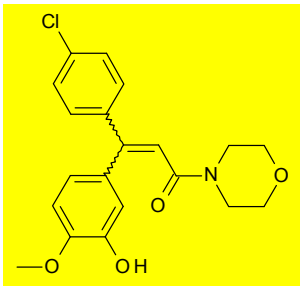
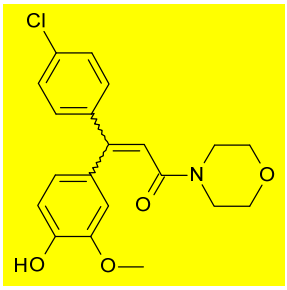
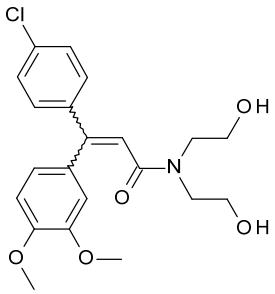
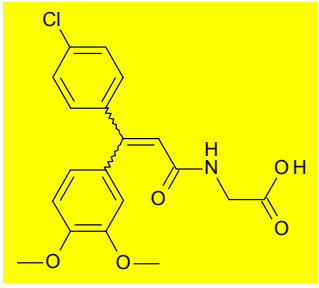
In bile, only very polar metabolite fractions (most likely conjugates) were found. After glucuronidase-treatment, besides the major metabolite M550F006 (Z67, 3.613-5.232 mg/kg), M550F005 (Z43, 1.187-1.584 mg/kg) and M550F008 (CUR 7117, 1.114-2.433 mg/kg) were found. Additionally, only one unknown metabolite fraction (1.072 mg/kg) was detected. After HCl-hydrolysis, besides M550F006 (Z67, 2.682-5.232 mg/kg) and M550F005 (Z43, 0.785-1.584 mg/kg), five additional degradation products (0.345-2.432 mg/kg) were detected. One of them was identified as M550F010 (CUR 7586, 0.278-1.216 mg/kg).

Table 6.2.3-7: Summary of identified and HPLC-characterized metabolites in milk, plasma and extracts of edible organs/tissues matrices of lactating goats after dosing with ^{14}C-BAS 550 F

Components	[mg/kg] (% TRR)							
	Milk	Plasma	Kidney	Liver	Heart	Muscle	Fat	Bile
Goat 1								
BAS 550 F (*E*+*Z*)	ND	0.004 (7.3)	0.034 (12.2)	5.767 (75.7)	0.015 (19.7)	0.006 (22.9)	0.065 (85.8)	ND
M550F006 (Z67)	ND	0.005 (8.6)	0.035 (12.8)	0.137 (1.8)	ND	ND	ND	5.232 (32.7)
M550F007 (Z69)	ND		ND	0.328 (4.3)	ND	ND	ND	ND
M550F005 (Z43)	ND	ND	ND	ND	ND	ND	ND	1.584 (9.9)
M550F009 (WL 376084)	ND	ND	0.016 (5.9)	ND	ND	ND	ND	ND
M550F008 (CUR 7117)	0.047 (46.0)	ND	ND	ND	ND	ND	ND	2.433* (15.2)
M550F010 (CUR 7586)	ND	ND	ND	ND	ND	ND	ND	1.216* (7.6)
Total identified	**0.047 (46.0)**	**0.009 (15.9)**	**0.085 (30.9)**	**6.232 (81.8)**	**0.015 (19.7)**	**0.006 (22.9)**	**0.065 (85.8)**	**11.025 (65.4)**
Number of characterized	*5*	*7*	*6*	*4*	*5*	*5*	*2*	*3*
Peak with the highest residue	*0.013 (12.5)*	*0.010 (17.3)*	*0.046 (16.7)*	*0.190 (2.5)*	*0.014 (19.2)*	*0.008 (29.7)*	*0.006 (7.5)*	*2.432 (15.2)*
Total characterized	**0.029 (28.4)**	**0.035 (61.5)**	**0.155 (56.5)**	**0.411 (5.4)**	**0.047 (64.8)**	**0.020 (70.5)**	**0.011 (13.9)**	**4.208 (29.8)**
Total identified and characterized	**0.076 (74.4)**	**0.044 (77.4)**	**0.240 (87.4)**	**6.643 (87.2)**	**0.062 (84.5)**	**0.026 (93.4)**	**0.076 (99.7)**	**15.233 (95.2)**
Goat 2								
BAS 550 F (*E*+*Z*)	ND	0.003 (4.6)	0.019 (7.1)	4.507 (70.6)	0.005 (11.9)	0.002 (12.9)	0.045 (74.6)	ND
M550F006 (Z67)	ND	0.005 (7.5)	0.030 (10.9)	0.421 (6.6)	ND	ND	ND	2.682 (28.0)
M550F007 (Z69)	ND	ND	ND	0.115 (1.8)	ND	ND	ND	ND
M550F005 (Z43)	ND	ND	ND	ND	ND	ND	ND	0.785 (8.2)
M550F009 (WL 376084)	ND	ND	0.021 (7.9)	ND	ND	ND	ND	ND
M550F008 (CUR 7117)	0.052 (49.6)	ND	ND	ND	ND	ND	ND	1.102* (11.5)
M550F010 (CUR 7586)	ND	ND	ND	ND	ND	ND	ND	0.278* (2.9)
Total identified	**0.052 (49.6)**	**0.008 (12.1)**	**0.070 (18.0)**	**5.043 (79.0)**	**0.005 (11.9)**	**0.002 (12.9)**	**0.045 (74.6)**	**4.847 (50.6)**
Number of characterized	*5*	*7*	*6*	*4*	*5*	*5*	*2*	*3*
Peak with the highest residue	*0.011 (10.6)*	*0.010 (16.3)*	*0.043 (15.9)*	*0.313 (4.9)*	*0.010 (23.1)*	*0.005 (27.5)*	*0.004 (7.0)*	*1.456 (15.2)*
Total characterized	**0.032 (29.7)**	**0.040 (65.2)**	**0.145 (53.5)**	**0.530 (8.3)**	**0.035 (76.8)**	**0.016 (79.6)**	**0.007 (12.3)**	**2.537 (26.5)**
Total identified and characterized	**0.084 (79.3)**	**0.048 (77.3)**	**0.215 (79.4)**	**5.573 (87.3)**	**0.040 (88.7)**	**0.018 (92.5)**	**0.052 (86.9)**	**7.384 (77.1)**

* Detected as degradation product after HCl-treatment, ND Not detected

Table 6.2.3-8: Summary of metabolite identities and quantities in edible matrices of goat tissues and milk after dosing with ^{14}C-BAS 550 F

Metabolite code (Reg.-No. of reference substance)	Metabolite Identity	Milk mg/kg (%TRR)	Muscle mg/kg (%TRR)	Fat mg/kg (%TRR)	Liver mg/kg (%TRR)	Kidney mg/kg (%TRR)
Dimethomorph BAS 550 F		ND	0.002 (7.41)	0.045 to 0.065 (61.6 to 89.0)	4.507 to 5.767 (63.2 to 80.8)	0.019 to 0.034 (6.79 to 12.1)
M550F006 (Z67)		ND	ND	ND	0.137 to 0.421 (1.92 to 5.90)	ND
M550F007 (Z69)		ND	ND	ND	0.115 to 0.328 (1.61 to 4.60)	
M550F009 (WL 376084)		ND	ND	ND	ND	0.016 to 0.021 (5.71 to 7.50)
M550F008 (CUR 7117)		0.047 to 0.052 (45.6 to 50.5)	ND	ND	ND	ND

ND Not detected

Storage stability
Analytical samples proved to be sufficiently stable for the study period at -20°C. Milk, liver, kidney, muscle and fat samples were re-extracted after 3-6 months of storage and metabolite patterns were compared to first extractions. However, the maximum storage interval did not exceed 6 months.

Metabolic pathway
The proposed metabolic pathway of BAS 550 F (Reg. No. 247723) in the lactating goat is provided in Figure 6.2.3-1**: Proposed metabolic pathway for dimethomorph in the lactating goat (considering peer reviewed and new data)**. Mainly M550F006 (Z67, kidney and liver) and M550F007 (Z69, liver) were detected indicating that dimethomorph in the lactating goat was initially metabolized via demethylation of one of the phenolic methoxy-groups. Alternatively, dimethomorph was metabolized via morpholine-ring opening and degradation resulting in M550F009 (WL 376084, kidney) and M550F008 (CUR 7117, milk).

III. CONCLUSION

In conclusion, the major component of the extractable residue in kidney, liver, muscle and fat was unchanged parent. In addition, mainly M550F006 (Z67, liver) and M550F007 (Z69, liver) were detected indicating that dimethomorph in the lactating goat was initially metabolized via demethylation of one of the phenolic methoxy groups. Alternatively, dimethomorph was metabolized via morpholine-ring opening and degradation resulting in M550F009 (WL 376084, kidney) and M550F008 (CUR 7117, milk).

Report: CA 6.2.3/3
[redacted] 2015 b
BAS 550 F: Metabolism in the lactating goat
2015/1000603
Guidelines: EPA 860.1300: Nature of the Residue in Plants Livestock, OECD Test Guideline 503 - Metabolism in livestock, EEC 91/414 (7030(VI/95 Rev. 3)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report: CA 6.2.3/4
[redacted] 2016 b
Report Amendment 1 - BAS 550 F: Metabolism in the lactating goat
2016/1002112
Guidelines: EPA 860.1300: Nature of the Residue in Plants Livestock, OECD Test Guideline 503 - Metabolism in livestock, EEC 91/414 (7030(VI/95 Rev. 3)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Description: [p-chlorophenyl-^{14}C]-BAS 550 F (chlorophenyl label, *E:Z* 45:55)
[Morpholine-2,3-^{14}C]-BAS 550 F (morpholine label, *E*:*Z* 46:54)
[Acrolyl-^{13}C]-BAS 550 F (carbon-13 label; E:Z 38:62)
[Morpholine-^{15}N]-BAS 550 F (nitrogen-15 label, *E:Z* 44:56)
BAS 550 F (unlabeled, *E:Z* 44:56)

Lot/batch #: 1068-0101 (chlorpophenyl label)
858-0101 (morpholine label)
1070-0101 (carbon-13 label)
1071-0105 (nitrogen-15 label)
AC9978-68A (unlabeled)

Purity: Radiochemical purity:
Chlorophenyl label: 96.4%
Morpholine label: 98.6%
Chemical purity:
Carbon-13 label: 91.3%
Nitrogen-15 label: 97.3%
Unlabeled BAS 550 F: 97.6%
Specific activity:
Chlorophenyl label: 7.70 MBq/mg
Morpholine label: 5.74 MBq/mg
Chlorophenyl dose: 1.959 MBq/mg
Morpholine dose: 1.931 MBq/mg

CAS#: 110488-70-5
Development code: Not reported
Stability of test compound: The test item was stable for the test period for both labels.

2. Test Animals

Species:	Goat
Variety:	"British Saanen"
Gender:	Female
Age:	Adult
Weight at dosing:	56 kg (chlorpophenyl label), 51 kg (morpholine label)
Number of animals:	4 (1 control, 2 per label)
Acclimation period:	at least 10 days (except for control: 2 days)
Diet:	2 x 0.5 kg non-medicated commercially available concentrate (Flockmaster 18 ewe nuts supplied by Heygate and Sons, Bugbrooke, Northampton, NN7 3QH) and 1 kg hay
Water:	Tap water, *ad libitum*
Housing:	Metabolic cage with a mesh floor suitable for the separate collection of urine and feces prior to first dose administration

Environmental conditions

Temperature:	16-26°C
Humidity:	16-82%
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:	**Amount of dose:**	0.37 mg/kg bw/day (chlorophenyl label) 0.42 mg/kg bw/day (morpholine label) 12.07 mg/kg feed (chlorophenyl label) 13.25 mg/kg feed (morpholine label)
	Food consumption:	1.580 kg DM / day (chlorophenyl label) 1.585 kg DM / day (morpholine label)
	Vehicle:	Gelatin capsule by dosing gun
	Timing:	Once daily
	Duration:	10 consecutive days

2. Sample collection

Milk collection:	Twice daily (pooled per day)
Urine and feces collection:	Daily
Interval from last dose to sacrifice:	6 h
Tissues harvested & analyzed:	Blood, urine, feces, milk, bile, liver, kidney, muscle (flank and loin), fat (omental, subcutaneous and renal), GI tract and contents, carcass

3. Test system

The metabolism and distribution of dimethomorph was investigated in four lactating goats following a repeated oral administration of [p-chlorophenyl-^{14}C]-BAS 550 F or [morpholine-2,3-^{14}C]-BAS 550 F at respective mean dose levels of 0.37 mg/kg bw/day (12.1 mg/kg feed) or 0.42 mg/kg bw/day (13.2 mg/kg feed) for ten consecutive days. The test item was prepared in gelatin capsules and administered orally by dosing gun. Details of the study outline are summarized in Table 6.2.3-9.

Table 6.2.3-9: Dose level following administration of [^{14}C]-BAS 550 F to lactating goats at a target dose level of 12 mg /kg dry weight diet/day

Goat number	Radio-label	Mean daily dose		Treatment days	Sacrifice	Sampling
		[mg/kg feed]	[mg/kg bw]			
Goat 1+2	U-^{14}C-chlorophenyl label	12.07	0.37	10	6 h after last dosing (according to maximal plasma concentration at Day 1)	Day 1: Blood: pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours (immediately prior to the second dose administration) Milk: twice daily prior following administration Urine/Feces: at 24 hour intervals Final: Blood, urine, feces, milk, bile, liver, kidney, muscle (flank and loin), fat (omental, subcutaneous and renal), GI tract and contents, carcass
Goat 4+5	2,3-^{14}C-morpholine label	13.25	0.42	10		

4. Sampling and storage

Blood samples were taken prior and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post first dose. Urine, feces and cage wash (using methanol:water 1:1, v:v) samples were collected prior to first dose and for each 24 h period until sacrifice. The goat was milked twice daily and immediately prior to sacrifice. At approximately 6 h (corresponding to maximal plasma concentration of test item in plasma) post final dose the goat was humanely killed and edible tissues (liver, kidney, muscle and fat), bile, blood, the GI tract and parts of the carcass were removed *post mortem*. All samples were stored at ca. –20°C.

5. Description of analytical methods

The radioactivity of homogenized extracts of the liquid samples (milk, bile, urine, blood mixed with lithium heparin) was determined by LSC (liquid scintillation counting) analysis. Solid debris (feces, GI tract content, tissues) were analyzed for radioactivity content by combustion analysis followed by LSC. Liver, kidney, muscle (each type) and fat (each type) were pooled across both animals per label before combustion. Since the different muscle types and the fat types showed comparable total radioactive residues, flank and loin sample and omental, subcutaneous and renal fat were pooled to a muscle and fat composite sample, respectively.

Edible matrices were homogenized and extracted repeatedly with different solvents (see Table 6.2.3-4). Liver, kidney and composite muscle were homogenized and extracted repeatedly with acetonitrile and in case of the morpholine label additionally with methanol, ethanol, acetonitrile:water and water. Composite fat samples were extracted repeatedly with dichloromethane. Whole milk, cream and skimmed milk were extracted repeatedly using dichloromethane and ethyl acetate and in case of the morpholine label additionally with acetone, methanol and ethanol. For whole milk and cream samples two more extraction regimes were performed to compare dichloromethane-, acetone- and acetonitrile based extractions (see Table 6.2.3-10).

Further investigation of non-solvent extractable residues in liver, kidney and cream (RRR) was conducted using protease enzyme. Cream was additional subjected to lipase digestion.

Table 6.2.3-10: Overview of extractions performed

Matrix	Extraction solvent (repetitions)				
	Procedure 1	Procedure 2	Procedure 3	Procedure 4	Procedure 5
Chlorphenyl label					
Whole milk	Dichloromethane (x3)	Ethyl acetate (x3)	-	-	-
Cream (Dichloromethane)	Dichloromethane (x2)	Dichloromethane (x2)	-	-	-
Cream (Acetone)	Acetone (x1)	Acetone (x2)	-	-	-
Cream (Acetonitrile)*	Acetonitrile (x3)	-	-	-	-
Skimmed milk	Dichloromethane (x3)	Ethyl acetate (x3)	Acetone (x2)	-	-
Liver	Acetonitrile (x2)	-	-	-	-
Kidney	Acetonitrile (x3)	-	-	-	-
Muscle	Acetonitrile (x2)	-	-	-	-
Fat	Dichloromethane (x2)	-	-	-	-
Morpholine label					
Whole milk (Dichloromethane)	Dichloromethane (x4)	Ethyl acetate (x2)	Acetone (x3)	Methanol (x4)	Ethanol (x2)
Whole milk (Acetone)	Acetone (x3)	Acetone (x1)			
Whole milk (Acetonitrile)*	Acetonitrile (x7)				
Cream (Dichloromethane)	Dichloromethane (x5)	Ethyl acetate(x2)	Acetone (x4)	Methanol (x2)	
Cream (Acetone)	Acetone (x5)	-	-	-	-
Cream (Acetonitrile)*	Acetonitrile (x9)	-	-	-	-
Skimmed milk	Dichloromethane (x3)	Ethyl Acetate (x3)	Acetone (x3)	Methanol(x4)	Ethanol (x2)
Liver	Acetonitrile (x3)	Methanol (x2)	Ethanol (x1)	Acetonitrile:water (50:50) (x2)	-
Kidney	Acetonitrile (x3)	Methanol (x3)	Ethanol (x1)	Acetonitrile:water (50:50) (x2)	Water (x4)
Muscle	Acetonitrile (x3)	Methanol (x4)	Ethanol (x2)	Water (x3)	-
Fat	Dichloromethane (x2)	-	-	-	-

* Selected goat samples were extracted using only acetonitrile in order to further assess the extractability of the radioactive residue in fatty matrices.

HPLC analysis was performed on relevant samples and extracts with a significant residue. Residues present in milk and tissues following solvent extraction were investigated by HPLC and HPLC-MS/MS using both the same HPLC method (HPLC 2). Identification of metabolites was performed by accurate mass HPLC-MS/MS analyses. On-line radio-detection for quantification and fraction collection and TopCount analysis were used to confirm the assignment and quantification. Moreover the identity of some radiolabeled components was tested also by co-chromatography with the authentic reference items. The unretained polar fraction observed in the morpholine label samples was isolated and analyzed by a second HPLC method, HPLC 3.

Metabolites were defined as identified, where a positive identification of a particular component was made using at least one chromatographic system in conjunction with mass spectrometric detection (accurate mass positive ion full scan and product ion evaluation including direct comparison with the fraction collected [^{14}C]-radio-chromatograms). Where the residue was unable to be identified or the residue was not large enough to warrant identification analysis, then the residue was classified as characterized.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in all matrices are summarized in Table 6.2.3-12. The radioactivity recovered in all matrices accounted for 80.25% (chlorophenyl label) and 84.27% (morpholine label) of the total dose (see Table 6.2.3-11). Until sacrifice, the radioactive residues excreted via urine, feces and cage wash amounted to 53.35%, 16.33% and 0.43%, respectively for the chlorophenyl label and 19.24%, 53.05% and 0.36%, respectively for the morpholine label. Furthermore in the gastro intestinal tract, its contents and in bile a total of 9.44 % (chlorophenyl label) and 10.4% (morpholine label) was found. Radioactivity associated with muscle, fat and whole blood accounted for both labels for 0.01% of the administered dose or below. Radioactive residues in whole milk accounted for 0.06% (chlorophenyl label) or 0.46% (morpholine label) of the dosed radioactivity and reached a plateau concentration in the range of approximately 0.06 mg/kg (chlorophenyl label) to 0.045 mg/kg (morpholine label) within 2 days. The radioactive residues in liver accounted for 0.64% and in kidney for 0.01% of the dosed radioactivity (both labels).

Table 6.2.3-11: Recovery of radioactivity in lactating goats following ten consecutive administrations of [^{14}C]-BAS 550 F at a target dose level of 12 mg /kg dry weight diet/day

Sample	Dose recovered in sample [%]	
	Chlorophenyl label	Morpholine label
Faces	53.35	53.05
Urine	16.33	19.24
Cage wash	0.43	0.36
Milk	0.06	0.46
Liver	0.64	0.64
Kidney	0.01	0.01
Flank muscle	<0.01	0.01
Loin muscle	<0.01	0.01
Omental fat	0.01	0.01
Subcutaneous fat	<0.01	<0.01
Renal fat	<0.01	<0.01
G. I. Tract	2.36	1.76
G. I. Tract contents	6.98	8.59
Whole blood	<0.01	<0.01
Bile	0.10	0.15
Total	**80.25**	**84.27**

Table 6.2.3-12: Total radioactive residues in edible goat matrices

Matrix	Timing	Mean TRR [mg/kg][1]			
		Measured	Calculated	Measured	Calculated
		Chlorphenyl label		Morpholine label	
Liver	Terminal	1.509	1.512	1.413	1.362
Kidney	Terminal	0.073	0.070	0.166	0.166
Flank muscle	Terminal	0.006	-	0.028	-
Loin muscle	Terminal	0.008	-	0.030	-
Composite muscle	Terminal	0.007	0.008	0.029	0.036
Omental fat	Terminal	0.008	-	0.011	-
Subcutaneous fat	Terminal	0.007	-	0.016	-
Renal fat	Terminal	0.007	-	0.010	-
Composite fat	Terminal	0.006	0.008	0.009	0.014
Whole milk pool	72-216 hours	0.006	0.006	0.045	0.048 / 0.034*
Cream pool	72-216 hours	0.012	0.017 / 0.014*	0.134	0.137 / 0.143*
Skim milk pool	72-216 hours	0.005	0.005	0.031	0.033

TRR Total radioactive residue (calculated TRR = sum of ERR + RRR)
1 Mean of two animals per label
* First value for dichloromethane based extraction, second value for acetone based extraction

B. EXTRACTION OF RESIDUES

The extractability of the edible tissues was high, ranging from 73.5% (muscle, morpholine label) to 109.6% (fat, chlorophenyl label) of the combusted TRR (see Table 6.2.3-13). In liver, where radioactivity accounted for 1.509 or 1.413 mg/kg (chlorophenyl label or morpholine label), 88.0% or 82.2% of the combusted TRR were extracted. In whole milk, cream and skimmed milk extracted radioactivity ranged from 86% to 110.4% for the chlorophenyl label and 40.2% to 87.4% for the morpholine label depending on the extraction solvent. The residual radioactive residue after solvent extraction ranged from <0.1% to 65.4%, the high values partly caused by low residue amounts and multiple extraction steps. Residual radioactive residues in liver and kidney of both labels and cream of the morpholine label were further investigated with protease digestion (both labels) and lipase digestion (morpholine label). An additional 8.7% and 31.8% of the TRR was released by enzymatic digestion and final residues in matrices of liver, kidney and cream accounted 1.7% to 5.6% (see Table 6.2.3-14).

Table 6.2.3-13: Extractability of goat matrices after [^{14}C]-BAS 550 F treatment

Matrix	TRR combusted		ERR[1]		RRR[2]		Recovery[3]
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Chlorophenyl label							
Liver	1.509	100	1.328	88.0	0.184	12.2	100
Kidney	0.073	100	0.060	82.6	0.010	13.8	96
Muscle	0.007	100	0.006	88.7	0.002	21.6	110
Fat	0.006	100	0.007	109.6	0.001	12.1	122[4]
Whole milk	0.006	100	0.006	102.2	<0.001	4.3	107
Cream (dichloromethane extr.)	0.012	100	0.010	86.9	0.007	55.3	142[4]
Cream (acetone extr.)			0.013	110.4	0.001	12.1	123[4]
Cream (acetonitrile extr.)*			0.012	103.5	-	-	-
Skimmed milk	0.005	100	0.005	92.3	<0.001	<0.1	92
Morpholine label							
Liver	1.413	100	1.161	82.2	0.202	14.3	97
Kidney	0.166	100	0.125	75.4	0.041	24.4	100
Muscle	0.029	100	0.021	73.5	0.014	47.1	121[4]
Fat	0.009	100	0.009	98.2	0.006	65.4	164[4]
Whole milk (dichloromethane extr)	0.045	100	0.039	87.4	0.009	20.8	108
Whole milk (acetone extr.)			0.029	65.0	0.005	10.9	76[4]
Whole milk (acetonitrile extr.)*			0.026	58.2	-	-	-
Cream (dichloromethane extr)	0.134	100	0.097	72.5	0.040	29.7	102
Cream (acetone extr.)			0.097	72.7	0.046	34.0	107
Cream (acetonitrile extr.)*			0.054	40.2	-	-	-
Skimmed milk	0.031	100	0.018	57.6	0.015	49.4	107

1 ERR = Extractable radioactive residue
2 RRR = Residual radioactive residues (alternatively post extraction solid: PES)
3 Sum of ERR + RRR
4 Recovery based on low residues and multiple extraction steps
* The extraction was performed in order to assess the extractability of the radioactive residue in fatty matrices by acetonitrile. Analysis of the solid debris was not performed.

Table 6.2.3-14: Summary of released non extractable radioactive residues

Matrix	RRR[1]		Protease digestion		Lipase digestion		Final residue		Recovery
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Chlorophenyl label									
Liver	0.184	12.2	0.131	8.7	-	-	0.062	4.1	105
Kidney	0.010	13.8	0.008	10.3	-	-	0.002	2.9	96
Morpholine label									
Liver	0.202	14.3	0.143	10.1	-	-	0.079	5.6	110
Kidney	0.041	24.4	0.036	21.4	-	-	0.007	4.0	104
Cream (dichloromethane extraction)	0.040	29.7	0.035	26.0	-	-	0.003	2.3	95
Cream (acetone extraction)	0.046	34.0	0.038	28.1	0.005	3.7	0.002	1.7	99

1 Residual radioactive residues (alternatively post extraction solid: PES)

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

Summaries of identified and characterized residues are shown in Table 6.2.3-15 to Table 6.2.3-16 for the chlorophenyl label and Table 6.2.3-17 to Table 6.2.3-18 for the morpholine label. A summary of all identified metabolites and their distribution in edible matrices is given in Table 6.2.3-19 (chlorophenyl label) and Table 6.2.3-20 (morpholine label). Identification was accomplished by HPLC in conjunction with mass spectrometric detection. On occasion, the identification process was aided by comparison with components observed in the corresponding BAS 550F rat (see M-CA 5.1.1/1, DocID 2015/1000602) or hen [see KCA 6.2.2/3 2015/1000604] studies. For quantification of labeled components, concentrated extracts of milk, cream, skimmed milk, liver, kidney and composite muscle were analyzed directly by HPLC (using the same HPLC method as for LC-MS analysis).
In those cases where metabolites were co-eluting, each metabolite was assigned the entire peak amount as a worst case assumption.

Chlorophenyl label
The acetone extraction of cream (ERR: 0.013 mg/kg; see Table 6.2.3-15) revealed the following metabolites: unchanged BAS 550 F *E*-isomer (0.001 mg/kg; 8.1% TRR); *Z*-isomer (0.001 mg/kg; 10.5% TRR), M550F011 (0.002 mg/kg; 13.4% TRR) and M550F008 (*Z*) (0.002 mg/kg; 13.4% TRR). In addition, a co-elution of the following metabolites M550F008 (*E*), M550F031 and M550F018 (0.004 mg/kg; 35.3% TRR) was observed. A further 0.002 mg/kg; 22.2% TRR was characterized by HPLC, consisting of three components, none accounting for more than 0.001 mg/kg.

Analysis of the acetonitrile liver extract (ERR: 1.328 mg/kg, see Table 6.2.3-16) identified the unchanged BAS 550 F as the most abundant component, accounting for 54.1% TRR (0.817 mg/kg) as the sum of the *E*- and the *Z*-isomer. Other identified metabolites in the liver were M550F007 (sum of isomers) accounting for 6.8% TRR (0.103 mg/kg), M550F009 (0.017 mg/kg; 1.1% TRR) M550F029 (0.014 mg/kg; 0.9% TRR) and M550F016 (0.038 mg/kg; 2.5% TRR; sum of isomers).
Metabolites M550F006, M550F008 and M550F012 (sum of isomers) accounted each for 2.2% TRR and 0.034 mg/kg, since they were co-eluting.
Other co-eluting metabolites were M550F028, M550F011 and M550F030 (0.029 mg/kg; 1.9% TRR); M550F030, M550F031 and M550F011 (0.029 mg/kg; 1.9% TRR); M550F018, and M550F031 (0.018 mg/kg; 1.2% TRR) and M550F015 and M550F013 (0.009 mg/kg; 0.6% TRR).
A residue of 0.014 mg/kg (0.9% TRR) remained unidentified following MS analysis, but this was consisting of three components, the largest of which represented a residue of 0.006 mg/kg (0.4% TRR).

Analysis of the acetonitrile kidney extract (ERR: 0.060 mg/kg, see Table 6.2.3-16) identified the residues as unchanged BAS 550 F (*Z*-isomer) (0.006 mg/kg; 7.8% TRR) and the metabolite M550F008 (sum of isomers) accounted for 0.013 mg/kg (18.7% TRR). The most abundant peak consisted of a co-elution of the following metabolites M550F031, M550F011 and M550F007 (*E*) (0.022 mg/kg; 29.8% TRR); the other major peak consisted of a co-elution of the metabolites M550F029, M550F015 and M550F013 (0.013 mg/kg; 17.8% TRR). Further identified metabolites were M550F018 and M550F031, each accounting for 0.007 mg/kg (10.0% TRR), as they co-eluted.

The whole milk extract (ERR: 0.006 mg/kg), the skimmed milk extract (ERR: 0.005 mg/kg), the muscle extract (ERR: 0.006 mg/kg) and the fat extract (ERR: 0.007 mg/kg) were not further analyzed due to their low levels of radioactivity (all below 0.010 mg/kg).

The non-extractable residues after solvent extraction of liver and kidney were 12.2 and 13.8% TRR, respectively and were therefore further investigated with protease enzyme, whereby an additional 8.7 and 10.3% of the TRR was released, respectively. This shows that the majority of the non-extractable radioactivity was bound, or incorporated, into the endogenous protein fraction. Solubilized radioactivity was characterized by HPLC, but warranted no further identification information.

Morpholine label
Whole milk was extracted according to two different extraction regimes; dichloromethane (Extraction 1) and acetone (Extraction 2).
Residues in the dichloromethane extract were identified as unchanged BAS 550 F (Z-isomer; <0.001 mg/kg; 0.5% TRR), M550F011 (<0.001 mg/kg; 0.5% TRR) and M550F008 (<0.002 mg/kg; 1.3% TRR as the sum of isomers). In addition, a co-elution of the metabolites M550F031 and M550F018 was observed, (<0.001 mg/kg; 0.8% TRR). A further 2.1% TRR (<0.001 mg/kg) was characterized (see Table 6.2.3-19) distributed on 4 peaks.
An additional residue of 0.008 mg/kg (17.6% TRR), unretained on the first chromatographic system (the polar fraction), co-chromatographed with [^{14}C]-morpholine on the second chromatographic system (HPLC 3), strongly indicating that this component was M550F021 (morpholine).
Extraction with acetone (Extraction 2) liberated a residue of 65% TRR (0.029 mg/kg). This entire residue consisted of one polar fraction (unretained on the first chromatographic system, HPLC 2). Analysis of this fraction on the second chromatographic system, HPLC 3, did not bring any further identification as no retention time match with morpholine was observed.

Cream was extracted according to two different extraction regimes; dichloromethane (Extraction 1) and acetone (Extraction 2).
In the dichloromethane extract (ERR: 0.097 mg/kg) residues were identified as unchanged BAS 550 F, the sum of *E*- and *Z*-isomers, (0.004 mg/kg; 3.0% TRR) and M550F007 (*Z*) (0.003 mg/kg; 2.1% TRR).
A further residue of 0.029 mg/kg, 21.3% TRR, in the solubilized radioactivity following protease hydrolysis, co-chromatographed with morpholine (see Table 6.2.3-17).
In the acetone extract (ERR: 0.097 mg/kg) identified residues were M550F008 (0.019 mg/kg; 14.6% TRR) as a sum of isomers, M550F031 (0.021 mg/kg; 16.1% TRR), M550F018 (0.012 mg/kg; 9.3% TRR) and a co-elution of M550F011 and M550F053, accounting for 0.009 mg/kg (6.8% TRR).
A fourth component, representing 0.058 mg/kg (43.2% TRR), was unretained on the first chromatographic system. Following analysis on the second chromatographic system, HPLC 3, this polar peak was split into two separate components, accounting for a maximum residue of 0.033 mg/kg (24.9% TRR). Neither of these components could be identified.

Analysis of the skimmed milk (ERR: 0.018 mg/kg, see Table 6.2.3-17) acetone extract revealed a single polar peak, accounting for 0.005 mg/kg (15.8% TRR). Following analysis on the second chromatographic system, HPLC 3, one single component was observed, which co-chromatographed with [^{14}C]-morpholine.

Analysis of the liver samples (ERR: 1.161 mg/kg, see Table 6.2.3-18) provided the following identifications: the major component was the unchanged parent compound, BAS 550 F representing a residue of 0.640 mg/kg, 45.4% TRR (as the sum of the *E*- and *Z* -isomers). In addition, the metabolite M550F007 was observed, accounting for 0.153 mg/kg, 10.8% TRR, as the sum of both isomers. Metabolite M550F016 represented 0.080 mg/kg; 5.7% TRR, as the sum of both isomers and metabolites M550F030 and M550F011 each represented 0.082 mg/kg; 5.8% TRR, as the sum of both isomers. Also, metabolite M550F006 was found in minor amounts, representing 0.020 mg/kg (1.5% TRR).

An additional component representing 0.059 mg/kg (4.2% TRR), was unretained on the first chromatographic system. Chromatography on the second HPLC system, HPLC 3, revealed that this polar fraction consisted of at least five different components, one of which had the same retention time as M550F021, and represented 0.021 mg/kg (1.5% TRR).
The residual extracted radioactivity was distributed among 18 peaks, the largest of which accounted for 0.031 mg/kg and 2.2% TRR.
A further residue of 0.131 mg/kg (9.3% TRR) in the solubilized radioactivity after protease treatment was tentatively identified as morpholine.

Analysis of the kidney sample (ERR: 0.125 mg/kg, see Table 6.2.3-18) showed the following residues: unchanged parent, BAS 550 F (*Z*) (0.002 mg/kg; 1.3% TRR) and metabolite M550F007, accounting for 0.039 mg/kg, 23.4% TRR, as a sum of both isomers. A residue of 0.023 mg/kg (13.8% TRR) consisting of at least six components (no single component represented a residue of ≥0.007 mg/kg), was characterized by HPLC analysis.
Again, a polar component, representing 0.039 mg/kg (23.3% TRR), was unretained on the first chromatographic system, HPLC 2. Chromatographic analysis on a second system, HPLC 3, showed that this polar fraction contained at least three components, of which one, representing 0.019 mg/kg and 11.2% TRR, co-chromatographed with morpholine.
A further residue of 0.036 mg/kg, 21.4% TRR, released following protease hydrolysis, co-chromatographed with morpholine as well.

Extraction of the muscle sample (ERR: 0.021 mg/kg, see Table 6.2.3-18) provided two extracts suitable for chromatography (acetonitrile and methanol extract), which contained a residue of 0.014 mg/kg (49.4% TRR). Radioactivity was distributed equally among two peaks, one of which represented the polar fraction. No identifications were achieved however, each single component represented only 0.007 mg/kg.

No further analysis was performed on the fat sample, since the residue was very low (0.009 mg/kg).

The non-extractable residues after solvent extraction of liver, kidney and cream were 14.3, 24.4 and 29.7% TRR, respectively and were therefore further investigated with protease enzyme, whereby an additional 10.1, 21.4 and 26.0% of the TRR was released, respectively. Re-extraction of the cream sample using acetone provided a residue of 34.0% TRR, which was further investigated using protease and lipase enzymes, whereby an additional 31.8% TRR was released. This demonstrates that the majority of the non-extractable residues are bound to or incorporated into the endogenous protein or lipid fraction. The solubilized radioactivity was further characterized by HPLC. In all cases, the enzyme treatment released a polar fraction, which was then shown (by HPLC 3) to contain in some cases free morpholine as a minor component.

Table 6.2.3-15: Summary of identified and characterized residues in milk matrices of lactating goats after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Components	Whole milk		Cream (dichlormethane based)		Cream (acetone based)		Skimmed milk	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified								
BAS 550 F (*E*)	-	-	2.4	<0.01	8.1	0.001	-	-
BAS 550 F (*Z*)	-	-	7.5	0.01	10.5	0.001	-	-
M550F008 (*Z*)	-	-	-	-	13.4	0.002	-	-
M550F009	-	-	3.3	<0.001	-	-	-	-
M550F011	-	-	11.8	0.001	13.4	0.002	-	-
Co-elutions of: M550F012 [42.1&44.7 min] M550F008 (*E*) [42.4 min] M550F008 (*Z*) [44.5 min] M550F031 [42.8 min]	-	-	14.5	0.002	-	-	-	-
M550F008 (*E*) [42.4 min] M550F031 [42.8 min] M550F018 [43.2 min]	-	-			35.3	0.004	-	-
Total identified	**NA**	**NA**	**39.5**	**0.004**	**80.7**	**0.010**	**NA**	**NA**
Characterized								
Number of peaks	-		-		3		-	
Peak with highest radioactive residue	-	-	-	-	10.3	0.001	-	-
Characterized by extraction	102.2	0.006	16.8	0.002	7.4	0.001	92.3	0.005
Total characterized	102.2	0.006	16.8	0.002	29.6	0.003	92.3	0.005
Total identified and/or characterized	**102.2**	**0.006**	**56.3**	**0.007**	**110.4**	**0.013**	**92.3**	**0.005**
Final residue	4.3	<0.001	55.3	0.007	12.1	0.001	<0.1	<0.001
Grand total*	**106.5**	**0.006**	**142.2**	**0.017**	**122.5**	**0.014**	**92.3**	**0.005**

* Including radioactive losses during extraction procedure (data not shown)
NA Not analyzed by HPLC; residue in extract <0.010 mg/kg

Table 6.2.3-16: Summary of identified and characterized residues in tissues of lactating goats after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Components	Liver		Kidney		Composite muscle		Composite fat	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified								
BAS 550 F (*E*)	30.3	0.457	-	-	-	-	-	-
BAS 550 F (*Z*)	23.8	0.360	7.8	0.006	-	-	-	-
M550F007 (*E*)	5.2	0.078	-	-	-	-	-	-
M550F007 (*Z*)	1.6	0.025	-	-	-	-	-	-
M550F008 (*Z*)	-	-	8.7	0.006	-	-	-	-
M550F009	1.1	0.017	-	-	-	-	-	-
M550F016	0.6	0.009	-	-	-	-	-	-
M550F029	0.9	0.014	-	-	-	-	-	-
Co-elutions of: M550F028 [38.4 min] M550F011 [38.7 min] M550F030 [38.9 min] M550F016 [39.3 min]	1.9	0.029	-	-	-	-	-	-
M550F030 [40.7 min] M550F031 [40.8 min] M550F011 [40.9 min]	1.9	0.029	-	-	-	-	-	-
M550F018 [42.0 min] M550F008 (*E*) [42.4 min] M550F006 (*E*) [42.5 min] M550F012 [42.1 min] M550F031 [42.8 min] M550F018 [43.2 min]	1.2	0.018	-	-	-	-	-	-
M550F006 (*Z*) [44.5 min] M550F008 (*Z*) [44.5 min] M550F012 [44.7 min]	1.0	0.016	-	-	-	-	-	-
M550F015 [32.4 min] M550F013 [32.7 min]	0.6	0.009	-	-	-	-	-	-
M550F031 [40.8 min] M550F011 [40.9 min] M550F007 (*E*) [41.5 min]	-	-	29.8	0.022	-	-	-	-
M550F029 [32.0 min] M550F015 [32.4 min] M550F013 [32.7 min]	-	-	17.8	0.013	-	-	-	-
M550F008 (*E*) [42.4 min] M550F018 [43.2 min] M550F031 [42.8 min]	-	-	10.0	0.007	-	-	-	-
Total identified	**70.3**	**1.060**	**74.1**	**0.054**	**NA**	**NA**	**NA**	**NA**
Characterized								
Number of peaks	3		0		-		-	
Peak with highest radioactive residue	0.4	0.006	-	-	-	-	-	-
Characterized by extraction	-	-	-	-	88.7	0.006	109.6	0.007
Total identified and/or characterized	**71.2**	**1.074**	**74.1**	**0.054**	**88.7**	**0.006**	**109.6**	**0.007**
Extracted by enzyme digestion	8.7	0.131	10.3	0.008	-	-	-	-
Final residue	4.1	0.062	2.9	0.002	21.6	0.002	12.1	0.001
Grand total*	**100.2**	**1.512**	**96.4**	**0.070**	**110.3**	**0.008**	**121.6**	**0.008**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)

Table 6.2.3-17: Summary of identified and characterized residues in milk matrices of lactating goats after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Components	Whole milk (dichlormethane based)		Whole milk (acetone based)		Cream (dichlormethane based)		Cream (acetone based)		Skimmed milk	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified										
BAS 550 F (*E*)	-	-	-	-	1.2	0.002	-	-	-	-
BAS 550 F (*Z*)	0.5	<0.001	-	-	1.8	0.002	-	-	-	-
M550F007 (*Z*)	-	-	-	-	2.1	0.003	-	-	-	-
M550F008 (*Z*)	0.5	<0.001	-	-	-	-	5.3	0.007	-	-
M550F011	0.5	<0.001	-	-	-	-	-	-	-	-
Co-elutions of: M550F008 (*E*) [42.4 min] M550F031 [42.8 min] M550F018 [43.2 min]	0.8	<0.01	-	-	-	-	9.3	0.012	-	-
M550F011 [40.9 min] M550F031 [40.8 min] M550F053 [41.1 min]	-	-	-	-	-	-	6.8	0.009	-	-
Total identified	**2.3**	**<0.001**	**NA**	**NA**	**5.1**	**0.007**	**21.4**	**0.028**	**NA**	**NA**
Characterized										
Number of peaks-HPLC 2	3		0		3		0		0	
Peak with highest radio-active residue	0.9	<0.001	-	-	3.7	0.005	-	-	-	-
Number of peaks-HPLC 3	1		1		-		2		1	
Peak with highest radioactive residue	17.6	0.008	45.6	0.021	-	-	24.9	0.033	15.8	0.005
Characterized by extraction	17.2	0.007	0.9	<0.001	4.3	0.006	-	-	23.1	0.007
Total identified and/or characterized	**39.2**	**0.015**	**46.5**	**0.021**	**14.7**	**0.020**	**64.6**	**0.087**	**38.9**	**0.012**
Extracted by enzyme digestion	-	-	-	-	26.0	0.035	31.8	0.043	-	-
Final residue	20.8	0.009	10.9	0.005	2.3	0.003	1.7	0.002	49.4	0.015
Grand total*	**108.2**	**0.048**	**75.9**	**0.034**	**102.2**	**0.137**	**106.7**	**0.143**	**107.0**	**0.033**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)
Number of peaks - HPLC 2 do not contain peaks, which were included in Number of peaks - HPLC3

Table 6.2.3-18: Summary of identified and characterized residues in tissues of lactating goats after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Components	Liver		Kidney		Composite muscle		Composite fat	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Identified								
BAS 550 F (*E*)	18.1	0.255	-	-	-	-	-	-
BAS 550 F (*Z*)	27.3	0.385	1.3	0.002	-	-	-	-
M550F006 (*Z*)	1.5	0.020	-	-	-	-	-	-
M550F007 (*E*)	7.7	0.108	22.8	0.038	-	-	-	-
M550F007 (*Z*)	3.1	0.045	0.6	0.001	-	-	-	-
M550F016	1.4	0.019	-	-	-	-	-	-
Co-elutions of: M550F016 [39.3 min] M550F011 [38.7 min] M550F030 [38.9 min]	4.3	0.061	-	-	-	-	-	-
M550F030 [40.7 min] M550F011 [40.9 min]	1.5	0.021	-	-	-	-	-	-
Total identified	**64.9**	**0.917**	**24.7**	**0.041**	**NA**	**NA**	**NA**	**NA**
Characterized								
Number of peaks-HPLC 2	18		6		2		-	
Peak with the highest residue	2.2	0.031	4.3	0.007	25.1	0.007	-	-
Number of peaks-HPLC 3	5		3		-		-	
Peak with highest residue	1.7	0.024	11.2	0.019	-	-	-	-
Characterized by extraction	-	-	4.2	0.007	5.4	0.001	98.2	0.009
Total identified and/or characterized	**81.5**	**1.151**	**66.6**	**0.110**	**54.8**	**0.015**	**98.2**	**0.009**
Extraction by enzyme digestion	10.1	0.143	21.2	0.036	-	-	-	-
Final residue	5.6	0.079	4.0	0.007	47.1	0.014	65.4	0.006
Grand total*	**96.5**	**1.362**	**99.8**	**0.166**	**120.6**	**0.036**	**163.6**	**0.014**

NA Not analyzed by HPLC; residue in extract <0.010 mg/kg
* Including radioactive losses during extraction procedure (data not shown)
Number of peaks - HPLC 2 do not contain peaks, which were included in Number of peaks - HPLC3

Table 6.2.3-19: Summary of identified residues in goat after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

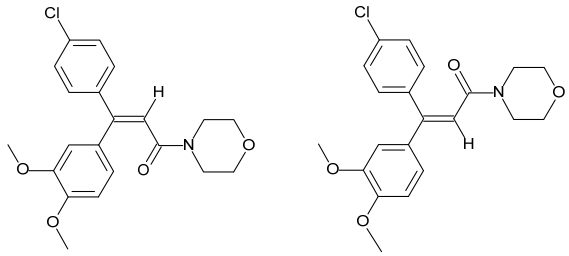
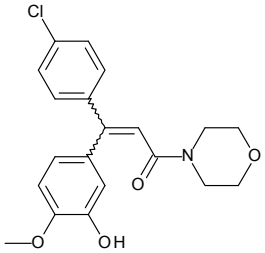
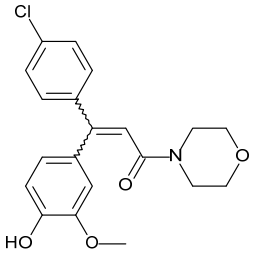
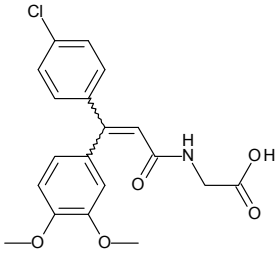
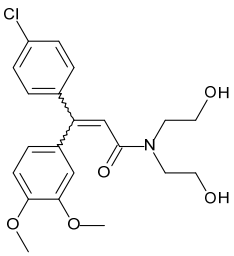
Metabolite	Structure	Cream (dichlor-methane)	Cream (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 550 F (*E*)		<0.001 (2.4)	0.001 (8.1)	0.457 (30.3)	ND
BAS 550 F (*Z*)		0.001 (7.5)	0.001 (10.5)	0.360 (23.8)	0.006 (7.8)
Sum E +Z		*0.001 (9.9)*	*0.002 (18.6)*	*0.817 (54.1)*	*0.006 (7.8)*
M550F006 (*E*)		ND	ND	0.018[A] (1.2)	ND
M550F006 (*Z*)		ND	ND	0.016[A] (1.0)	ND
M550F007 (*E*)		ND	ND	0.078 (5.2)	0.022[A] (29.8)
M550F007 (*Z*)		ND	ND	0.025 (1.6)	ND
M550F008 (*E*)		0.002[A] (14.5)	0.004[A] (35.3)	0.018[A] (1.2)	0.007[A] (10.0)
M550F008 (*Z*)		0.002[A] (14.5)	0.002 (13.4)	0.016[A] (1.0)	0.006 (8.7)
M550F009 (sum of isomers)		<0.001 (3.3)	ND	0.017 (1.1)	ND

Table 6.2.3-19: Summary of identified residues in goat after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

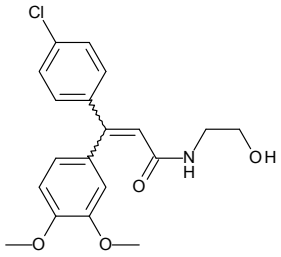
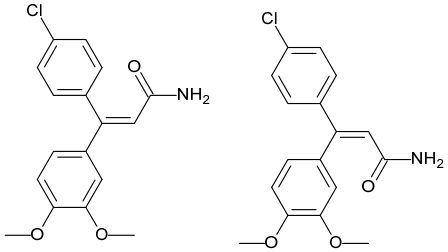
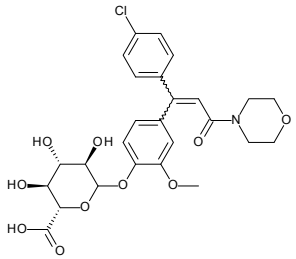
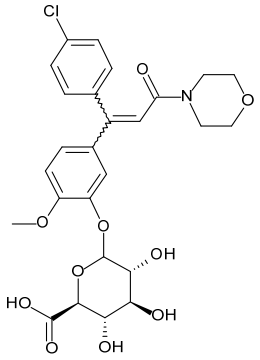
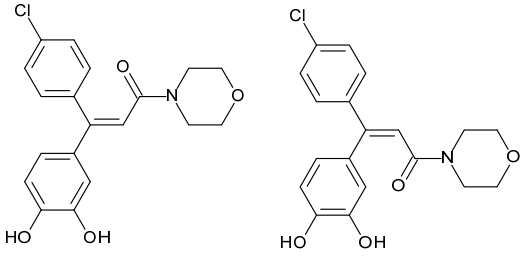
Metabolite	Structure	Cream (dichlor-methane)	Cream (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F011 (sum of isomers)		0.001 (11.8)	0.002 (13.4)	0·058[A] (3.8)	0.022[A] (29.8)
M550F012 (sum of isomers)		0.002[A] (14.5)	ND	0.034[A] (2.2)	ND
M550F013 (sum of isomers)		ND	ND	0.009[A] (0.6)	0.013[A] (17.8)
M550F015 (sum of isomers)		ND	ND	0.009[A] (0.6)	0.013[A] (17.8)
M550F016 (sum of isomers)		ND	ND	0.038[A] (2.5)	ND

Table 6.2.3-19: Summary of identified residues in goat after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

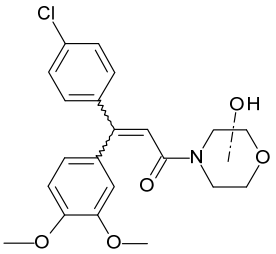
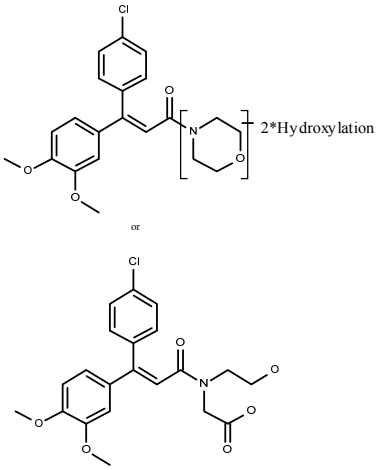
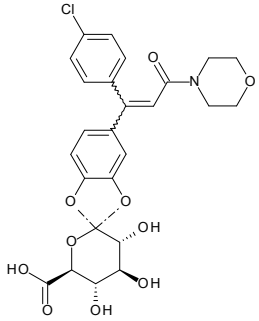
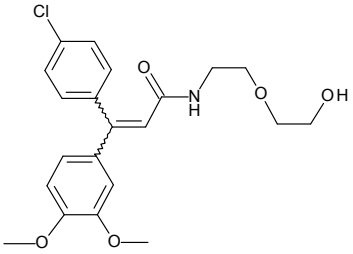
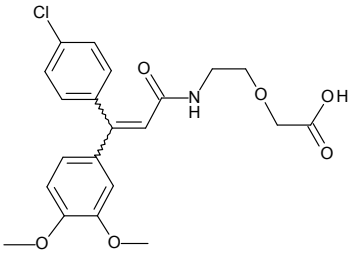
Metabolite	Structure	Cream (dichlor-methane)	Cream (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F018 (sum of isomers)		ND	0.004[A] (35.3)	0.036[A] (2.4)	0.007[A] (10.0)
M550F028 (sum of isomers)		ND	ND	0.029[A] (1.9)	ND
M550F029 (sum of isomers)		ND	ND	0.014 (0.9)	0.013[A] (17.8)
M550F030 (sum of isomers)		ND	ND	0.058[A] (3.8)	ND

Table 6.2.3-19: Summary of identified residues in goat after dosing with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Metabolite	Structure	Cream (dichlor-methane)	Cream (acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F031 (sum of isomers)		0.002[A] (14.5)	0.004[A] (35.3)	0.047[A] (3.1)	0.029[A] (39.8)

ND Not Detected
A Co-eluting component

Table 6.2.3-20: Summary of identified residues in goat after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

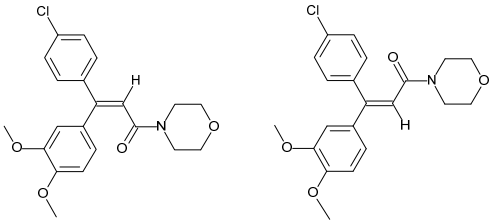
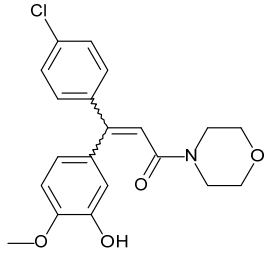
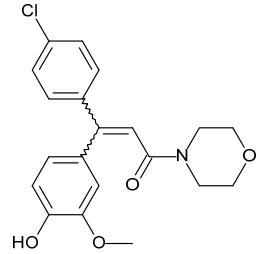
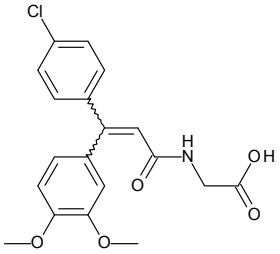
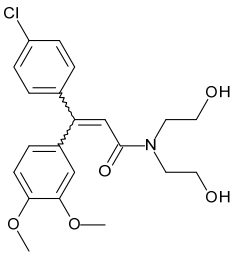
Metabolite	Structure	Milk (dichlor-methane)	Cream (dichlor-methane)	Cream acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 550 F (*E*)		ND	0.002 (1.2)	ND	0.255 (18.1)	ND
BAS 550 F (*Z*)		<0.001 (0.5)	0.002 (1.8)	ND	0.385 (27.3)	0.002 (1.3)
Sum E+Z		*<0.001 (0.5)*	*0.004 (3.0)*	*ND*	*0.640 (45.4)*	*0.002 (1.3)*
M550F006 (*E*)		ND	ND	ND	ND	ND
M550F006 (*Z*)		ND	ND	ND	0.020 (1.5)	ND
M550F007 (*E*)		ND	ND	ND	0.108 (7.7)	0.038 (22.8)
M550F007 (*Z*)		ND	0.003 (2.1)	ND	0.045 (3.1)	0.001 (0.6)
M550F008 (*E*)		<0.001[A] (0.8)	ND	0.012[A] (9.3)	ND	ND
M550F008 (*Z*)		<0.001 (0.5)	ND	0.007 (5.3)	ND	ND
M550F009 (sum of isomers)		ND	ND	ND	ND	ND

Table 6.2.3-20: Summary of identified residues in goat after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

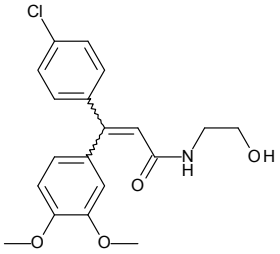
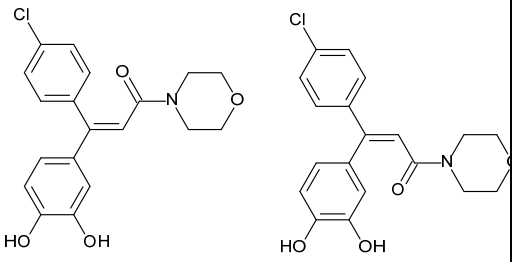
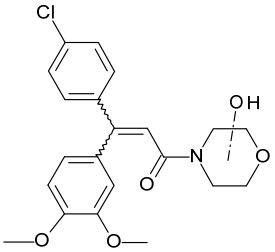
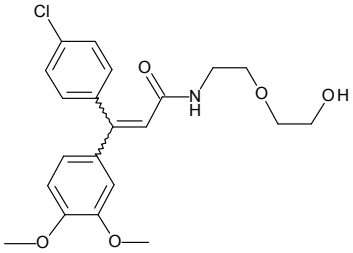
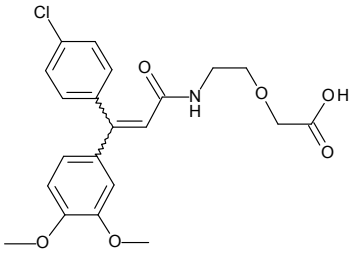
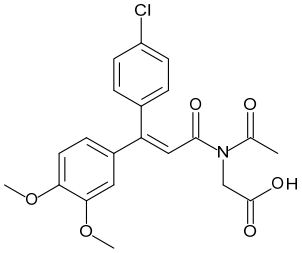
Metabolite	Structure	Milk (dichlor-methane)	Cream (dichlor-methane)	Cream acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F011 (sum of isomers)		<0.001 (0.5)	ND	0.009[A] (6.8)	0.082[A] (5.8)	ND
M550F016		ND	ND	ND	0.080[A] (5.7)	ND
M550F018 (sum of isomers)		<0.001[A] (0.8)	ND	0.012[A] (9.3)	ND	ND
M550F030 (sum of isomers)		ND	ND	ND	0.082[A] (5.8)	ND
M550F031 (sum of isomers)		<0.001[A] (0.8)	ND	0.021[A] (16.1)	ND	ND

Table 6.2.3-20: Summary of identified residues in goat after dosing with [morpholine-2,3-^{14}C]-BAS 550 F

Metabolite	Structure	Milk (dichlor-methane)	Cream (dichlor-methane)	Cream acetone)	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
M550F053 (sum of isomers)		ND	ND	0.009[A] (6.8)	ND	ND

ND Not detected
A Co-eluting component

Identification of the polar region

In the samples of the morpholine label (with HPLC method 2) a polar fraction was observed through all matrices (either in extracts or in released radioactivity after enzyme treatment of non-extractable residues, see Table 6.2.3-21. It was shown to consist in most cases of multiple components, which could not clearly be identified despite significant effort using several methods. Hereby several LC-MS/MS techniques (HILIC), co-chromatography with potential metabolites, TLC methods and enzyme deconjugation techniques (glucoronidase, sulphatase, and hydrolysis) were used to identify the polar fraction. Since this remained unsuccessful, additionally an in-vitro-study in goat hepatocytes was performed to support the identification of the polar region [see KCA 6.2.3/5 2015/1231130]. However, no sufficient amount of the polar region was produced in the hepatocytes to allow MS identification.

Table 6.2.3-21: Summary of quantification of polar region and M550F021 (morpholine)

Matrix	Additional information	Polar region (0-6 min; HPLC 2)		M550F021 (HPLC 3)	
		mg/kg	%TRR	mg/kg	%TRR
Morpholine label					
Whole milk	Dichloromethane extr.	0.009	18.5	0.008	17.6
	Acetone extr.	0.021	45.6	n.d.	n.d.
Cream	Dichloromethane extr.	0.005	3.7	n.d.	n.d.
	Dichloromethane extr./ PES-hydrolysate	0.029	21.3	0.029	21.3
	Acetone extr.	0.058	43.2	n.d.	n.d.
	Acetone extr./ PES-hyrdolysate	0.038	28.1	n.d.	n.d.
Skimmed milk	Extraction	0.005	15.8	0.005	15.8
Liver	Extraction	0.101	6.9	0.021	1.5
	PES-hydrolysate	0.143	10.1	0.131	9.3
Kidney	Extraction	0.039	23.3	0.019	11.2
	PES-hydrolysate	0.036	21.4	0.036	21.4
Muscle	Extraction	0.007	25.1	ND	ND

ND Not detected
PES Post extraction solid

An alternative HPLC method (HPLC 3) was used to analyze the isolated polar fraction. Co-chromatography with the radioactive reference substance morpholine confirmed the assumption that M550F021 (morpholine) might be part of the polar fraction (see Table 6.2.3-21).
Considering all analytical results from this study it is hypothesized that an incorporation of C2 fragments (stemming from the degradation of the morpholine moiety) into natural biosynthetic pathways is a relevant decomposition mechanism for BAS 550 F, resulting in small endogenous molecules of polar nature.

Extraction efficiency

In the study several extraction procedures were performed. For the tissues (liver, kidney, muscle), usually acetonitrile was used and provided good extractability. Where extractability with acetonitrile was not yet sufficient, sequential solvents were used to increase the extractability, but still the majority of residues was found in the acetonitrile extract in all cases (see Table 6.2.3-22 and Table 6.2.3-23). The data generation methods for animal tissues and fat, FAMS 023-01, uses acetonitrile as extraction solvent, thus the identical solvent as employed in the metabolism study (except for fat). For this reason, no further investigations on extraction efficiency in muscle, liver, and kidney were considered necessary. For fat, additional investigation of the extraction efficiency of acetonitrile (data generation method) vs. dichloromethane (metabolism) was considered not useful due to the very low overall residue levels in this matrix.

For the matrices milk, fat and cream initial extraction was based on dichloromethane. For whole milk and cream (as matrices showing sufficiently high residue levels) additionally extractions with acetone and acetonitrile were performed. Acetone is the solvent used by the data generation method for milk and milk products (FAMS 024-01). All results of this solvent comparison are shown in Table 6.2.3-22 and Table 6.2.3-24. Acetone proved to be the most effective solvent in all cases, with a total of 72.7% TRR extracted compared to 61.8% TRR for the dichloromethane in cream (morpholine label) or 86.9% TRR (dichloromethane) vs. >100% TRR (acetone) extractability in cream of the chlorophenyl label. In whole milk (morpholine label) acetone was able to extract 65% TRR while dichloromethane only extracted 42.9% TRR. This proves the suitability of method FAMS 024-01 to efficiently extract residues of dimethomorph from milk and milk products.

Table 6.2.3-22: Extraction efficiency of tissues and milk matrices treated with [p-chlorophenyl-U-^{14}C]-BAS 550 F

Matrix	Acetonitrile (Solvent for data generation method tissues)		Dichloromethane		Ethyl Acetate		Acetone (Solvent of data generation method for milk)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Liver	1.328	88.0	-	-	-	-	-	-
Kidney	0.060	82.6	-	-	-	-	-	-
Muscle	0.006	88.7	-	-	-	-	-	-
Fat	-	-	0.007	109.6	-	-	-	-
Whole milk	-	-	0.003	55.9	0.003	46.3	-	-
Skimmed milk	-	-	0.002	42.0	0.002	39.6	0.001	10.6
Cream	0.012	103.5	0.010	86.9	-	-	0.013	110.4

Table 6.2.3-23: Extraction efficiency of tissues treated with [morpholine-2,3-^{14}C]-BAS 550 F

Matrix	Acetonitrile (Solvent for data generation method tissues)		Methanol		Ethanol		Acetonitrile:Water		Water	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Liver	1.000	70.8	0.119	8.4	0.011	0.8	0.031	2.2	-	-
Kidney	0.090	54.5	0.028	16.7	0.002	1.1	0.003	1.9	0.002	1.2
Muscle	0.011	36.3	0.009	31.8	< 0.001	1.4	0.001	4.0	-	-

Table 6.2.3-24: Extraction efficiency of milk treated with [morpholine-2,3-^{14}C]-BAS 550 F

Matrix	Dichloromethane		Acetonitrile		Acetone (Solvent of data generation method for milk)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fat	0.009	98.2	-	-	-	-
Whole milk	0.019	42.9	0.026	58.2	0.029	65.0
Skimmed milk	0.002	7.8			0.012	35.7
Cream	0.083	61.8	0.054	40.2	0.097	72.7

Storage stability
As the extraction and analysis phase of the study exceeded 6 months after sacrifice, a storage stability assessment was performed. Extracts of cream, liver and kidney for the chlorphenyl label and whole milk, liver and kidney for the morpholine label were re-analyzed at the end of the experimental phase using HPLC method 2, and the initial and stability chromatograms were compared to assess whether the components showed stability during the course of the study. The profiles were comparable showing stability over the course of the study.

Metabolic pathway
The proposed metabolic pathway of BAS 550 F (Reg. No. 247723) in the lactating goat is provided in Figure 6.2.3-1**: Proposed metabolic pathway for dimethomorph in the lactating goat (considering peer reviewed and new data)**. BAS 550 F was extensively metabolized in the goat. An overview on quantified metabolites in numerial order is provided in Table 6.2.3-19 and Table 6.2.3-20. The identified metabolites comprise the following Phase I and Phase II conversions of the parent compound, BAS 550 F:
Phase I metabolism is characterized by a demethylation of the dimethyoxyphenyl-ring (M550F006, M500F007 and M550F016) or hydroxylation of the morpholine ring (M550F018 and M550F028) including several consecutive and sequential metabolites of oxidative degradation of the morpholine ring (M550F009, M550F030, M550F031, M550F011, M550F012, M550F008 and M550F053). Data shows that a cleavage of the molecule and release of free morpholine (M550F021) is probable, although no unambiguous identification could be provided. Phase II metabolism is characterized by conjugation of de-methylated metabolites to their respective glucuronides (M550F013, M550F015 and M550F029).

The polar fraction that was observed through all matrices (either in extracts or in released radioactivity after enzyme treatment of non-extractable residues) was shown to consist in most cases of multiple components, which could not clearly be identified despite much effort. One of the polar components seems to be free morpholine (M550F021). Considering all analytical results it is hypothesized that an incorporation of C2 fragments (stemming from the degradation of the morpholine moiety) into biosynthetic pathways is a relevant decomposition mechanism for BAS 550 F, resulting in small endogenous molecules of polar nature.

Stereoisomer analysis
The dose formulation of BAS 550 F is a mixture of *E* and *Z*-isomers in a ratio of approx. 45:55. Therefore the *E*/*Z*-isomer ratio was investigated in all edible matrices, which contained BAS 550 F (see Table 6.2.3-25).
In liver, the E/Z ratio ranged from 56:44 to 40:60, thus very similar to the application solution. In whole milk and kidney, only low amounts of dimethomorph were found, exclusively as the *Z*-isomer. The isomeric ratio in cream depended on the extraction solvent and ranged from 24:76 to 44:56, however it has to be kept in mind that the absolute amounts of BAS 550 F in cream were very low (≤0.002 mg/kg) for all extraction solvents, therefore a constant isomeric ratio can be claimed.
Overall, all matrices containing BAS 550 F showed an isomeric ratio comparable to the dose formulation. Therefore absorption and distribution of BAS 550 F did not change the isomeric ratio within the goat.

Table 6.2.3-25: Summary of E/Z ratio of BAS 550 F in edible matrices

Application formulation / matrix	Additional information	%TRR		Ratio (%)	
		E-isomer	*Z*-isomer	*E*-isomer	*Z*-isomer
Chlorophenyl label					
Application formulation	Radiolabeled test item (CoA)	N/A	N/A	43	57
	Dose formulation*	44.8**	52.4**	46	54
Cream	Dichloromethane based extraction	2.4	7.5	24	76
	Acetone based extraction	8.1	10.5	44	56
Liver		30.3	23.8	56	44
Kidney		n.d	7.8	N/A	N/A
Morpholine label					
Application formulation	Radiolabeled test item (CoA)	N/A	N/A	44	56
	Dose formulation*	42.9**	53.0**	45	55
Whole milk		ND	0.5	N/A	N/A
Cream	Dichloromethane based extraction	1.2	1.8	40	60
	Acetone based extraction	ND	5.3	N/A	N/A
Liver		18.1	27.3	40	60
Kidney		ND	1.3	N/A	N/A

ND Not detected
N/A Not applicable
* Method 2; prior dosing
** Area (%)

III. CONCLUSION

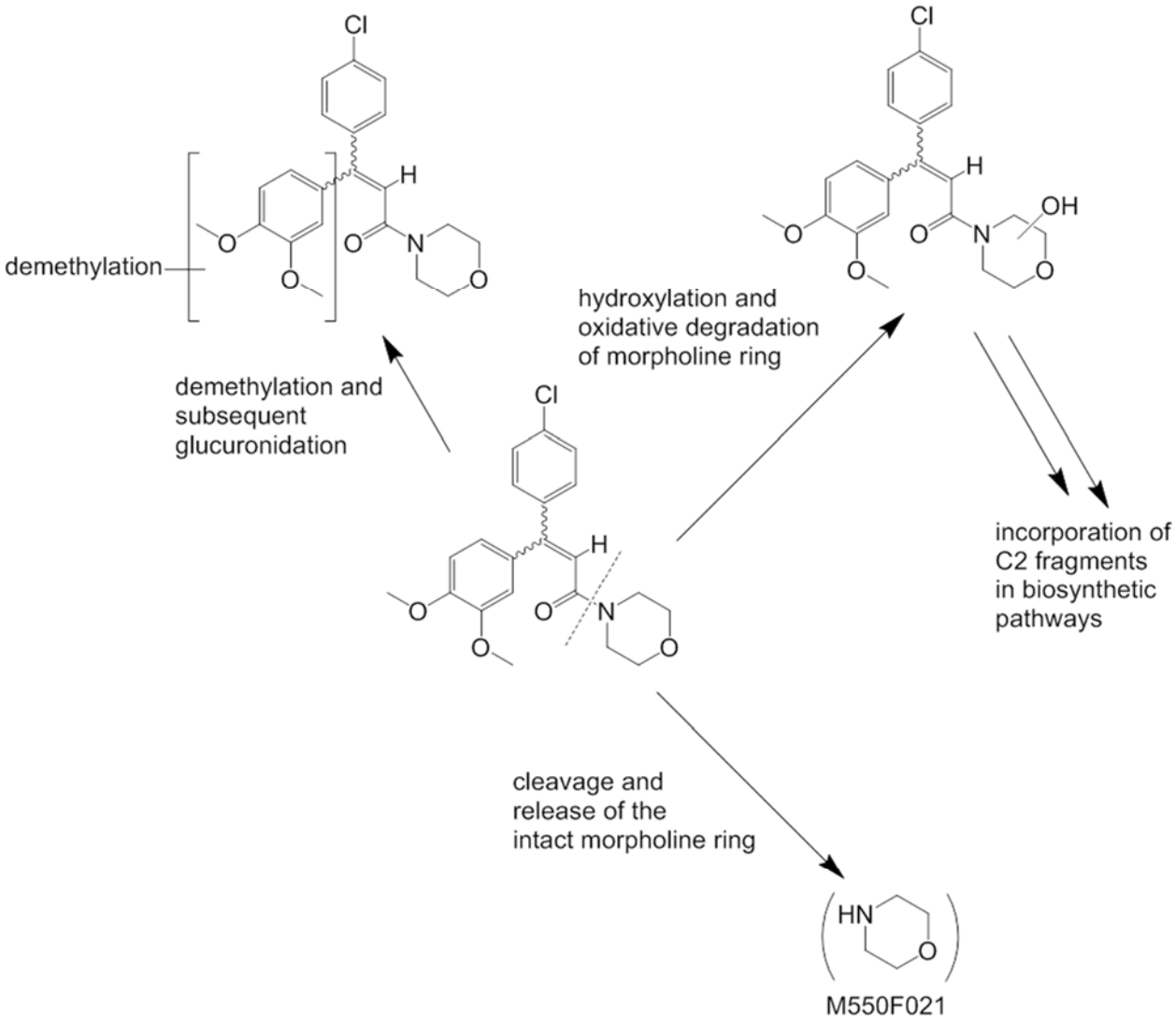
After oral dosing of BAS 550 F (Reg. No. 247723) to lactating goats for 10 consecutive days, residues were rapidly and efficiently excreted. Approximately 80-84% of the total mean dose was recovered, the majority of which was present in the feces (53.1-53.4%), urine (16.3-19.2%) and GI tract and contents (9.3-10.4%). Relatively low amounts were recovered in cage wash (0.4%). Radioactivity associated with edible portions (milk and tissues) accounted for approximately 0.7-1.1% of the administered dose. Radioactive residues in milk were mostly associated with the fat fraction (cream) and reached a plateau within 2 days (whole milk). Total radioactive residues were mostly higher in samples from the morpholine label as compared to the chlorophenyl label except in liver, where they had a similar level.

Parent BAS 550 F was extensively metabolized in the lactating goat. In all analyzed matrices from goats dosed with chlorophenyl labeled BAS 550 F, the unchanged parent was detected, accounting for 54.1% TRR in liver, 7.8% TRR in kidney and for 18.6% TRR in cream. The main component in the extract of cream was M550F008 (48.7% TRR, as the sum of the isomers), formed by hydroxylation and oxidative degradation of the morpholine ring. In addition, metabolite M550F011, also formed by hydroxylation and oxidative degradation of the morpholine ring, was present in the cream at 13.4% TRR. In the liver, the major proportion of the radioactivity was due to the unchanged parent (54.1% TRR) and M550F007 (6.8% TRR). A large number of metabolites representing much smaller residues were also observed. In the kidney, the major metabolites were M550F008 (18.7% TRR), M550F007, co-eluting with M550F031 and M550F011 (29.8% TRR) and the glucuronide metabolites of M550F013 and M550F015 (17.8% TRR).

In goats dosed with morpholine labeled BAS 550 F, the unchanged parent was detected in liver samples (up to 45.4%TRR) and also at lower concentrations (up to 3%) in the kidney, cream and whole milk. A major metabolite, observed in extracts of kidney, liver and cream (up to 23.4% TRR, as the sum of isomers) was M550F007, formed by demethylation. M550F008, formed by hydroxylation and oxidative degradation of the morpholine ring, was observed in cream and whole milk extracts (up to 14.6% TRR, as the sum of isomers). The metabolite M550F0011, also formed by hydroxylation and oxidative degradation of the morpholine ring, was also present in cream, liver and whole milk (up to 6.8% TRR). The polar fraction that was observed through all matrices (either in extracts or in released radioactivity after enzyme treatment of non-extractable residues) was shown to consist in most cases of multiple components, which could not be identified unambiguously despite much effort. One of the components seems to be free morpholine (M550F021) resulting from a cleavage of the molecule. Considering all analytical results it is hypothesized that an incorporation of C2 fragments (stemming from the degradation of the morpholine moiety) into biosynthetic pathways is a relevant decomposition mechanism for BAS 550 F, resulting in small endogenous molecules of polar nature.

It can be concluded, that metabolism of dimethomorph in goat is dominated by demethylation at the dimethoxy ring and conjugation to glucuronides, and also by the hydroxylation and opening and step-wise degradation of the morpholine ring, including complete degradation to C2 fragments, which are then subsequently incorporated into natural biosynthetic pathways, rendering small endogenous molecules of polar nature.

Figure 6.2.3-1: Proposed metabolic pathway for dimethomorph in the lactating goat (considering peer reviewed and new data)



( ) indicates metabolite is characterised

Figure 6.2.3-1: Proposed metabolic pathway for dimethomorph in the lactating goat **(considering peer reviewed and new data)**

Metabolites from demethylation and glucuronidation of the dimethoxyphenyl ring:

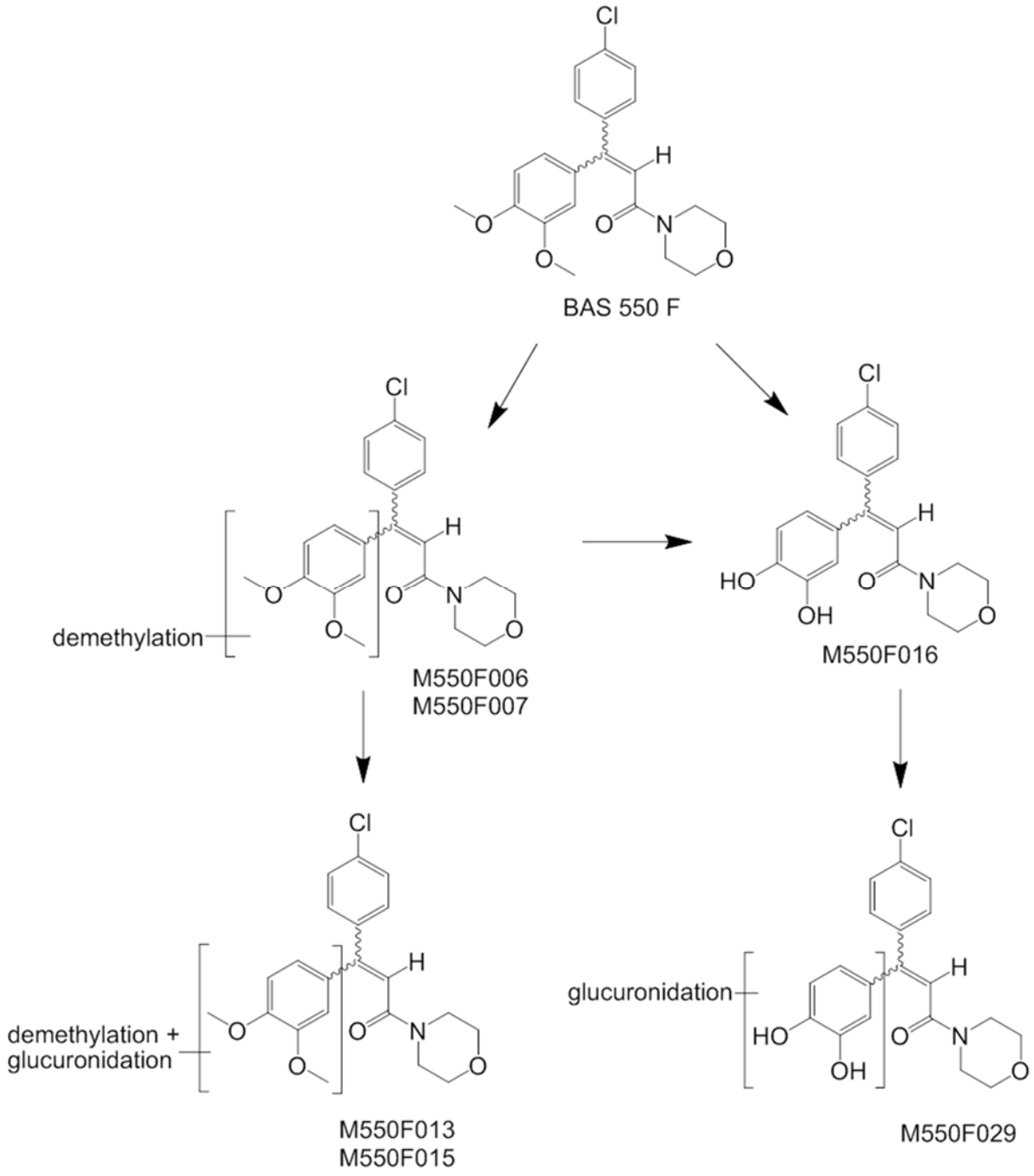
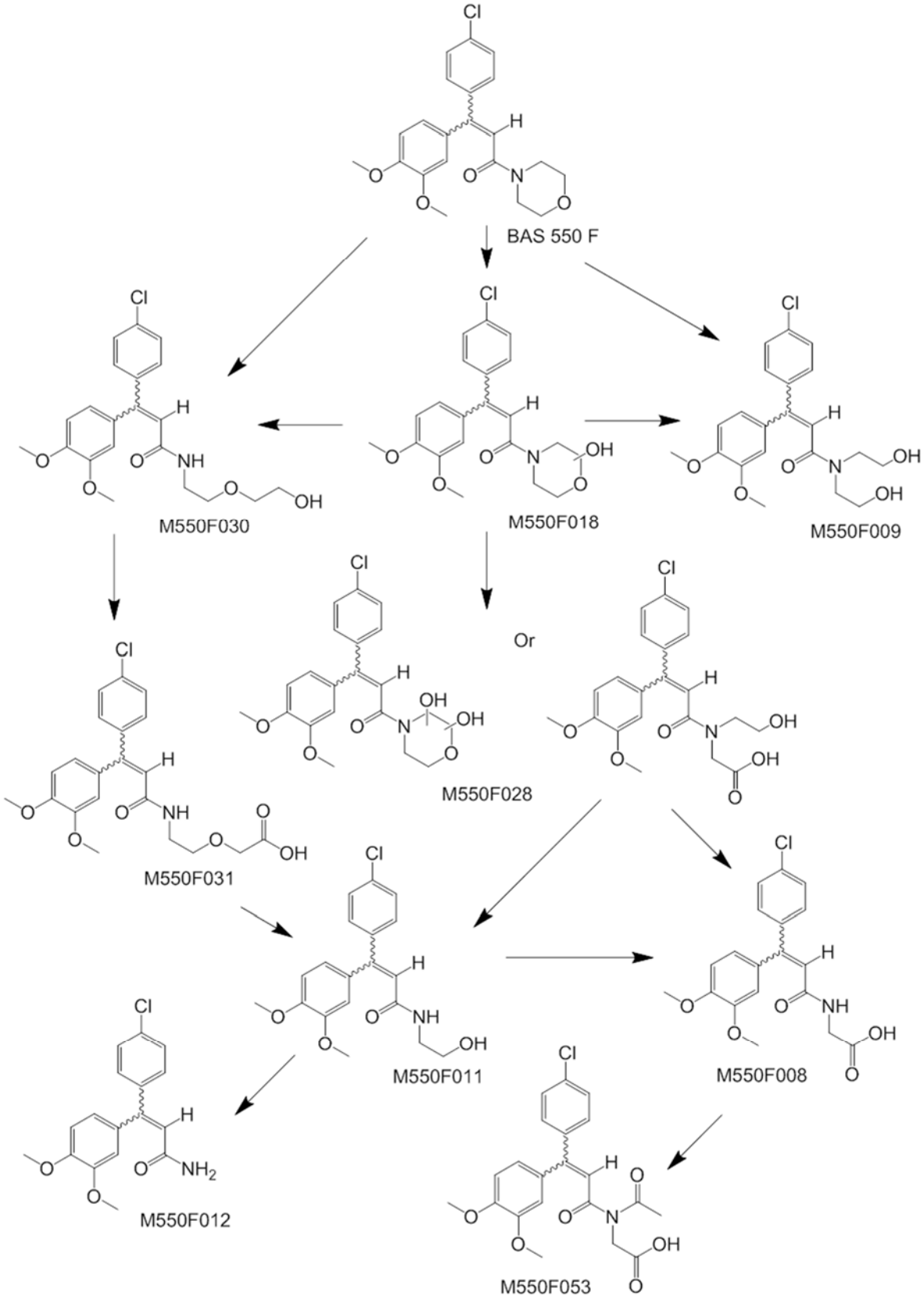


Figure 6.2.3-1: Proposed metabolic pathway for dimethomorph in the lactating goat **(considering peer reviewed and new data)**

Metabolites from hydroxylation and oxidative degradation of the morpholine ring:



In livestock metabolism studies for lactating goat [see KCA 6.2.3/3 2015/1000603] and laying hen [see KCA 6.2.2/3 2015/1000604] a polar region was detected after administration of 2,3-morpholine ^{14}C-BAS 550 F. In order to facilitate the identification of this polar region an additional study in hepatocytes was set up. Therefore ^{14}C-morpholine-BAS 550 F and ^{14}C-morpholine was applied to goat and hen hepatocytes in order to elucidate the metabolic behavior of this polar region *in vitro*. The results of these investigations are summarized in the following report.

Report: CA 6.2.3/5
Birks V., 2015 a
14C-BAS 550 F and 14C-Morpholine: In-vitro metabolism using freshly isolated goat and hen hepatocytes
2015/1231130
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 550 F
Description: [morpholine-2,3-^{14}C]-BAS 550 F (morpholine label)
[morpholine-^{15}N]-BAS 550 F
Unlabeled BAS 550 F
Lot/Batch #: 858-1101 (morpholine label)
1071-0105 (morpholine-^{15}N)
AC9978-68A (unlabeled)
Purity: Chemical purity 97.6% (unlabeled)
Chemical purity 97.3% (morpholine ^{15}N label)
Radiochemical purity: 95.4% (morpholine label)
Specific activity: 5.63 MBq/mg
CAS#: 110488-70-5

Test Material: Morpholine
Description: Morpholine-[2-^{14}C]HCl
Unlabeled morpholine
Lot/Batch #: 150130 (labeled)
STBD8343V (unlabeled)
Purity: 99.9% (unlabeled)
Specific activity: 55 mCi/mmol (16.46 MBq/mg)
CAS#: 110-91-8

Stability of test compound:
The test item was stable over the test period. Stability controls without cells showed nearly identical HPLC profiles that contained only peaks corresponding to the unchanged active substance BAS 550 F or morpholine.

2. Vehicle and/or positive control:

Vehicle: hepatocytes
Positive control: testosterone or 7-ethoxycoumarin instead of the test substances were incubated with hepatocytes from the two different species to indicate the metabolic activity of the different hepatocytes.

3. Test animals:

Species: Goat, hen
Strain: British Saanen, Lohman Brown

B. STUDY DESIGN AND METHODS

1. Dates of work: 5-May-2015 - 26-June-2015

This study was carried out at Quotient Bioresearch (Rushden) Ltd., United Kingdom.

Test procedure

In livestock metabolism studies for lactating goat [see KCA 6.2.3/3 2015/1000603] and laying hen [see KCA 6.2.2/3 2015/1000604] a polar region was detected after administration of ^{14}C-morpholine label. In order to facilitate the identification of this polar region an additional study in hepatocytes was set up. Therefore ^{14}C-morpholine-BAS 550 F and ^{14}C-morpholine was applied to goat and hen hepatocytes in order to elucidate the metabolic behavior of this polar region *in vitro*. Particular attention was paid to early eluting peaks following radio-HPLC (RHPLC) analysis of ^{14}C-morpholine-BAS 550 F samples, especially whether these corresponded to morpholine or morpholine-related metabolites.

To address this question, the radiolabeled test items were incubated with hepatocytes from goat and hen at final concentrations of 1 and 10 µM.

After incubation for 0, 2 or 4 hours, the reaction was terminated by addition of acetonitrile (25% of incubation sample volume) and the resulting supernatant was analyzed by HPLC.

All the supernatants contained >80% of the applied radioactivity (% AR) and therefore pellet extraction was not required.

Negative and positive controls were run in parallel to demonstrate the absence of non-metabolic degradation and the metabolic activity of the hepatocytes (Phase I and Phase II metabolic reactions), respectively. The control experiments yielded the expected results.

Test design and analytical procedures

Test solutions
A 0.2 mM and 2 mM solution of $^{14}C/^{15}N/^{12}C$-BAS 550 F and a 0.2 mM and 2mM solution of ^{14}C-morpholine were prepared in DMSO for application, leading to final concentrations after dilution with hepatocyte incubation medium (WME) of 1 and 10 µM $^{14}C/^{15}N/^{12}C$-BAS 550 F and ^{14}C-morpholine, respectively.

Negative controls:
In the negative controls no metabolism should occur. For the "stability control", the application medium was mixed with incubation medium instead of the cell suspension. For the "zero incubation control" (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.

Preparation of hepatocytes
Hepatocytes were isolated from liver taken from female control animals. Williams' Medium E (without phenol red, pre-gassed with a 95:5% (v/v) oxygen : carbon dioxide mixture; WME) was used as the hepatocyte incubation medium and was supplemented with L-glutamine (4 mM) and magnesium sulphate (2 mM).
Liver lobes were excised from the abdominal cavity and rinsed in cold heparinized saline (0.9%, w/v) and kept in saline on ice until use, respectively. Hepatocytes were isolated from liver lobes using a collagenase perfusion procedure.

Viability tests
Prior to use, the initial cell integrity of the hepatocyte suspension was investigated. This was achieved by using Trypan Blue dye exclusion to confirm the initial cell viability was ≥80%.

The hepatocyte suspension was diluted with hepatocyte incubation medium (WME) to produce a suspension of approximately 1 million viable cells/mL. Isolated hepatocytes were stored at approximately 4°C (on ice) prior to use in the incubation experiments.

In vitro assays
Goat or hen hepatocytes were incubated with application solutions at final concentrations of 1 and 10 µM of either ^{14}C-morpholine-BAS 550 F or ^{14}C-morpholine. In the case of testosterone and 7-ethoxycoumarin, the incubations were performed at 150 µM and 25 µM, respectively. Purity checks were performed after preparation of the application media using HPLC.

Each sample with goat hepatocytes (2.01 mL total incubation volume) comprised 0.01 mL of application solution in DMSO and 2 mL of hepatocyte cell suspension (in WME) in one of the wells of a 24-well plate. Each sample with hen hepatocytes (4.02 mL total incubation volume) comprised 0.02 mL of application solution in DMSO and 4 mL of hepatocyte cell suspension (in WME) in one of the wells of a 24-well plate. The final cell concentration was approximately 1 x 10^6 cells/mL for both goat and hen incubations. The reactions were performed for 0, 2 and 4 h at 37°C in an incubator.

Two negative control incubations ("no cell" and "zero incubation") were performed for each species and test item. Two positive control incubations (testosterone and 7-ethoxycoumarin) were also performed for each species.

In each experimental setup, the incubation of the ^{14}C-BAS 550 F and ^{14}C-morpholine was performed in duplicate. "No cell" control assays and incubations with ^{14}C-testosterone or ^{14}C-7-ethoxycoumarin were performed in singlicate.

Sampling and sample storage
Incubations with ^{14}C-morpholine-BAS 550 F and ^{14}C-morpholine were terminated by pipetting the incubation mixture into a tube containing cold acetonitrile to adjust the sample to an acetonitrile concentration of approximately 20% (v/v). The incubations containing testosterone and 7-ethoxycoumarin were terminated by pipetting the incubation mixture into a tube containing methanol or acetonitrile, respectively.

The recovery of radioactivity in the samples was determined by LSC analysis of aliquots pre and post centrifugation. In the supernatant the radioactive residues were quantified and ranged from 80% to 109% and therefore no further extraction of the remaining pellet was performed.
No further processing of the pellets was required assuming that a metabolite identification was not feasible. All samples were stored in a freezer at -20°C.

II. RESULTS AND DISCUSSION

For the stability controls without cells and zero incubation controls, all replicates showed nearly identical HPLC profiles that contained only peaks corresponding to the unchanged active substance BAS 550 F or morpholine. Therefore no significant metabolism or degradation of BAS 550 F or morpholine occurred without the influence of hepatocytes.

The positive controls with testosterone showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. Testosterone was metabolized (>20%) after incubation with goat and hen hepatocytes.

HPLC analyses of the positive controls with 7-ethoxycoumarin revealed mean portions of the metabolized 7-ethoxycoumarin reaching values of 41.3% AR after incubation with goat hepatocytes and 98.8% AR after incubation with hen hepatocytes. However, these incubations confirm that these hepatocytes are capable of phase II metabolism.
Hydroxylated, sulphated and glucuronidated metabolites of 7-ethoxycoumarin were detected in goat and hen. Similar levels of 7HC glucuronide were formed in both species (3.39 - 5.95% AR). In goat and hen, 7HC sulphate was the major metabolite (29.4 and 75.8% AR, respectively).

^{14}C-morpholine-BAS 550 F was metabolized by goat and hen hepatocytes, the extent of which being greater in the goat (10 metabolites) than in hen (8 metabolites). A polar peak eluting was seen in both species, which was larger in goat than in hen. This polar fraction in goat was collected (2.5 - 5.5 min fraction) for analysis using HPLC to evaluate if morpholine was present. A small peak was present with a similar retention time to morpholine, but this was a minor component compared to the other two peaks seen. Due to the low absolute levels of these metabolites formed by goat hepatocytes, it was not considered productive to submit samples for metabolite identification investigation. In addition, for hen the levels of radioactivity in the polar peak were too low for HPLC analysis using the same HPLC method. Therefore it was not possible to assign a distinct structure.
Since the focus in this study was on the polar region, no further identification/characterization work was done on the mid-polar region.

Morpholine was stable in the presence of goat and hen hepatocytes for up to 4 hours at 37°C with no metabolism observed.

III. CONCLUSION

In livestock metabolism studies for lactating goat [see KCA 6.2.3/3 2015/1000603] and laying hen [see KCA 6.2.2/3 2015/1000604] a polar region was detected after administration of 2,3-morpholine ^{14}C-BAS 550 F. In order to facilitate the identification of this polar region an additional study in hepatocytes was set up. Therefore ^{14}C-morpholine-BAS 550 F and ^{14}C-morpholine were applied to goat and hen hepatocytes over a period of 4 hours in order to elucidate the metabolic behavior of this polar region *in vitro*.

Dimethomorph (^{14}C-morpholine-BAS 550 F) was metabolized by both goat and hen hepatocytes, with the extent of metabolism being greater in goat. An early eluting peak was seen in both species, which was larger in goat than in hen. Further analysis of this polar fraction suggested that morpholine was present as a minor component, with two other peaks being major components. Due to the low levels of these metabolites formed by goat hepatocytes it was not considered productive to submit samples for metabolite identification and to assign a distinct structure. Since the focus in this study was on the polar region, no further identification/characterization work was done on the mid-polar region.

Morpholine, which seems to be a minor metabolite of BAS 550 F, was stable following incubation with goat and hen hepatocytes for up to 4 hours and showed no metabolic conversion.

Overall conclusions animal metabolism

The new metabolism studies in goat and hen (carried out with the chlorophenyl and morpholine ring radiolabel) show a metabolic fate of dimethomorph that is well in agreement with the already available data. They confirm the main metabolic steps and significantly enlarge the previous understanding of the metabolism of dimethomorph in livestock. The data also shows that the metabolism is qualitatively similar for both livestock species and the rat.

The metabolic pathways in goats and hens proceed along the following main routes:

1) Demethylation of the two methoxy groups of the dimethoxyphenyl ring to produce M550F006, M550F007 andM550F016. These metabolites were confirmed to be further conjugated in Phase II reactions to form the respective glucuronides M550F15, M550F013, M550F029 and M550F074/M550F076 (latter ones observed in poultry only).

2) Hydroxylation of the morpholine ring (M550F018 and M550F028)

Previous studies have not identified this metabolic step, probably due to the reason that no synthesized reference standard had been available. With the better technology used for identification (MS/MS) in the new studies, single (M550F018) and double hdyoxylation (M550F028, one isomer) could be identified in both livestock species. Possibly this hydroxylation is the initial step of the morpholine ring opening and subsequent degradation, which was an observed metabolic step in the previous and new metabolism studies.

3) Opening and stepwise degradation of the morpholine ring producing metabolites M550F009 (WL 376084), M550F011 (CUR 7216), M550F008 (CUR 7117), M550F028 (other isomer), M550F030, M550F031, M550F053, and M550F012 in both species. In poultry additionally M550F005 (Z43), M550F022, M550F033, M550F069, M550F038, M550F091, M550F062, M550F035, and M550F049 were observed. All these metabolites represent parent-like structures with the morpholine ring opened at either the oxygen or nitrogen atom, oxidized (hydroxyl or carboxy groups) and degraded to only the nitrogen group remaining, all this in combination with demethylation of the dimethoxy ring and glucuronidation.

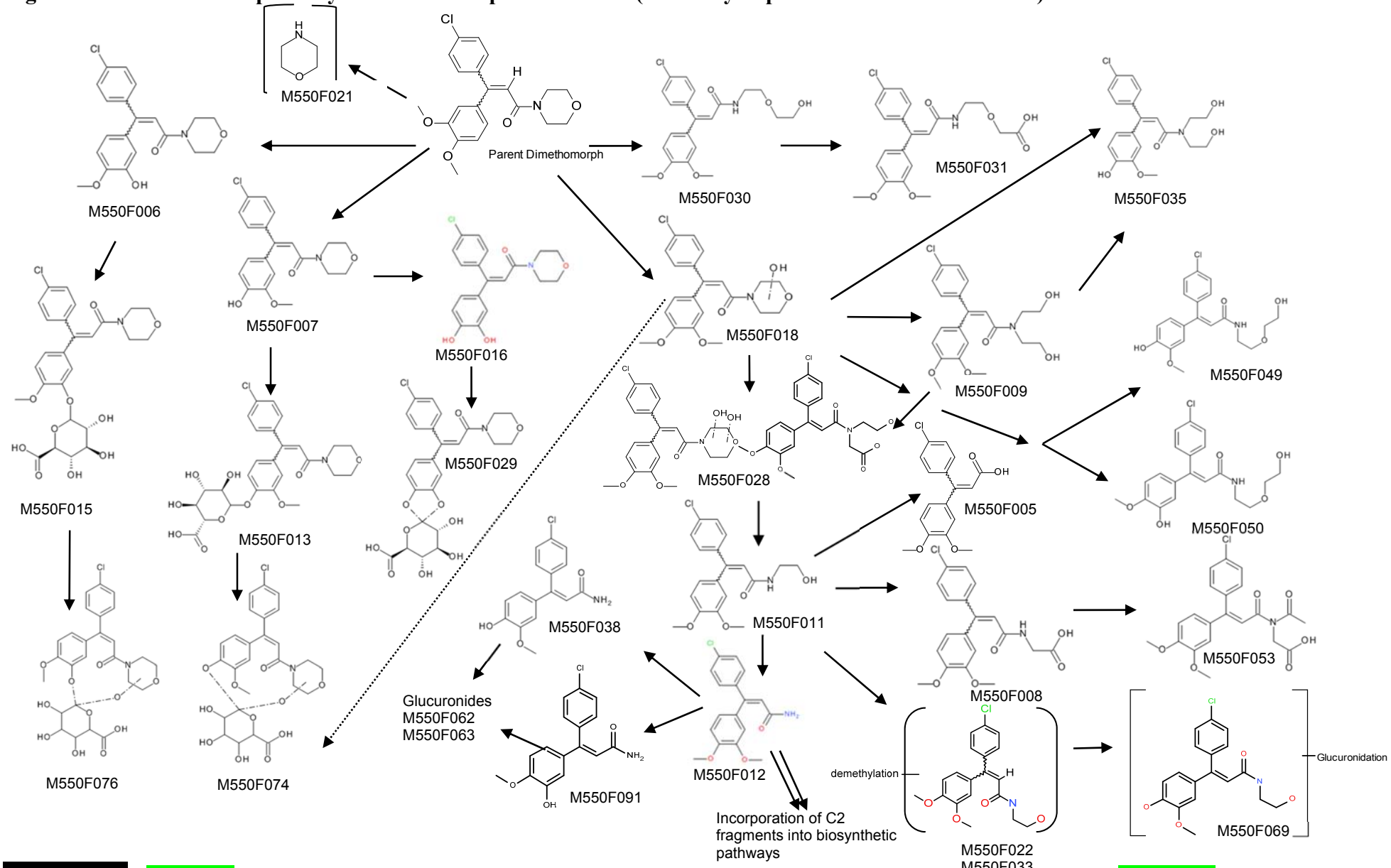
Better technology used in the new metabolism studies (MS/MS) allowed the identification of multiple metabolites arising from this metabolic step that was already known from previous studies. Metabolite M550F005 (Z43), which was identified in the previous poultry study was not found in quantifiable amounts in the new poultry study (although in traces in the MS analysis). This difference is thought to be due to the different approaches used for identification. The previous studies used TLC in combination with synthesized reference standards. This approach implies that only those metabolites can be assigned as identified which were also available as reference standards. The new studies showed that many metabolites had very similar polarity (often co-eluting) and thus it is probable that the peak that was assigned in the previous studies to be M550F005 is rather one of the identified metabolites in the new studies with similar polarity (e.g. M550F012).

4) Cleavage and release of the intact morpholine ring (M550F021).

This pathway was shown to be of subordinate significance. Metabolite M550F021 (morpholine) was not observed in the previous studies due to the position of the radiolabel. In the new studies with the morpholine label, a significant amount of the total radioactive residue occurred in form of a polar fraction, which was demonstrated to be comprised of multiple components, one of which was tentatively assigned as morpholine (based on co-chromatography on one HPLC system). This accounted in most cases for only a minor share of the entire polar fraction, showing that the cleavage and release of the intact morpholine moiety is not of major significance in quantitative terms. The amount of the total polar fraction was much more pronounced in poultry than in the goat. Although it was tried by many different approaches to identify the components of this polar fraction, it was not successful in terms of unambiguous identification. However, all generated data support the hypothesis that the polar fraction is comprised of many different compounds, which probably arise from the degradation of the morpholine ring into C2 fragments and their subsequent incorporation into endogenous biosynthetic pathways (fatty acid synthesis and TCA cycle), thus representing endogenous molecules rather than dimethomorph specific metabolites.

In conclusion, the previous and new metabolism studies in livestock species show an extensive metabolization of dimethomorph. The demethylation of the dimethoxy ring and the opening and degradation of the morpholine ring are the major pathways in quantitative terms. A significant amount of radioactivity also occurs as small polar natural endogenous molecules arising from the degradation of the morpholine ring into C2 fragments and subsequent incorporation into the biosynthetic pathways.

Figure 6.2.3-2: Metabolic pathway of dimethomorph in livestock (summary of peer reviewed and new data)



CA 6.2.4 Pigs

No separate metabolism study for pigs is required since the metabolic pathways in rodents (rats) and ruminants (goats) are comparable. Metabolism data for pigs can be extrapolated from ruminants.

CA 6.2.5 Fish

Fish

According to the new data requirements as laid down in EU regulation 283/2013, metabolism studies in fish may be required "where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications."

The working document on the nature of pesticide residues in fish (SANCO/11187/2013 rev. 3 of 31 January 2013) further specifies that metabolism studies in fish are required only if an active substance has a log P_{ow} equal or greater than 3. For substances with rather low lipophilicity (log P_{ow}<3), accumulation of residues in fish via the diet is known to be negligible. It further lists the crops that are typically considered to be fed to fish. These are mainly cereal grains/crop seeds, by-products of cereals and oilseeds, and vegetable oil.

Based on this available information, no fish metabolism study is required for dimethomorph according to the following rationale:

1. The log P_{ow} of dimethomorph is <3 and
2. The crops in which dimethomorph products are mainly registered (grapes, strawberry, potatoes, leafy vegetables, fruiting vegetables, cucurbits, hops) are no typical fish feed items. Only potato is listed as the by-product "potato protein" with a max. contribution of 3% for carp, however, residues of dimethomorph in potatoes are typically below the LOQ (<0.05 mg/kg, EU MRL is 0.05 mg/kg). It is thus unlikely, that fish will be exposed to substantial amounts of dimethomorph via the diet.

CA 6.3 Magnitude of residues trials in plants

Dimethomorph is registered in a wide variety of crops (grapes, potatoes, hops, strawberries, orange, brassica, onions, leafy vegetables, fruiting vegetables, and cucurbits) belonging to different EU crop groups. Within this dossier residue data are provided for the representative uses grapes, strawberries and lettuce supporting the renewal of approval. The solo formulations BAS 550 01 F (strawberry, lettuce) and BAS 550 02 F (grapes) have been chosen as representative formulation.
The following table summarizes the critical use patterns for the selected representative crops.

Table 6.3-1: Overview of critical GAP supported in the representative crops

Crop	End use product	Pests controlled	F/P/G	Application						PHI (days)
				Method	Timing (BBCH)	Max number (interval)	Kg or L product /ha	Kg a.s./ha or g a.s./plant	Water L/ha	
Grapes SEU	BAS 550 02 F	*Plasmopara viticola*	F	Spray	15-83	5 (10)	1.67	0.250	200-1500	28
Lettuce CEU	BAS 550 01 F	*Bremia lactucae*	G	Spray	10-39	2 (7)	0.36	0.180	1000	14
Strawberry CEU	BAS 550 01 F	*Phytophthora cactorum*	F	Spray	At trans-planting ~~or~~ BBCH 15-42	1	3	1.5	200-1200	*
Strawberry CEU	BAS 550 01 F	*Phytophthora cactorum*	F+G	Drench	At trans-planting ~~or~~ BBCH 15-42	1	3	1.5 0.05 g a.s./plant	5000-10000	*
Strawberry CEU	BAS 550 01 F	*Phytophthora cactorum*	G	Drip	At trans-planting ~~or~~ BBCH 15-42	1	10	5 0.05 g a.s./plant	n.a.	*
Strawberry CEU	BAS 550 01 F	*Phytophthora cactorum*	G	Drip	At trans-planting ~~or~~ BBCH 15-42	1	3	1.5 0.05 g a.s./plant	n.a.	*

n.a. Not applicable
* PHI defined by intended use, i.e. time between last application and harvest at crop maturity

Regarding the timing of application in strawberry, the originally added BBCH growth stage was recognized to lead to misunderstandings since it mixes different developmental stages. The development in strawberry plants is complex in the way that the different growth stages related to leaf development (BBCH10-19), development of daughter plants (BBCH 41-49) and development of flower buds (BBCH 55-59) can be present at the same time and do not appear in strict chronological order. The description of growth stages at application in the residue trial reports however refer always to the leaf development only. Consequently, for clarification it is more appropriate to give the application timing only relating to the leaf development stage. The wording "at transplanting" is deemed sufficient as it comprises young plants at a growth stage of typically BBCH 03-15 and reflects the instructions on the label. A clarification of the strawberry use patterns with regard to the relevant growth stages at application is provided in a separate document (DocID 2017/1078262) to demonstrate that the submitted residue trials are suitable for supporting the intended use.

Table 6.3-2: Overview of residue trials included in the dossier

Crop	Formulation	Applied dose	DALA	No of trials	Trial location	Reference
Strawberries						
Studies supporting the cGAP						
Drip/drench use						
Field: drip/drench	BAS 550 01 F (WP)	1 x 0.065 g a.s./plant	43-44	3	EU North	M-CA 6.3.1/1 2005/1004964 M-CA 6.3.1/2 2010/1051738
Field: drip/drench	SBO 8801 (WP)	1 x 0.050 g ai/plant	39-55	4	EU North	M-CA 6.3.1/3 DK-713-018
Field: drip/drench	BAS 550 01 F (WP)	3 x 0.05 g ai/plant	35-42	4	EU North	M-CA 6.3.1/4 2015/1000641
Protected: drip/drench	BAS 550 01 F (WP)	1 x 0.125 g ai/plant	34-36	4	EU North (Protected)	M-CA 6.3.1/5 2008/1051524
Protected drip/drench	BAS 550 01 F (WP)	1 x 0.125 g ai/plant	34-43	4	EU North (Protected)	M-CA 6.3.1/1 2005/1004964 M-CA 6.3.1/2 2010/1051738
Spray use						
Field: spray	SBO 8801 (WP)	1 x 1.5 kg/ha	39-55	4	EU North	M-CA 6.3.1/3 DK-713-018
Field: spray	BAS 550 01 F (WP)	1 x 1.5 kg/ha	35-36	4	EU North	M-CA 6.3.1/6 2009/1112576
Studies included as supportive information						
Field: drip/drench	CME 151 (WP Paraat (WP)	3 x 0.05 g ai/plant	58-64	1	EU North	M-CA 6.3.1/7 DK-713-039
Field: drip/drench	Paraat (WP)	3 x 0.05 g ai/plant	64	1	EU North	M-CA 6.3.1/8 DK-713-040
Field: drip/drench	Paraat (WP)	3 x 0.05 g ai/plant	64	1	EU North	M-CA 6.3.1/9 DK-713-041
Field: drip/drench	Paraat (WP)	3 x 0.05 g ai/plant	76	1	EU North	M-CA 6.3.1/10 DK-713-042
Lettuce						
Studies supporting the cGAP						
Protected: spray	BAS 550 01 F (WP)	2 x 0.18 kg/ha	14	8	EU North EU South (Protected)	M-CA 6.3.2/1 2014/1109986 M-CA 6.3.2/2 2015/1000642
Grapes						
Studies supporting the cGAP						
Field: spray	BAS 551 11 F (SC)	5 x 0.270 kg/ha	28-29	2	EU North	M-CA 6.3.3/1 2008/1005539
	BAS 550 01 F (WP)	5 x 0.300 kg/ha	28-29	2	EU South	
Field: spray	BAS 550 01 F (WP)	5 x 0.300 kg/ha	27-28	2 2	EU North EU South	M-CA 6.3.3/2 2012/1135760
Field: spray	BAS 550 09 (DC)	5 x 0.300 kg/ha		8 8	EU North EU South	See Table 6.3.3-1
Field: spray	BAS 550 02 (DC)	5 x 0.225 kg/ha		4 4	EU North EU South	M-CA 6.3.3/3 2015/1241719

DALA Days after last treatment

CA 6.3.1 Strawberry

Drip/drench use

Report:	CA 6.3.1/1 Schulz H., 2007 a Study on the residue behaviour of BAS 550 F in strawberries after treatment of different application rates of BAS 550 01 F under field conditions and under glasshouse conditions in Germany, Belgium, The Netherlands and Denmark, 2004 2005/1004964
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report:	CA 6.3.1/2 Schulz H., 2010a Study on the residue behaviour of BAS 550 F in strawberries after treatment of different application rates of BAS 550 01 F under field conditions and under glasshouse conditions in Germany, Belgium, The Netherlands and Denmark, 2004 2010/1051738
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 550 01 F (WP)
Lot/Batch #: 92007 (dimethomorph 500 g/kg nominal); 1006 (dimethomorph 50% nominal); 1007 (dimethomorph 50% nominal)
Purity: Not relevant
CAS#: 110488-70-5 (dimethomorph)
Development code:
Spiking levels: 0.0044-0.55 mg/kg

2. Test Commodity:

Crop: Strawberry
Type: Berries and small fruit
Variety: Elsanta, Everst
Botanical name: *Fragaria x ananassa*
Crop parts(s) or processed commodity: Fruits
Sample size: ≥0.5 kg

B. STUDY DESIGN

1. Test procedure

In 2004, 7 trials (3 field trials, 4 protected trials) were conducted with strawberries in Northern Europe (Germany, Belgium, the Netherlands and Denmark). The trials were conducted with BAS 550 01 F.
BAS 550 01 F was applied once as drench application at planting/transplanting at application rates of 0.125 g a.s./plant and 0.0625 g a.s./plant with a water volume of 250 mL/plant. Samples of strawberry fruits were taken 35 days after drench application (±25%, corresponding to 26-44 days after treatment), and in case the fruits were still green at that stage, an additional sampling was carried out at BBCH 87.

Table 6.3.1-1: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (ga.s./plant)	Water volume (mL/plant)	Target date/ Timing
2004	3	1	F	BAS 550 01 F (WP)	Dimethomorph	0.125 0.0625	250	At planting / transplanting
2004	4	1	P*	BAS 550 01 F (WP)	Dimethomorph	0.125 0.0625	250	At planting / transplanting

* Plastic tunnels or glasshouse

2. Description of analytical procedures

The specimens were analyzed for residues of dimethomorph (*cis*- and *trans*-isomers, i.e. Z and E isomer) using method DFG S19 (extended version) with a limit of quantitation (LOQ) of 0.005 mg/kg per isomer.

Specimen material was extracted with acetone according to extraction module E 1. Water was added beforehand in an amount that took full account of the natural water content of the specimen so that during extraction the acetone/water ratio remained constant at 2/1 (v/v). For liquid-liquid partition ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added and after repeated mixing excess water was separated.

The evaporated residue of an aliquot of the organic phase was cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluant (module GPC). The residue containing fraction was concentrated and analyzed for residues of dimethomorph by gas chromatography (GC) using mass selective detection (MSD, module D 4).

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-2: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method No DFG S19		BAS 550 F *cis*-isomer (Z)			BAS 550 F *trans*-isomer (E)		
Fruit	0.0044-0.55	8	99.6	18	8	101	14

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-3. Details are shown in Table 6.3.1-4 and Table 6.3.1-5.
After application of 0.125 g a.s./plant, residues of total dimethomorph in mature strawberries ranged from <0.01 to 0.03 mg/kg in field trials. Under protected conditions, residues ranged from 0.03 to 0.51 mg/kg.
After application of 0.0625 g a.s./plant, residues of total dimethomorph in mature strawberries ranged from <0.01 to 0.02 mg/kg in field trials. Under protected conditions, residues ranged from 0.03 to 0.3 mg/kg.

Table 6.3.1-3: Summary of residues in strawberry after application of BAS 550 01 F

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)			
				Matrix	BAS 550 F *cis*-isomer (Z isomer)	BAS 550 F *trans*-isomer (E isomer)	Total BAS 550 F
Strawberry (field)	2004	BAS 550 01 F 1 x 0.125	43-61	Fruit	<0.0056-0.033	<0.0044-0.007	<0.01-0.03
		BAS 550 01 F 1 x 0.0625			<0.0056-0.017	<0.0044	<0.01-0.02
Strawberry (protected)		BAS 550 01 F 1 x 0.125	34-80	Fruit	0.027-0.217	0.005-0.294	0.03-0.51
		BAS 550 01 F 1 x 0.0625			0.024-0.190	<0.0044-0.114	0.03-0.3

1 Days after last application

III. CONCLUSION

After application of 0.0625 g a.s./plant, residues of total dimethomorph (sum of E and Z isomer) in mature strawberries ranged from <0.01 to 0.02 mg/kg in field trials. Under protected conditions, residues ranged from 0.03 to 0.3 mg/kg.

Table 6.3.1-4: Residues of dimethomorph (BAS 550 F) in outdoor strawberries after drench treatment in Northern Europe

Trial details		Crop	Country	Formulation application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*cis*-isomer (Z)	*trans*-isomer (E)	Total
Study code:	181087	Strawberry	Belgium	BAS 550 01 F	15					
Doc ID:	2005/1004964			1 x 0.125		43	Fruit	0.033	<0.0044	0.04
Trial No:	AGR/19/04					57		0.024	0.007	0.03
GLP:	yes			1 x 0.0625		43		0.017	<0.0044	<u>0.02</u>
Year:	2004					57		0.016	<0.0044	0.02
Study code:	181087	Strawberry	Netherlands	1 x 0.125	15	43	Fruit	0.009	<0.0044	0.01
Doc ID:	2005/1004964					61		<0.0056	<0.0044	<0.01
Trial No:	AGR/20/04			1 x 0.0625		43		<0.0056	<0.0044	<u><0.01</u>
GLP:	yes					61		<0.0056	<0.0044	<0.01
Year:	2004									
Study code:	181087	Strawberry	Germany	BAS 550 01 F	10	44	Fruit	0.014	<0.0044	0.02
Doc ID:	2005/1004964			1 x 0.125		56		0.016	<0.0044	0.02
Trial No:	ACK/11/04			1 x 0.0625		44		0.009	<0.0044	0.01
GLP:	yes					56		0.014	<0.0044	<u>0.02</u>
Year:	2004									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Table 6.3.1-5: Residues of dimethomorph (BAS 550 F) in protected strawberries after drench treatment

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*cis*-isomer (Z)	*trans*-isomer (E)	Total
Study code:	181087	Strawberry	Belgium	BAS 550 01 F	19					
Doc ID:	2005/1004964			1 x 0.125		34	Fruit	0.217	0.294	0.51
Trial No:	AGR/21/04[3]									
GLP:	yes			1 x 0.0625		34		0.190	0.114	<u>0.3</u>
Year:	2004									
Study code:	181087	Strawberry	Netherlands	BAS 550 01 F	19					
Doc ID:	2005/1004964			1 x 0.125		34	Fruit	0.120	0.169	0.29
Trial No:	AGR/22/04[3]									
GLP:	yes			1 x 0.0625		34		0.177	0.080	<u>0.26</u>
Year:	2004									
Study code:	181087	Strawberry	Germany	BAS 550 01 F	10					
Doc ID:	2005/1004964			1 x 0.125		43	Fruit	0.039	0.008	0.05
Trial No:	ACK/12/04[4]					50		0.027	0.005	0.03
GLP:	yes			1 x 0.0625		43		0.026	<0.0044	<u>0.03</u>
Year:	2004					50		0.024	<0.0044	0.03
Study code:	181087	Strawberry	Denmark	BAS 550 01 F	12					
Doc ID:	2005/1004964			1 x 0.125		42	Fruit	0.163	0.065	0.23
Trial No:	ALB/14/04[4]					80		0.055	0.043	0.06
GLP:	yes			1 x 0.0625		42		0.151	0.030	<u>0.18</u>
Year:	2004					80		0.024	0.006	0.03

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Plastic tunnel
4 Glasshouse
_ Underlined values were used for MRL derivation

Report: CA 6.3.1/3
Weitzel R., 1994a
Dimethomorph: Residues in strawberries following treatment with PARAAT, 500 g/kg wettable powder, under field conditions (the Netherlands, 1994)
DK-713-018
Guidelines: none
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: SBO 8801 (PARAAT; WP)
Lot/Batch #: 92007 (dimethomorph 500 g/kg, nominal)
Purity: Not relevant
CAS#: 110488-70-5 (dimethomorph)
Development code:
Spiking levels: 0.01-0.10 mg/kg

2. Test Commodity:

Crop: Strawberry
Type: Berries and small fruit
Variety: Elsanta
Botanical name: *Fragaria x ananassa*
Crop parts(s) or processed commodity: Fruits
Sample size: 2.0 kg

B. STUDY DESIGN

1. Test procedure

In 1994, 4 comparative field trials were conducted with strawberries in Northern Europe (Netherlands). Formulation SBO 8801 was applied once just after planting as a foliar spray at a rate of 1.5 kg a.s./ha, 3.0 kg a.s./ha or as a root drench application of 0.05 g a.s./plant in spray volumes of 1000 L/ha and 100 mL/plant, respectively. Samples of strawberries were collected 39-55 days after the application. In the following only the results of the trials with drench application are shown in more detail. Samples were stored deep frozen at or below -18°C from harvest until analysis for a maximum of 119 days.

Table 6.3.1-6: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha[1] g a.s./pant[2])	Water volume (L/ha[3] ml/plant[4])	Target date/ Timing
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	1.5[1]	1000[1]	Just after planting, young transplants, no fruits present
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	3.0[1]	1000[1]	Just after planting, young transplants, no fruits present
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	0.05[2]	100[2]	Just after planting, young transplants, no fruits present

1 Spray application
2 Root drench

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method No FAMS 002-02.
Dimethomorph residues were extracted with acetone and then partitioned with dichloromethane. An aliquot of the extract was cleaned up using GPC followed by clean-up on a silica gel column. The purified residue was taken up in a methanol for HPLC-UV determination. The limit of quantitation for the tested matrix is 0.01 mg/kg. The two isomer peaks were determined together. The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-7: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method No FAMS 002-02		**BAS 550 F**		
Fruit	0.01-0.10	3	94	9

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-8. Details are shown in Table 6.3.1-9.

Only the results of the drench application are shown here. The results of the spray application are shown under the chapter for spray application.

Table 6.3.1-8: Summary of residues in strawberry after drench application of BAS 550 01 F

Crop	Year	Application	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (drench; field)	1994	SBO 8801 1 x 0.05 g a.s./plant	39-55	Fruit	0.01-0.02

1 Days after last application

III. CONCLUSION

Residues of dimethomorph in strawberries were in the range of 0.01-0.02 mg/kg for the drench application.

Table 6.3.1-9: Residues of dimethomorph (BAS 550 F) in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801	Just after	45	Fruit	0.02
Doc ID:	DK-713-018			1 x 0.05	planting			
Trial No:	CYNF94143							
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801	Just after	55	Fruit	0.01
Doc ID:	DK-713-018			1 x 0.05	planting			
Trial No:	CYNF94320							
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801	Just after	41	Fruit	0.01
Doc ID:	DK-713-018			1 x 0.05	planting			
Trial No:	CYNF94323							
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801	Just after	39	Fruit	0.01
Doc ID:	DK-713-018			1 x 0.05	planting			
Trial No:	CYNF94324							
GLP:	yes							
Year:	1994							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL calculations

The following study provides also data on the residue levels of the metabolite M550F002 (the most abundant metabolite in plant metabolism studies) and its aglycon M550F007 in the representative crop strawberry.

Report:	CA 6.3.1/4 Plier S., 2015 a Study on the residue behaviour of Dimethomorph (BAS 550 F) in strawberries after treatment with BAS 550 01 F under field conditions in Northern Europe, 2014/2015 2015/1000641
Guidelines:	EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009), EC 1107/2009 (14 June 2011), EEC 7029/VI/95 rev. 5 (July 22 1997)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 01 F (WP)
Lot/Batch #:	FRE-001045, FRE-001184 (dimethomorph 500 g/kg, nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.005-250 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and small fruit
Variety:	Clery, Polka, Ostara, Christine
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Leaves, inflorescences, fruits
Sample size:	Min. 0.2 kg

B. STUDY DESIGN

1. Test procedure

In 2014/15, 4 field trials were conducted with strawberries in Northern Europe (Germany, The Netherlands, Belgium and United Kingdom). Formulation BAS 550 01 F was applied three times as root drench applications at individual rates of 0.05 g a.s./plant in 100 mL water. The first application was directly after planting, the second was 27-28 days thereafter, and the third was 35-36 days before harvest.
On a separate plot, the third application was a spray application of 1.5 kg a.s./ha in a spray volume of 400 L/ha instead. Data related to this plot are not summarized any further as they are not relevant here.
For the drench plot there were four samplings. Sampling 1 was immediately before the last application (leaves), sampling 2 was at growth stage BBCH 65 (leaves and inflorescences) and sampling 3 was 35-36 days after the last application (DALA; fruits). A fourth sampling was only needed in one trial because the growth stage BBCH 87/89 was not yet reached at sampling 3.
Samples were stored deep frozen from harvest until analysis for a maximum of 209 days.

Table 6.3.1-10: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./pant^2)	Water volume (mL/plant)	Target date/ Timing
2014/15	4	3	F	BAS 550 01 F (WP)	Dimethomorph	0.05	100	1. Directly after planting 2. 28 days after 1st appl. 3. 35 DBH

DBH Days before harvest

2. Description of analytical procedures

All specimens were analyzed for dimethomorph *E*-isomer and *Z*-isomer using BASF method No L0013/02 and for metabolites M550F002 and M550F007 using BASF method No L0013/03.
Both methods have a limit of quantitation (LOQ) of 0.01 mg/kg for each metabolite. For BASF method No L0013/02, 0.01 mg/kg is the sum of both isomers (LOQ 0.005 mg/kg for each isomer). The limit of detection (LOD) is 0.001 mg/kg for each isomer and 0.0025 mg/kg for each metabolite.
Principle of BASF Method No. L0013/02
Dimethomorph *E*- and *Z*-Isomer are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination is performed by HPLC-MS/MS.
Principle of BASF Method No. L0013/03
M550F002 and M550F007 are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned twice against dichloromethane. The final determination is performed by HPLC-MS/MS.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-11: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method No L0013/02		***E*-BAS 550 F**			***Z*-BAS 550 F**		
Leaves	0.005-250	14	92.0	9.0	14	94.9	13
Inflorescences	0.005-50	4	91.2	3.1	4	82.9	3.7
Fruit	0.005-0.5	6	96.4	5.3	6	96.3	16
Method No L0013/03		**M550F002**			**M550F007**		
Leaves	0.01-50	9	78.5	6.7	9	103	4.7
Inflorescences	0.01-10	4	77.2	9.2	4	103	9.9
Fruit	0.01-0.1	8	74.1	2.9	8	89.6	2.3

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-12 and Table 6.3.1-13. Details are shown in Table 6.3.1-14 and Table 6.3.1-15.

Dimethomorph *E*- and *Z*-isomers

In inflorescences specimens the sum of residues of dimethomorph *E*- and *Z*-isomers ranged between 0.033-2.0 mg/kg.

In fruits specimens the sum of residues of dimethomorph *E*- and *Z*-isomers were at 0.069 mg/kg at growth stage BBCH 81-85 and ranged between <0.01-0.058 mg/kg at growth stage BBCH 87-89.

No residues of dimethomorph *E*- and *Z*-isomers above the LOQ were found in any control specimen, except for one inflorescences sample with 0.10 mg/kg and one leaf sample with 0.36 mg/kg.

Residues of M550F002 and M550F007

In inflorescences specimens the residues of M550F002 (expressed as parent equivalent) ranged between <0.007-0.020 mg/kg and the residues of M550F007 (expressed as parent equivalent) ranged between <0.01-0.018 mg/kg.

In fruits specimens the residues of M550F002 (expressed as parent equivalent) were at 0.008 mg/kg at growth stage BBCH 81-85 and ranged between <0.007-0.008 mg/kg at growth stage BBCH 87-89 and the residues of M550F007 (expressed as parent equivalent) were <0.01 mg/kg at growth stage BBCH 81-85 and growth stage BBCH 87-89.

No residues of M550F002 and M550F007 above the LOQ were found in any control specimen.

Table 6.3.1-12: Summary of dimethomorph residues in strawberry after drench application of BAS 550 01 F

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	E-BAS 550 F	Z-BAS 550 F	Total BAS 550 F
Strawberry (drench; field)	2014/15	BAS 550 01 F 3 x 0.05 g a.s./plant	0	55-61	Leaves	0.010-0.40	0.057-1.5	0.066-1.9
			4-9	65	Leaves	0.008-1.2	0.057-3.4	0.065-4.5
			4-9	65	Inflorescences	0.011-1.0	0.022-1.0	0.033-2.0
			36	81-85	Fruits	0.006	0.062	0.069
			35/42	87-89	Fruits	<0.005-0.014	<0.005-0.050	<0.01-0.058

1 Days after last application

Table 6.3.1-13: Summary of dimethomorph metabolite residues in strawberry after drench application of BAS 550 01 F

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)		
					Matrix	M550F002[2]	M550F007[3]
Strawberry (drench; field)	2014/15	BAS 550 01 F 3 x 0.05 g a.s./plant	-0	55-61	Leaves	0.12-0.28	<0.01-0.020
			4-9	65	Leaves	0.075-0.36	<0.01-0.028
			4-9	65	Inflorescences	<0.007-0.020	<0.01-0.018
			36	81-85	Fruits	0.008	<0.01
			35/42	87-89	Fruits	<0.007-0.008	<0.01

1 Days after last application
2 Expressed as parent equivalent; conversion factor 0.723654
3 Expressed as parent equivalent; conversion factor 1.03752

III. CONCLUSION

Residues of dimethomorph in strawberry fruits were in the range of <0.01-0.058 mg/kg at harvest (BBCH 87-89). Residues of M550F002 ranged from <0.007 to 0.008 mg/kg, and M550F007 residues were below the LOQ of 0.01 mg/kg (<0.01 mg/kg).

Based on these data, a conversion factor from enforcement to dietary risk assessment can be derived for strawberry from the two field trials that showed residues for either parent or metabolite M550F002 above the LOQ. These were the trial L140723 from Germany and trial L140726 from UK.

Trial	Residues according to RD for MRL setting (Dimethomorph) in mg/kg	Residues according to RD for risk assessment (dimethomorph+M550F002+M550F007)*	Conversion factor
L140723	0.050	0.067	1.34
L140726	0.058	0.076	1.31
		Median CF	**1.33**

*for the purpose of calculating the sum, residues of metabolites <LOQ were set at the LOQ

Table 6.3.1-14: Residues of dimethomorph (BAS 550 F) in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-BAS 550 F	*Z*-BAS 550 F	Total BAS 550 F
Study code:	425661	Strawberry	Germany	BAS 550 01 F	61	-0	Leaves	0.40	1.5	1.9
Doc ID:	2015/1000641			3 x 0.05		7	Inflorescences	1.0	0.97	2.0
Trial No:	L140723					7	Leaves	1.2	3.4	4.5
GLP:	yes					36	Fruits	0.0062	0.062[3]	0.069
Year:	2014/15					42	Fruits	0.014	0.036	<u>0.050</u>
Study code:	425661	Strawberry	The Netherlands	BAS 550 01 F	55	-0	Leaves	0.0097	0.057	0.066
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	0.074	0.13[3]	0.20
Trial No:	L140724					9	Leaves	0.0078	0.057	0.065
GLP:	yes					35	Fruits	<0.005	<0.005	<u><0.01</u>
Year:	2014/15									
Study code:	425661	Strawberry	Belgium	BAS 550 01 F	55	-0	Leaves	0.10	0.33	0.43
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	0.011	0.022	0.033
Trial No:	L140725					9	Leaves	0.044	0.17[3]	0.21
GLP:	yes					35	Fruits	<0.005	<0.005	<u><0.01</u>
Year:	2014/15									
Study code:	425661	Strawberry	United Kingdom	BAS 550 01 F	61	-0	Leaves	0.082	0.36	0.44
Doc ID:	2015/1000641			3 x 0.05		4	Inflorescences	0.52[4]	1.0[5]	1.5[6]
Trial No:	L140726					4	Leaves	0.64[7]	1.6[8]	2.2[9]
GLP:	yes					35	Fruits	0.0079	0.050	<u>0.058</u>
Year:	2014/15									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two injections
4 A residue of 0.035 mg/kg was found in the untreated control sample
5 A residue of 0.066 mg/kg was found in the untreated control sample
6 A residue of 0.10 mg/kg was found in the untreated control sample
7 A residue of 0.12 mg/kg was found in the untreated control sample
8 A residue of 0.24 mg/kg was found in the untreated control sample
9 A residue of 0.36 mg/kg was found in the untreated control sample
_ Underlined values were used for MRL derivation

Table 6.3.1-15: Residues of dimethomorph metabolites in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)		
							Matrix	M550F002[4]	M550F007[5]
Study code:	425661	Strawberry	Germany	BAS 550 01 F	61	-0	Leaves	0.28	0.020
Doc ID:	2015/1000641			3 x 0.05		7	Inflorescences	0.015	<0.01
Trial No:	L140723					7	Leaves	0.22	0.021
GLP:	yes					36	Fruits	0.0084	<0.01
Year:	2014/15					42	Fruits	<0.007	<0.01
Study code:	425661	Strawberry	The Netherlands	BAS 550 01 F	55	-0	Leaves	0.12	<0.01
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	<0.007	<0.01
Trial No:	L140724					9	Leaves	0.085[3]	<0.01
GLP:	yes					35	Fruits	<0.007	<0.01
Year:	2014/15								
Study code:	425661	Strawberry	Belgium	BAS 550 01 F	55	-0	Leaves	0.13	<0.01
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	<0.007	<0.01
Trial No:	L140725					9	Leaves	0.075	<0.01
GLP:	yes					35	Fruits	<0.007	<0.01
Year:	2014/15								
Study code:	425661	Strawberry	United Kingdom	BAS 550 01 F	61	-0	Leaves	0.20	0.011
Doc ID:	2015/1000641			3 x 0.05		4	Inflorescences	0.020	0.018
Trial No:	L140726					4	Leaves	0.36	0.028
GLP:	yes					35	Fruits	0.008	<0.01
Year:	2014/15								

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two injections
4 Expressed as parent equivalent; conversion factor 0.723654
5 Expressed as parent equivalent; conversion factor 1.03752

Report:	CA 6.3.1/5 Simek I., 2008a Study on the residue behavior of Dimethomorph in protected strawberries after treatment with BAS 550 01 F under plastic tunnel in Northern France, Germany, Netherlands and Belgium, 2007 2008/1051524
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004)
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 01 F (WP)
Lot/Batch #:	1007 (dimethomorph 50% w/w)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.01-0.5 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and small fruit
Variety:	Elsanta, Matis
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, 4 trials with strawberries under plastic tunnels were conducted in Northern Europe (France, Germany, Netherlands and Belgium) to determine the residue levels of dimethomorph (BAS 550 F) in strawberries.
BAS 550 01 F was applied once as a drench application with a rate of 0.125 g dimethomorph per plant and a water volume of 250 mL/plant at planting/transplanting. Strawberries were collected 35±1 days after the application and at maturity (BBCH 87; 43-46 DALA).

Table 6.3.1-16: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./plant)	Water volume (mL/plant)	Target date/ Timing
2007	4	1	P	BAS 550 01 F (WP)	Dimethomorph	0.125	250	At planting / transplanting

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to BASF method No 575/0.
Dimethomorph was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of BAS 550 F was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for dimethomorph is 0.01 mg/kg.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-17: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 575/0		**BAS 550 F**		
Fruit	0.01-0.50	2	91.5	N/A

N/A Not applicable

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-18. Details are shown in Table 6.3.1-19.

The residues of dimethomorph in strawberries were 0.04-0.18 mg/kg at 35±1 DALA. They remained at this level (0.02-0.21 mg/kg) at the time of harvest (87 BBCH).

Table 6.3.1-18: Summary of residues in strawberry after application of BAS 550 01 F

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (protected)	2007	BAS 550 01 F 1 x 0.125	34-36	Fruit	0.04-0.18
			43-46		0.02-0.21

1 Days after last application

III. CONCLUSION

Residues of dimethomorph in strawberries ranged from 0.04 to 0.18 mg/kg at 35±1 days after the last application. Residues in mature strawberries ranged from 0.02 to 0.21 mg/kg.

Table 6.3.1-19: Residues of dimethomorph (BAS 550 F) in protected strawberries after drench application

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	256522	Strawberry	France	BAS 550 01 F	11	36	Fruit	0.18
Doc ID:	2008/1051524			1 x 0.125		46		<u>0.21</u>
Trial No:	A7047 OB1							
GLP:	yes							
Year:	2007							
Study code:	256522	Strawberry	Germany	BAS 550 01 F	At planting	35	Fruit	0.05
Doc ID:	2008/1051524			1 x 0.125		44		<u>0.03</u>
Trial No:	A7047 GE1							
GLP:	yes							
Year:	2007							
Study code:	256522	Strawberry	Netherlands	BAS 550 01 F	At planting	34	Fruit	0.04
Doc ID:	2008/1051524			1 x 0.125		43		<u>0.03</u>
Trial No:	A7047 NL1							
GLP:	yes							
Year:	2007							
Study code:	256522	Strawberry	Belgium	BAS 550 01 F	At planting	34	Fruit	0.04
Doc ID:	2008/1051524			1 x 0.125		45		<u>0.02</u>
Trial No:	A7047 BE1							
GLP:	yes							
Year:	2007							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Spray application

Report:	CA 6.3.1/3 Weitzel R., 1994a Dimethomorph: Residues in strawberries following treatment with PARAAT, 500 g/kg wettable powder, under field conditions (the Netherlands, 1994) DK-713-018
Guidelines:	none
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SBO 8801 (PARAAT; WP)
Lot/Batch #:	92007 (dimethomorph 500 g/kg, nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.01-0.10 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and small fruit
Variety:	Elsanta
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	2.0 kg

B. STUDY DESIGN

1. Test procedure

In 1994, 4 comparative field trials were conducted with strawberries in Northern Europe (Netherlands). Formulation SBO 8801 was applied once just after planting as a foliar spray at a rate of 1.5 kg a.s./ha, 3.0 kg a.s./ha or as a rootdrench application of 0.05 g a.s./plant in spray volumes of 1000 L/ha and 100 mL/plant, respectively. Samples of strawberries were collected 39-55 days after the application.
Samples were stored deep frozen at or below -18°C from harvest until analysis for a maximum of 119 days.

Table 6.3.1-20: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha[1] g a.s./pant[2])	Water volume (L/ha[3] ml/plant[4])	Target date/ Timing
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	1.5[1]	1000[3]	Just after planting, young transplants, no fruits present
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	3.0[1]	1000[3]	Just after planting, young transplants, no fruits present
1994	4	1	F	SBO 8801 (WP)	Dimethomorph	0.05[2]	100[4]	Just after planting, young transplants, no fruits present

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method No FAMS 002-02.
Dimethomorph residues were extracted with acetone and then partitioned with dichloromethane. An aliquot of the extract was cleaned up using GPC followed by clean-up on a silica gel column. The purified residue was taken up in a methanol for HPLC-UV determination. The limit of quantitation for the tested matrix is 0.01 mg/kg. The two isomer peaks were determined together.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-21: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method No FAMS 002-02		**BAS 550 F**		
Fruit	0.01-0.10	3	94	9

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-22. Details are shown in Table 6.3.1-23.
No dimethomorph residues above the LOQ of 0.01 mg/kg were detected in strawberries.

Table 6.3.1-22: Summary of residues in strawberry after spray application of BAS 550 01 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (spray; field)	1994	SBO 8801 1 x 1.5 kg a.s./ha	39-55	Fruit	<0.01

1 Days after last application

III. CONCLUSION

Residues of dimethomorph in strawberries were below the LOQ of 0.01 mg/kg after the spray application.

Table 6.3.1-23: Residues of dimethomorph (BAS 550 F) in strawberry after spray application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801 1 x 1.5	Just after planting	45	Fruit	<u>≤0.01</u>
Doc ID:	DK-713-018							
Trial No:	CYNF94143			SBO 8801 1 x 3.0				<0.01
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801 1 x 1.5	Just after planting	55	Fruit	<u>≤0.01</u>
Doc ID:	DK-713-018							
Trial No:	CYNF94320			SBO 8801 1 x 3.0				<0.01
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801 1 x 1.5	Just after planting	41	Fruit	<u>≤0.01</u>
Doc ID:	DK-713-018							
Trial No:	CYNF94323			SBO 8801 1 x 3.0				<0.01
GLP:	yes							
Year:	1994							
Study code:	CFS 1994-137	Strawberry	Netherlands	SBO 8801 1 x 1.5	Just after planting	39	Fruit	<u>≤0.01</u>
Doc ID:	DK-713-018							
Trial No:	CYNF94324			SBO 8801 1 x 3.0				<0.01
GLP:	yes							
Year:	1994							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Report: CA 6.3.1/6
Oxspring S., 2009a
Study on the residue behaviour of Dimethomorph in strawberry after treatment with BAS 550 01 F under field conditions in Northern Europe during 2009
2009/1112576
Guidelines: none
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 550 01 F (WP)
Lot/Batch #: 1007 (dimethomorph 50% w/w, nominal)
Purity: Not relevant
CAS#: 110488-70-5 (dimethomorph)
Development code:
Spiking levels: 0.01-1.0 mg/kg

2. Test Commodity:

Crop: Strawberry
Type: Berries and small fruit
Variety: Elsanta, Mara des Bois, Dorselect, Charlotte
Botanical name: *Fragaria x ananassa*
Crop parts(s) or processed commodity: Fruits
Sample size: 2.0 kg

B. STUDY DESIGN

1. Test procedure

In 2009, 4 field trials were conducted with strawberries in Northern Europe (Netherlands, United Kingdom, Belgium and Germany). BAS 550 01 F was applied once as foliar spray at a rate of about 1.5 kg a.s./ha in a water volume of about 200 L/ha. Samples of strawberry fruit were collected 28-29, 35-36 and 42-43 days after the application.
Samples were stored deep frozen at or below -18°C from harvest until analysis for a maximum of 164 days.

Table 6.3.1-24: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2009	4	1	F	BAS 550 01 F (WP)	Dimethomorph	1.5	200	35±1 days before expected harvest

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method No L0013/01 (575/0).
Dimethomorph was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS. The limit of quantitation for the tested matrix is 0.01 mg/kg. The two isomer peaks were determined together.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-25: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No L0013/01 (575/0)		**BAS 550 F**		
Fruit	0.01-1.0	3	101	2.7

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-26. Details are shown in Table 6.3.1-27. Residues in strawberries ranged from <0.01-0.061 mg/kg at 28-29 DALA, from <0.01 to 0.028 mg/kg at 35-36 DALA and from <0.01-0.013 mg/kg at 42-43 DALA.

Table 6.3.1-26: Summary of residues in strawberry after application of BAS 550 01 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (field)	2009	BAS 550 01 F 1 x 1.5	28-29	Fruit	<0.01-0.061
			35-36		<0.01-0.028
			42-43		<0.01-0.013

1 Days after last application

III. CONCLUSION

Residues of dimethomorph in strawberries ranged from <0.01 to 0.028 mg/kg at a PHI of 35 days after application and from <0.01 to 0.013 mg/kg at harvest of the mature fruits.

Table 6.3.1-27: Residues of dimethomorph (BAS 550 F) in strawberry in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	358794	Strawberry	United Kingdom	BAS 550 01 F	65-71	28	Fruit	<0.01
Doc ID:	2009/1112576			1 x 1.5		35		0.018
Trial No:	L090258					42		0.013
GLP:	yes							
Year:	2009							
Study code:	358794	Strawberry	Netherlands	BAS 550 01 F	61	29	Fruit	<0.01
Doc ID:	2009/1112576			1 x 1.5		36		<0.01
Trial No:	L090259					43		<0.01
GLP:	yes							
Year:	2009							
Study code:	358794	Strawberry	Germany	BAS 550 01 F	61	28	Fruit	0.014
Doc ID:	2009/1112576			1 x 1.5		35		0.028[3]
Trial No:	L090260					42		0.013[3]
GLP:	yes							
Year:	2009							
Study code:	358794	Strawberry	Belgium	BAS 550 01 F	65-81	28	Fruit	0.061
Doc ID:	2009/1112576			1 x 1.5		35		0.012
Trial No:	L090261					42		0.012
GLP:	yes							
Year:	2009							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Value from untreated specimen since untreated and treated specimens were mixed up during study
_ Underlined values were used for MRL derivation

Supplementary studies

Report: CA 6.3.1/7
Roland L., 1993a
Dosage de residus de Dimethomorph dans des fraises (Essai: 1991-1992 - Opzoekingsstation van Gorsem)
DK-713-039
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: CME 151 (WP); Paraat (WP)
   Lot/Batch #: Not reported (dimethomorph 25%); not reported (dimethomorph 50%)
   Purity: Not relevant
   CAS#: 110488-70-5 (dimethomorph)
   Development code:
   Spiking levels: 0.01-0.05 mg/kg

2. **Test Commodity:**
   Crop: Strawberry
   Type: Berries and small fruit
   Variety: Elsanta
   Botanical name: *Fragaria x ananassa*
   Crop parts(s) or processed commodity: Fruits
   Sample size: ≥0.35 kg

B. STUDY DESIGN

1. Test procedure

During the 1991/1992 growing season, one field trial with strawberries was conducted in Northern Europe (Belgium) to determine the residue levels of dimethomorph (BAS 550 F) in strawberries. CME 151 was applied twice and Paraat was applied once with individual application rates of 0.05 g dimethomorph per plant and a water volume of 250 mL/plant. The first application was performed four days after plantation, the second one was done 35 days thereafter and the last application was performed at restart of plant growth, about six months (182 days) after the second treatment. Strawberries were collected 58 days after the last application and at harvest (64 DALA). Four field samples were taken at each sampling. Samples were stored frozen at or below -18°C until analysis.

Table 6.3.1-28: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./plant)	Water volume (mL/plant)	Target date/ Timing
1991/ 1992	1	3	F	CME 151 (WP) Paraat (WP)	Dimethomorph	0.05	250	4 days after planting 35 days after 1st appl. At restart of plant growth

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method SOP MR 029.

Dimethomorph was extracted with a mixture of dichloromethane and acetone. The extract was purified with partitioning with petrol ether and on a silica gel column. The final determination was performed by HPLC-UV. The limit of quantitation (LOQ) of the method for dimethomorph is 0.01 mg/kg. Samples were analyzed in duplicate.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-29: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method SOP MR 029		**BAS 550 F**		
Fruit	0.01/0.02/0.05	n.r.	90	n.r.

n.r. Not reported

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-30. Details are shown in Table 6.3.1-31.
Mean residues of dimethomorph in strawberries were 0.012 mg/kg at 58 days after the last application and 0.013 mg/kg at harvest (64 DALA).
No residues at or above the LOQ of 0.01 mg/kg were found in the untreated control sample.

Table 6.3.1-30: Summary of residues in strawberry after application of CME 151 and Paraat

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (field)	1991/1992	CME 151/Paraat 3 x 0.05	58	Fruit	0.012
			64		0.013

1 Days after last application

III. CONCLUSION

Mean residues of dimethomorph in strawberries were 0.012 mg/kg at 58 days after the last application and 0.013 mg/kg at harvest (64 DALA).

Table 6.3.1-31: Residues of dimethomorph (BAS 550 F) in strawberry in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CRP/93/1143	Strawberry	Belgium	CME 151	At restart of	58	Fruit	0.012
Doc ID:	DK-713-039			2 x 0.05	plant growth	64		0.013
Trial No:	B1-D3-C6-			Paraat				
GLP:	no			1 x 0.05				
Year:	1991/1992							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Report: CA 6.3.1/8
Roland L., 1994a
Residus de Dimethomorph sur fraises - Essai 1993-1994 - Melsele
DK-713-040
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: Paraat (WP)
   Lot/Batch #: Not reported (dimethomorph 50%)
   Purity: Not relevant
   CAS#: 110488-70-5 (dimethomorph)
   Development code:
   Spiking levels: 0.01-0.05 mg/kg

2. **Test Commodity:**
   Crop: Strawberry
   Type: Berries and small fruit
   Variety: Elsanta
   Botanical name: *Fragaria x ananassa*
   Crop parts(s) or processed commodity: Fruits
   Sample size: About 1 kg

B. STUDY DESIGN

1. Test procedure

During the 1993/1994 growing season, one field trial with strawberries was conducted in Northern Europe (Belgium) to determine the residue levels of dimethomorph (BAS 550 F) in strawberries.
Paraat was applied three times with individual application rates of 0.05 g dimethomorph per plant and a water volume of 100 mL/plant. The first application was performed on August 11th, 1993, the second one was done 45 days thereafter and the last application was performed at restart of plant growth, about six months (194 days) after the second treatment. Strawberries were collected in duplicate samples at harvest (64 DALA).
Samples were stored frozen at or below -18°C until analysis.

Table 6.3.1-32: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./plant)	Water volume (mL/plant)	Target date/ Timing
1993/ 1994	1	3	F	Paraat (WP)	Dimethomorph	0.05	100	Not stated 45 days after 1st appl. At restart of plant growth

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method SOP MR 029.
Dimethomorph was extracted with a mixture of dichloromethane and acetone. The extract was purified with partitioning with petrol ether and on a silica gel column. The final determination was performed by HPLC-UV. The limit of quantitation (LOQ) of the method for dimethomorph is 0.01 mg/kg. Samples were analyzed in duplicate.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-33: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method SOP MR 029		**BAS 550 F**		
Fruit	0.01/0.05	n.r.	90	n.r.

n.r. Not reported

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-34. Details are shown in Table 6.3.1-35.
Mean residues of dimethomorph in strawberries were 0.01 mg/kg at harvest (64 DALA).
No residues at or above the LOQ of 0.01 mg/kg were found in the untreated control sample.

Table 6.3.1-34: Summary of residues in strawberry after application of Paraat

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (field)	1993/1994	Paraat 3 x 0.05	64	Fruit	0.01

1 Days after last application

III. CONCLUSION

Mean residues of dimethomorph in strawberries were 0.01 mg/kg at harvest (64 DALA).

Table 6.3.1-35: Residues of dimethomorph (BAS 550 F) in strawberry in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CRP/94/1297	Strawberry	Belgium	Paraat 3 x 0.05	At restart of plant growth	64	Fruit	0.01
Doc ID:	DK-713-040							
Trial No:	36634A/B							
GLP:	no							
Year:	1993/1994							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Report: CA 6.3.1/9
Roland L., 1994b
Residus de Dimethomorph sur fraises - Essai 1993-1994 - Zwijndrecht
DK-713-041
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Paraat (WP)
Lot/Batch #: Not reported (dimethomorph 50%)
Purity: Not relevant
CAS#: 110488-70-5 (dimethomorph)
Development code:
Spiking levels: 0.01-0.05 mg/kg

2. Test Commodity:

Crop: Strawberry
Type: Berries and small fruit
Variety: Elsanta
Botanical name: *Fragaria x ananassa*
Crop parts(s) or processed commodity: Fruits
Sample size: About 1 kg

B. STUDY DESIGN

1. Test procedure

During the 1993/1994 growing season, one field trial with strawberries was conducted in Northern Europe (Belgium) to determine the residue levels of dimethomorph (BAS 550 F) in strawberries.
Paraat was applied three times with individual application rates of 0.05 g dimethomorph per plant and a water volume of 100 mL/plant. The first application was performed on August 12th, 1993, the second one was done 46 days thereafter and the last application was performed at restart of plant growth, about six months (192 days) after the second treatment. Strawberries were collected in duplicate samples at harvest (64 DALA).
Samples were stored frozen at or below -18°C until analysis.

Table 6.3.1-36: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./plant)	Water volume (mL/plant)	Target date/ Timing
1993/ 1994	1	3	F	Paraat (WP)	Dimethomorph	0.05	100	Not stated 46 days after 1st appl. At restart of plant growth

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method SOP MR 029.
Dimethomorph was extracted with a mixture of dichloromethane and acetone. The extract was purified with partitioning with petrol ether and on a silica gel column. The final determination was performed by HPLC-UV. The limit of quantitation (LOQ) of the method for dimethomorph is 0.01 mg/kg. Samples were analyzed in duplicate.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-37: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method SOP MR 029		**BAS 550 F**		
Fruit	0.01/0.05	n.r.	90	n.r.

n.r. Not reported

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-38. Details are shown in Table 6.3.1-39.
Mean residues of dimethomorph in strawberries were 0.016 mg/kg at harvest (64 DALA).
No residues at or above the LOQ of 0.01 mg/kg were found in the untreated control sample.

Table 6.3.1-38: Summary of residues in strawberry after application of Paraat

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (field)	1993/1994	Paraat 3 x 0.05	64	Fruit	0.016

1 Days after last application

III. CONCLUSION

Mean residues of dimethomorph in strawberries were 0.016 mg/kg at harvest (64 DALA).

Table 6.3.1-39: Residues of dimethomorph (BAS 550 F) in strawberry in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CRP/94/1298	Strawberry	Belgium	Paraat 3 x 0.05	At restart of plant growth	64	Fruit	0.016
Doc ID:	DK-713-041							
Trial No:	36632A/B							
GLP:	no							
Year:	1993/1994							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Report: CA 6.3.1/10
Roland L., 1994c
Residus de Dimethomorph sur fraises - Essai 1993-1994 - Stekene
DK-713-042
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: Paraat (WP)
   Lot/Batch #: Not reported (dimethomorph 50%)
   Purity: Not relevant
   CAS#: 110488-70-5 (dimethomorph)
   Development code:
   Spiking levels: 0.01-0.05 mg/kg

2. **Test Commodity:**
   Crop: Strawberry
   Type: Berries and small fruit
   Variety: Valeta
   Botanical name: *Fragaria x ananassa*
   Crop parts(s) or processed commodity: Fruits
   Sample size: About 1 kg

B. STUDY DESIGN

1. Test procedure

During the 1993/1994 growing season, one field trial with strawberries was conducted in Northern Europe (Belgium) to determine the residue levels of dimethomorph (BAS 550 F) in strawberries.
Paraat was applied three times with individual application rates of 0.05 g dimethomorph per plant and a water volume of 100 mL/plant. The first application was performed on August 12th, 1993, the second one was done 49 days thereafter and the last application was performed at restart of plant growth, about six months (189 days) after the second treatment. Strawberries were collected in duplicate samples at harvest (76 DALA).
Samples were stored frozen at or below -18°C until analysis.

Table 6.3.1-40: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./plant)	Water volume (mL/plant)	Target date/ Timing
1993/ 1994	1	3	F	Paraat (WP)	Dimethomorph	0.05	100	Not stated 49 days after 1st appl. At restart of plant growth

2. Description of analytical procedures

The determination of the dimethomorph residues was performed according to method SOP MR 029.
Dimethomorph was extracted with a mixture of dichloromethane and acetone. The extract was purified with partitioning with petrol ether and on a silica gel column. The final determination was performed by HPLC-UV. The limit of quantitation (LOQ) of the method for dimethomorph is 0.01 mg/kg. Samples were analyzed in duplicate.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-41: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Method SOP MR 029		**BAS 550 F**		
Fruit	0.01/0.05	n.r.	90	n.r.

n.r. Not reported

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.1-42. Details are shown in Table 6.3.1-43.
Mean residues of dimethomorph in strawberries were 0.017 mg/kg at harvest (76 DALA).
No residues at or above the LOQ of 0.01 mg/kg were found in the untreated control sample.

Table 6.3.1-42: Summary of residues in strawberry after application of Paraat

Crop	Year	Application (g a.s./plant)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Strawberry (field)	1993/1994	Paraat 3 x 0.05	76	Fruit	0.017

1 Days after last application

III. CONCLUSION

Mean residues of dimethomorph in strawberries were 0.017 mg/kg at harvest (76 DALA).

Table 6.3.1-43: Residues of dimethomorph (BAS 550 F) in strawberry in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	CRP/94/1299	Strawberry	Belgium	Paraat 3 x 0.05	At restart of plant growth	76	Fruit	0.017
Doc ID:	DK-713-042							
Trial No:	36659A/B							
GLP:	no							
Year:	1993/1994							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

CA 6.3.2 Lettuce

Report: CA 6.3.2/1
Plier S., 2014a
Determination of residues of BAS 550 F (Dimethomorph) in lettuce (greenhouse) after two applications of BAS 550 01 F in Germany, Belgium, The Netherlands, United Kingdom, Italy and Spain, 2013
2014/1109986

Guidelines: EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/3029/99 rev. 4 (11 July 2000), EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 550 01 F (WP)
Lot/Batch #: 01638729U0 (dimethomorph 50% nominal)
Purity: Not relevant
CAS#: 110488-70-5 (dimethomorph)
Development code:
Spiking levels: 0.005-20 mg/kg

2. Test Commodity:

Crop: Head lettuce (open leaf varieties); rucola (cutting lettuce, arugula); lamb's lettuce
Type: Leafy vegetables
Variety: Lollo rosso (Klausia), Solmar, Concorde RZ, Paradai RZ; Grazia; Trophy
Botanical name: *Lactuca sativa; Eruca sativa; Valerianella locusta*
Crop parts(s) or processed commodity: Heads; leaves; whole plants without roots
Sample size: ≥0.5 kg except for one head lettuce sample at 0 DALA (0.35 kg)

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season, eight greenhouse trials in lettuce were performed with the formulation BAS 550 01 F to determine the residue levels of dimethomorph (BAS 550 F). The plants were treated twice with nominal application rates of 0.18 kg dimethomorph/ha at BBCH stages 19 and 45 for the first and second application. The spray interval was 7 days and the spray volume about 400-1000 L/ha. Lettuce specimens were sampled on the day of the last application as well as 6-7, 13-14 and 20-21 days thereafter. The target harvest time was PHI 14 days. Specimens were stored frozen at or below -18°C. The maximum storage interval from harvest until analysis for plant samples was 310 days.

Table 6.3.2-1: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2013	8	2	G	BAS 550 01 F (WP)	Dimethomorph	0.18	400-1000	21±1 DBH 14±1 DBH

DBH days before harvest

2. Description of analytical procedures

The specimens were analyzed for residues of the dimethomorph *Z*- and *E*-isomers using BASF method No L0013/02 which has a limit of quantitation of 0.005 mg/kg per isomer.
Residues of dimethomorph (*E*- and *Z*-isomers) were extracted using an acidified methanol/water solution. After sample extraction and clean-up by partitioning against cyclohexane, residues were determined by HPLC-MS/MS. The results of procedural recovery experiments are summarized in the following table:

Table 6.3.2-2: Summary of recoveries for dimethomorph in lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0013/02		**BAS 550 F *E*-isomer**			**BAS 550 F *Z*-isomer**		
Head lettuce (open leaf varieties)	0.005/0.05/0.5/20	11	96.4	15	11	88.5	20
Rucola leaves	0.005/0.05/0.5/10	6	76.1	6.2	6	75.1	11
Lamb's lettuce whole plants without roots	0.005/10	6	82.6	11	6	85.8	7.5

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.2-3. Details are shown in Table 6.3.2-4.
At the intended PHI of 14 days total residues of dimethomorph (sum of *E*- and *Z*-isomer) ranged between 0.029 and 2.2 mg/kg in lettuce while in rucola and lamb's lettuce residue levels of 0.053 and 0.14 mg/kg were observed at the intended PHI of 14 days.
In the control samples no residues at or above the limit of quantitation (0.005 mg/kg per isomer) were found.

Table 6.3.2-3: Summary of residues in lettuce after application of BAS 550 01 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			
				Matrix	BAS 550 F *E*-isomer	BAS 550 F *Z*-isomer	Total BAS 550 F
Head lettuce (open leaf)	2013	BAS 550 01 F 2 x 0.18	0	Head	0.015-6.9	0.023-9.4	0.038-16
			6-7		0.24-3.3	0.36-4.2	0.60-7.5
			13-14		0.011-1.1	0.018-1.1	0.029-2.2
			20-21		0.0089-0.21	<0.005-0.29	0.020-0.50
Rucola			0	Leaves	2.7	3.3	5.9
			7		0.38	0.41	0.79
			14		0.029	0.024	0.053
			21		0.029	0.022	0.051
Lamb's lettuce			0	Whole plants without roots	4.6	5.1	9.7
			7		0.55	3.8	4.3
			14		0.092	0.050	0.14
			21		0.034	0.019	0.053

1 Days after last application

III. CONCLUSION

The results show that the residues of dimethomorph in lettuce generally decline over time. At the intended PHI of 14 days total dimethomorph residues were between 0.029 and 2.2 mg/kg.

Table 6.3.2-4: Residues of dimethomorph (BAS 550 F) in lettuce (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-isomer	*Z*-isomer	Total
Study code:	428189	Head	Germany	BAS 550 01 F	19	0	Head	6.9	9.4	16
Doc ID:	2014/1109986	lettuce		2 x 0.18		7		0.74	1.1	1.8
Trial No:	L130502	(open				14		0.27	0.29	0.56
GLP:	yes	leaf)				21		0.011	0.023	0.035
Year:	2013									
Study code:	428189	Head	Belgium	BAS 550 01 F	43	0	Head	6.9	6.6	14
Doc ID:	2014/1109986	lettuce		2 x 0.18		6		0.51	0.92	1.4
Trial No:	L130503	(open				13		0.16	0.29	0.44
GLP:	yes	leaf)				20		0.054	0.12	0.17
Year:	2013									
Study code:	428189	Head	Belgium	BAS 550 01 F	43	0	Head	4.5	3.3	7.9
Doc ID:	2014/1109986	lettuce		2 x 0.18		7		0.24	0.36	0.60
Trial No:	L130504	(open				14		0.011	0.018	0.029
GLP:	yes	leaf)				20		0.0089	0.012	0.021
Year:	2013									
Study code:	428189	Rucola	Netherlands	BAS 550 01 F	43	0	Leaves	2.7	3.3	5.9
Doc ID:	2014/1109986			2 x 0.18		7		0.38	0.41	0.79
Trial No:	L130505					14		0.029	0.024	0.053
GLP:	yes					21		0.029	0.022	0.051
Year:	2013									
Study code:	428189	Lamb's	Netherlands	BAS 550 01 F	33	0	Whole plant[3]	4.6	5.1	9.7
Doc ID:	2014/1109986	lettuce		2 x 0.18		7		0.55	3.8	4.3
Trial No:	L130506					14		0.092	0.050	0.14
GLP:	yes					21		0.034	0.019	0.053
Year:	2013									
Study code:	428189	Head	United	BAS 550 01 F	42/43	0	Head	2.5	3.3	5.8
Doc ID:	2014/1109986	lettuce	Kingdom	2 x 0.18		7		0.84	1.2	2.0
Trial No:	L130507	(open				14		0.27	0.38	0.65
GLP:	yes	leaf)				21		0.21	0.29	0.50
Year:	2013									

Table 6.3.2-4: Residues of dimethomorph (BAS 550 F) in lettuce (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-isomer	*Z*-isomer	Total
Study code:	428189	Head	Italy	BAS 550 01 F	42	0	Head	0.015	0.023	0.038
Doc ID:	2014/1109986	lettuce		2 x 0.18		7		3.3	4.2	7.5
Trial No:	L130508	(open				14		1.1	1.1	2.2
GLP:	yes	leaf)				21		0.015	<0.005	0.02
Year:	2013									
Study code:	428189	Head	Spain	BAS 550 01 F	45	0	Head	2.4	2.9	5.3
Doc ID:	2014/1109986	lettuce		2 x 0.18		7		1.6	2.0	3.6
Trial No:	L130509	(open				14		1.0	1.1	2.1
GLP:	yes	leaf)				21		0.16	0.19	0.36
Year:	2013									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without roots
_ Underlined values were used for MRL derivation

The following study will provide data on the residue level of metabolite M550F002 (the most abundant metabolite in the plant metabolism studies) and its aglycon M550F007 for the representative crop lettuce. The samples analyzed in this study origin from the lettuce trials reported under CA 6.3.2/1, DocID 2014/1109986. ~~The analysis is currently still ongoing and the results will be available and included into the dossier update in March 2016~~.

Report:	CA 6.3.2/2 Sturm M.,Guedez Orozco A.-A., 2016 a Determination of the Dimethomorph metabolites M550F001 (Reg.No. 4388253), M550F00s (Reg.No. 4581886), M550F006 (Reg.No. 4060806) and M550F007 (Reg.No. 4060805) in plant matrices 2015/1000642
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 01 F (WP)
Lot/Batch #:	01638729U0 (dimethomorph 50% nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.01-10 mg/kg

2. Test Commodity:

Crop:	Head lettuce (open leaf varieties); rucola (cutting lettuce, arugula); lamb's lettuce
Type:	Leafy vegetables
Variety:	Lollo rosso (Klausia), Solmar, Concorde RZ, Paradai RZ; Grazia; Trophy
Botanical name:	*Lactuca sativa; Eruca sativa; Valerianella locusta*
Crop parts(s) or processed commodity:	Head, leaves, whole plant without roots

B. STUDY DESIGN

1. Test procedure

The present study describes the analysis of lettuce specimens regarding their content of dimethomorph metabolites M550F001 (Reg.No 4388253), M550F002 (Reg.No 4581886), M550F006 (Reg.No 4060806) and M550F007 (Reg.No 4060805) in field samples derived from the study reported under CA 6.3.2/1, DocID 2014/1109986.

During the 2013 growing season, eight greenhouse trials were performed on lettuce with the formulation BAS 550 01 F. The plants were treated twice with nominal application rates of 0.18 kg dimethomorph/ha at BBCH stages 19 (first application) and 45 (second application). The spray interval was 7 days and the spray volume was in a range from 400-1000 L/ha. Lettuce specimens were sampled on the day of the last application as well as 6-7, 13-14 and 20-21 days thereafter. The target harvest time was PHI 14 days. Specimens were stored frozen at or below -18°C at a maximum of 967 days from sampling until analysis.

Table 6.3.2-5: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./pant^2)	Water volume (mL/plant)	Target date/ Timing (DBH) [1]
2013	8	2	G	BAS 550 01 F (WP)	Dimethomorph	0.18	400-1000	21±1 14±1

1 Days before harvest

2. Description of analytical procedures

All specimens were analyzed for dimethomorph metabolites M550F001, M550F002, M550F006 and M550F007 using BASF method No L0013/03. The limit of quantitation (LOQ) was 0.01 mg/kg for each metabolite.

Metabolites were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned twice against dichloromethane. The final determination was performed by LC-MS/MS.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.2-6: Summary of recoveries for dimethomorph metabolites in lettuce

Matrix	Fortification level (mg/kg)	M550F001 (Reg.No 4388253)			M550F002 (Reg.No 4381886)		
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Lamb's lettuce	0.01-10	8	63.3	4.3	8	62.7	3.7
Rucola leaves	0.01-1	7	73.6	2.7	7	73.4	3.4
Head lettuce (open leaf)	0.01-10	15	72.5	6.1	15	73.7	5.5
Matrix	**Fortification level (mg/kg)**	**M550F006 (Reg.No 4060806)**			**M550F007 (Reg.No 4060805)**		
		n	**Mean (%)**	**RSD (%)**	**n**	**Mean (%)**	**RSD (%)**
Lamb's lettuce	0.01-10	8	91.7	2.1	8	101	3.0
Rucola leaves	0.01-1	7	101	2.5	7	100	2.7
Head lettuce (open leaf)	0.01-10	15	94.4	3.3	15	97.9	4.2

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.2-7. Details are shown in Table 6.3.2-8 and Table 6.3.2-9.

Residues in head lettuce (open leaf)

Residues of M550F002 (expressed as parent equivalent) ranged between <0.007 mg/kg and 0.070 mg/kg at DALA 0 (BBCH 19-45), between 0.0092 mg/kg and 0.11 mg/kg at DALA 6-7 (BBCH 35-47) and between <0.007 mg/kg and 0.096 mg/kg at BBCH 47-49 (DALA 13-14, 20-21).

Residues of metabolite M550F007 (expressed as parent equivalent) ranged from <0.01-0.025 mg/kg at DALA 0 (BBCH 19-45), from <0.01-0.025 mg/kg DALA 6-7 (BBCH 35-47), was <0.01 mg/kg at DALA 13-14 (BBCH 47-49) and ranged from <0.01-0.012 mg/kg at DALA 20-21 (BBCH 48-49).

No residues of M550F001 and M550F006 were detected in any head lettuce samples.

Residues in rucola leaves:

Metabolite M550F002 was the only metabolite detected in rucola leaves, in the sample at DALA 7 (BBCH 47) in amounts of 0.0088 mg/kg (expressed as parent equivalent). No residues above the LOQ were detected for the other metabolites in rucola leaves.

Residues in lamb's lettuce (whole plant without roots):

Residues of metabolite M550F002 (expressed as parent equivalent) accounted for 0.071 mg/kg at DALA 0 (BBCH 33), 0.080 mg/kg at DALA 7 (BBCH 45), 0.035 mg/kg at DALA 14 (BBCH 49) and 0.021 mg/kg at DALA 21 (BBCH 49).

No residues of metabolite M550F001, M550F006 and M550F007 above the LOQ were detected in lamb's lettuce samples.

Table 6.3.2-7: Summary of residues of dimethomorph metabolites in lettuce related matrices

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)				
					Matrix	M550F001	M550F002 [2]	M550F006	M550F007 [3]
Head lettuce (open leaf)	2013	BAS 550 01 F 2 x 0.18 g a.s./plant	0	19-45	Head	<0.01	<0.007-0.070	<0.01	<0.01-0.025
			6-7	35-47		<0.01	0.0092-0.11	<0.01	<0.01-0.025
			13-14	49		<0.01	<0.007-0.096	<0.01	<0.01
			20-21	49		<0.01	<0.007-0.096	<0.01	<0.01-0.012
Rucola leaves	2013	BAS 550 01 F 2 x 0.18 g a.s./plant	0	43	Leaves	<0.01	<0.007	<0.01	<0.01
			7	47		<0.01	0.0088	<0.01	<0.01
			14	49		<0.01	<0.007	<0.01	<0.01
			21	49		<0.01	<0.007	<0.01	<0.01
Lamb's lettuce	2013	BAS 550 01 F 2 x 0.18 g a.s./plant	0	33	Whole plant without roots	<0.01	0.071	<0.01	<0.01
			7	45		<0.01	0.080	<0.01	<0.01
			14	49		<0.01	0.035	<0.01	<0.01
			21	49		<0.01	0.021	<0.01	<0.01

1 Days after last application
2 Expressed as parent equivalent; conversion factor 0.723654
3 Expressed as parent equivalent; conversion factor 1.03752

III. CONCLUSION

No residues of metabolites M550F001 (Reg.No 4388253) and M550F006 (Reg.No 4060806) were detected in any sample.
Residues of metabolite M550F002 (Reg.No 4581886, expressed as parent equivalent) in lettuce related matrices were in a range from <0.007-0.096 mg/kg at DALA 13-14. Residues of M550F007 (Reg.No 4060805) were <LOQ (0.01 mg/kg) at DALA 13-14.
Based on these data and the residues of the parent dimethomorph (E- and Z-isomer) determined in study 2014/1109986 (CA 6.3.2/1), a conversion factor from enforcement to dietary risk assessment can be derived for lettuce related matrices from the eight field trials.

Trial	DALA	Matrix	Residues according to RD for MRL setting (dimethomorph) [mg/kg] [1]	Residues according to RD for risk assessment (dimethomorph+M550F002+M550F007)[2] [mg/kg]	Conversion factor
L130502	0	Head	16.3	16.359	1.00
	7		1.84	1.889	1.03
	14		**0.56**	**0.583**	**1.04**
	21		0.034	0.051	1.49
L130503	0	Head	13.5	13.592	1.01
	6		1.43	1.55	1.09
	13		**0.45**	**0.505**	**1.13**
	20		0.174	0.215	1.24
L130504	0	Head	7.8	7.864	1.01
	7		0.6	0.648	1.08
	14		**0.029**	**0.046**	**1.59**
	20		0.0209	0.0379	1.81
L130505	0	Leaves	6.0	6.017	1.00
	7		0.79	0.809	1.02
	14		**0.053**	**0.07**	**1.32**
	21		0.051	0.068	1.33
L130506	0	Whole plant without root	9.7	9.781	1.01
	7		4.35	4.44	1.02
	14		**0.142**	**0.187**	**1.32**
	21		0.053	0.084	1.58
L130507	0	Head	5.8	5.885	1.01
	7		2.04	2.152	1.06
	14		**0.65**	**0.756**	**1.16**
	21		0.5	0.608	1.22
L130508	0	Head	0.038	0.055	1.45
	7		7.5	7.625	1.02
	14		**2.2**	**2.246**	**1.02**
	21		0.015	0.032	1.85
L130509	0	Head	5.3	5.3182	1.00
	7		3.6	3.6212	1.01
	14		**2.1**	**2.1184**	**1.01**
	21		0.35	0.367	1.05
				Median conversion factor at PHI 14	**1.145**

1 Values derived from CA 6.3.2/1; unrounded values were taken for greater precision
2 For the purpose of calculating the sum, residues of metabolites <LOQ were set at the LOQ

Table 6.3.2-8: Residues of dimethomorph metabolites in lettuce related matrices after application in Northern Europe (greenhouse)

Trial details	Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[1] (BBCH)	DA-LA[2]	Residues found (mg/kg)					
						Matrix	I	II	III	IV	V
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130502 **GLP:** yes **Year:** 2013	Head lettuce (open leaf)	Germany	BAS 550 01 F 2 x 0.18	19	0 7 14 21	Head	<0.01 <0.01 <0.01 <0.01	0.038 0.039 0.013 <0.007	<0.01 <0.01 <0.01 <0.01	0.021 <0.01 <0.01 <0.01	16.359 1.889 0.583 0.051
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130503 **GLP:** yes **Year:** 2013	Head lettuce (open leaf)	Belgium	BAS 550 01 F 2 x 0.18	43	0 6 13 20	Head	<0.01 <0.01 <0.01 <0.01	0.067[3] 0.11[3] 0.045[3] 0.031[3]	<0.01 <0.01 <0.01 <0.01	0.025 <0.01 <0.01 <0.01	13.592 1.55 0.505 0.215
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130504 **GLP:** yes **Year:** 2013	Head lettuce (open leaf)	Belgium	BAS 550 01 F 2 x 0.18	43	0 7 14 20	Head	<0.01 <0.01 <0.01 <0.01	0.044[3] 0.038[3] <0.007 <0.007	<0.01 <0.01 <0.01 <0.01	0.020 <0.01 <0.01 <0.01	7.864 0.648 0.046 0.0379
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130505 **GLP:** yes **Year:** 2013	Rucola leaves	The Netherlands	BAS 550 01 F 2 x 0.18	43	0 7 14 21	Leaves	<0.01 <0.01 <0.01 <0.01	<0.007 0.009 <0.007 <0.007	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	6.017 0.809 0.07 0.068
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130506 **GLP:** yes **Year:** 2013	Lamb's lettuce	The Netherlands	BAS 550 01 F 2 x 0.18	33	0 7 14 21	Whole plant without roots	<0.01 <0.01 <0.01 <0.01	0.071[3] 0.080[3] 0.035[3] 0.021[3]	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	9.781 4.44 0.187 0.084
Study code: 428189 **Doc ID:** 2014/1109986 **Trial No:** L130507 **GLP:** yes **Year:** 2013	Head lettuce (open leaf)	United Kingdom	BAS 550 01 F 2 x 0.18	43	0 7 14 21	Head	<0.01 <0.01 <0.01 <0.01	0.070 0.10 0.096 0.096	<0.01 <0.01 <0.01 <0.01	0.015 0.012 <0.01 0.012	5.885 2.152 0.756 0.608

0 Actual application rates varied by 10% at most
1 At last application
2 Days after last application
3 Mean of two injections
I M550F001
II M550F002, expressed as parent equivalent using a conversion factor of 0.723654
III M550F006
IV M550F007, expressed as parent equivalent using a conversion factor of 1.03752
V Sum of the parent dimethomorph (total E/Z isomers, CA 6.3.2/1) and metabolites M550F002 and M550F007 (both expressed as parent equivalent) relevant for risk assessment. For calculation purposes, residues below or at the LOQ are set on the LOQ.

Table 6.3.2-9: Residues of dimethomorph metabolites in lettuce related matrices after application in Southern Europe (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[1] (BBCH)	DA-LA[2]	Residues [mg/kg]					
							Matrix	I	II	III	IV	V
Study code:	425661	Head	Italy	BAS 550 01 F	42	0		<0.01	<0.007	<0.01	<0.01	0.055
Doc ID:	2015/1000641	lettuce		2 x 0.18		7		<0.01	0.10	<0.01	0.025	7.625
Trial No:	L130508	(open				14	Head	<0.01	0.036	<0.01	<0.01	2.246
GLP:	yes	leaf)				21		<0.01	<0.007	<0.01	<0.01	0.032
Year:	2013											
Study code:	425661	Head	Spain	BAS 550 01 F	45	0		<0.01	0.0072	<0.01	0.011	5.3182
Doc ID:	2015/1000641	lettuce		2 x 0.18		7		<0.01	0.0092	<0.01	0.012	3.6212
Trial No:	L130509	(open				14	Head	<0.01	0.0084	<0.01	<0.01	2.1184
GLP:	yes	leaf)				21		<0.01	<0.007	<0.01	<0.01	0.367
Year:	2013											

0 Actual application rates varied by 10% at most
1 At last application
2 Days after last application
I M550F001
II M550F002, expressed as parent equivalent using a conversion factor of 0.723654
III M550F006
IV M550F007, expressed as parent equivalent using a conversion factor of 1.03752
V Sum of the parent dimethomorph (total E/Z isomers, CA 6.3.2/1) and metabolites M550F002 and M550F007 (both expressed as parent equivalent) relevant for risk assessment. For calculation purposes, residues below or at the LOQ are set on the LOQ.

The following residue trials in lettuce are included in the dossier to address the request for a further two trials in lettuce to support the representative use in lettuce. They were carried out with a mix formulation of dimethomorph and mancozeb, a WG formulation. The representative use in the renewal dossier for lettuce is with BAS 550 01F, a WP formulation. According to SANCO 7525, experience showed that WP and WG formulations produce similar residue levels especially when the last application is more than 7 days prior to harvest, which is the case here. Thus, the additionally included trials with the WG formulation are considered suitable to address the request for further data in lettuce supporting the cGAP of the renewal dossier.
[see LoA KCA 6.3.2/9 2017/1111621]

Report:	CA 6.3.2/3 Anonymous, 2002 Residue data summary from supervised trials (summary) - Rueckstandsbestimmung von Dimethomorph und Mancozeb in Salaten 2001/7002755
Guidelines:	IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992,EEC 96/46
GLP:	yes (certified by Ministerium fuer Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, 40190 Duesseldorf, Germany)

[see KCA 6.3.2/6 2017/1111624]

Report:	CA 6.3.2/4 Offenbaecher G.2002a Rueckstandsbestimmung von Dimethomorph und Mancozeb in Salaten 1997/1004021
Guidelines:	IVA Guidelines Residue Studies Part I Studies with plants (1992),EEC 96/46,BBA Leitlinie: Rueckstandsanalysenmethoden fuer die Ueberwachung. Heft 43 (1998)
GLP:	yes (certified by Ministerium fuer Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, 40190 Duesseldorf, Germany)

[see KCA 6.3.2/7 2017/1111623]

Report:	CA 6.3.2/5 Offenbaecher G.2002b Rueckstandsbestimmung von Dimethomorph und Mancozeb in Kopfsalat 1997/1004020
Guidelines:	IVA Guidelines Residue Studies Part I Studies with plants (1992),EEC 96/46,BBA Leitlinie: Rueckstandsanalysenmethoden fuer die Ueberwachung. Heft 43 (1998)
GLP:	yes (certified by Ministerium fuer Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, 40190 Duesseldorf, Germany))

[see KCA 6.3.2/8 2017/1111622]

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Acrobat plus (WG)
Lot/Batch #:	Not reported, BAS 550 F (dimethomorph): 90 g/kg BAS 266 F (mancozeb): 600 g/kg
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	BASF 550 F (dimethomorph): 0.02, 0.2 mg/kg BAS 266 F (mancozeb): 0.5, 2.0 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leafy vegetables
Variety:	Open leaf: Amandine (Lollo-rossa), Yorvik (Novita), Kendal, NUN 9672 Head: Rodderick
Botanical name:	*Lactuca sativa* L.
Crop parts(s) or processed Commodity:	Leaves, heads
Sample size:	≥0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2001 growing season, eight greenhouse trials in open leaf and head lettuce were performed with the WG formulation acrobat plus to determine the residue levels of dimethomorph (BAS 550 F) and mancozeb (BAS 266 F). The plants were treated twice (7-12 day interval) at an individual rate of 0.18 kg dimethomorph/ha and 1.2 kg mancozeb/ha in spray volume of 600-900 L/ha.

Lettuce specimens were sampled on the day of the last application as well as 7, 14, 21, and 28-29 days thereafter. Specimens were stored frozen at or below -18°C. The maximum storage interval from harvest until analysis for plant samples was 186 days.

Table 6.3.2-1: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2001	8	2	G	Acrobat plus (WG)	Dimethomorph	0.18	600-900	21-26 DBH 14 DBH

DBH days before harvest

2. Description of analytical procedures

The specimens were analyzed for residues of dimethomorph (BAS 550 F) using method DFG S19 with a limit of quantitation (LOQ) of 0.02 mg/kg.
Residues were extracted with water, acetone, dichloromethane, and sodium chloride. The organic phase was isolated and cleaned up by gel permeation chromatography on Bio Beads S-X3. The final determination of the analytes was performed by gas chromatography (GC) using phosphorus nitrogen detector (PND).

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.2-2: Summary of recoveries for dimethomorph in lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
DFG S19		BAS 550 F		
Leaves*	0.02	3	90	15
Leaves*	0.2	3	70	8
Head**	0.02	3	90	0
Head**	0.2	3	86	2

* Determined in control sample of U-RU-F-1601BW S 1/3
** Determined in control sample of RU-F-1601BW RE 1/1

II. RESULTS AND DISCUSSION

The residues ranges for dimethomorph are shown in Table 6.3.2-3 and detailed residues levels are shown in Table 6.3.2-4 for the different trials.

After application of acrobat plus lettuce head specimens taken at the day of the last application contained 2.1-5.3 mg/kg dimethomorph. The residues decreased to ranges of 0.31-7.2 mg/kg, <0.020-0.78 mg/kg, 0.070-0.60 mg/kg, and <0.020-0.14 mg/kg at 7, 14, 21, and 28 DALA respectively.

Residues of dimethomorph were not found above the limit of quantitation (0.02 mg/kg) in control samples.

Table 6.3.2-3: Summary of residues in lettuce after application of acrobat plus

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	
				Matrix	Total BAS 550 F
Lettuce	2001	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	0 7 14 21 28	Head	2.1-5.3 0.31-7.2 <0.020-0.78 0.070-0.60 <0.020-0.14

1 Days after last application

III. CONCLUSION

After application treatment with acrobat plus residues of BAS 550 F in lecture heads were between <0.020 mg/kg and 0.78 mg/kg at the intended PHI of 14 days.

Table 6.3.2-4: Residues of dimethomorph (BAS 550 F) in lettuce (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[1]	DALA[2]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	GLP 01/061	Lettuce (open leaves)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 28	0	Leaves	5.3
Doc ID:	2001/7002755 1997/100421					7		7.2
Trial No:	RU-F-1601BWS 1/1					14		0.37
GLP:	yes					21		0.31
Year:	2001					28		0.14
Study code:	GLP 01/062	Lettuce (open leaves)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 32	0	Leaves	3.9
Doc ID:	2001/7002755 1997/100421					7		0.58
Trial No:	RU-F-1601BWS 1/2					14		0.28
GLP:	yes					21		0.16
Year:	2001					28		0.090

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[1]	DALA[2]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	GLP 01/063	Lettuce (open leaves)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 31	14	Leaves	0.78
Doc ID:	2001/7002755					21		0.60
	1997/100421							
Trial No:	RU-F-1601BWS 1/3							
GLP:	yes							
Year:	2001							
Study code:	GLP 01/065	Lettuce (open leaves)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 31	14	Leaves	0.31
Doc ID:	2001/7002755					21		0.21
	1997/100421							
Trial No:	RU-F-1601BWS 1/4							
GLP:	yes							
Year:	2001							
Study code:	GLP 01/066	Lettuce (head)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 31	0	Head	3.9
Doc ID:	2001/7002755					7		0.80
	1997/100421					14		0.46
Trial No:	RU-F-1601BWS 1/5					21		0.17
GLP:	yes					28		0.050
Year:	2001							
Study code:	GLP 01/064	Lettuce (head)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 29	14	Head	0.74
Doc ID:	2001/7002755							
	1997/100429							
Trial No:	RU-F-1601BWS 1/6							
GLP:	yes							
Year:	2001							
Study code:	GLP 01/067	Lettuce (head)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	Head form.	0	Head	2.2
Doc ID:	2001/7002755					7		0.31
	1997/100429					14		0.17
Trial No:	RU-F-1601BW RE [redacted]					21		0.070
GLP:	yes					29		<0.020
Year:	2001							
Study code:	GLP 01/068	Lettuce (head)	Germany	BAS 550 F 2 x 0.18 BAS 266 F 2 x 1.20	DC 31	14	Head	<0.020
Doc ID:	2001/7002755							
	1997/100429							
Trial No:	RU-F-1601BW RE [redacted]							
GLP:	yes							
Year:	2001							

0 Actual application rates varied by 10% at most
1 At last application
2 Days after last application

_ Underlined values were used for MRL derivation

CA 6.3.3 Grape

For the representative use of BAS 550 02 F (150 g/L DC) in grapes, 16 trials performed in the Northern and Southern EU during 2000 and 2001 are available. The trials were conducted with the formulation BAS 550 09 F, which is equivalent to the formulation BAS 550 02 F (minor change). It is identical to BAS 550 02 F with regard to a.s. content (150 g/L) and formulation type (DC). These trials were all included in the previous dossier for EU Annex I inclusion of dimethomorph and are thus already peer reviewed. The following table summarizes the relevant information for the convenience of the reviewer. The trials were all carried out with the previous cGAP of 5 x 300 g a.s./ha. Within the 25% rule they support also the intended new cGAP of 5 x 250 g a.s./ha for BAS 550 02 F.

Table 6.3.3-1: Peer-reviewed residue studies in grape treated with BAS 550 09 F

CROP **Country, Year** ***Location*** **(variety)** **Trial No**	**Formulation**	**Appli-cation method**	**Rate [kg a.s./ha]**	**Spray volume [L/ha]**	**No.**	**PHI [d]**	**Sample material**	**Residue dimetho-morph [mg/kg]**	**Author** **Report Year** **Study No.** **DocID.**
France, 2000 *Cauroy-les-Hermonvolle* (Pineau Meunier) 00-503-439	BAS 550 09F 150g/L DC	foliar	0.300-0.315	500-525	5	28 28	Grapes	**0.38** 0.33	H. Devine 2002 DK-FR-00-503 DK-713-082
France, 2000 *Merfy* (Pineau Meunier) 00-504-440	BAS 550 09F 150g/L DC	foliar	0.293-0.302	489-503	5	-0 0 7 10 14 21 28	Grapes	0.81 0.80 0.60 0.56 0.55 0.53 **0.50**	H. Devine 2002 DK-FR-00-504 DK-713-083
Italy, 2000 *Imola* (Sangiovese) 00-505-01	BAS 550 09F 150g/L DC	foliar	0.299-0.312	998-1039	5	-0 0 7 9 15 21 28	Grapes	0.29 0.57 0.61 0.83 0.44 0.53 **0.42**	H. Devine 2002 DM-IT-00-505 DK-713-084
Spain, 2000 *Madrid* (Airen) 00-506-12	BAS 550 09F 150g/L DC	foliar	0.289-0.307	724-769	5	10 28 28	Grapes	3.27 **2.28** 0.98	H. Devine 2002 DM-SP-00-506 DK-713-087
Spain, 2000 *Ricote* (Monastrell 00-507-09	BAS 550 09F 150g/L DC	foliar	0.279-0.327	929-1090	5	-0 0 7 10 14 21 28	Grapes	6.07 12.37 5.66 7.27 4.03 5.70 4.60$^{\Delta}$	H. Devine 2002 DM-ES-00-507 DK-713-085

Greece, 2000 *Thessaloniki* (Razaki) 00-508-01	BAS 550 09F 150g/L DC	foliar	0.780- 0.299	926- 998	5	10 28	Grapes	0.66 **0.36**	H.Devine 2002 DM-HE-00-508 DK-713-088
Spain, 2001 *Palacios* (Airen) ALO/43/01	BAS 550 09F 150g/L DC	foliar	0.302- 0.312	1005- 1039	5	0 14 21 27 34	Grapes	1.11 0.38 0.30 **0.24** 0.24	S. Jones 2002 83333 DK-713-089
Germany, 2001 *Wiesloch* (Portugieser) DU2/04/01	BAS 550 09F 150g/L DC	foliar	0.296- 0.315	987- 1050	5	0 14 21 28 35	Grapes	2.41 1.30 1.61 **1.30** 0.97	
Germany, 2001 *Eschbach* (Scheurebe)	BAS 550 09F 150g/L DC	foliar	0.292- 0.306	975- 1020	5	0 14 21 28 35	Grapes	1.94 1-52 1.21 **1.08** 0.81	
France, 2001 *Steinseltz* (Sylvaner) FAN/11/01	BAS 550 09F 150g/L DC	foliar	0.278- 0.324	925- 1080	5	0 14 21 28	Grapes	1.43 1.06 0.65 **0.62**	
France, 2001 *Handschuheim* (Auxerrois) FAN/22/01	BAS 550 09F 150g/L DC	foliar	0.278- 0.318	925- 1061	5	0 14 21 28 35	Grapes	4.25 2.71 1.81 **1.65** 1.43	
France, 2001 *Pont de l'Iere* (Syrah-red FBD/09/01	BAS 550 09F 150g/L DC	foliar	0.271- 0.327	905- 1092	5	0 14 22 29 35	Grapes	1.99 0.75 0.66 **0.38** 0.35	
France, 2001 *Saint Aubin de Luigne* (Grolleau)	BAS 550 09F 150g/L DC	foliar	0.293- 0.317	978- 1057	5	0 15 21 29 35	Grapes	0.87 0.77 0.69 **0.38** 0.32	
France, 2001 *Martigne-Briand* (Gamay) FBM/09/01	BAS 550 09F 150g/L DC	foliar	0.292- 0.319	974- 1063	5	0 15 21 27 35	Grapes	0.82 0.65 0.64 **0.51** 0.39	
Macedonia, 2001 *Arnissa* (Mosxato) HEL/05/01	BAS 550 09F 150g/L DC	foliar	0.284- 0.321	945- 1069	5	0 14	Grapes	0.87 **0.44**	
Italy, 2001 *Pozzol Groppo* (Cortese) ITA/22/01	BAS 550 09F 150g/L DC	foliar	0.288- 0.317	987- 1057	5	0 16 22 29 35	Grapes	0.82 0.39 0.32 **0.21** 0.19	

$^{\Delta}$ trial was run under extreme drought weather conditions yielding unusually small berries. The consequently higher ratio of skin area to fruit volume might explain the exceptionally high residues. Also, application mistake possible due to very high residues at Day 0. Value was not used for MRL calculation. .Values shown in bold indicate samples harvested at PHI±1 day.

The following residue trials are included as supportive information since they also support the cGAP of 5 x 250 g ai/ha. They were conducted with the formulation BAS 550 01 F.

Report:	CA 6.3.3/1 Schaeufele M., 2009a Residue study (decline) with BAS 551 11 F, BAS 550 01 F and BAS 266 10 F applied to wine grapes in Northern France, Germany, Spain and Southern France in 2007 2008/1005539
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 96/68
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

This study report contains also data on mancozeb (BAS 266 F). They are not summarized here since they are not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 551 11 F (WG); BAS 550 01 F (WP)
Lot/Batch #:	BAS 551 11 F: FRE-000455 (mancozeb 60%, dimethomorph 9% nominal); BAS 550 01 F: 1007 (dimethomorph 50% nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.01-10 mg/kg

2. Test Commodity:

Crop:	Grape (wine)
Type:	Berries and small fruit
Variety:	Gris meunier, Regent, Bobal, Grenache
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed commodity:	Fruit
Sample size:	>1 kg (12 bunches)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four comparative trials were performed in Northern and Southern Europe with the formulations BAS 551 11 F and BAS 550 01 F to determine the residue levels of dimethomorph (BAS 550 F). Two trials were performed in Northern Europe (Northern France, Germany) and two in Southern Europe (Spain, Southern France).
The plots were treated five times with nominal application rates of 0.27 or 0.30 kg dimethomorph/ha in a water volume of about 800 L/ha at intervals of about 10 (±1) days. Grape samples were taken at 0, 21, 23, 28-29, and 35-36 days after the last application.

Table 6.3.3-2: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2007	4	5	F	BAS 551 11 F (WG)	Dimethomorph/Mancozeb	0.27	800	68±1 DBH 58±1 DBH 48±1 DBH 38±1 DBH 28±1 DBH
		5		BAS 550 01 F (WP)	Dimethomorph	0.30	800	

DBH Days before harvest

2. Description of analytical procedures

Residues of dimethomorph were analyzed according to the BASF method No 575/0.
BAS 550 F was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of BAS 550 F was performed by HPLC-MS/MS. The two isomer peaks were determined together. The limit of quantitation for the tested matrix is 0.01 mg/kg (sum of *cis*- and *trans*-isomers of dimethomorph). The results of procedural recovery experiments are summarized in the following table:

Table 6.3.3-3: Summary of recoveries for dimethomorph in grape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 575/0 (L0013/01)		**BAS 550 F**		
Grape (wine) fruit	0.01/0.1/10	6	84.8	17.8

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.3-4 and Table 6.3.3-5. Details are shown in Table 6.3.3-6 and Table 6.3.3-7.
Residues of dimethomorph in grapes collected immediately after the last application (DALA 0) ranged from 0.81 to 4.94 mg/kg for BAS 551 11 F (WG) and from 0.77 to 3.33 mg/kg for BAS 550 01 F (WP). Residues declined over time. At the intended pre-harvest interval of 28 days, dimethomorph residues were found in a range of 0.35-1.82 mg/kg and 0.46-1.65 mg/kg for grape specimens.

Table 6.3.3-4: Summary of residues in grape after application of BAS 551 11 F

Crop	Year	Application[0] (kg a.s./ha)	DALA[1]	Residues found(mg/kg)	
				Matrix	BAS 550 F
Grape (North EU)	2007	BAS 551 11 F 5 x 0.27	0	Fruit	0.81-2.90
			21-23		0.34-2.84
			28-29		0.35-1.82
			35-36		0.38-1.96
Grape (South EU)	2007	BAS 551 11 F 5 x 0.27	0	Fruit	1.20-4.94
			21-23		0.90-2.84
			28-29		0.90-1.24
			35-36		1.22-1.83

0 Actual application rates varied by 10% at most
1 Days after last application

Table 6.3.3-5: Summary of residues in grape after application of BAS 550 01 F

Crop	Year	Application[0] (kg a.s./ha)	DALA[1]	Residues found(mg/kg)	
				Matrix	BAS 550 F
Grape (North EU)	2007	BAS 550 01F 5 x 0.30	0	Fruit	0.77-1.82
			21-23		0.34-1.54
			28-29		0.46-1.01
			35-36		0.48-1.33
Grape (South EU)	2007	BAS 550 01 F 5 x 0.30	0	Fruit	1.47-3.33
			21-23		0.98-2.93
			28-29		0.71-1.65
			35-36		0.80-1.85

0 Actual application rates varied by 10% at most
1 Days after last application

III. CONCLUSION

The results show that the residues of dimethomorph in grapes decline over time. At the intended PHI of 28 days dimethomorph residues were between 0.35 and 1.65 mg/kg. Both tested formulations yielded similar residue levels.

Table 6.3.3-6: Residues of dimethomorph (BAS 550 F) in grape in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	256513	Wine grape	France	BAS 551 11 F 5 x 0.27	79-81	0	Fruit	2.90
Doc ID:	2008/1005539					21		2.84
Trial No:	L070775					28		1.82
GLP:	yes					35		<u>1.96</u>
Year:	2007			BAS 550 01 F 5 x 0.30	79-81	0	Fruit	1.82
						21		1.54
						28		1.01
						35		1.33
Study code:	256513	Wine grape	Germany	BAS 551 11 F 5 x 0.27	85	0	Fruit	0.81
Doc ID:	2008/1005539					23		0.34
Trial No:	L070776					29		0.35
GLP:	yes					36		0.38
Year:	2007			BAS 550 01 F 5 x 0.30	85	0	Fruit	0.77
						23		0.34
						29		0.46
						36		<u>0.48</u>

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Table 6.3.3-7: Residues of dimethomorph (BAS 550 F) in grape in Southern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	256513	Wine grape	Spain	BAS 551 11 F 5 x 0.27	81	0	Fruit	4.94
Doc ID:	2008/1005539					21		2.28
Trial No:	L070777					28		1.24
GLP:	yes					35		1.83
Year:	2007			BAS 550 01 F 5 x 0.30	81	0	Fruit	3.33
						21		2.93
						28		1.65
						35		<u>1.85</u>
Study code:	256513	Wine grape	France	BAS 551 11 F 5 x 0.27	83	0	Fruit	1.20
Doc ID:	2008/1005539					23		0.90
Trial No:	L070778					29		0.90
GLP:	yes					36		<u>1.22</u>
Year:	2007			BAS 550 01 F 5 x 0.30	83	0	Fruit	1.47
						21		0.98
						28		0.71
						35		0.80

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Report:	CA 6.3.3/2 Martin T., 2012a Study on the residue behavior of Ametoctradin and Dimethomorph on grapes (wine) after the application of either BAS 550 01, BAS 651 00 F or BAS 650 00 F under field conditions in France (North), Germany, Greece, Italy and Spain, 2011 2012/1135760
Guidelines:	EEC 91/414 Annex III (Part A Section 8), EEC 91/414 Annex II (Part A Section 6), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

This study report contains also data on ametoctradin (BAS 650 F). They are not summarized here since they are not relevant for this dossier.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	BAS 651 00 F (SC); BAS 550 01 F (WP)
Lot/Batch #:	BAS 651 00 F: 0004369608 (ametoctradin 300 g/L, dimethomorph 225 g/L nominal); BAS 550 01 F: 01638729U0 (dimethomorph 50% nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.01-10 mg/kg

2. **Test Commodity:**

Crop:	Grape (wine)
Type:	Berries and small fruit
Variety:	Johanniter, Chardonnay, Riesling, Cabernet Franc, Fortana (Uvad'Oro), Merlot, Muscat, Trebbiano Romagnolo (pergula variety)
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed commodity:	Fruit
Sample size:	Min. 1 kg / 12 bunches (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season, four trials in grapes (wine) were conducted in different representative growing areas in France (North), Germany, Greece, Italy and Spain to determine the residue level of dimethomorph (BAS 550 F) in or on raw agricultural commodities (RAC).
The formulation BAS 550 01 F was applied as foliar sprays as outlined in Table 6.3.3-8. Specimens of fruits were collected at the day of the last application, and 28±1, 35±1 and 42±1 days thereafter. Samples were generally stored frozen at or below -18°C for a maximum of 201 days until analysis of dimethomorph.

Table 6.3.3-8: Target application rates and timings for BAS 550 01 F in grape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2011	4	5	F	BAS 550 01 F (WP)	Dimethomorph	0.30	800	75 ± 1 65 ± 1 55 ± 1 45 ± 1 35 ± 1

2. Description of analytical procedures

Specimens were analyzed for dimethomorph with BASF method No L0013/01 (identical with method 575/0). The method has a limit of quantitation of 0.01 mg/kg.
Dimethomorph was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. Partitioning was performed twice and the respective aliquots are combined. The final determination of dimethomorph was performed by HPLC-MS/MS. The two isomer peaks are determined together.
The methods were validated concurrently with fortified untreated specimens. The results of procedural recovery experiments are summarized in the following table:

Table 6.3.3-9: Summary of recoveries for dimethomorph in grape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 575/0 (L0013/01)		**BAS 550 F**		
Grape (wine) fruit	0.01/0.1/10	15	98.8	10.1

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.3-10. Details are shown in Table 6.3.3-11 and Table 6.3.3-12.
After application of BAS 550 01 F, dimethomorph residues in grape specimens taken at 0 DALA ranged from 0.40 to 1.61 mg/kg. They decreased to 0.27-1.09 mg/kg at the intended PHI at 27-28 DALA and further to 0.13-1.06 mg/kg at 35 DALA. In the specimens from the last sampling at 42 DALA the residues were 0.13-0.98 mg/kg.
In the untreated samples no residues at or above the limit of quantitation of dimethomorph were found.

Table 6.3.3-10: Summary of residues in grape after application of BAS 550 01 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	
				Matrix	BAS 550 F
Grape (North EU)	2011	BAS 550 01 F 5 x 0.30	0 28±1 35 42	Fruit	1.08-1.61 0.52-1.09 0.57-1.06 0.35-0.98
Grape (South EU)	2011	BAS 550 01 F 5 x 0.30	0 28±1 35 42	Fruit	0.40-0.88 0.27-0.52 0.13-0.64 0.13-0.35

1 Days after last application

III. CONCLUSION

At the pre-harvest interval (PHI) of about 28 days residues of dimethomorph ranged between 0.27 and 1.09 mg/kg after application of BAS 550 01 F.

Table 6.3.3-11: Residues of dimethomorph (BAS 550 F) in grape in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	398476	Wine grape	Germany	BAS 550 01 F	85	0	Fruit	1.61
Doc ID:	2012/1135760			5 x 0.30		27		<u>1.09</u>
Trial No:	L110391					35		1.06
GLP:	Yes					42		0.98
Year:	2011							
Study code:	398476	Wine grape	France	BAS 550 01 F	79	0	Fruit	1.08
Doc ID:	2012/1135760			5 x 0.30		28		0.52
Trial No:	L110392					35		<u>0.57</u>
GLP:	Yes					42		0.35
Year:	2011							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Table 6.3.3-12: Residues of dimethomorph (BAS 550 F) in grape in Southern Europe

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 550 F
Study code:	398476	Wine grape	Italy	BAS 550 01 F	83	0	Fruit	0.40
Doc ID:	2012/1135760			5 x 0.30		28		<u>0.27</u>
Trial No:	L110395					35		0.13
GLP:	Yes					42		0.13
Year:	2011							
Study code:	398476	Wine grape	Spain	BAS 550 01 F	81	0	Fruit	0.88
Doc ID:	2012/1135760			5 x 0.30		28		0.52
Trial No:	L110396					35		<u>0.64</u>
GLP:	Yes					42		0.35
Year:	2011							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

The following study provides data on the residue level of metabolite M550F002 (the most abundant metabolite in the plant metabolism studies) and its aglycon M550F007 for the representative crop grapes. Residue levels of metabolites M550F001 and M550F006 were monitored as well.

An additional 8 trials (4 in EU-N and 4 in EU-S) were performed in the field season 2016 and are currently being analyzed for dimethomorph and its metabolites M550F002 and M550F007 and morpholine, in order to provide a complete data package for the metabolites included in the proposed residue definition for risk assessment. The study report will be available under DocID 2017/1078549 in August 2017.

Report:	CA 6.3.3/3 Schneider E., 2016 a Study on the residue behaviour of Dimthomorph (BAS 550 F) in wine grapes and table grapes after treatments with BAS 550 02 F under field conditions in Northern and Southern Europe, 2015 2015/1241719
Guidelines:	OECD-ENV/JM/MONO(99)22, OECD-ENV/JM/MONO(2002)/9, ENV/MC/CHEM(98)17, The GLP Principles of the German Chemikaliengesetz (Chemicals Act) Anhang 1 zu §19 a Abs. ChemG vom 25.07.1994, Article Annexe II a l article D523-8 du code de l environnement, Chemikaliengesetz § 19a-d, Presidential Order No. 273/2000, Real Decreto 1369/2000, Decreto legislativo 2 marzo 2007 n°50, EC 1107/2009 of the European Parliament, 2004/10/EC of 11 February 2004, EEC 7525/VI/95 rev. 9 (March 2011), EEC 7029/VI/95 rev. 5 Appendix B, OECD 509 Crop Field Trial (2009), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4, EEC 91/414 Annex III (Part A Section 5), OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 02 F (DC)
Lot/Batch #:	0008416300 (dimethomorph 0.15 kg a.i. nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.005-5 mg/kg

2. Test Commodity:

Crop:	Grapes, wine, table (fruit)
Type:	Berries and other small fruits
Variety:	Johanniter, Weissburgunder, Pinot Noir, Chardonnay, Carignan, Xinomavro, Italia, Palieri
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed commodity:	Fruit
Sample size:	1 kg (12 bunches)

B. STUDY DESIGN

1. Test procedure

During the growing season of 2015, eight field trials with grapes (wine and table) were conducted in Germany, Northern France and Southern France, Greece, Italy and Spain with the formulation BAS 550 02 F (DC) in order to determine the residue levels of *E*-Dimethomorph (M550F000E), *Z*-Dimethomorph (M550F000Z), as well as its metabolites M550F001, M550F002, M550F006, and M550F007 after foliar application. Six trials were performed on wine grapes and two trials were performed on table grapes (trial L150362 in Italy and L150363 in Spain).

The trials consisted of one control (untreated) and one treated plot (plot 2), except for trial L150360 (Southern France), which consisted of three plots, one untreated and two treated plots (plots 2 and 3). In the trials in Northern Europe, plants were treated twice at 39-41 and 27-28 days before harvest (DBH) with nominal application rates of 0.225 kg dimethomorph/ha. In the trials in Southern Europe, plants were treated five times at 66-70 DBH, 56-60 DBH, 46-50 DBH, 36-39 DBH and at 27-28 DBH with nominal application rates of 0.225 kg dimethomorph/ha. Trial L150360 (Southern France) consisted of two treated plots, on plot 2 two applications were performed at 42 DBH and 29 DBH and on plot 3 five applications were carried out at 70 DBH, 60 DBH, 49 DBH, 39 DBH and 29 DBH, all applications at a nominal rate of 0.225 kg dimethomorph/ha. The spray volume was about 400-800 L/ha. Grape specimens were sampled on the day of the last application as well as 21, 28 (at harvest) and 35 days thereafter. Specimens were stored frozen at or below -18°C. The maximum storage interval from harvest until analysis for plant samples was 161 to 177 days.

Table 6.3.3-13: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2015	4	2	F	BAS 550 02 F (DC)	Dimethomorph	0.225	400-800	40±1 DBH 28±1 DBH
	5	5	F	BAS 550 02 F (DC)	Dimethomorph	0.225	400-800	68±2 DBH 58±2 DBH 48±2 DBH 38±1 DBH 28±1 DBH

DBH days before harvest

2. Description of analytical procedures

The specimens were analyzed for residues of the dimethomorph *Z*- and *E*-isomers using BASF method No L0013/02 with a limit of quantitation of 0.005 mg/kg per isomer and for the metabolites M550F001, M550F002, M550F006 and M550F007 using BASF method No L0013/03 with a limit of quantitation of 0.01 mg/kg for each metabolite.

Residues of dimethomorph (*E*- and *Z*-isomers) were extracted using an acidified methanol/water solution. After sample extraction, clean-up by partitioning against cyclohexane, evaporation to dryness, residues were dissolved in methanol and water and determined by HPLC-MS/MS.
Metabolites M550F001, M550F002, M550F006 and M550F007 were extracted using an acidified methanol/water solution. After sample extraction, partitioning against dichloromethane, evaporation to dryness, residues were dissolved in methanol and water and determined by HPLC-MS/MS.
The results of procedural recovery experiments are summarized in the table below.

Table 6.3.3-14: Summary of recoveries for dimethomorph and its metabolites in grapes

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0013/02		**BAS 550 F *E*-isomer**			**BAS 550 F *Z*-isomer**		
Grape fruit	0.005/0.50/5.0	10	82.1	5.4	10	85.1	5.6
BASF method No L0013/03		**M550F001**			**M550F002**		
Grape fruit	0.010/0.10/1.0	12	82.1	7.1	12	82.5	6.6
BASF method No L0013/03		**M550F006**			**M550F007**		
Grape fruit	0.010/0.10/1.0	12	99.8	5.9	12	98.8	6.0

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.3-15 and Table 6.3.3-16. Details are shown in Table 6.3.3-18 to Table 6.3.3-21.

Dimethomorph (sum of *E*- and *Z*-isomer)
In treated grapes specimens of northern Europe (two applications), total residues of dimethomorph (sum of *E*- and *Z*-isomer) ranged from 1.0 to 2.2 mg/kg directly after application (DALA 0). At the intended PHI of 28 days, total residues ranged from 0.33 to 0.62 mg/kg (27 – 29 DALA).
In southern Europe (five applications), at 0 DALA total residues of dimethomorph (sum of *E*- and *Z*-isomer) ranged from 0.50 to 3.3 mg/kg. At the intended PHI of 28 days, residue levels of 0.059 to 2.6 mg/kg were observed in treated grapes specimens. In treated grapes from Trial 150360 (South France) Plot 2 (two applications), residues of dimethomorph (sum of *E*- and *Z*-isomer) were 0.30 mg/kg, 0.21 mg/kg, 0.080 mg/kg, and 0.072 mg/kg at 0, 22, 29 and 36 DALA.
No residues at or above the limit of quantitation (0.005 mg/kg per isomer) were found in any of the control samples, except for specimen number L1503600003 where residues of dimethomorph (sum of *E*- and *Z*-isomer) with 0.039 mg/kg for the sum of both isomers were found.

M550F001 and M550F002
In treated grapes specimens of northern Europe residues of M550F001 were below the limit of quantification, whereas residues of M550F002 ranged between <0.007 and 0.033 mg/kg over 0 to 36 DALA.
In southern Europe at 0 to 36 DALA, residues of M550F001 (expressed as parent equivalent) ranged from <0.001 to 0.042 mg/kg and M550F002 (expressed as parent equivalent) from <0.007 to 0.023 mg/kg in treated grapes specimens, respectively.
No residues at or above the limit of quantitation were found in any of the control samples, except for specimen number L1503620003 from sampling time 27 DALA where residues of M550F002 (expressed as parent equivalent) with 0.008 mg/kg were found.

M550F006 and M550F007

In treated grapes specimens of northern Europe residues of M550F006 were below the limit of quantification, whereas residues of M550F007 (expressed as parent equivalent) ranged between <0.010 and 0.017 mg/kg over 0 to 36 DALA.

In southern Europe at 0 to 36 DALA residues of M550F006 ranged from <0.010 to 0.028 mg/kg and M550F007 (expressed as parent equivalent) from <0.010 to 0.058 mg/kg in treated grapes specimens, respectively.

No residues at or above the limit of quantitation were found in any of the control samples.

Table 6.3.3-15: Summary of residues of dimethomorph (E- and Z-isomer) in grapes after application of BAS 550 02 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 550 F *E*-isomer	BAS 550 F *Z*-isomer	Sum BAS 550 F
Grape (North EU)	2015	BAS 550 02 F 2 x 0.225	0	81-85	Fruit	0.30-0.69	0.70-1.5	1.0-2.2
			20-22	85-89		0.13-0.17	0.48-0.59	0.60-0.76
			27-28	88-89		0.061-0.14	0.27-0.48	0.33-0.62
			34-35	89		0.093-0.17	0.30-0.55	0.39-0.72
Grape (South EU)		BAS 550 02 F 2 x 0.225	0	85	Fruit	0.093	0.21	0.30
			22	89		0.047	0.16	0.21
			29	89		0.019	0.061	0.080
			36	89		0.016	0.056	0.072
Grape (South EU)		BAS 550 02 F 5 x 0.225	0	83-85	Fruit	0.15-1.1	0.35-2.2	0.50-3.3
			20-22	85-89		0.022-1.1	0.066-2.1	0.089-3.2
			27-29	85-89		0.012-0.88	0.047-1.8	0.059-2.6
			34-36	89		0.043-0.37	0.16-0.79	0.21-1.2

1 DALA = Days After Last Application

Table 6.3.3-16: Summary of residues of dimethomorph metabolites M550F001, M550F002, M550F006, and M550F007 in grapes after application of BAS 550 02 F

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)				
					Matrix	M550F001	M550F002	M550F006	M550F007
Grape (North EU)	2015	BAS 550 02 F 2 x 0.225	0	81-85	Fruit	<0.010	<0.010-0.014	<0.010	<0.010-0.016
			20-22	85-89		<0.010	0.027-0.040	<0.010	<0.010-0.014
			27-29	88-89		<0.010	0.017-0.039	<0.010	<0.010
			34-36	89		<0.010	0.022-0.046	<0.010	<0.010-0.012
Grape (South EU)	2015	BAS 550 02 F 2 x 0.225	0	85	Fruit	<0.010	<0.010	<0.010	<0.010
			22	89		<0.010	0.019	<0.010	<0.010
			29	89		<0.010	<0.010	<0.010	<0.010
			36	89		<0.010	0.010	<0.010	<0.010
Grape (South EU)	2015	BAS 550 02 F 5 x 0.225	0	83-85	Fruit	<0.010-0.018	0.020-0.18	<0.010-0.016	<0.010-0.035
			20-22	85-89		<0.010-0.031	<0.010-0.24	<0.010-0.028	<0.010-0.056
			27-29	85-89		<0.010-0.042	<0.010-0.31	<0.010-0.019	<0.010-0.051
			34-36	89		<0.010-0.015	0.026-0.15	<0.010	<0.010-0.028

1 Days after last application

Table 6.3.3-17: Summary of residues of dimethomorph metabolites expressed as parent equivalent in grapes after application of BAS 550 02 F,

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)[2]				
					Matrix	M550F001	M550F002	M550F006	M550F007
Grape (North EU)	2015	BAS 550 02 F 2 x 0.225	0	81-85	Fruit	<0.007	<0.007-0.010	<0.010	<0.010-0.017
			20-22	85-89		<0.007	0.019-0.029	<0.010	<0.010-0.015
			27-29	88-89		<0.007	0.012-0.028	<0.010	<0.010
			34-36	89		<0.007	0.016-0.033	<0.010	<0.010-0.013
Grape (South EU)	2015	BAS 550 02 F 2 x 0.225	0	85	Fruit	<0.007	<0.007	<0.010	<0.010
			22	89		<0.007	0.014	<0.010	<0.010
			29	89		<0.007	<0.007	<0.010	<0.010
			36	89		<0.007	0.0072	<0.010	<0.010
Grape (South EU)	2015	BAS 550 02 F 5 x 0.225	0	83-85	Fruit	<0.007-0.013	0.014-0.13	<0.010-0.017	<0.010-0.037
			20-22	85-89		<0.007-0.022	<0.007-0.17	<0.010-0.029	<0.010-0.058
			27-29	85-89		<0.007-0.030	<0.007-0.23	<0.010-0.020	<0.010-0.053
			34-36	89		<0.007-0.011	0.019-0.11	<0.010	<0.010-0.029

1 Days after last application
2 Conversion factor of 0.724 (for M550F001 and M550F002) and 1.038 (for M550F006 and M550F007)

III. CONCLUSION

In treated grape specimens of northern Europe (two applications), total residues of dimethomorph (sum of *E*- and *Z*-isomer) ranged from 1.0-2.2 mg/kg, while in grape specimens from Southern Europe (five applications), total residues ranged from 0.50 to 3.3 mg/kg directly after application (DALA 0).
In specimens sampled at harvest (PHI 28) residues of dimethomorph (sum of *E*- and *Z*-isomer) declined to 0.059-2.6 mg/kg and 0.33-0.62 mg/kg in Southern and Northern trials, respectively. Residues of M550F002 and M550F007 showed an initial increase and later on decreased again. At the intended PHI of 28 days, residues of M550F002 ranged from <0.01-0.31 mg/kg and 0.017-0.039 mg/kg in grape samples from South and North EU; respectively.

The results from the trials in Southern France demonstrates that independent of the treatment frequency (2 or 5 times,) similar residue levels were observed directly after application (DALA 0) and at PHI 28.

Table 6.3.3-18: Residues of dimethomorph (BAS 550 F) in grapes (Northern Europe)

Trial details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-isomer	*Z*-isomer	Sum
Study code:	773021	Grapes	Germany	BAS 550 02 F	85	0	Fruit	0.33	0.73	1.1
Doc ID:	2015/1241719			2 x 0.225		20		0.17	0.59	0.76
Trial No:	L150356					27		0.14	0.48	0.62
GLP:	yes					34		0.16	0.48	0.64
Year:	2016									
Study code:	773021	Grapes	Germany	BAS 550 02 F	81	0	Fruit	0.69	1.5	2.2
Doc ID:	2015/1241719			2 x 0.225		21		0.15	0.55	0.70
Trial No:	L150357					28		0.11	0.40	0.51
GLP:	yes					35		0.17	0.55	0.72
Year:	2016									
Study code:	773021	Grapes	Northern France	BAS 550 02 F	81	0	Fruit	0.30	0.70	1.0
Doc ID:	2015/1241719			2 x 0.225		22		0.13	0.51	0.63
Trial No:	L150358					28		0.061	0.27	0.33
GLP:	yes					34		0.096	0.35	0.45
Year:	2016									
Study code:	773021	Grapes	Northern France	BAS 550 02 F	85	0	Fruit	0.33	0.78	1.1
Doc ID:	2015/1241719			2 x 0.225		21		0.13	0.48	0.60
Trial No:	L150359					27		0.12	0.41	0.53
GLP:	yes					34		0.093	0.30	0.39
Year:	2016									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.3-19: Residues of dimethomorph (BAS 550 F) in grapes (Southern Europe)

Trial details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-isomer	Z-isomer	Total
Study code:	773021	Grapes	Southern France	BAS 550 02 F	85	0	Fruit	0.093	0.21	0.30
Doc ID:	2015/1241719			2 x 0.225		22		0.047	0.16	0.21
Trial No:	L150360					29		0.019	0.061	0.080
GLP:	yes					36		0.016	0.056	0.072
Year:	2016									
				BAS 550 02 F	85	0	Fruit	0.15	0.35	0.50
				5 x 0.225		22		0.022	0.066	0.089
						29		0.012	0.047	0.059
						36		0.043	0.16	0.21
Study code:	773021	Grapes	Greece	BAS 550 02 F	81	0	Fruit	0.22	0.72	0.94
Doc ID:	2015/1241719			5 x 0.225		20		0.066	0.30	0.37
Trial No:	L150361					28		0.067	0.31	0.38
GLP:	yes					34		0.070	0.27	0.34
Year:	2016									
Study code:	773021	Grapes	Italy	BAS 550 02 F	81	0	Fruit	0.52	0.98	1.5
Doc ID:	2015/1241719			5 x 0.225		20		0.33	0.77	1.1
Trial No:	L150362					27		0.27	0.63	0.90
GLP:	Yes					34		0.18	0.46	0.64
Year:	2016									
Study code:	773021	Grapes	Spain	BAS 550 02 F	85	0	Fruit	1.1	2.2	3.3
Doc ID:	2015/1241719			5 x 0.225		21		1.1	2.1	3.2
Trial No:	L150363					28		0.88	1.8	2.6
GLP:	yes					35		0.37	0.79	1.2
Year:	2016									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.3-20: Residues of dimethomorph metabolites in grapes related matrices after application in Northern Europe

Trial details	Crop	Country	Formulation application rate[0] (g a.s./plant)	Crop growth stage[1] (BBCH)	DA-LA[2]	Residues found (mg/kg)					
						Matrix	I	II	III	IV	V
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150356 **GLP:** yes **Year:** 2016	Grapes	Germany	BAS 550 02 F 2 x 0.225	85	0	Fruit	<0.01	<0.007	<0.01	0.014	1.08
					20		<0.01	0.029	<0.01	0.013	0.802
					27		<0.01	0.028	<0.01	<0.01	0.658
					34		<0.01	0.032	<0.01	<0.01	0.682
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150357 **GLP:** yes **Year:** 2016	Grapes	Germany	BAS 550 02 F 2 x 0.225	81	0	Fruit	<0.01	0.010	<0.01	0.017	2.22
					21		<0.01	0.022	<0.01	0.015	0.737
					28		<0.01	0.020	<0.01	<0.01	0.54
					35		<0.01	0.033	<0.01	0.013	0.766
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150358 **GLP:** yes **Year:** 2016	Grapes	Northern France	BAS 550 02 F 2 x 0.225	81	0	Fruit	<0.01	<0.007	<0.01	<0.01	1.017
					22		<0.01	0.019	<0.01	<0.01	0.659
					28		<0.01	0.012	<0.01	<0.01	0.352
					34		<0.01	0.016	<0.01	<0.01	0.472
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150359 **GLP:** yes **Year:** 2016	Grapes	Northern France	BAS 550 02 F 2 x 0.225	85	0	Fruit	<0.01	<0.007	<0.01	<0.01	1.127
					21		<0.01	0.021	<0.01	<0.01	0.641
					27		<0.01	0.024	<0.01	<0.01	0.564
					34		<0.01	0.024	<0.01	<0.01	0.427

0 Actual application rates varied by 10% at most
1 At last application
2 Days after last application
I M550F001
II M550F002, expressed as parent equivalent using a conversion factor of 0.723654
III M550F006
IV M550F007, expressed as parent equivalent using a conversion factor of 1.03752
V Sum of the parent dimethomorph (E/Z isomers) and metabolites M550F002 and M550F007 (both expressed as parent equivalent) relevant for risk assessment. For calculation purposes, residues below the LOQ are set on the LOQ.

Table 6.3.3-21: Residues of dimethomorph metabolites in grapes related matrices after application in Southern Europe

Trial details	Crop	Country	Formulation application rate[0] (g a.s./plant)	Crop growth stage[1] (BBCH)	DA-LA[2]	Residues found (mg/kg)					
						Matrix	I	II	III	IV	V
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150360 **GLP:** yes **Year:** 2016	Grapes	Southern France	BAS 550 02 F 2 x 0.225	85	0	Fruit	<0.01	<0.007	<0.01	<0.01	0.320
					22		<0.01	0.014	<0.01	<0.01	0.231
					29		<0.01	<0.007	<0.01	<0.01	0.097
					36		<0.01	0.0072	<0.01	<0.01	0.0892
			BAS 550 02 F 5 x 0.225	85	0	Fruit	<0.01	0.024	<0.01	<0.01	0.534
					22		<0.01	<0.007	<0.01	<0.01	0.105
					29		<0.01	<0.007	<0.01	<0.01	0.076
					36		<0.01	0.039	<0.01	<0.01	0.252
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150361 **GLP:** yes **Year:** 2016	Grapes	Greece	BAS 550 02 F 5 x 0.225	81	0	Fruit	<0.01	0.034	0.011	0.019	0.993
					20		<0.01	0.048	<0.01	0.011	0.425
					28		<0.01	0.035	<0.01	0.010	0.422
					34		<0.01	0.030	<0.01	<0.01	0.38
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150362 **GLP:** Yes **Year:** 2016	Grapes	Italy	BAS 550 02 F 5 x 0.225	81	0	Fruit	<0.01	0.014	<0.01	0.012	1.526
					20		<0.01	0.020	<0.01	0.010	1.13
					27		<0.01	0.021[3]	<0.01	<0.01	0.9314
					34		<0.01	0.019	<0.01	<0.01	0.669
Study code: 773021 **Doc ID:** 2015/1241719 **Trial No:** L150363 **GLP:** yes **Year:** 2016	Grapes	Spain	BAS 550 02 F 5 x 0.225	85	0	Fruit	0.018	0.13	0.016	0.037	3.467
					21		0.031	0.17	0.028	0.058	3.428
					28		0.042[3]	0.023[3]	0.019[3]	0.052[3]	2.7562
					35		0.015	0.11	<0.01	0.029	1.299

0 Actual application rates varied by 10% at most
1 At last application
2 Days after last application
3 Average of 2 values
I M550F001
II M550F002, expressed as parent equivalent using a conversion factor of 0.723654
III M550F006
IV M550F007, expressed as parent equivalent using a conversion factor of 1.03752
V Sum of the parent dimethomorph (E/Z isomers) and metabolites M550F002 and M550F007 (both expressed as parent equivalent) relevant for risk assessment. For calculation purposes, residues below the LOQ are set on the LOQ.

Trial	DALA	Matrix	Residues according to RD for MRL setting (dimethomorph) [mg/kg][1]	Residues according to RD for risk assessment (dimethomorph+M550F002+M550F007)[2] [mg/kg]	Conversion factor
L150356	0	Fruit	1.060	1.0810	1.02
	20		0.760	0.8020	1.06
	27		0.620	0.6580	**1.06**
	34		0.640	0.6820	1.07
L150357	0	Fruit	2.190	2.2170	1.01
	21		0.700	0.7370	1.05
	28		0.510	0.5400	**1.06**
	35		0.720	0.7660	1.06
L150358	0	Fruit	1.000	1.0170	1.02
	22		0.640	0.6690	1.05
	28		0.331	0.3530	**1.07**
	34		0.446	0.4720	1.06
L150359	0	Fruit	1.110	1.1270	1.02
	21		0.610	0.6410	1.05
	27		0.530	0.5640	**1.06**
	34		0.393	0.4270	1.09
L150360*	0	Fruit	0.500	0.5340	1.07
	22		0.088	0.1050	1.19
	29		0.059	0.0760	**1.29**
	36		0.203	0.2520	1.23
L150360**	0	Fruit	0.303	0.3200	1.06
	22		0.207	0.2310	1.11
	29		0.080	0.0970	**1.21**
	36		0.072	0.0892	1.24
L150361	0	Fruit	0.940	0.9930	1.06
	20		0.366	0.4250	1.16
	28		0.377	0.4220	**1.12**
	34		0.340	0.3800	1.12
L150362	0	Fruit	1.500	1.5260	1.02
	20		1.100	1.1300	1.03
	27		0.900	0.9314	**1.03**
	34		0.640	0.6690	1.05
L150363	0	Fruit	3.300	3.4670	1.05
	21		3.200	3.4280	1.07
	28		2.680	2.7562	**1.03**
	35		1.160	1.2990	1.12
				Median conversion factor at PHI 28	**1.06**

1 Unrounded values were taken for higher precision
2 For the purpose of calculating the sum, residues of metabolites <LOQ were set at the LOQ

CA 6.3.4 Other

The following studies ~~will~~ provide residues data for the plant metabolite M550F021 (morpholine) in the representative crops lettuce, strawberry and grapes. ~~They are currently still ongoing and will be provided with the dossier update in March 2016~~. The results confirm that no significant residues of morpholine occur in plant matrices.

~~The studies were scheduled to be provided in this dossier update in March 2016. However, since with the validation of the analytical method for the determination of morpholine severe analytical problems were faced, the analysis of the samples for both above mentioned studies could not be completed in time of this dossier update. Due to problems with blank values and contamination from other sources (via lab equipment/gloves) no reliable residue data can be provided at this point. The data can be provided to the Rapporteur Member State in September 2016.~~

Report:	CA 6.3.4/1 Sturm M.,Wotske M., 2016 a Determination of the Dimethomorph metabolite Morpholin (Reg.No. 21322) in plant matrices 2015/1243750
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

The objective of this study was to determine residues of dimethomorph metabolite M550F021 (morpholine) in field samples derived from BASF studies 2015/1000641 (strawberry; see CA 6.3.1/4) and 2014/1109986 (lettuce; see CA 6.3.2/1).

I. MATERIAL AND METHODS

A. MATERIALS

Please refer to BASF studies 2015/1000641 (see CA 6.3.1/4) and 2014/1109986 (see CA 6.3.2/1).

B. STUDY DESIGN

1. Test procedure

Please refer to BASF studies 2015/1000641 (see CA 6.3.1/4) and 2014/1109986 (see CA 6.3.2/1).

2. Description of analytical procedures

The specimens were analyzed for residues of the dimethomorph metabolite M550F021 using BASF method No L0013/04 which has a limit of quantitation of 0.02 mg/kg.
The water contents of the homogenized crop samples were adjusted and the samples were extracted with acidified methanol after addition of an internal standard. An aliquot of the extract was filtered and diluted (for acidic matrices the pH value was adjusted to 5-6 using NaOH). The final determination of M550F021 was performed by LC MS/MS.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.4-1: Summary of procedural recoveries for M550F021 in lettuce and strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No L0013/04		**M550F021**		
Lettuce (Lamb) whole plant without roots	0.020/0.20/2.0	7	86.0	7.0
Lettuce (Cutting) leaves	0.020/0.20/2.0	7	93.8	1.4
Lettuce (Head) head	0.020/0.20/2.0	12	89.9	6.7
Strawberry fruit	0.020/0.20	6	92.2	7.8
Strawberry leaves	0.020/0.20/2.0	8	94.3	5.5
Strawberry inflorescences	0.020/0.20	6	89.4	7.0

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.4-2. Details are shown in Table 6.3.4-3 and 6.3.4-4.

No residues of M550F021 were found in any strawberry related matrix of plot 2 (drench application). Residues of M550F021 in plot 3 (drench + foliar spray application) were below the LOQ of 0.020 mg/kg in leaves at 0 DBLA (days before last application) (BBCH 55-61). At 0 DALA (BBCH 55-61), the residues ranged between 0.041 and 0.15 mg/kg. 4-9 days after the last application (BBCH 65), the residues ranged between 0.041 and 0.87 mg/kg. In strawberry inflorescences, residues of M550F021 ranged between <0.020 and 0.062 mg/kg 4-9 DALA (BBCH 65). In strawberry fruit, no residues of M550F021 were found.

No residues of M550F021 were found in lettuce (cutting) and lettuce (lamb). In lettuce (head), residues of M550F021 were below the LOQ of 0.020 mg/kg at 0 DALA (BBCH 19-45). At 6-7 DALA (BBCH 35-47), the residues ranged between <0.020 and 0.023 mg/kg. 13-14 days after the last application (BBCH 39-49), the residues ranged between <0.020 and 0.027 mg/kg. At 20-21 DALA (BBCH 39-49), the residues ranged between <0.020 and 0.024 mg/kg.

In the control samples no residues at or above the limit of quantitation (0.02 mg/kg) were found.

Table 6.3.4-2: Summary of M550F021 residues in lettuce and strawberry after application of BAS 550 01 F

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	
				Matrix	M550F021
Head lettuce	2013	BAS 550 01 F 2 x 0.18	0 6-7 13-14 20-21	Head	<0.020 <0.020-0.023 <0.020-0.027 <0.020-0.024
Cutting lettuce			0 7 14 21	Leaves	<0.020 <0.020 <0.020 <0.020
Lamb's lettuce			0 7 14 21	Whole plants without roots	<0.020 <0.020 <0.020 <0.020
Strawberry (plot 2: drench; field plot 3: drench + spray; field)	2014/15	BAS 550 01 F 3 x 0.05 g a.s./plant (plot 2)	-0 4-9 4-9 36 35/42	Leaves Leaves Inflorescences Fruits Fruits	<0.020 <0.020 <0.020 <0.020 <0.020
		BAS 550 01 F 2 x 0.05 g a.s./plant + 1 x 1.5 kg a.s./ha (plot 3)	-0 0 4-9 4-9 36 35/42	Leaves Leaves Leaves Inflorescences Fruits Fruits	<0.020 0.041-0.15 0.041-0.87 <0.020-0.062 <0.020 <0.020

1 Days after last application

III. CONCLUSION

The results show that no residues of M550F021 occur in strawberry fruit and lettuce. Low residues just above the LOQ were only found in one single head lettuce trial.

Table 6.3.4-3: Residues of M550F021 in lettuce (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	801797	Head lettuce (open leaf)	Germany	BAS 550 01 F 2 x 0.18	19	0	Head	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130502					14		<0.020
GLP:	yes					21		<0.020
Year:	2013							
Study code:	801797	Head lettuce (open leaf)	Belgium	BAS 550 01 F 2 x 0.18	43	0	Head	<0.020
Doc ID:	2015/1243750					6		<0.020
Trial No:	L130503					13		<0.020
GLP:	yes					20		<0.020
Year:	2013							
Study code:	801797	Head lettuce (open leaf)	Belgium	BAS 550 01 F 2 x 0.18	43	0	Head	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130504					14		<0.020
GLP:	yes					20		<0.020
Year:	2013							
Study code:	801797	Rucola	Netherlands	BAS 550 01 F 2 x 0.18	43	0	Leaves	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130505					14		<0.020
GLP:	yes					21		<0.020
Year:	2013							
Study code:	801797	Lamb's lettuce	Netherlands	BAS 550 01 F 2 x 0.18	33	0	Whole plant[3]	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130506					14		<0.020
GLP:	yes					21		<0.020
Year:	2013							
Study code:	801797	Head lettuce (open leaf)	United Kingdom	BAS 550 01 F 2 x 0.18	42/43	0	Head	<0.020
Doc ID:	2015/1243750					7		0.023
Trial No:	L130507					14		0.027
GLP:	yes					21		0.024
Year:	2013							

Table 6.3.4-3: Residues of M550F021 in lettuce (greenhouse)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	801797	Head lettuce (open leaf)	Italy	BAS 550 01 F 2 x 0.18	42	0	Head	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130508					14		<0.020
GLP:	yes					21		<0.020
Year:	2013							
Study code:	801797	Head lettuce (open leaf)	Spain	BAS 550 01 F 2 x 0.18	45	0	Head	<0.020
Doc ID:	2015/1243750					7		<0.020
Trial No:	L130509					14		<0.020
GLP:	yes					21		<0.020
Year:	2013							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without roots

Table 6.3.4-4: Residues of M550F021 in in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	801797	Strawberry	Germany	BAS 550 01 F	61	-0	Leaves	<0.020
Doc ID:	2015/1243750			3 x 0.05		7	Inflorescences	<0.020
Trial No:	L140723			(plot 2)		7	Leaves	<0.020
GLP:	yes					36	Fruits	<0.020
Year:	2014/15					42	Fruits	<0.020
				BAS 550 01 F	61	-0	Leaves	<0.020
				2 x 0.05		0	Leaves	0.15
				1 x 1.5 kg		7	Inflorescences	0.060[3]
				a.s./ha		7	Leaves	0.087
				(plot 3)		36	Fruits	<0.020
						42	Fruits	<0.020
Study code:	801797	Strawberry	The Netherlands	BAS 550 01 F	55	-0	Leaves	<0.020
Doc ID:	2015/1243750			3 x 0.05		9	Inflorescences	<0.020
Trial No:	L140724			(plot 2)		9	Leaves	<0.020
GLP:	yes					35	Fruits	<0.020
Year:	2014/15			BAS 550 01 F	55	-0	Leaves	<0.020
				2 x 0.05		0	Leaves	0.075
				1 x 1.5 kg		9	Inflorescences	<0.020
				a.s./ha		9	Leaves	0.041
				(plot 3)		35	Fruits	<0.020
Study code:	801797	Strawberry	Belgium	BAS 550 01 F	55	-0	Leaves	<0.020
Doc ID:	2015/1243750			3 x 0.05		9	Inflorescences	<0.020
Trial No:	L140725			(plot 2)		9	Leaves	<0.020
GLP:	yes					35	Fruits	<0.020
Year:	2014/15			BAS 550 01 F	55	-0	Leaves	<0.020
				2 x 0.05		0	Leaves	0.077
				1 x 1.5 kg		9	Inflorescences	0.020
				a.s./ha		9	Leaves	0.043
				(plot 3)		35	Fruits	<0.020
Study code:	801797	Strawberry	United Kingdom	BAS 550 01 F	61	-0	Leaves	<0.020
Doc ID:	2015/1243750			3 x 0.05		4	Inflorescences	<0.020
Trial No:	L140726			(plot 2)		4	Leaves	<0.020
GLP:	yes					35	Fruits	<0.020
Year:	2014/15			BAS 550 01 F	61	-0	Leaves	<0.020
				2 x 0.05		0	Leaves	0.041
				1 x 1.5 kg		4	Inflorescences	0.062[3]
				a.s./ha		4	Leaves	0.080
				(plot 3)		35	Fruits	<0.020

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two injections

Report:	CA 6.3.4/2 Richter S., 2016 b Study on the residue behavior of Dimethomorph metabolite Morpholine (M550F021) on rotational crops after one application of BAS 550 01 F to bare soil, on wine grapes and table grapes after treatment with BAS 550 02 F, field conditions N and S Europe, 2015 2015/1243751
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

The objective of this study was to determine residues of dimethomorph metabolite M550F021 (morpholine) in rotational crop samples originating from field trials after application of BAS 550 01 F to bare soil 30 days prior planting (BASF study 2015/1241720; see CA 6.6.2/2) and in grape samples after 2 or 5 applications with BAS 550 02 F (BASF study 2015/1241719; see CA 6.3.3/3).

I. MATERIAL AND METHODS

A. MATERIALS

Please refer to BASF studies 2015/1241719 (see CA 6.3.3/3) and 2015/1241720 (see CA 6.6.2/2).

B. STUDY DESIGN

1. Test procedure

Please refer to BASF studies 2015/1241719 (see CA 6.3.3/3) and 2015/1241720 (see CA 6.6.2/2).

2. Description of analytical procedures

The specimens were analyzed for residues of the dimethomorph metabolite M550F021 using BASF method No L0013/04 which has a limit of quantitation of 0.02 mg/kg.
The water contents of the homogenized crop samples were adjusted and the samples were extracted with acidified methanol. An aliquot of the extract was filtered and diluted. The final determination of morpholine (M550F021) was performed by LC-MS/MS.
The results of procedural recovery experiments are summarized in the following table:

Table 6.3.4-5: Summary of procedural recoveries for M550F021 in grapes and rotational crops

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No L0013/04		**M550F021**		
Zucchini fruit	0.020/0.20	6	84.4	9.9
Pea rest of plant without roots	0.020/0.20	6	103	4.7
Pea pods with seeds	0.020/0.20	6	84.8	11
Pea pods without seeds	0.020/0.20	6	88.8	9.0
Pea seeds	0.020/0.20	6	104	7.6
Bean rest of plant without roots	0.020/0.20	6	94.4	11
Bean pods with seeds	0.020/0.20	6	87.5	8.9
Bean pods without seeds	0.020/0.20	6	85.5	12
Bean seeds	0.020/0.20	6	102	5.6
Potato tuber	0.020/0.20	6	97.4	5.6
Grape fruit	0.020/0.20	10	87.6	16

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.3.4-6. Details are shown in Tables 6.3.4-7 to 6.3.4-9.

No residues of morpholine (M550F021) at or above the LOQ (0.020 mg/kg) were detected in any of the treated grape and rotational crop specimens.

In the control samples no residues at or above the limit of quantitation (0.02 mg/kg) were found.

Table 6.3.4-6: Summary of M550F021 residues in grapes and rotational crops

Crop	Year	Application (kg a.s./ha)	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)	
					Matrix	M550F021
Grape (North EU)	2015	BAS 550 02 F 2 x 0.225	0	81-85	Fruit	<0.020
			20-22	85-89		<0.020
			27-28	88-89		<0.020
			34-35	89		<0.020
Grape (South EU)		BAS 550 02 F 2 x 0.225	0	85	Fruit	<0.020
			22	89		<0.020
			29	89		<0.020
			36	89		<0.020
Grape (South EU)		BAS 550 02 F 5 x 0.225	0	83-85	Fruit	<0.020
			20-22	85-89		<0.020
			27-29	85-89		<0.020
			34-36	89		<0.020
Zucchini	2015	BAS 550 01 F 1 x 1.5	64-87	89	Fruit	<0.020
Peas			108	79	Rest of plant w/o roots	<0.020
			108	79	Pods with seeds	<0.020
			108	79	Pods without seeds	<0.020
			108	79	Seeds	<0.020
			148	89	Rest of plant w/o roots	<0.020
			148	89	Seeds	<0.020
Beans			105-120	79	Rest of plant w/o roots	<0.020
			105-120	79	Pods with seeds	<0.020
			105-120	79	Pods without seeds	<0.020
			105-120	79	Seeds	<0.020
			118-147	89	Rest of plant w/o roots	<0.020
			118-147	89	Seeds	<0.020
Potato			108-124	49	Tuber	<0.020

1 Days after last application

III. CONCLUSION

No residues of morpholine (M550F021) at or above the LOQ (0.020 mg/kg) were detected in any of the treated grape and rotational crop specimens.

Table 6.3.4-7: Residues of M550F021 in grapes (Northern Europe)

Trial details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	422754	Grapes	Germany	BAS 550 02 F	85	0	Fruit	<0.020
Doc ID:	2015/1243751			2 x 0.225		20		<0.020
Trial No:	L150356					27		<0.020
GLP:	yes					34		<0.020
Year:	2016							
Study code:	422754	Grapes	Germany	BAS 550 02 F	81	0	Fruit	<0.020
Doc ID:	2015/1243751			2 x 0.225		21		<0.020
Trial No:	L150357					28		<0.020
GLP:	yes					35		<0.020
Year:	2016							
Study code:	422754	Grapes	Northern France	BAS 550 02 F	81	0	Fruit	<0.020
Doc ID:	2015/1243751			2 x 0.225		22		<0.020
Trial No:	L150358					28		<0.020
GLP:	yes					34		<0.020
Year:	2016							
Study code:	422754	Grapes	Northern France	BAS 550 02 F	81	0	Fruit	<0.020
Doc ID:	2015/1243751			2 x 0.225		21		<0.020
Trial No:	L150359					27		<0.020
GLP:	yes					34		<0.020
Year:	2016							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.4-8: Residues of M550F021 in grapes (Southern Europe)

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	422754	Grapes	Southern France	BAS 550 02 F 2 x 0.225	85	0	Fruit	<0.020
Doc ID:	2015/1243751					22		<0.020
Trial No:	L150360					29		<0.020
GLP:	yes					36		<0.020
Year:	2016							
				BAS 550 02 F 5 x 0.225	85	0	Fruit	<0.020
						22		<0.020
						29		<0.020
						36		<0.020
Study code:	422754	Grapes	Greece	BAS 550 02 F 5 x 0.225	83	0	Fruit	<0.020
Doc ID:	2015/1243751					20		<0.020
Trial No:	L150361					28		<0.020
GLP:	yes					34		<0.020
Year:	2016							
Study code:	422754	Grapes	Italy	BAS 550 02 F 5 x 0.225	83	0	Fruit	<0.020
Doc ID:	2015/1243751					20		<0.020
Trial No:	L150362					27		<0.020
GLP:	Yes					34		<0.020
Year:	2016							
Study code:	422754	Grapes	Spain	BAS 550 02 F 5 x 0.225	83	0	Fruit	<0.020
Doc ID:	2015/1243751					21		<0.020
Trial No:	L150363					28		<0.020
GLP:	yes					35		<0.020
Year:	2016							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.4-9: Residues of M550F021 in succeeding crops

Trial details		Crop	Country	Formulation Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	M550F021
Study code:	422754	Zucchini	Germany	BAS 550 01 F 1 x 1.5 to bare soil	89	69	Fruit	<0.020
Doc ID:	2015/1243751	Potato			49	124	Tuber	<0.020
Trial No:	Yes							
GLP:	2015							
Year:	L150167							
Study code:	422754	Zucchini	Denmark	BAS 550 01 F 1 x 1.5 to bare soil	89	87	Fruit	<0.020
Doc ID:	2015/1243751	Peas			79	108	Rest of plant w/o roots	<0.020
Trial No:	Yes				79	148	Rest of plant w/o roots	<0.020
GLP:	2015				79	108	Pods with seeds	<0.020
Year:	L150168				79	108	Pods without seeds	<0.020
					89	108	Seeds	<0.020
					89	148	Seeds	<0.020
		Potato			89	108	Tuber	<0.020
Study code:	422754	Zucchini	Italy	BAS 550 01 F 1 x 1.5 to bare soil	89	73	Fruit	<0.020
Doc ID:	2015/1243751	Beans			79	120	Rest of plant w/o roots	<0.020
Trial No:	Yes				79	147	Rest of plant w/o roots	<0.020
GLP:	2015				79	120	Pods with seeds	<0.020
Year:	L150169				79	120	Pods without seeds	<0.020
					89	120	Seeds	<0.020
					89	147	Seeds	<0.020
		Potato			49	120	Tuber	<0.020
Study code:	422754	Zucchini	Spain	BAS 550 01 F 1 x 1.5 to bare soil	89	64	Fruit	<0.020
Doc ID:	2015/1243751	Beans			79	105	Rest of plant w/o roots	<0.020
Trial No:	Yes				79	118	Rest of plant w/o roots	<0.020
GLP:	2015				79	105	Pods with seeds	<0.020
Year:	L150170				79	105	Pods without seeds	<0.020
					89	105	Seeds	<0.020
					89	118	Seeds	<0.020
		Potato			49	113	Tuber	<0.020

0 Actual application rates varied by 10% at most
1 Days after last application
2 At sampling

CA 6.4 Feeding studies

CA 6.4.1 Poultry

A feeding study in livestock is only required if the estimated maximum feed burden (1x level) is above 0.004 mg/kg bw/d *and* if the metabolism study shows that detectable residues have to be expected in animal products upon consumption of feed containing residues at the 1x level.

No feeding study in laying hens has been performed for dimethomorph for the following reasons:

The estimated maximum feed burden considering all registered uses for dimethomorph in the EU resulted in values of 0.002 mg/kg bw/d for broiler, 0.004 mg/kg bw/d for laying hens and 0.005 mg/kg bw/d for turkeys. Thus the estimated intake is only slightly above the trigger value for only one species, the turkey. The new metabolism study on laying hens was performed at an actual dose level of 1.121 mg/kg bw/d (morpholine label) representing a 224-fold overdosing with respect to the estimated feedburden for turkey and a 280-fold overdosing with respect to the estimated feedburden for laying hens.

The following table shows the total radioactive residues observed in the relevant tissues and eggs in the metabolism study and the anticipated residues at the 1x level obtained by extrapolation using the overdosing factor of 224 for tissues and the factor of 280 for eggs. The TRR values shown are the ones observed for the morpholine label since these were consistently higher than after dosing of the chlorophenyl label.

Matrix	TRR in metabolism study [mg/kg]	Anticipated residue at 1x level [mg/kg]
Eggs (white/yolk/whole)	0.147* / 1.290*/ 0.517**	0.0005/ 0.0046 / 0.0018
Muscle	0.123	0.0005
Fat (pool)	0.334	0.0015
Kidney	0.730	0.0033
Liver	1.184	0.0053

*Represents values in egg white and yolk (pool sample Day 7-13)
** Calculated value for whole egg (pool sample Day 7-13)

The extrapolation shows that no residues above the LOQ of 0.01 mg/kg would be detectable even when considering the entire TRR as worst case. If only the components of the proposed residue definition for risk assessments are considered (parent, metabolites M550F006/M550F007), anticipated residue levels in liver and egg yolk would be even much lower. It can be summarized that at the estimated maximum dietary burden for poultry, no residues above 0.01 mg/kg are anticipated to occur in tissues and eggs. Therefore, the conduct of a poultry feeding study is not necessary.

CA 6.4.2 Ruminants

The following feeding study in lactating cows has already been evaluated in the last EU peer review and was found acceptable. An extensive summary is included in this dossier for the reviewer's convenience.

Report: CA 6.4.2/1
[redacted] 1991 a
CME 151 (Dimethomorph) technical - Residues in milk and tissues of dairy cows - Volume 1
DK-705-007
Guidelines: EPA 171-4(c)
GLP: yes
(certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	Dimethomorph (CME 151) technical
Lot/Batch #:	53/47 DW 11/86
CAS Number:	BAS 550 F: 110488-70-5
Purity:	96.6 % ± 0.8 %
Spiking levels:	Milk + milk products: 0.02-0.10 mg/kg Tissue: 0.01-0.10 mg/kg

2. **Test Animals:** Lactating dairy cows

Species:	Friesian dairy cows
Gender:	Females in mid lactation
Age:	4 - 6 years
Weight at dosing:	429.5 to 618.0 kg (at the start of dosing phase)
Number of animals:	15 (all dosing groups)
Acclimation period:	14 days (except one cow, which was exchanged on day -10)
Diet:	At milking (twice daily) 2 kg of HRC Standard Dairy Ration (Supplier: Whitworth Bros. Ltd., Eye Mill, Peterborough, U.K.).

	%
Rolled barley	34.0
Rolled wheat	5.0
Rolled oats	1.0
Ground beans (coarse)	10.0
Extracted soya bean meal	20.5
Flaked maize	15.5
Beet pulp	3.3
Molasses	5.0
Dicalcium phosphate	0.7
Minsal 375*	5.0

*Mineral/vitamin supplement

Good quality meadow hay was given in the covered pens after each milking at an approximate overall feeding rate of 16 kg per animal per day.

Water: Fresh drinking water (Anglian Water mains supply), *ad libitum*

Housing: Animals were group-housed in covered pens with concrete walls and floors in an open-fronted building. Each pen contained hay racks and automatic drinkers, and straw was provided as bedding material and replenished as necessary to maintain a deep straw litter system. The control animals were housed separately from test animals to avoid possible contamination via urine or feces.

Environmental conditions:

Temperature: 8 – 18°C

Humidity: Not reported

Air change: Natural ventilation

Photoperiod: Natural light supplemented with fluorescent lighting during working hours as necessary

B. STUDY DESIGN

1. Dosing regime-

Oral: Amount of dose: Group A, control, 0 mg Dimethomorph/animal/day
Group B, 50 mg Dimethomorph / animal / day
Group C, 150 mg Dimethomorph / animal / day
Group D, 500 mg Dimethomorph / animal / day

Food consumption: Feed consumption was recorded daily. In general, all concentrate feed offered from Day -7 to termination of the study was consumed.

Vehicle: Premixes of the test substance (5 mg a.s./mL) were prepared weekly in corn oil. The test substance was predissolved in acetone (12% of final volume) and made up with corn oil to final volume. (In week 1 this was followed by an intermediate evaporation step, which was later omitted due to variable evaporation rates.) Appropriate aliquots of the premix were added to the concentrate feed ration (i.e. 5, 15, 50 mL to groups B, C and D, respectively). 50 mL of corn oil only (containing 12% acetone) was similarly added to the feed ration for control cows.

Timing: Twice daily, during feeding at milking

Duration: 28±1 to 35±1 days

Observations: All animals generally remained in good health throughout the study, and no treatment-related clinical abnormalities were observed.

2. Sample collection

Milk collection: Twice daily; evening and morning milk pooled per cow
Milk for analysis taken on study days: -1, 1, 3, 5, 6, 7, 10, 12, 14, 18, 21, 23, 25, 28, 30, 32, 35, 37, 40, 42, 45, 47, 49, 52, 54, 56 (see Table 6.4-4 for details)
Additionally part of the samples of each individual cow on study day 14 and 28 were separated into skimmed milk and cream.
On Day 28 only, the remaining 24-hour milk production of each cow was pooled (after sampling) within treatment groups and mixed thoroughly. The four composite samples were used to prepare pateurized milk, skimmed milk, acid whey and cream (for description see below).

Interval from last dose to sacrifice: 16-24 h for "zero" withdrawal animals; 7, 14, 21 days after last dose

Dosing and sacrifice schedule:

Animal numbers				Days of dosing	Day of sacrifice	Withdrawal time (days)
A	B	C	D			
1, 2, 3				1-27	28	0 (Control)
	4, 6	8	10	1-28	29	0
	5	7,9		1-29	30	0
			11, 12	1-35	36	0
			13	1-35*	43	7
			14	1-35*	50	14
			15	1-35*	57	21

*Not dosed on day 29 am

Samples collected and analyzed: Skeletal muscle (pooled from pectoralis/adductor muscle of thigh) Peritoneal fat (perirenal/omental fat pooled sample), Subcutaneous fat
Liver
Kidney

3. Storage of samples: Milk and tissue samples were stored frozen at ≤18° C in plastic screw-topped containers (milk) and polythene bags (tissues)

4. Extraction and characterization-Analytical method & type:

Milk samples: FAMS 017-01 (a)
FAMS 024-02 (b)
Tissue samples: FAMS 023-01

Analysis of milk samples was carried out according to method FAMS 017-01 (determination of dimethomorph and M550F006/M550F007) during the first phase of analysis and according to FAMS 024-02 (determination of dimethomorph, M550F006/M550F007 and M550F008) during the second phase of analysis.
Processed milk samples (pasteurized milk, skimmed milk, acid whey and cream) were analyzed according to method FAMS 024-02 (determination of dimethomorph, M550F006/M550F007 and M550F008).

Principle of the method: A 20 g representative aliquot of milk or processed milk was extracted with 60 mL of acetone for 1 min using an Ultra-Turrax homogenizer. After centrifugation the liquid phase was decanted off the solid pellet and the acetone was evaporated. The aqueous phase was partitioned three times with 20 mL, each, of ethyl acetate. The organic solutions were drained through sodium sulfate and then rotary evaporated to almost dryness. The concentrated residue was dissolved in methanol and injected into a gel permeation chromatography system equipped with a column packed with 250 mL Fractogel TSX-HW-40 (S). The sample was eluted with methanol (a) or methanol + 0.01 mol acetic acid/L (b), at a flow rate of 3.3 mL/min. The eluate was concentrated by rotary evaporation and then dissolved in methanol for final HPLC determination.
Concentrations of E- and Z-isomers of dimethomorph were determined as one combined peak (no separation on the RPLC column used). Due to their very similar retention times, metabolites M550F006 and M550F007 were also determined together.
The limit of determination in milk and processed milk samples is 0.01 mg/kg for dimethomorph and M550F008 and 0.02 mg/kg for M550F006 and M550F007.

The analytical methods used for whole milk samples were validated at levels of 0.02, 0.05 and 0.10 mg/kg for dimethomorph and CUR 7117 and at 0.02 mg/kg for M550F006 and M550F007 with every series of analysis, where appropriate. The average of all milk recoveries was 93% for dimethomorph (CME 151) (ranging from 74% to 124%), 89% for M550F006/M550F007 (ranging from 67% to 113%) and 89% for CUR 7117 (ranging from 73% to 103%).
The analytical method used for processed milk samples was validated at levels of 0.01, 0.05 and 0.10 mg/kg for dimethomorph (CME 151) and M550F008 and at 0.02 + 0.02 and 0.1 + 0.1 mg/kg for M550F006 + M550F007, together, with every series of analysis. The average recoveries of all processed milk samples were 96% for dimethomorph (ranging from 90% to 105%), 95% for M550F006 + M550F007 (ranging from 84% to 105%) and 100% for M550F008 (ranging from 87% to 126%).
The average recoveries by matrix are listed in Table 6.4.2-1.

Analysis of tissue samples was performed according to method FAMS 023-01 (determination of dimethomorph (CME 151) and metabolites M550F006 and M550F007).

Principle of the method: A 30 g representative aliquot of tissue sample was extracted with 100 mL of acetonitrile for 1 min using an Ultra-Turrax homogenizer (muscle, liver, kidney) or, after melting at 50 °C, for 10 min using an automatic shaker, respectively (fat). After centrifugation (muscle, liver, kidney) or cooling (fat), the liquid phase was decanted off the solid pellet.
The organic phase remaining was partitioned once with 30 mL of n-hexane, which was discarded. After addition of 40 mL of saturated sodium chloride solution and 400 mL of water, the acetonitrile phase was then partitioned twice with 50 mL of dichloromethane. The combined dichloromethane phases were rotary evaporated to almost dryness. The concentrated residue of the partitions was dissolved in methanol and injected into a gel permeation chromatography system equipped with a column packed with 250 mL Fractogel TSK-HW-40 (S). The sample was eluted with methanol at a flow rate of 33 mL/min. The eluate was concentrated by rotary evaporation and then dissolved in cyclo-hexanone.
Residues were finally determined by gas chromatography (GC) with thermionic detection (NPD). For some samples, confirmatory measurements by GC with mass selective detection (MSD) were carried out. Residues of CME 151 were evaluated with the aid of separate calibration curves for E- and Z-isomer based on an E/Z-ratio of about 50/50 in the analytical standard. As the analytical standards of M550F006 and M550F007 consisted almost only of the Z-isomer, the respective calibration curves were also used for evaluation of the E-isomer, if residues were found.
The limit of determination in tissues is 0.01 mg/kg for dimethomorph as well as for M550F006 and M550F007.
The analytical method used was validated at levels of 0.01, 0.05 and 0.10 mg/kg for CME 151, M550F006 and M550F007 with every series of analysis. The average of all tissue recoveries was 105% for CME 151 (ranging from 86% to 131%), 97% for M550F006 (ranging from 71% to 132%) and 95% for M550F007 (ranging from 65% to 114%). The average recoveries by matrix are listed in Table 6.4.2-2. Additional validation data for this method have been generated to further support this study (please refer to DocID 2015/1000643 in chapter 4.1.2 of this dossier.

Table 6.4.2-1: Accuracy and precision data for methods FAMS 017-01 and FAMS 024-02 in milk and for FAMS 024-02 in processed milk samples – procedural recoveries

Matrix	Compound	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
Milk	Dimethomorph (CME 151)	0.02 0.05 0.10	95 92 91	19 (n=10) 6 (n = 9) 7 (n = 10)
	M550F006 / M550F007 (Z67/Z69)	0.02 + 0.02	89	13 (n = 26)
	M550F008 (CUR 7117)	0.02 0.05 0.10	82 94 90	13 (n = 5) 8 (n = 5) 7 (n = 5)
Pasteurized milk	Dimethomorph (CME 151)	0.01 0.05 0.10	90 92 95	NA (n=1) NA (n=1) NA (n=1)
	M550F006 / M550F007 (Z67/Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	95 100 95	NA (n=1) NA (n=1) NA (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	92 99 91	NA (n=1) NA (n=1) NA (n=1)
Skimmed milk	Dimethomorph (CME 151)	0.01 0.05 0.10	105 93 91	NA (n=1) NA (n=1) NA (n=1)
	M550F006 / M550F007 (Z67/Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	88 94 91	NA (n=1) NA (n=1) NA (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	126 94 87	NA (n=1) NA (n=1) NA (n=1)
Acid whey	Dimethomorph (CME 151)	0.01 0.05 0.10	101 94 95	NA (n=1) NA (n=1) NA (n=1)
	M550F006/ M550F007 (Z67/Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	91 96 84	NA (n=1) NA (n=1) NA (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	124 110 94	NA (n=1) NA (n=1) NA (n=1)
Cream	Dimethomorph (CME 151)	0.01 0.05 0.10	104 95 99	NA (n=1) NA (n=1) NA (n=1)
	M550F006/ M550F007 (Z67/Z69)	0.02 + 0.02 0.02 + 0.02 0.10 + 0.10	105 98 99	NA (n=1) NA (n=1) NA (n=1)
	M550F008 (CUR 7117)	0.01 0.05 0.10	101 90 89	NA (n=1) NA (n=1) NA (n=1)

RSD Relative standard deviation
NA Not applicable

Table 6.4.2-2: Accuracy and precision data for method FAMS 023-01 in tissue samples obtained during the study (procedural recoveries)

Matrix	Compound	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
Skeletal muscle	Dimethomorph (CME 151)	0.01 0.05 0.10	131 86 91	NA (n=1) NA (n=1) NA (n=1)
	M550F006 (Z67)	0.01 0.05 0.10	103 71 76	NA (n=1) NA (n=1) NA (n=1)
	M550F007	0.01 0.05 0.10	85 65 71	NA (n=1) NA (n=1) NA (n=1)
Subcutaneous fat	Dimethomorph (CME 151)	0.01 0.05 0.10	99 112 108	NA (n=1) NA (n=1) NA (n=1)
	M550F006 (Z67)	0.01 0.05 0.10	89 112 102	NA (n=1) NA (n=1) NA (n=1)
	M550F007 (Z69)	0.01 0.05 0.10	112 107 104	NA (n=1) NA (n=1) NA (n=1)
Peritoneal fat	Dimethomorph (CME 151)	0.01 0.05 0.10	114 100 99	NA (n=1) NA (n=1) NA (n=1)
	M550F006 (Z67)	0.01 0.05 0.10	132 90 86	NA (n=1) NA (n=1) NA (n=1)
	M550F007 (Z69)	0.01 0.05 0.10	n.r.* 91 85	NA (n=1) NA (n=1) NA (n=1)
Liver	Dimethomorph (CME 151)	0.01 0.05 0.10	114 100 111	NA (n=1) NA (n=1) NA (n=1)
	M550F006 (Z67)	0.01 0.05 0.10	112 89 102	NA (n=1) NA (n=1) NA (n=1)
	M550F007 (Z69)	0.01 0.05 0.10	103 94 109	NA (n=1) NA (n=1) NA (n=1)
Kidney	Dimethomorph (CME 151)	0.01 0.05 0.10	123 91 91	NA (n=1) NA (n=1) NA (n=1)
	M550F006 (Z67)	0.01 0.05 0.10	120 88 90	NA (n=1) NA (n=1) NA (n=1)
	M550F007 (Z69)	0.01 0.05 0.10	114 94 96	NA (n=1) NA (n=1) NA (n=1)

RSD Relative standard deviation
NA Not applicable
* n.r. Not reported (outlier)

Dose administration

Animals were dosed twice daily. The achieved actual daily intake is listed in Table 6.4.2-3. The actual doses are calculated on a mg/kg/bodyweight, and a mg/animal/day basis, taking into account the averaged individual body weights in the beginning, during and at the end of the dosing period and the actual average individual feed intake. The actual amount of the test item consumed by the animals was assumed to be the complete administered dose since only in rare cases refusal of the feed containing the test item was observed (only cows 2,3, 6 and 10 and most occasions were before dosing was started). After the dosing period of 27-29 days, the cows of test groups A, B and C and one cow of test group D were sacrificed (zero withdrawal). For two animal of the highest dose group (D) the dosing period was extended by one week. Three animals of the highest dose group were maintained on a base level diet for further 7, 14 and 21 days to provide depletion data.

Table 6.4.2-3: Nominal and actual dose levels

Animal	**Nominal dose**	**Actual dose**	
	[mg/animal/day] 2)	**[mg/kg feed] 1)**	**[mg/kg bw] 3)**
1	0	0	0
2	0	0	0
3	0	0	0
Average Group A (control)	**0**	**0**	**0**
4	50	2.5	0.091
5	50	2.5	0.080
6	50	2.5	0.115
Average Group B (1x)	**50**	**2.5**	**0.095**
7	150	7.5	0.289
8	150	7.5	0.273
9	150	7.5	0.274
Average Group C (3x)	**150**	**7.5**	**0.279**
10	500	25	0.985
11	500	25	1.030
12	500	25	0.954
13	500	25	0.966
14	500	25	0.872
15	500	25	0.956
Average Group D (10x)	**500**	**25**	**0.961**

1 The actual feed intake was not specifically monitored, thus it is assumed to be identical with the nominally administered ration of 20 kg feed (2 x 2 kg of concentrate food and 16 kg of meadow hay per day)

2 It was assumed that each animal received the intended amount of test item throughout the dosing period by taking up the whole food ration

3 Based on averaged individual body weights, determined on the beginning, middle and end of the dosing period

Milking and milk sampling

All cows were machine-milked twice daily into individual bucket units and the milk yield (kg) recorded. Standard dairy hygiene procedures were observed and the cows were milked in a set order (Control → High dose) to minimise any risk of cross-contamination of milk samples. The morning milk production of each cow was retained in a separate, closed container at +4°C until the evening (pm) milking. The pm milk production for that animal was then added (after recording yield) and mixed thoroughly. Individual milk samples (2 x 200 mL) were taken from each 24-hour milk production.

On Days 14, and 28 of dosing, additional two-liter samples were retained from the milk production of each individual cow. Samples were separated into cream and skimmed milk by centrifugation, divided into 2 subsamples and stored frozen at -20°C.

On Day 28 only, the remaining 24-hour milk production of each cow was pooled (after sampling) within treatment groups A to D and mixed thoroughly. The four composite samples thus produced were stored overnight at +4°C before submitting for processing. If any composite sample exceeded 50 liters, the excess was discarded, i.e. 50 liters maximum were submitted for each group. Each composite sample was then treated as follows:

1. A 3000 mL sample was removed and pasteurized (batch pasteurisation at 63°C for 30 minutes), cooled to approximately 5°C and 4 x 500 mL subsamples were taken.
2. The remaining bulk sample was separated at a temperature of 45°C to 50°C using an Elecrem Separator, to a cream with a fat content of approximately 35% butterfat; skimmed milk fat content approximately 0.1%. 4 x 500 mL subsamples of cream and of skim were retained.
3. From the bulked sample of the remaining skim, 4000 mL were removed and cooled to approximately 15°C. The pH was reduced to approximately 4.6 by the addition of lactic acid diluted 50/50 with water. The acidified skim was then heated to 51.5°C and the curd removed by filtering through cheese cloth. The resulting whey was put through a small-scale separator at 3000 rpm and 4 x 500 mL subsamples retained.

All milk samples were stored frozen in plastic screw-topped containers.

Mean milk production showed a slight overall decline in all groups over the experimental period; no treatment-related group differences were apparent.

Terminal procedures

All cows were sedated by intravenous injection of xylazine, then sacrificed by intravenous injection of sodium pentobarbitone and exsanguination. A macroscopic post mortem examination was carried out and quadruplicate samples of the following tissues were retained:

subcutaneous fat, skeletal muscle (pooled from pectoralis/adductor muscle of thigh), peritoneal fat (perirenal/omental fat pooled sample), liver, kidneys .

All cows were examined post mortem. No macroscopic abnormalities were noted which could be associated with experimental treatment.

All samples were wrapped in polythene bags and transferred to frozen storage (-20°C) within one hour of sampling. Two complete sets of tissue subsamples were sent to the Shell Forschung GmbH Residue Laboratory (SFS-CUA) for analysis.

II. RESULTS AND DISCUSSION

Residues in milk, pasteurised milk, skimmed milk, acid whey and cream

No residues of dimethomorph or M550F008 at or above the limit of quantitation of 0.01 mg/kg or of M550F006/M550F007 (LOQ = 0.02 mg/kg) could be detected in any milk sample. Isolated minor residues found in the milk of two cows during the depletion phase appear to stem from contaminations. For detailed results see Table 6.4.2-4.

On day 28 of the study, milk from each cow was pooled (after taking of individual samples) within treatment groups A - D and mixed thoroughly. The four combined samples thus produced were submitted for processing to pasteurized milk, skimmed milk, acid whey and cream. Two parallel sets of group samples of processed milk were analyzed for dimethomorph (CME 151) and relevant metabolites (M550F006, M550F007 and M550F008).
No residues of dimethomorph or its metabolites (M550F006, M550F007 and M550F008) at or above the LOQ of 0.01 mg/kg (dimethomorph, M550F008) or 0.02 mg/kg (M550F006, M550F007) could be detected in pasteurized milk, skimmed milk, and acid whey samples of all treatment groups. In cream, also no residues of all analytes were found for all dose groups except for the highest treatment group (D), where very low residues of parent dimethomorph at the LOQ were detected.
The results demonstrated no necessity to analyze the additional individual cream and skim milk samples obtained on days 14 and 28 of the study.

The results for processed milk samples of each treatment group are summarized in Table 6.4.2-5.

Table 6.4.2-4: Summary of group mean results for whole milk

Day of dosing	Group	Group mean residues of analytes in whole milk [mg/kg]		
		Dimethomorph	M550F006/ M550F007	M550F008
-1	A (control)	<0.01 (3)	<0.02 (3)	<0.01 (2)
-1	B (1x, 50 mg)	<0.01 (3)	<0.02 (3)	<0.01 (3)
-1	C (3x, 150 mg)	<0.01 (3)	<0.02 (3)	<0.01 (3)
-1	D (10x, 500 mg)	<0.01 (6)	<0.02 (3)	<0.01 (3)
1	D	<0.01 (3)	<0.02 (3)	
3	D	<0.01 (3)	<0.02 (3)	
5	D	<0.01 (3)	<0.02 (3)	
6	D	<0.01 (3)	<0.02 (3)	
7	A	<0.01 (2)	<0.02 (2)	<0.01 (2)
7	B	<0.01 (3)	<0.02 (3)	<0.01 (3)
7	C	<0.01 (3)	<0.02 (3)	<0.01 (3)
7	D	<0.01 (6)	<0.02 (6)	<0.01 (3)
10	A	<0.01 (1)	<0.02 (1)	
10	D	<0.01 (3)	<0.02 (3)	
12	D	<0.01 (3)	<0.02 (3)	
14	A	<0.01 (2)	<0.02 (2)	<0.01 (2)
14	B	<0.01 (3)	<0.02(3)	<0.01 (3)
14	C	<0.01 (3)	<0.02 (3)	<0.01 (3)
14	D	<0.01 (6)	<0.02 (6)	<0.01 (3)
18	A	<0.01 (1)	<0.02 (1)	
18	D	<0.01 (3)	<0.02 (3)	
21	A	<0.01 (2)	<0.02 (2)	<0.01 (2)
21	B	<0.01 (3)	<0.02 (3)	<0.01 (3)
21	C	<0.01 (3)	<0.02 (3)	<0.01 (3)
21	D	<0.01 (6)	<0.02 (6)	<0.01 (3)
23	A	<0.01 (1)	<0.02 (1)	
23	D	<0.01 (3)	<0.02 (3)	
25	D	<0.01 (3)	<0.02 (3)	
28	A	<0.01 (3)	<0.02 (3)	<0.01 (3)
28	B	<0.01 (3)	<0.02 (3)	<0.01 (3)
28	C	<0.01 (3)	<0.02 (3)	<0.01 (3)
28	D	<0.01 (6)	<0.02 (6)	<0.01 (3)
30	D	<0.01 (3)	<0.02 (3)	<0.01 (3)
32	D	<0.01 (3)	<0.02 (3)	<0.01 (3)
35	D	<0.01 (5)	<0.02 (5)	<0.01 (5)
37	D	<0.01 (3)	<0.02 (3)	<0.01 (3)
40	D	<0.01 (3)	<0.02 (3)	<0.01 (3)
42	D	<0.01 (3)	<0.02 (3)	<0.01 (3)
45	D	0.01 (2)	0.02 (2)	0.01 (2)
47	D	<0.01 (2)	<0.02 (2)	<0.01 (2)
49	D	<0.01 (2)	<0.02 (2)	<0.01 (2)
52	D	<0.01 (1)	<0.02 (1)	<0.01 (1)
54	D	0.01 (1)	<0.02 (1)	<0.01 (1)
56	A	---	---	---
56	B	---	---	---
56	D	<0.01 (1)	<0.02 (1)	<0.01 (1)

The number of animals included is given in parentheses.

Table 6.4.2-5:Summary of mean results for processed milk samples*

Compound	Group A (Control)	Group B	Group C	Group D
	Residues in pasteurized milk [mg/kg] (mean of two sample sets)			
Dimethomorph	<0.01	<0.01	<0.01	<0.01
M550F006 + M550F007	<0.02	<0.02	<0.02	<0.02
M550F008	<0.01	<0.01	<0.01	<0.01
	Residues in skimmed milk [mg/kg] (mean of two sample sets)			
Dimethomorph	<0.01	<0.01	<0.01	<0.01
M550F006 + M550F007	<0.02	<0.02	<0.02	<0.02
M550F008	<0.01	<0.01	<0.01	<0.01
	Residues in acid whey [mg/kg] (mean of two sample sets)			
Dimethomorph	<0.01	<0.01	<0.01	<0.01
M550F006 + M550F007	<0.02	<0.02	<0.02	<0.02
M550F008	<0.01	<0.01	<0.01	<0.01
	Residues in cream [mg/kg] (mean of two sample sets)			
Dimethomorph	<0.01	<0.01	<0.01	0.01
M550F006 + M550F007	<0.02	<0.02	<0.02	<0.02
M550F008	<0.01	<0.01	<0.01	<0.01

* Milk was taken on day 28. Milk from each cow was pooled (after individual sampling) within treatment groups A - D and mixed thoroughly. The four combined samples thus produced were submitted for processing to pasteurized milk, skimmed milk, acid whey and cream. Two parallel sets of group samples of processed milk were analyzed for dimethomorph (CME 151) and relevant metabolites (M550F006, M550F007 and M550F008). Both measurements obtained the same results.

Residues in tissues

The group mean values (and in parentheses the highest individual residues) of each treatment group are summarized in Table 6.4.2-6 and Table 6.4.2-7.
No residues at all of M550F006 could be detected in any sample analyzed. Also, no residues of dimethomorph and M550F007 were detected in any tissue sample of the control group.

Table 6.4.2-6: Summary of dimethomorph residues in tissues

Treatment group	Individual and group mean residues of dimethomorph (sum of E + Z – isomers) in mg/kg*				
	Skeletal muscle	Subcutaneous fat	Peritoneal fat	Kidney	Liver
A (control)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)
B (1x)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)
C (3x)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)
D (10x) animals 10-12	<0.01 (3)	<0.01 <0.01 0.03 Av. 0.02	<0.01 <0.01 0.04 Av. 0.02	<0.01 (3)	0.01 0.01 0.05 Av. 0.02
D (10x) animals 13-15 (7, 14, and 21 days withdrawal)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01

n.d. Not detected
* For calculating the mean value, residues below the LOQ (<0.01 mg/kg) were assigned a value at the LOQ
Some samples were measured using GC-NPD and GC-MSD. In case values differed, the higher value was used for calculating the mean value

Table 6.4.2-7:Summary of residues of M550F007 (Z69) in tissues

Treatment group	Individual and group mean residues of M550F007 (sum of E + Z – isomers) in mg/kg*				
	Skeletal muscle	Subcutaneous fat	Peritoneal fat	Kidney	Liver
A (control)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)
B (1x)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 0.01 0.02 Av. 0.01	<0.01 <0.01 0.02 Av. 0.01
C (3x)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 <0.01 0.02 Av. 0.01	0.02 0.01 0.02 Av. 0.02
D (10x) animals 10-12	<0.01 (3)	<0.01 (3)	0.01 0.01 0.01 Av. 0.01	0.05 0.07 0.14 Av. 0.09	0.06 0.07 0.15 Av. 0.09
D (10x) animals 13-15 (7, 14, and 21 days withdrawal)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)

n.d. Not detected
* For calculating the mean value, residues below the LOQ (<0.01 mg/kg) were assigned a value at the LOQ
Some samples were measured using GC-NPD and GC-MSD. In case values differed, the higher value was used for calculating the mean value

In samples from the lowest dose group (B, 50 mg/animal), no residues of parent dimethomorph were detected in any tissue. For metabolite M550F007, also no residues were found in muscle and fat while low residues at the method LOQ were detected in liver and kidney.

Regarding the middle treatment group (C, 150 mg/animal), again no residues of dimethomorph and M550F007 were detected in any tissue sample except for low residues of M550F007 at a level of 0.02 mg/kg in liver.

The highest treatment group (D, 500 mg/animal) showed low residues of parent dimethomorph in subcutaneous and peritoneal fat and in liver (mean residues of 0.02 mg/kg). Low residues of M550F007 at 0.01 mg/kg were found in peritoneal fat and in kidney and liver somewhat higher residues of 0.09 mg/kg M550F007 were detected.

Residues were eliminated fast as demonstrated by the fact that no residues of all analytes were detected in residues of the depletion animals.

III. CONCLUSION

A cattle feeding study was carried out to determine residues of dimethomorph and metabolites M550F006 (Z67), M550F007 (Z69) and M550F008 (CUR 7117) in tissue, milk and processed milk of dairy cows. Groups of lactating dairy cows received dose levels of 50 (group B), 150 (group C) or 500 (group D) mg/animal/day for 28 or 35 days, which is equivalent to a dose of 0.095, 0.279 and 0.961 mg/kg bw/d.

Milk residues: no dose-related residues of dimethomorph or relevant metabolites (M550F006, M550F007 or M550F008) were found in any samples of raw whole milk, pasteurized milk, skimmed milk or acid whey. Very low residues of dimethomorph at the LOQ (0.01 mg/kg) were found in composite cream sample taken from the high dose group (Group D) at Day 28.

Tissue residues: no residues of M550F006 were found in any samples analyzed. Low mean residues of dimethomorph at a level of 0.02 mg/kg were detected in fat and liver samples of the highest treatment group (D). Low residues of M550F007 in the range of 0.01-0.02 mg/kg were seen in liver and kidney samples of the low (B) and middle (C) dose group while the residues in these tissues from the highest treatment group (D) were somewhat higher at 0.09 mg/kg. Residues were not detected in any of the 3 cows sacrificed after a withdrawal period of 7, 14 or 21 days showing a fast depuration of residues.

CA 6.4.3 Pigs

No feeding study in pigs is necessary since the metabolic pathways in rat and livestock are similar. Residue levels in pig tissues can thus be anticipated based on the available residue data generated in cattle.

CA 6.4.4 Fish

No metabolism study in fish is necessary because the log P_{ow} <3 and there are no registered uses (and thus no residues) of dimethomorph in typical fish feed items (as specified in the working document SANCO/11187/2013 rev. 3 of 31 January 2013). The crops in which dimethomorph products are mainly registered (grapes, strawberry, potatoes, leafy vegetables, fruiting vegetables, cucurbits, hops) are no typical fish feed items. Only potato is listed as the by-product "potato protein" with a max. contribution of 3% for carp; however, residues of dimethomorph in potatoes are typically below the LOQ (<0.05 mg/kg, EU MRL is 0.05 mg/kg). It is thus unlikely that fish will be exposed to substantial amounts of dimethomorph via the diet.
Furthermore, all available data on the residue behavior of dimethomorph in animals shows that the compound does not accumulate in tissues. Consequently, no feeding study in fish is necessary.

CA 6.5 Effects of Processing

Processing studies in grapes, strawberries, oranges and lettuce are presented in this dossier to cover the typical processing steps for the representative crops grapes (juice, wine, must, raisins), strawberry (juice, jam) and lettuce (washing).

CA 6.5.1 Nature of the residue

A high temperature hydrolysis study for dimethomoph was already reviewed during the previous Annex I process. The suitability of this study was confirmed by EFSA during peer review of the active substance (see EFSA Scientific Report (2006) 82, 1-69). Dimethomorph was found to be hydrolytically stable under the simulated processing conditions at pH 4, 5 and 6 at 90°C, 100°C and 120 °C, respectively.

An OECD summary of the study is provided in the following:

Report:	CA 6.5.1/1 Afzal J., 2002 a BAS 550 F (Dimethomorph): Effects of processing on the nature of the residues due to hydrolysis DK-790-062
Guidelines:	EEC 96/68, EEC 91/414
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

BAS 550 F was not degraded neither during the simulation of pasteurisation (pH 4, 90°C) nor during the simulation of baking, boiling, brewing (pH 5, 100°C) or during sterilisation (pH 6, 120°C). Because no degradation occurred, no degradation products were observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

Internal code:	BAS 550 F, dimethomorph
Reg.No.:	247723
Chem. name:	(E,Z) 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl) acryloylimorpholine
Molar mass:	387.9 g/mol
Label:	p-chlorophenyl-U-^{14}C BAS 550 F (uniformyl labeled at p-chlorophenyl ring)
Batch-No.:	AC 12694-5A
Specific act.:	41.3 Ci/mg
Radiochem. Purity:	98.7%

2. Test system

The hydrolysis of dimethomorph, (E,Z) 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl) acryloyl]morpholine was conducted using p-chlorophenyl-U-^{14}C dimethomorph. The test substance had a radiochemical purity of 98.7% and a specific activity of 41.3 µCi/mg. The experiments were carried out in a closed system under the simulated processing conditions specified below. The test system consisting of a series of solutions of the test substance at pH 4, 5, and 6 was fortified at the nominal rate of 10 ppm. The temperatures were maintained at 90±2°C, 100±2°C, and 120±3°C, respectively, during the course of the experiments.

B. STUDY DESIGN

pH 4 and 90°C - pasteurisation

The test solutions were pasteurised for 20 min at 90°C. To avoid an influence of light, the glassware was wrapped.

pH 5 and 100°C - baking, brewing, boiling

The test solutions were treated at 100°C for 60 min. To avoid an influence of light, the glassware was wrapped.

pH 6 and 120°C - sterilisation

The test solutions were sterilised at about 120°C in an autoclave for 20 min.

Radioactivity was quantified by liquid scintillation counting. The duplicate samples were measured by HPLC analysis without any extraction and further clean up.

II. RESULTS & DISCUSSION

Test samples were assayed by liquid scintillation counting at 0-time (before incubation) and after incubation. The recoveries of radioactivity were determined based on the dose applied. Recoveries of radioactivity after incubation for the treated test solutions were found to be in the range of 96%-105% of the dose applied. An exaggerated nominal treatment rate of 10 ppm was chosen to facilitate characterization of significant breakdown products. Analyses of each solution at the initiation and termination of the study indicated that no significant change in pH (<0.05 pH units) had occurred in any of the solutions and that the systems were sterile during the course of the study.
The before and after incubation samples were directly amenable to HPLC analysis without extraction and further clean up. The HPLC analysis of the after incubation samples (replicates A and B) at pH 4, 5 and 6 indicated that the only significant component was the unchanged parent accounting for 98.0% - 8.8% of the applied dose.

The overall radioactivity before and after each test performance is given in Table 6.5.1-1. The HPLC analysis of the after incubation samples (replicates A and B) at pH 4, 5 and 6 indicated that the only significant component was the unchanged parent accounting for 98.0%-98.8% of the applied dose. No major loss of radioactive material occurred.

Table 6.5.1-1 Material balance before and after processing simulation tests with ^{14}C-BAS 550F

Test	pH 4, 90°C	pH 5, 100°C	pH 6, 120°C
before test [%TAR]	100	100	100
after test [% TAR]	98.00 (replicate A) 98.38 (replicate B)	98.52 (replicate A) 98.79 (replicate B)	98.31 (replicate A) 98.14 (replicate B)

TAR = total applied radioactivity

III. CONCLUSION

Dimethomorph is found to be hydrolytically stable under the simulated processing conditions at pH 4, 5 and 6 at 90°C, 100°C and 120 °C, respectively.

According to the new data requirements, for studies on the nature of residues in processing the residue definition for risk assessment for the raw commodity should be considered. In this case, residues of parent dimethomorph and its metabolites M550F002 and M550F007 have to be considered. Metabolite M550F002 is a glucoside metabolite, which most likely will only degrade to its aglycon M550F007 upon the typical conditions that are tested for simulated processing. Metabolite M550F007 is almost identical to the structure of dimethomorph, the only difference being a demethylation of the *p*-methoxygroup at the dimethoxy ring. As dimethomorph itself was found to be stable, the same behavior can be deduced for the metabolite M550F007. Also, according to the OECD Guideline 507, point 22 it is appropriate to use only the parent compound for the study if this is the major residue in crops, which is the case for dimethomorph.

Nevertheless, a high temperature hydrolysis study was conducted for metabolite M550F002, which proved to be stable under standard processing conditions. A summary of the study is given below.

Report:	CA 6.5.1/2 Wijntjes C., 2016 a [14C] Reg.No. 4581886: Simulated processing - Hydrolysis at 90°C, 100 °C and 120°C 2016/1209107
Guidelines:	OECD 507 - Nature of the residues in processed commodities - High temperature hydrolysis, EPA 860.1520, EC 1107/2009 of the European Parliament, European Commission Regulation No 283/2013 Part A Section 6
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

Internal code:	M550F002
Reg.No.:	4581886
Chem. name:	4-[(1E,Z)-1-(4-chlorophenyl)-3-(morpholin-4-yl)-3-oxoprop-1-en-1-yl]-2-methoxyphenyl D-glucopyranoside
Molar mass:	535.98 g/mol (unlabelled)
Label:	Morpholin-2,3-^{14}C
Batch-No.:	1162-1022
Specific act.:	7.16 MBq/mg
Radiochem. Purity:	99.4% (CoA), 97.4% as re-determined before use

2. Test system

The test item was suspended in sterile aqueous acetate buffer solutions at three pH values and temperatures to give an initial concentration of 0.209 to 0.238 mg/L.

B. STUDY DESIGN

The experiment was conducted using triplicate samples of 10 mL sterile buffer solution containing M550F002. The samples were incubated under the following conditions.

pH 4 and 90 ± 5°C - pasteurisation
20 minutes in closed high pressure stainless steel vessels using a water bath, representing the process of pasteurisation

pH 5 and 100 ± 5°C - baking, brewing, boiling
60 minutes in high pressure stainless steel vessels using a water bath, representing the processes of baking, brewing and boiling

pH 6 and 120 ± 5°C - sterilisation
20 minutes in closed high pressure stainless steel vessels using an autoclave, representing the process of sterilisation

The temperature of the solutions was continuously monitored and recorded at 1 min intervals for pH 4 and pH 5 experiments, and at 5 min intervals for pH 6 experiments.

The weight of each sample was determined prior to and immediately after incubation.

Before (0 min) and after incubation, an aliquot of the samples was measured by LSC to check for recovery and subjected to HPLC analysis, in order to determine the amount of M550F002 and potential hydrolysis products. TLC of selected samples was performed as secondary analytical method. The pH values of the samples were measured directly in the test vessel after sampling.

Besides, the radiochemical purity of [^{14}C]-M550F002 in the application solution was determined by HPLC prior to the first and after the final treatment.

II. RESULTS & DISCUSSION

The mean recovery of applied radioactivity (AR) was 93.6-105.8% (see Table 6.5.1-2) after incubation compared to the theoretical radioactivity before incubation. At the end of the incubation periods, M550F002 was stable after 20 minutes of incubation in buffer solution at pH 4 and 90°C, representing a mean amount of 96.4% AR. Up to four minor unknown metabolites were detected with a maximum mean value of 1.4% AR. M550F002 was stable after 60 minutes of incubation in buffer solution at pH 5 and 100°C, representing a mean amount of 103.0% AR. Up to five minor unknown metabolites were detected with a maximum mean value of 1.3% AR. M550F002 was basically stable after 20 minutes of incubation in buffer solution at pH 6 and 120°C, representing a mean amount of 89.8% AR. Up to five minor unknown metabolites were detected with a maximum mean value of 1.4% AR.
Since all seven minor unknown metabolites were detected in approximate amounts in the non-incubated samples and/or application solution, and did not significantly increase under all three incubation conditions, its appearance was ascribed to impurity of the application solution.

Table 6.5.1-2 Recovery after processing simulation tests with ^{14}C-M550F002

Process represented	Test conditions	Mean recovery %								
		Total	M550 F002	Un-known 1	Un-known 2	Un-known 3	Un-known 4	Un-known 5	Un-known 6	Un-known 7
Pasteuri-sation	pH 4, 90°C, 20 minutes	99.8 (98.6, 102.3, 98.4)	96.4 (94.7, 98.6, 96.0)	1.4 (1.4, 1.3, 1.5)	0.4 (1.1, n.d., n.d.)	1.3 (1.4, 1.3, 0.9)	0.3 (n.d., 1.0, n.d.)	-	-	-
Baking/ brewing/ boiling	pH 5, 100°C, 60 minutes	105.8 (97.0, 109.9, 110.4)	103.0 (97.0, 105.9, 106.1)	1.3 (n.d., 2.0, 2.0)	-	0.4 (n.d., n.d., 1.1)	-	0.4 (n.d., n.d., 1.2)	0.3 (n.d., 1.0, n.d.)	0.3 (n.d., 1.0, n.d.)
Sterili-sation	pH 6, 120°C, 20 minutes	93.6 (93.2, 90.4, 97.1)	89.8 (89.1, 89.2, 91.1)	1.4 (1.9, 1.2, 1.2)	-	-	-	1.0 (0.9, n.d., 2.0)	0.4 (n.d., n.d., 1.2)	1.0 (1.3, n.d., 1.6)

Values of the three single replicates are shown in parentheses
n.d. Not detected

The test item was applied as a mixture of two structural isomers at a defined *E* and *Z* isomer ratio of about 63:37. This isomeric ratio did not change under any of the test conditions, namely during incubation in buffer solutions at pH 4 (90°C), pH 5 (100°C) and pH 6 (120°C) for 20 and 60 minutes, respectively.

III. CONCLUSION

M550F002 was hydrolytically stable in sterile buffer solution at pH 4 at a temperature simulating pasteurization (90°C) after 20 minutes, at pH 5 at a temperature simulating baking/brewing/boiling (100°C) after 60 minutes, and at pH 6 at a temperature simulating sterilization (120°C) after 20 minutes. The test item did not degrade under any of these test conditions. Additionally, the ratio of structural isomers remained constant throughout incubation.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

The distribution between peel and pulp is not applicable for the representative uses strawberry, grape and lettuce crops in this dossier. However, since the processing study on orange is used for extrapolation to grape and strawberry juice the transfer factors for peel and pulp derived are shown in chapter 6.5.3, report 6.5.3/2.

CA 6.5.3 Magnitude of residues in processed commodities

Two studies investigating the residues in processed fractions of grapes have already been reviewed according to the Uniform Principles during the active substance evaluation in the EU. The studies investigating dimethomorph during wine processing indicated that residues are transferred into pomace and wine (40-60% and 16-50% of the initial residues in raw grapes, respectively). Twenty-four follow-up studies on wine processing demonstrated that the average transfer factor from grapes to wine is 0.34 and 0.23 for red and white wine, respectively (see EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph).

A study analyzing processing of grapes into raisins is presented in the following. It indicates a processing factor of 1.78 from grapes to raisins.

Furthermore, a study in oranges is included in order to address the processing step juice production and a processing study in strawberries is presented. Mean transfer factors calculated for strawberries processed into jam and canned fruits as well as for oranges processed into juice, marmalade, dried pulp and oil were all below 1, indicating that dimethomorph residues do not accumulate in strawberry and orange processed fractions destined for human consumption.

In addition, the transfer factors for dimethomorph in processed fractions of lettuce destined for human consumption (inner leaves) were below 1. The following table summarizes the relevant processing factors for the representative crops.

An additional processing study in grapes is currently ongoing and will be analyzed according to the proposed residue definition for risk assessment, providing data for dimethomorph and its metabolites M550F002 and M550F007 and additionally morpholine. The study report will be available under DocID 2017/1000589 in October 2017.

Summary of processing factors

Crop	Processed fraction	Mean PF	Median PF	Reference	Review status
Grapes	Raisins	1.78	1.78	6.5.3/1 (DK-713-031)	Submission AIR3 Dossier
	Pomace	2.4	2.6	EFSA Scientific Report (2006) 82,1-69	EU peer reviewed
	Red wine	0.36	0.31	EFSA Scientific Report (2006) 82,1-69	EU peer reviewed
	White wine	0.30	0.30	EFSA Scientific Report (2006) 82,1-69	EU peer reviewed
	Juice	<0.08	<0.08	6.5.3/2 (2012/1002401)	Extrapolation from orange processing submitted with AIR3 Dossier
Orange	Juice	<0.08	<0.08	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
	Wet pomace	1.38	1.37	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
	Dry pomace	5.64	4.87	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
	Marmalade	<0.09	<0.09	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
	Dried pulp	0.22	0.21	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
	Oil	<0.08	<0.08	6.5.3/2 (2012/1002401)	Submission AIR3 Dossier
Strawberries	Jam (cooked)	0.41	0.44	6.5.3/3 (2009/1041521)	Submission AIR3 Dossier
	Canned fruit	1.08	1.11	6.5.3/3 (2009/1041521)	Submission AIR3 Dossier
	Juice	<0.08	<0.08	6.5.3/2 (2012/1002401)	Extrapolation from orange processing submitted with AIR3 Dossier
Hops	Beer	0.002	0.002	EFSA Scientific Report (2006) 82,1-69	EU peer reviewed
Lettuce	Inner leaves	0.17	0.17	6.5.3/4 (2008/1004853)	Submission AIR3 Dossier
	Inner leaves, washed	0.09	0.09	6.5.3/4 (2008/1004853)	Submission AIR3 Dossier

Grapes

Report:	CA 6.5.3/1 Bleif J. et al., 1997 a Dimethomorph/Mancozeb (CL 336379/CL903067) 90/600 g ai/kg WP (CY 50586): At harvest residue study of Dimethomorph (CL336379) in grapes and raisins (Spain, 1996) DK-713-031
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EPA 171-4, EPA 860.1000, EPA 860.1520, IVA-Leitlinie Rueckstandsversuche Teil I (1992), BBA I 1-2, DFG Method Series for Pesticide Residue Analysis V and VIII (1991)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 55101F
Lot/Batch #:	F067295, Dimethomorph: 90 g/kg; Mancozeb: 600 g/kg
Purity:	
CAS#:	BAS 550 F (dimethomorph): 110488-70-5
Development code:	
Spiking levels:	BAS 550 F (dimethomorph): 0.02 - 2.0 mg/kg

2. Test Commodity:

Crop:	Grape
Type:	Wine grape
Variety:	Moscatel
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed commodity:	Grapes, must, stalks, pomace, dregs, young wine, wine
Sample size:	Grapes and stalks (fresh) 9.8-10.5 kg, raisins (dry) 3.4-3.9 kg

B. STUDY DESIGN

1. Test procedure

During the growing seasons 1996, two field trials were conducted in Southern Europe (Spain) to determine the residue levels of dimethomorph (BAS 550 F) in grapes and in their processed product raisins. The trials were performed with the formulation CY 50586 containing 90 g/kg BAS 550 F (dimethomorph). All trials were treated six times with a dose rate of 0.169-0.259 kg dimethomorph/ha each in spray volumes of 940 to 1150 L/ha. As an exception, during one application 750 L/ha were sprayed, only. The applications were made in both trials starting from growth stage BBCH 65 (full flowering) up to BBCH 81 (beginning of ripening). Samples of grapes were taken at growth stage BBCH 89 (berries ripe for harvest), 28 days after the last application and either frozen for later analysis or used for processing into raisins. Local commercial processing procedures were followed. For drying the fresh harvested bunches of grapes were laid on a dryer surface consisting of compacted soil, mainly slates. Here they were exposed to sunlight for a time period of 29 days. All samples were put into a freezer within 24 hours of sampling or processing.

2. Description of analytical procedures

FAMS 002-04 (Cyanamid Forschung GmbI-I) of December 15th, 1995 was used for analysis of residues in grapes. The precursor of this method, FAMS 002-02 (Shell Forschung GmbH) of 1st February 1989, which is identical to FAMS 002-04 with respect to the analytical procedure, has been validated as an independent laboratory validation (ILV) study (Mirbach and Huber, 1991). For raisins, method FAMS 076-01 was used.

Principle of method FAMS-002-02 (used for grapes)

Dimethomorph residues are extracted with acetone and then partitioned with dichloromethane. An aliquot of the extract is cleaned up using GPC followed by cleanup on a silica gel column. The purified residue is taken up in methanol for HPLC-UV determination. The method was used with the following changes of solvents for purity reasons: n-hexane for organic residue analysis (Baker no 9262), dichloromethane for organic residue analysis (Baker no 9264-54).

Principle of method FAMS-076-01 (used for raisins)

Dimethomorph residues are extracted with acetone and water and then partitioned with dichloromethane. An aliquot of the extract is cleaned up using a Florisil column followed by cleanup with GPC. The purified residue is taken up in methanol for HPLC-UV determination. The limit of quantitation is 0.02 mg/kg. Procedural recoveries for BAS 550 F obtained with the analytical series averaged at 97% in grapes, at 98% in raisins. The fortifications levels varied from 0.02 to 2.0 mg/kg.

II. RESULTS AND DISCUSSION

No residues of dimethomorph were detected in any of the untreated samples. Dimethomorph residues in treated berries were 0.09 and 0.11 mg/kg. Residues determined in the corresponding treated raisins were 0.19 and 0.16 mg/kg.

The residue levels detected in the treated specimens and processed fractions as well as the calculated transfer factors are presented in the following table:

Table 6.5.3-1: Summary of dimethomorph residues and transfer factors

Matrix	DALA	Total residue dimethomorph (mg/kg)		Transfer factors BAS 550 F		
		96-214-03 (Trial 1)	96-214-36 (Trial 2)	96-214-03 (Trial 1)	96-214-36 (Trial 2)	Mean
Grapes (berries fresh)	28	0.09	0.11	-	-	-
Raisins* (berries dry)	28	0.19	0.16	2.11	1.45	1.78

* Mean of three single values

For dimethomorph a mean transfer factor of 1.78 has been calculated, indicating a slight concentration of residues during processing from grapes to raisins.

III. CONCLUSION

For dimethomorph a mean transfer factor of 1.78 has been calculated, indicating a concentration of residues during processing from grapes to raisins.

Oranges

The following study on processing of oranges is included in this submission in order to address the processing step juice production. Since the production of juice from different crops is, in principal, comparable, the resulting processing factors in this study can also be applied for grape and strawberry juice production.

Report:	CA 6.5.3/2 Plier S., 2013 a Determination of residues of BAS 550 F (Dimethomorph) in oranges (sweet) and their processed products after two applications of BAS 550 02 F in Spain 2012/1002401
Guidelines:	BBA IV 3-3, BBA IV 3-4, OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD 509 Crop Field Trial (2009), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, EEC 7029/VI/95 rev. 5, EEC 7035/VI/95 rev. 5, Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix E
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 02 F
Lot/Batch #:	1023, BAS 550 F: 150.0 g/L nominal; 145.1 g/L analysed
CAS#:	Dimethomorph: 110488-70-5

2. Test Commodity:

Crop:	Oranges
Type:	Citrus fruits
Variety:	Navelina
Botanical name:	*Citrus x aurantium*
Crop parts(s) or processed commodity:	Juice, wet pomace, dried pomace, marmalade, peel after oil extraction, pulp, peel, dried pulp and oil

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season, a total of four field trials were conducted in order to investigate the residue behaviour of BAS 550 F (dimethomorph) in oranges (sweet) and their processed products previously treated with BAS 550 02 F. Each field trial consisted of a treated plot. Two field trials (L110357 and L110359) also included a control plot. The product (DC formulation) was foliar applied twice at an exaggerated target rate of 8.1 L product/ha (1.215 kg a.s./ha) for each application. The applications took place at 28 days and 14-15 days before harvest (DBH) with a spray volume of 3000 L/ha, respectively.
Orange specimens for analysis were sampled on the day of the last application (DALA) and 14-15 DALA. Raw Agricultural Commodities (RAC) specimens for analysis were taken from the treated specimens directly before the start of processing. The processing of oranges was conducted with orange whole fruit specimens taken at the last sampling. After processing, nine different fractions of orange products or intermediates were collected for analysis, namely juice, wet pomace, dried pomace, marmalade, peel after oil extraction, pulp, peel, dried pulp and oil.

2. Description of analytical procedures

All orange specimens and their processed products were analyzed for residues of BAS 550 F according to BASF method No 575/0 (L0013/01) which determined the analyte by means of HPLC-MS/MS. In principle, BAS 550 F was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. Partitioning was performed twice and the respective aliquots were combined. The final determination of BAS 550 F was performed with HPLC-MS/MS. The limit of quantitation for BAS 550 F was 0.01 mg/kg. The two isomer peaks were determined together.

II. RESULTS AND DISCUSSION

Orange (sweet) whole fruit specimens sampled 14-15 days after last application contained BAS 550 F (dimethomorph) between 0.058 mg/kg and 0.15 mg/kg, RAC specimens taken directly before the start of processing contained 0.079 - 0.25 mg/kg.
After processing, the portions destined for human consumption, juice (<0.01 mg/kg), marmalade (<0.01 - 0.012 mg/kg), pulp (<0.01 mg/kg) and oil (<0.01 mg/kg) contained less BAS 550 F (dimethomorph) than the RAC. The amount of BAS 550 F (dimethomorph) in the waste fraction dried pulp (0.014 - 0.048 mg/kg) was lower and of wet pomace (0.12-0.35 mg/kg), dried pomace (0.34 - 1.2 mg/kg), peel after oil extraction (0.14 - 0.78 mg/kg) and peel (0.11 - 0.53 mg/kg) was higher than in the unprocessed oranges.

The residue levels detected in the treated specimens and processed fractions as well as the calculated transfer factors are presented in the following table:

Table 6.5.3-2: Summary of total residues in process fractions and transfer factors

Portion analyzed	Residue BAS 550 F (mg/kg)					Transfer factor (TF) [1] BAS 550 F				
Trial [2)]	DALA	1*	2*	3*	4*	1	2	3	4	Mean TF
Whole fruit	0	0.34	0.31	0.32	0.73	-	-	-	-	-
Whole fruit[3]	14-15	0.12	0.13	0.058	0.15	-	-	-	-	-
Peel	14-15	0.30	0.35	0.23	0.41	-	-	-	-	-
Pulp	14-15	0.022	0.013	<0.010	0.013	-	-	-	-	-
Whole fruit, RAC[4]		0.079	0.12	0.12	0.25	-	-	-	-	-
Juice		<0.010	<0.010	<0.010	<0.010	<0.13	<0.08	<0.08	<0.04	<0.08
Wet pomace		0.14	0.12	0.16	0.35	1.77	1.00	1.33	1.40	1.38
Dried pomace		0.39	0.34	1.2	1.2	4.94	2.83	10.00	4.80	5.64
Marmalade		<0.010	0.012	<0.010	<0.010	<0.13	0.10	<0.08	<0.04	<0.09
Peel after oil extraction		0.14	0.19	0.21	0.78	1.77	1.58	1.75	3.12	2.06
Pulp		<0.010	<0.010	<0.010	<0.010	<0.13	<0.08	<0.08	<0.04	<0.08
Peel		0.11	0.18	0.18	0.53	1.39	1.50	1.50	2.12	1.63
Dried pulp		0.026	0.014	0.026	0.048	0.33	0.12	0.22	0.19	0.22
Oil		<0.010	<0.010	<0.010	<0.010	<0.13	<0.08	<0.08	<0.04	<0.08

1 Transfer factor = residue in processed fraction (PF) / residue in RAC
2 Trial 1: L110357, Trial 2: L110358, Trial 3: L110359, Trial 4: L110360
* For calculation purposes <0.01 mg/kg is set 0.01 mg/kg
3 Specimens were taken on the field
4 Specimens were taken directly before start of processing

The mean transfer factors representing the different processing steps were below 1 for juice, marmalade, pulp, dried pulp and oil. Therefore it can be concluded, that BAS 550 F is not being accumulated in these processed fractions.
The mean transfer factor of wet pomace, dried pomace, peel after oil extraction and peel (>1) indicates that the BAS 550 F residues are mainly located in the peel of the oranges.

III. CONCLUSION

The calculated mean transfer factors were below 1 for juice, marmalade, pulp and oil, indicating that dimethomorph residues do not accumulate in any of the processed fractions destined for human consumption.

Strawberries

Report:	CA 6.5.3/3 Braun D., 2009 a Determination of residues of Dimethomorph in strawberries and their processed products after one application of BAS 550 01 F in Germany 2009/1041521
Guidelines:	Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, EEC 7029/VI/95 rev. 5, BBA IV 3-3, IVA Guideline IA-II (1992)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 55001F
   Lot/Batch #: 1007, Dimethomorph: 50% nominal
   CAS#: 110488-70-5

2. **Test Commodity:**
   Crop: Strawberries
   Type: Berries and small fruit
   Variety: Elsanta
   Botanical name: *Fragaria x. ananassa*
   Crop parts(s) or processed commodity: Strawberry fruits, washed fruits, jam, canned fruit
   Sample size: 1-12 kg fruit

B. STUDY DESIGN

1. Test procedure

In 2007, the residue level of dimethomorph was determined in strawberries after processing from field trials at four representative strawberry growing areas in Germany. BAS 550 01 F, an WP formulation containing 500 g/kg dimethomorph, was foliar applied once at an exaggerated target rate of 0.375 g/plant (3.75-fold GAP) in a drench volume of 250 mL/plant. The application was made 42±1 days before harvest using a spray volume of 300 L/ha. Strawberry specimens were sampled 35 days after application. The samples were processed into washed fruits, jam and canned fruit. All intermediates, fractions and end products involved in these processes were analyzed.

2. Description of analytical procedures

Principle of the BASF analytical method No 575/0

BAS 550 F is extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of BAS 550 F is performed by HPLC-MS/MS. The two isomer peaks are determined together. The limit of quantitation for strawberries and their processed products is 0.01 mg/kg (sum of cis and trans).

II. RESULTS AND DISCUSSION

35 days after the application, residues between 0.091 and 0.25 mg/kg were found in strawberry fruit (RAC). The residues found in washed strawberries ranged between 0.11 and 0.27 mg/kg and the residues found in wash water were 0.065-0.19 mg/kg. The residues found in jam before cooking ranged between 0.037 and 0.19 mg/kg and the residues found in jam after cooking ranged between 0.034 and 0.11 mg/kg. In canned strawberries, residues were between 0.08 and 0.25 mg/kg; and in vegetable stock, residues were between 0.08 and 0.17 mg/kg. No residues of the analytes at or above the limit of quantitation were found in the untreated (control) specimens.

The residue levels determined in the treated specimens and its processed fractions as well as the calculated transfer factors are presented Table 6.5.3-3. Due to relatively high difference of residue levels between the RAC samples 1 and 3 and 2 and 4 transfer factors that are calculated on the basis of all RAC samples are not regarded as realistic. Therefore mean transfer factors were additionally calculated on the basis of only RAC samples 1 and 3 (see Table 6.5.3-3) as well as on the basis of residue contents in washed fruits (see Table 6.5.3-4). Mean transfer factors calculated according to the latter two approaches ranged from 0.34-0.94 (based on RAC samples 1 and 3) and from 0.30-0.76 (based on washed fruits) and were thus below 1 for all processed fractions.

Table 6.5.3-3: Summary of total residues in process fractions and transfer factors

Portion analysed	**Residue BAS 550 F (mg/kg)**				**Transfer factor (TF) [1] BAS 550 F**					
Trial [3]	**1**	**2***	**3**	**4***	**1**	**2**	**3**	**4**	**Mean TF**	**Mean TF[2]**
strawberry fruit (RAC)	0.14	0.092	0.25	0.091	-	-	-	-	-	-
washed fruits	0.11	0.17	0.27	0.20	0.79	1.85	1.08	2.20	1.48	**0.94**
wash water	0.13	0.070	0.19	0.065	0.76	0.76	0.76	0.71	0.79	**0.85**
jam before cooking	0.037	0.051	0.19	0.054	0.26	0.55	0.76	0.59	0.54	**0.51**
jam after cooking	0.034	0.050	0.11	0.039	0.24	0.54	0.44	0.43	0.41	**0.34**
canned fruits	0.080	0.14	0.25	0.11	0.57	1.52	1.00	1.21	1.08	**0.79**
vegetable stock	0.055	0.080	0.17	0.057	0.39	0.87	0.68	0.63	0.64	**0.54**

1) Transfer factor = residue in PF / residue in RAC
2) mean transfer factor calculated from residues in trial 1 and trial 3 only
3) Trial 1: FR 24/07/50, Trial 2: FR 24/07/70, Trial 3: FR 24/07/30, Trial 4: FR 24/07/75
* Samples (RAC) from trials 2 and 4 contained unusually low values (maybe due to analytical problems)

Table 6.5.3-4: Summary of total residues in process fractions and transfer factors based on residue levels determined in washed strawberries

Portion analysed	Residue BAS 550 F (mg/kg)				Transfer factor (TF) [1] BAS 550 F				
Trial [3]	1	2*	3	4*	1	2	3	4	Mean TF
washed fruits	0.11	0.17	0.27	0.20	-	-	-	-	-
wash water	0.13	0.070	0.19	0.065	1.18	0.41	0.70	0.32	**0.65**
jam before cooking	0.037	0.051	0.19	0.054	0.34	0.30	0.70	0.27	**0.40**
jam after cooking	0.034	0.050	0.11	0.039	0.31	0.29	0.40	0.195	**0.30**
canned fruits	0.080	0.14	0.25	0.11	0.73	0.82	0.93	0.55	**0.76**
vegetable stock	0.055	0.080	0.17	0.057	0.50	0.47	0.63	0.28	**0.47**

1) Transfer factor = residue in PF / residue in RAC
2) mean transfer factor calculated from residues in trial 1 and trial 3 only
3) Trial 1: FR 24/07/50, Trial 2: FR 24/07/70, Trial 3: FR 24/07/30, Trial 4: FR 24/07/75
* Samples (RAC) form trials 2 and 4 contained unusually low values (maybe due to analytical problems)

III. CONCLUSION

Mean transfer factors calculated on the basis of RAC samples from trial 1 and 3 as well as on the basis of washed strawberries were all below 1, indicating that dimethomorph residues do not accumulate in any strawberry processed fractions destined for human consumption.

Lettuce

Report:	CA 6.5.3/4 Klimmek S.,Gizler A., 2008 a Study on the residue behaviour of BAS 650 F and BAS 550 F in lettuce after three applications of BAS 651 00 F under field conditions in Denmark, Northern and Southern France, Germany, Greece, Italy, the Netherlands and Spain, 2007 2008/1004853
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 7 (17 March 2004)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Wissenschaft und Gesundheit, Hamburg, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 65100F
Lot/Batch #:	40013, BAS 650 F: 300 g/L nominal; BAS 550 F: 225 g/L nominal
Purity:	
CAS#:	110488-70-5
Development code:	
Spiking levels:	BAS 550 F (dimethomorph): 0.01 - 20.0 mg/kg

2. Test Commodity:

Crop:	Head lettuce
Type:	Leafy vegetables and fresh herbs
Variety:	Imagination, Einstein, Carolus, Canasta
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Head, outer leaves, inner leaves, outer leaves (washed), inner leaves (washed), wash water (inner leaves), wash water (outer leaves)
Sample size:	≥ 0.50-1 kg min. (lettuce: ≥ 12 heads)

B. STUDY DESIGN

1. Test procedure

During the growing season 2007, four field trials were conducted in Northern France, Germany, Spain and Italy to determine the residue levels of dimethomorph (BAS 550 F) in head lettuce and its processed specimens. The trials were performed with the mix-formulation BAS 651 00 F containing 300 g a.s./L BAS 650 F and 225 g a.s./L BAS 550 F (dimethomorph). The trials were treated three times with a dose rate of 0.18 kg dimethomorph/ha. The spray volume was 300 L/ha. Lettuce heads were collected 0, 7, 14 and 21 days after the last application for analysis of the raw agricultural commodity (RAC) and the samples for processing were taken at 7 DALA. During processing the lettuce heads were divided into inner and outer leaves and the leaves were washed.

2. Description of analytical procedures

Residues of dimethomorph were analyzed according to the BASF method No. 575/0. This method involved the extraction of dimethomorph residues from lettuce heads with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analyte was performed by LC-MS/MS. The limit of quantification was 0.01 mg/kg.

II. RESULTS AND DISCUSSION

The dimethomorph residues in the raw commodity range between 0.15 mg/kg and 1.46 mg/kg. In outer leaves the residues ranged from 0.27 mg/kg to 3.11 mg/kg and in inner leaves from 0.012 mg/kg to 0.14 mg/kg. The washing step decreases the residues in outer leaves to between 0.083 mg/kg and 1.45 mg/kg and in inner leaves to between <0.01mg/kg and 0.21 mg/kg.

In Table 6.5.3-5 the residues and the transfer factors are shown in detail and in Table 6.5.3-6 a mass balance is given.

Table 6.5.3-5: Summary of dimethomorph residues and transfer factors

Matrix	DALA	Residue dimethomorph / mg/kg				Transfer factor dimethomorph			
Trial		**L070028**	**L070029**	**L070034**	**L070035**	**L070028**	**L070029**	**L070034**	**L070035**
Head, RAC	7	0.28	0.15	1.46	0.59	1	1	1	1
Inner leaves		0.071	0.012	0.14	0.14	0.25	0.08	0.10	0.24
Inner leaves, washed		<0.01	<0.01	0.21	0.063	0.04	0.07	0.14	0.11
Outer leaves		0.27	0.28	3.11	1.03	0.96	1.9	2.1	1.75
Outer leaves, washed		0.083	0.11	1.45	0.39	0.30	0.73	0.99	0.66
Wash water (inner Leaves)		0.016	0.014	0.028	0.025	0.06	0.09	0.02	0.04
Wash water (outer Leaves)		0.21	0.049	0.23	0.23	0.75	0.33	0.16	0.39

$$\text{Transfer factor} = \frac{\text{residue in the processing product}}{\text{residue in RAC}}$$

Taking into account the weight of the starting material, the weight of the processed products and the analytical results, the whole amount of dimethomorph residues were calculated as follows:

Table 6.5.3-6: Mass balance of dimethomorph residues in lettuce

Matrix	L070028		L070029		L070034		L070035		Mean Rec. (%)
	BAS 550 F (mg)	Rec. (%)	BAS 550 F (mg)	Rec. (%)	BAS 550 F (mg)	Rec. (%)	BAS 550 F (mg)	Rec. (%)	
Head, RAC	0.85	100	0.72	100	2.74	100	3.08	100	100
Inner leaves	0.060	7.0	0.027	3.7	0.11	3.8	0.31	10	6.2
Outer leaves	0.53	62	0.71	99	3.50	128	3.16	103	98
Sum	0.059	69	0.74	102	3.60	132	3.47	113	104
Inner Leaves, washed	0.007	0.8	0.025	3.5	0.18	6.7	0.16	5.2	4.0
Outer Leaves, washed	0.19	22	0.35	49	1.99	73	1.60	52	49
Wash water (inner leaves)	0.032	3.7	0.070	9.7	0.015	0.5	0.50	16	7.6
Wash water (outer leaves)	0.42	49	0.49	68	0.13	4.6	4.60	149	68
Sum	0.65	76	0.94	130	2.32	85	6.86	223	128

III. CONCLUSION

The transfer factors for dimethomorph indicate higher residues on the outer leaves. Dimethomorph was not enriched in processed fractions destined for human consumption (inner leaves). The washing step resulted in a significant decrease of the residue levels of dimethomorph in the leaves.

CA 6.6 Residues in Rotational Crops

According to the soil degradation studies (evaluated during the previous EU Review of the active substance), the DT90 value of dimethomorph is expected to exceed 1 year which is higher than the trigger value of 100 days (EFSA Scientific Report, 2006). Thus, according to the European guidelines on rotational crops further investigation of residues in rotational crops was relevant.

CA 6.6.1 Metabolism in rotational crops

Two confined rotational crop studies were peer-reviewed during the last Annex I inclusion. The study DK-640-008 investigates only the residue levels, while study DK-790-028 provides also information on the nature of residues. The residue levels and the nature of residues were investigated in four different succeeding crop groups - root & tuber (carrot, radish), leafy vegetables (lettuce), cereals (wheat) and pulses & oilseeds (soybean) - after application rates of 1.7 kg a.s./ha and 4 kg as/ha. Studies were conducted with ^{14}C-chlorophenyl ring labeled dimethomorph. The studies were considered acceptable and on the basis of these results it was concluded that the metabolism of dimethomorph in rotational crops is similar to the metabolism in primary crops. This assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Reg. 396/2005, Art. 12.

The following conclusion is directly copied from the Reasoned Opinion (see EFSA Journal 2011, 9(8):2348):

> "….thus indicating that dimethomorph was taken up by the roots and that the metabolism in following crops is similar to that observed in primary crops. Consequently, the peer review concluded that for rotational crops the same residue definition as for primary crops applies (EFSA, 2006)."

In order to address the fate of the morpholine moiety during crop rotation (especially the question to what extent free morpholine occurs in follow crops), a new confined rotational crop study was performed with the test item radiolabeled in the morpholine ring.
In the following, ~~short summaries~~ full OECD summaries of the already evaluated studies are shown for reasons of convenience, along with the summary of the new study.

Schlüter H. 1990, ^{14}C-dimethomorph (CME 151) – Confined Accumulation study on rotational crops (DocID DK-640-008)

The test substance dimethomorph (BAS 550 F) labeled in the chlorophenyl ring with an E/Z ratio of 46.9/53.1, blended with unlabeled dimethomorph was used for the treatment. ^{14}C-dimethomorph was applied to sandy loam soil at an exaggerated rate of 4 kg dimethomorph/ha (2.6N compared to the maximum seasonal application rate of 1.5 kg/ha). Soil was aged for 29, 120 and 361 days prior planting of rotational crops. The treated soil was mixed with untreated soil simulating tilling. The rotational crops carrot (root & tuber group), lettuce (leafy group) and wheat (cereal group), were grown under laboratory conditions. Soil samples were taken after treatment and at harvest for analysis of the level of radioactivity (LSC) and for the nature of residue (TLC/HPLC). Crops were sampled at harvest and analyzed for the level of radioactivity (LSC) and nature of residue (TLC/HPLC). Wheat samples were also taken at earing stage (feed item).

Findings:

Residues in soil

The level of radioactivity in soil after treatment was equivalent to the expected concentration of 12 mg/kg. The radioactivity levels were 105%, 80.4% and 66% of applied radioactivity 29, 120 and 361 days after treatment, respectively. $^{14}CO_2$ was found at 7.4, 6.8 and 2.5% of applied radioactivity for the 29, 120 and 361 days aging period, respectively.

Residues in rotational crops

The extractability ranged from 67% to 83% of the total radioactivity residue (TRR) in lettuce, wheat grain, green wheat plants and wheat straw, except for carrots leaves with lower extractability. This may have been related to contamination with soil particles containing non-extractable radioactivity. The nature of residue was identified as dimethomorph and compounds more polar than dimethomorph. No quantitation was possible because of low radioactivity level. Due to limited plant material, further identification was not possible.

Table 6.6.1-1 TRR in rotational crops after different plant back intervals

Rotational crops		TRR after aging period (mg/kg FW)		
		29 days	120 days	361 days
Lettuce		0.205	0.038	0.009
Carrots	Leaves	0.143	0.069	0.055
	Peelings	0.049	0.026	0.009*
	Peeled roots	0.016	0.014	0.005*
	Total roots	0.025	0.017	0.006*
Wheat	Green plants (earing stage)	1.007	0.243	0.028
	Straw (maturity)	4.828	0.775	0.145
	Kernels (maturity)	0.057	0.062	0.014*

FW Fresh weight

* Mean data of less than twice background

Table 6.6.1-2 ERR in rotational crops after different plant back intervals

Rotational crops		ERR (%) after aging period		
		29 days	120 days	361 days
Lettuce		72.3	NA	NA
Carrots	Leaves	50.8	34.8	47
Wheat	Green plants (earing stage)	74.3	75.8	NA
	Straw (maturity)	68.1	66.6	82.7

Conclusion:

Radioactive residues declined in all sample materials (soil and crops) with increasing aging time. Dimethomorph was the only identified compound from the ERR. Therefore, it can be concluded that dimethomorph was itself taken up by the crops, which is in accordance with the tomato hydroponic study (see Chapter 6.2; DK-640-020) where dimethomorph was taken up via roots when applied in a nutrient solution.

Report:	CA 6.6.1/1 Schlueter H., 1990 b 14C-Dimethomorph (CME 151) - Confined accumulation study on rotational crops DK-640-008
Guidelines:	EPA 165-1
GLP:	yes (certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	^{14}C-dimethomorph (chlorophenyl label) Dimethomorph (unlabeled)
	Description:	Ratio of *E*/*Z*-isomers: 47:53 (labeled dimethomorph) 50:50 (unlabeled dimethomorph)
	Lot/batch #:	2271-040 (labeled dimethomorph) H 7879 (unlabeled dimethomorph)
	Purity:	Radiochemical purity: ≥98.5% (dimethomorph) Chemical purity: 99.2% (unlabeled dimethomorph) Specific activity: 1.669 MBq/g (45.12 µCi/mg (labeled dimethomorph) 82.57 - 86.95 MBq/g (application mixture)
	CAS#:	110488-70-5 (dimethomorph)
	Stability of test compound:	-

2.	**Test commodity:**	
	Crop:	Lettuce Carrots Wheat
	Type:	Leafy vegetables Root and tuber vegetables Cereals
	Variety:	Lettuce: Attraktion Carrots: Nantaise Wheat: Telpek
	Botanical name:	*Lactuca sativa* *Daucus carota* *Triticum aestivum*

Crop parts(s) or processed commodity:	Lettuce (leaves) Carrots (leaves, peelings, peeled roots, total roots) Wheat (green plants, straw and kernels)
Sample size:	Not relevant

Table 6.6.1-1: Soil physicochemical properties

Soil series	Soil type	pH	OM %	> 0.2 mm %	0.02-0.2 mm %	0.002-0.02 mm %	< 0.002 mm %	Moisture at 1/3 bar	CEC[1] cmol/kg
Moers II	Sandy loam	7.0	1.86	23.7	36.0	17.2	23.1	28.2	12.5

1 Cation exchange capacity

B. STUDY DESIGN AND METHODS

The test substance dimethomorph (BAS 550 F) labeled in the chlorophenyl ring, blended with unlabeled dimethomorph was used for the treatment. The study was performed during the period 1987 to 1990 at Shell Forschung GmbH, D-6501 Schwabenheim, Germany.

1. Test procedure

^{14}C-dimethomorph was applied to sandy loam soil at an exaggerated rate of 4 kg dimethomorph/ha (2.6N compared to the maximum seasonal application rate of 1.5 kg/ha). Soil was aged for 29, 120 and 361 days prior planting of rotational crops. The treated soil was mixed with untreated soil simulating tilling. The rotational crops carrot, lettuce and wheat were grown under laboratory conditions. Soil samples were taken after treatment and at harvest. Crops were sampled at harvest and freeze-dried. Wheat samples were also taken at earing stage (feed item).

The maintenance of the growing crops was performed in accordance with normal agricultural practice.

2. Description of analytical procedures

Samples were analyzed for the determination of the total radioactive residue (TRR) in soil and crops, and the nature of the residue in crops. Amounts of radioactivity of less than twice the background levels were considered to be below the limit of accurate measurement. The detection limit of the radio-assay was approximately 0.01 µg/g for peeled roots (high water content) and approximately. 0.05 µg/g for wheat kernels (dry sample material).

Radioanalysis: For the determination of TRR, soil and plant subsamples were combusted followed by liquid scintillation counting (LSC). Aliquot samples of each solution or extract were also counted on a LSC.

Extraction: Plant materials containing >0.05 mg/kg were extracted to determine the extractable radioactive residues (ERR). The homogenized plant material was extracted with acetone, methanol:water (4:1) and water. After determination of extractable radioactivity, extracts containing sufficient amounts of radioactivity were subjected to radio thin-layer chromatography.

Each soil sample was extracted twice with acetone, twice with methanol/water (4:1) and twice with water. For the combined extracts, the radioactivity was determined after a concentration step by liquid scintillation counting. Extracts containing sufficient amounts of radioactivity were subjected to radio thin-layer chromatography. Non-extractable radioactivity was determined by combustion of at least three aliquots of the air-dried soil samples using a sample oxidizer followed by liquid scintillation counting.

Identification of metabolites:
Unlabeled dimethomorph and putative degradation products (as reference substances) were applied to the TLC plates for reference purposes. The Rf-values for dimethomorph and the reference substances were determined. The chromatograms were evaluated using TLC linear analyzers. Results were processed by means of programmable on-line calculators.

II. RESULTS AND DISCUSSION

TRR in plant matrices

In the case of almost all sample materials, the highest amounts of radioactive residues were found in those samples originating from the soil which had been aged over a period of 29 days after treatment with dimethomorph (Table 6.6.1-2). The results also demonstrate that, in almost all sample materials, radioactive residues declined significantly with increasing periods of soil aging.

As a consequence of the decline in the radioactivity uptake occurring with increasing soil aging periods, edible parts of crops (lettuce, carrot roots, wheat kernels) grown in soils aged over a period of 120 days contained only small amounts of radioactive residues (≤0.06 mg/kg). Following an aging period of one year, only ≤0.01 mg/kg could be detected in these materials. Comparison of the residue values found in green wheat plants with those found in leaves of carrots and lettuce, shows clearly that the amount of radioactivity taken up from the soil was highest in the case of wheat. The even higher residue concentrations found in wheat straw, however, were due to the high degree of dryness of this sample material at the time of harvesting as compared to green wheat plants.

Table 6.6.1-2: TRR in rotational crops after different plant back intervals

Rotational crops		TRR after aging period (mg/kg fresh weight)		
		29 days	120 days	361 days
Lettuce		0.205	0.038	0.009
Carrots	Leaves	0.143	0.069	0.055
	Peelings	0.049	0.026	0.009*
	Peeled roots	0.016	0.014	0.005*
	Total roots	0.025	0.017	0.006*
Wheat	Green plants (earing stage)	1.007	0.243	0.028
	Straw (maturity)	4.828	0.775	0.145
	Kernels (maturity)	0.057	0.062	0.014*

* Mean data of less than twice background

TRR in soil

The level of radioactivity in soil after treatment was equivalent to the expected concentration of 12 mg/kg. The radioactivity levels were 105%, 80.4% and 66% of applied radioactivity 29, 120 and 361 days after treatment, respectively. $^{14}CO_2$ was found at 7.4, 6.8 and 2.5% of applied radioactivity for the 29, 120 and 361 days aging period, respectively.

Extraction and characterization of residues in rotational crops

The extractability ranged from 67% to 83% of the total radioactivity residue (TRR) in lettuce, wheat grain, green wheat plants and wheat straw, except for carrots leaves with lower extractability (Table 6.6.1-3). This may have been related to contamination with soil particles containing non-extractable radioactivity.

Table 6.6.1-3: ERR in rotational crops after different plant back intervals

Rotational crops		ERR after aging period					
		29 days		120 days		361 days	
		% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Lettuce		72.3	0.148	NA	NA	NA	NA
Carrots	Leaves	50.8	0.073	34.8	0.024	47	0.026
Wheat	Green plants (earing stage)	74.3	0.749	75.8	0.185	NA	NA
	Straw (maturity)	68.1	3.288	66.6	0.516	82.7	0.120

NA Not applicable

Identification and quantification of extractable residues in rotational crops

Investigation of the extractable radioactive residues resulted in the identification of dimethomorph and the characterization of compounds more polar than dimethomorph. No quantitation was possible because of low radioactivity level. Due to limited plant material, further identification was not possible.

Proposed metabolic pathway

As the parent compound was the only important extractable residue in soils from which considerable quantities of radioactive residues were taken up by rotational crops (soils aged 29 or 120 days), it can be concluded that dimethomorph itself was taken up by the plants. On being taken up by the plant, dimethomorph is metabolized to different, mainly polar, compounds.

Storage stability

The study was initiated 30 June 1987 and completed 23 November 1990. The analytical phase was completed 5 October 1990. Information on the storage stability is sufficiently addressed within the AIR 3 Dossier.

III. CONCLUSION

Radioactive residues declined in all sample materials (soil and crops) with increasing aging time. Dimethomorph was the only identified compound from the ERR. Therefore, it can be concluded that dimethomorph was itself taken up by the crops, which is in accordance with the tomato hydroponic study [see KCA 6.2.1/11 DK-640-020] where dimethomorph was taken up via roots when applied in a nutrient solution.

~~Afzal J. 1999, Dimethomorph (AC 336379) Metabolism of Carbon-14 Labeled AC336379 Using Lettuce, Radishes, Soybeans, and Wheat as Rotational Crops (DocID DK-790-028)~~

~~This study was performed to determine the level of residues from the chlorophenyl-ring-labeled ^{14}C-dimethomorph taken up from the soil by lettuce, radishes, soybean and wheat when used as rotational crops and to determine the nature of any significant residues found in these crops. The ^{14}C-labeled dimethomorph (EC formulation) was applied three times in intervals of two weeks to bare soil (sandy loam) for a maximum seasonal application of 1.7 kg a.s./ha (1.1N compared to the maximum seasonal application rate of 1.5 kg/ha). Winter wheat was planted 30, 60 and 394 days after last application (DALA), spring wheat at 181 DALA. Radishes and lettuce were planted at 30, 60, 274 and 394 DALA and soybeans were planted at 274 DALA. Crop samples were collected at mid-maturity and at harvest. Soil samples were collected from all plots at -1 DAA (pretreatment) and 0 DAA (within two hours) following the first and third applications of the test substance. Soil in the treated and control plots were sampled to a depth of 45 cm at -1 DAA and to a depth of 30 cm after the first and third applications. Samples were analyzed for the determination of the total radioactive residue (TRR) in soil and crops, and the nature of the residue in crops. Soil samples were collected and analyzed with the sole objective to ascertain the application rate of the test substance. Therefore, no effort was made to determine the nature of the residue in soil. The detection limit of the radio-assay was approximately 0.01 mg/kg.~~

~~Findings:~~
~~Wheat, soybean, lettuce and radishes showed some uptake of dimethomorph-derived residue from soil when these rotational crops were planted in sandy loam soil which had been treated three times in intervals of two weeks for a maximum seasonal application of 1.7 kg a.s./ha. Soil residues were largely confined to the 0-8 cm (0-3 inch) horizon. The total radioactive residues (TRR) in the edible parts of succeeding crops destined for human consumption were very low (radish roots, lettuce, soybean and cereal grains).~~

Table 6.6.1-6 TRR (expressed as dimethomorph equivalent), extractability and nature of the ^{14}C residue profile in crops at different planting intervals (expressed as mg/kg of TRR)

Wheat												
PBI	**30**			**60**			**181**			**394**		
Matrix	Forage	Straw	Grain	Forage	Straw	Grain	Forage	Straw	Grain	Forage	Straw	Grain
TRR	0.05	0.15	0.01	0.04	0.13	0.01	0.03	0.13	0.02	0.01	0.02	0.01
ERR	0.04	0.09	NA	0.03	0.08	NA	0.02	0.07	NA	NA	NA	NA
Dimethomorph	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA
M550F007 (Z69)	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA
M550F002 (CL411266)	0.01	0.04	NA	<0.01	0.03	NA	<0.01	<0.01	NA	NA	NA	NA
Others	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA

Lettuce				
PBI	**30**	**60**	**274**	**394**
Matrix	Whole plant	Whole plant	Whole plant	Whole plant
TRR	0.09	0.05	0.06	0.01
ERR	0.06	0.03	0.03	NA
Dimethomorph	0.01	<0.01	<0.01	NA
M550F007 (Z69)	<0.01	<0.01	<0.01	NA
M550F002 (CL411266)	<0.01	0.02	<0.01	NA
Others	<0.01	<0.01	<0.01	NA

Soybean						
PBI	**30**	**60**	**181**	**274**		
Matrix	No sample	No sample	No sample	Forage	Straw	Seed
TRR				0.05	0.05	0.03
ERR				0.03	0.02	<0.01
Dimethomorph				<0.01	<0.01	NA
M550F007 (Z69)				<0.01	<0.01	NA
M550F002 (CL411266)				<0.01	<0.01	NA
Others				<0.01	<0.01	NA

Radishes								
PBI	**30**		**60**		**274**		**394**	
Matrix	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
TRR	0.07	0.02	0.04	0.03	0.04	0.06	0.02	0.01
ERR	0.04	<0.01	0.02	0.01	0.03	0.03	0.01	NA
Dimethomorph	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
M550F007 (Z69)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
M550F002 (CL411266)	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
Others	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA

NA = Not applicable. Not further characterized because either TRR was <0.01 mg/kg or due to low extractable residue and matrix inferences. ; PBI = Plant back interval (time from last application to planting)

~~Dimethomorph was extensively metabolized into a multitude of minor components in the crops studied and accounted for approximately <0.01–0.01 mg/kg in harvested wheat straw, soybean, lettuce and radish tops and roots. The *p*-desmethyl derivative of the parent compound (M550F007, Z69) and its glucose conjugate (M550F002, CL 411266), accounted for <0.01 mg/kg and <0.01–0.04 mg/kg in all crop samples, respectively. All other components of the residue were <0.01 mg/kg. For succeeding crops, the proposed metabolic pathway involves demethylation of the methoxy groups followed by glucose conjugation.~~

~~Conclusion~~

~~There is no accumulation of dimethomorph or its degradation products in the parts of plants used for human food or animal feed consumption. In the case of root vegetables (radish), leafy vegetables (lettuce), pulses/oilseeds (soybean) and wheat straw, the concentration of parent was <0.01 mg/kg at mid-maturity and at harvest. The levels of individual metabolites present were below 0.01 mg/kg. Besides the parent compound, the *p*-desmethyl metabolite M550F007 and its glucoside M550F002 were identified.~~

~~Based on these results, the following metabolic pathway was proposed for dimethomorph in rotational crops:~~

Report:	CA 6.6.1/2 Afzal J., 1999 a Dimethomorph (AC 336379): Metabolism of carbon-14 labeled AC 336379 using lettuce, radishes, soybeans, and wheat as rotational crops DK-790-028
Guidelines:	EEC 91/414, EEC 96/68, EPA 860.1850, EPA 40 CFR 158.290
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	^{14}C-dimethomorph (chlorophenyl label) Dimethomorph (unlabeled)
	Description:	Ratio of *E*/*Z*-isomers: approx. 50:50 (labeled dimethomorph) 44:56 (unlabeled dimethomorph)
	Lot/batch #:	AC 10011-58 (labeled dimethomorph) AC 9978-68A (unlabeled dimethomorph)
	Purity:	Radiochemical purity: 98.9% (dimethomorph) Chemical purity: 98.8% (labeled dimethomorph) 97.6% (unlabeled dimethomorph) Specific activity: 44.0 µCi/mg (labeled dimethomorph) 4.49 µCi/mg (application mixture)
	CAS#:	110488-70-5 (dimethomorph)
	Stability of test compound:	-

3.	**Test commodity:**	
	Crop:	Winter wheat Spring wheat Lettuce Soya bean Radishes
	Type:	Cereals Leafy vegetables Pulses and oilseeds Root and tuber vegetables
	Variety:	Winter wheat: Coker 9803 Spring wheat: 2375 (181 DAT) Lettuce: Black Seeded Simpson Soya bean: Clifford Radish: White Icicle
	Botanical name:	*Triticum aestivum* *Lactuca sativa* *Glycine max* *Raphanus sativus* L

Crop parts(s) or processed commodity:	Wheat (straw and grain) Lettuce (whole plant) Soya bean (forage, straw and seed) Radish (tops and roots)
Sample size:	Not relevant

Table 6.6.1-4: Soil physicochemical properties

Soil series	Soil type	pH	OM %	Sand %	Silt %	Clay %	Moisture at 1/3 bar	CEC[1] cmol/kg
Norfolk A	Sandy clay loam*	5.5	0.9	61*	19*	20*	16.2	8.8

1 Cation exchange capacity
* USDA scheme

B. STUDY DESIGN AND METHODS

This study was performed to determine the level of residues from the chlorophenyl-ring-labeled ^{14}C-dimethomorph taken up from the soil by lettuce, radishes, soya bean and wheat when used as rotational crops and to determine the nature of any significant residues found in these crops.
The study was performed during the period 1996 to 1998. The field phase was conducted at American Agricultural Services, Incorporated (AASI), Lucama, North Carolina, US. The ^{14}C-analyses and determination of the nature of residues in crops was conducted at American Cyanamid Company, Agricultural Products Research Division, Clarksville Road, Princeton, NJ, US.

1. Test procedure

The ^{14}C-labeled dimethomorph (EC formulation) was applied three times in intervals of two weeks to bare soil (sandy loam) for a total application rate of 1.7 kg a.s./ha (1.1N compared to the maximum seasonal application rate of 1.5 kg/ha).

Winter wheat was planted 30, 60 and 394 days after last application (DALA), spring wheat at 181 DALA (see Table 6.6.1-5 for time intervals of planting). Radishes and lettuce were planted at 30, 60, 274 and 394 DALA and soya beans were planted at 274 DALA. Crop samples were collected at mid-maturity and at harvest. Soil samples were collected from all plots at -1 DAA (pretreatment) and 0 DAA (within two hours) following the first and third applications of the test substance.

Table 6.6.1-5: Time intervals of planting

Time interval (DALA) [1]	Plot A	Plot B
30	Winter wheat, radishes, lettuce	-
60	-	Winter wheat, radishes, lettuce
181	-	Spring wheat
274	-	Soya bean, radishes, lettuce
394	Winter wheat, radishes, lettuce	-

1 DALA: Days after application

The maintenance of the growing crops was performed in accordance with normal agricultural practice. Soil in the treated and control plots were sampled to a depth of 45 cm at -1 DAA and to a depth of 30 cm after the first and third applications.

Samples were kept frozen until shipment and maintained frozen at approximately -20°C until taken for analysis. Extracts and fractions were stored refrigerated at approximately 4°C.

2. Description of analytical procedures

Samples were analyzed for the determination of the total radioactive residue (TRR) in soil and crops, and the nature of the residue in crops. Soil samples were collected and analyzed with the sole objective to ascertain the application rate of the test substance. Therefore, no effort was made to determine the nature of the residue in soil. The detection limit of the radio-assay was approximately 0.01 mg/kg.

Radioanalysis: For the determination of TRR, soil and plant subsamples were combusted. Background values were determined from control samples taken at the same sampling intervals. The residual radioactive residues after solvent extraction (RRR, also PES1) and the final residue after solubilization were air-dried and combusted to determine the extent of unextractable radioactivity.
The supernatants from each extraction step were either counted on a LSC (liquid scintillation counting) or combusted to determine the percent extractability.

Extraction: For the determination of the extractable radioactive residues (ERR), the homogenized plant material was extracted twice with methanol:water (70:30). Thereafter, extracts were concentrated and partitioned twice with methylene chloride. The organo- and water-soluble fractions were separated and each fraction analyzed separately.

Characterization of the RRR:
The residual radioactive residues (RRR) of representative crop matrices with the highest amounts of radioactivity in the residues (radish tops and roots, lettuce, wheat straw, wheat grain, soya bean straw and soya bean seed) were subjected to sequential hydrolytic procedures with 2% HCl (PES2), pepsin (PES3), cellulase (PES4) and 6N HCl (PES5).

Identification of metabolites:
The peak assignment of BAS 550 F (AC 336379) and its metabolites M550F002 (CL-411266) and M550F007 (CL-900986) was primarily based on comparison of the retention times of the components with the ^{14}C-signals of the HPLC analyses performed with the respective reference items. In addition, the parent compound and the desmethyl metabolite M550F007 (CL-900986) were isolated from organo-soluble wheat straw extracts using HPLC fractionation and were confirmed by mass spectrometry. The identity of the glucoside M550F002 (CL-411266) was confirmed by hydrolysis with HCl to the corresponding aglycone.

II. RESULTS AND DISCUSSION

Wheat, soya bean, lettuce and radishes showed some uptake of dimethomorph-derived residue from soil when these rotational crops were planted in sandy loam soil which had been treated three times in intervals of two weeks for a maximum seasonal application of 1.7 kg a.s./ha. Soil residues were largely confined to the 0-8 cm (0-3 inch) horizon. The total radioactive residues (TRR) in the edible parts of succeeding crops destined for human consumption were very low (radish roots, lettuce, soya bean and cereal grains).

TRR in plant matrices
The TRR found in crop samples at the different planting intervals indicate that BAS 550 F-derived residues were absorbed and translocated into crops from soil (Table 6.6.1-6).
In comparison to the respective wheat forage samples, the higher residue levels found at harvest in wheat straw at 30, 60 and 181 DALA planting intervals may be attributable to dehydration.

Table 6.6.1-6: TRR (expressed as dimethomorph equivalent), extractability and nature of the ^{14}C residue profile in crops at different planting intervals (expressed as mg/kg)

Wheat												
PBI	**30**			**60**			**181**			**394**		
Matrix	Forage	Straw	Grain	Forage	Straw	Grain	Forage	Straw	Grain	Forage	Straw	Grain
TRR*	0.05	0.15	0.01	0.04	0.13	0.01	0.03	0.13	0.02	0.01	0.02	0.01
ERR	0.04	0.09	NA	0.03	0.08	NA	0.02	0.07	<0.01	NA	<0.01	NA
Dimethomorph	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA
M550F007 (Z69)	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA
M550F002 (CL411266)	0.01	0.04	NA	<0.01	0.03	NA	<0.01	<0.01	NA	NA	NA	NA
Others	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	NA	NA	NA

Lettuce				
PBI	**30**	**60**	**274**	**394**
Matrix	Whole plant	Whole plant	Whole plant	Whole plant
TRR	0.09	0.05	0.06	0.01
ERR	0.06	0.03	0.03	NA
Dimethomorph	0.01	<0.01	<0.01	NA
M550F007 (Z69)	<0.01	<0.01	<0.01	NA
M550F002 (CL411266)	<0.01	0.02	<0.01	NA
Others	<0.01	<0.01	<0.01	NA

Soya bean						
PBI	**30**	**60**	**181**	**274**		
Matrix	No sample	No sample	No sample	Forage	Straw	Seed
TRR				0.05	0.05	0.03
ERR				0.03	0.02	<0.01
Dimethomorph				<0.01	<0.01	NA
M550F007 (Z69)				<0.01	<0.01	NA
M550F002 (CL411266)				<0.01	<0.01	NA
Others				<0.01	<0.01	NA

Radishes								
PBI	**30**		**60**		**274**		**394**	
Matrix	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
TRR	0.07	0.02	0.04	0.03	0.04	0.06	0.02	0.01
ERR	0.04	<0.01	0.02	0.01	0.03	0.03	0.01	NA
Dimethomorph	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
M550F007 (Z69)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
M550F002 (CL411266)	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA
Others	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA

NA Not applicable. Not further characterized because either TRR was <0.01 mg/kg or due to low extractable residue and matrix inferences. ; PBI = Plant back interval (time from last application to planting)
* TRR combusted

TRR in soil

TRR levels found in soil were confined largely to the 0-8 cm horizon (Table 6.6.1-7). TRR values found in the 0-8 cm depth soil samples ranged from 0.29-0.45 mg/kg in plot A and from 0.35-0.45 mg/kg in plot B following the first application. Similarly, TRR found in soil depth 0-8 cm ranged from 0.86-1.34 mg/kg in plot A and from 0.41-0.84 mg/kg in plot B following the third application. TRR levels found in soil depths 8-15 cm and 15-30 cm were low and generally ranged from <0.01-0.07 mg/kg with a maximum of 0.12 mg/kg at all sampling intervals.

Table 6.6.1-7: Total radioactive residues in soil within two hours after first and third treatment with ^{14}C-dimethomorph

Soil depths [cm]	TRR determined by direct combustion [mg/kg]		
	0-8	8-15	15-30
Plot A (0 DAA, first treatment)	0.36	0.08	0.03
Plot A (0 DAA, third treatment)	1.09	0.04	0.02
Plot B (0 DAA, first treatment)	0.41	0.03	0.01
Plot B (0 DAA, third treatment)	0.65	0.04	0.01

DAA: Days after application

1. Extraction and characterization of residues in rotational crops

The extraction efficiencies from all crops (after initial extraction with methanol:water) and a comparison of the nature of the residues in crops are summarized in Table 6.6.1-6.

In wheat forage planted 30, 60, and 181 DALA, sampled at mid-maturity, the ERR was 63.4-85.0% TRR (0.02-0.04 mg/kg). Thereby ≤0.01 mg/kg remained as unextractable radioactivity. In wheat forage planted at 394 DALA, the TRR sampled at mid-maturity accounted for 0.01 mg/kg. Therefore, no further extractions were undertaken.
For wheat straw at harvest, the ERR ranged from 25.0-61.6% TRR (<0.01-0.09 mg/kg).
For wheat grain, the TRR was 0.01 mg/kg at 30, 60 and 394 DALA planting intervals. For wheat grain of the 181 DALA planting interval, the ERR accounted for 20.0% TRR (<0.01 mg/kg).

For lettuce at harvest following the 30, 60 and 274 DALA planting intervals, the ERR were in the range from 48.4-68.0% TRR (0.03-0.06 mg/kg).

For radish tops at harvest following the 30, 60, 274 and 394 DALA plantings, the ERR were in the range from 50.0-72.5% TRR (0.01-0.04 mg/kg). For radish roots at harvest following the 30, 60 and 274 DALA plantings, the ERR were in the range from 41.7-46.7% TRR (<0.01-0.03 mg/kg). Since the TRR in radish roots at harvest following the 394 DALA planting was 0.01 mg/kg, no further extractions were undertaken to characterize the nature of the residue.

For soya bean forage and soya bean straw planted 274 DALA, the ERR accounted for 60% TRR (0.03 mg/kg) and 44.0% TRR (0.02 mg/kg). For soya bean seed of the same plant back interval, the ERR accounted for <0.01 mg/kg (TRR combusted: 0.03 mg/kg).

Characterization of the RRR

Using 2% HCl, mild acid hydrolysis released approximately 23.5-44.9% TRR (<0.01-0.03 mg/kg) of the remaining TRR. The residue after 2% HCl treatment (PES2) was further subjected to pepsin digestion, where approximately 11.9-37.8% TRR (<0.01 mg/kg) was released for all matrices with the exception of soya bean seed (78.4% TRR or 0.01 mg/kg). For soya bean seed, the pepsin extract was partitioned with methylene chloride, resulting in concentrations <0.01 mg/kg for both organo- and water-soluble fractions. Low extractable residues and interfering matrix precluded further characterization.

The residue after pepsin digest (PES3) was further subjected to cellulase hydrolysis, where 19.3-33.8% TRR (<0.01 mg/kg each) was released. The residue after cellulase hydrolysis (PES4), was subjected to hydrolysis using 6N HCl, resulting in the release of approximately 38.6-79.6% TRR (<0.01-0.01 mg/kg) of the remaining TRR. The solubilizates were concentrated and partitioned with methylene chloride to determine the distribution of residues into the organo- and water-soluble fraction. The radioactive residues in the water-soluble fractions ranged from 17.3-60.7% TRR (<0.01 mg/kg each) of the remaining TRR for all matrices. Therefore, no further characterization of these fraction was performed. The radioactive residues in the organo-soluble fractions ranged from 39.3-82.7% TRR (<0.01-0.01 mg/kg) of the remaining TRR.

In summary, the acid and enzymatic hydrolytic procedures demonstrated that approximately 65.0-96.0% TRR was extractable from representative crop matrices with ≤0.01-0.01 mg/kg remaining as unextractable.

Table 6.6.1-8: Characterization of residual radioactive residues (RRR) in rotational crop matrices

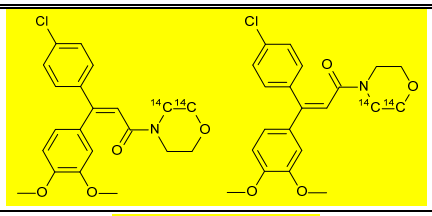
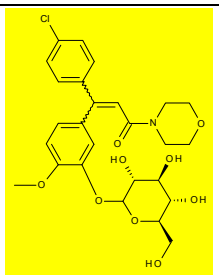
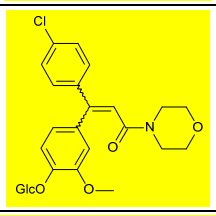
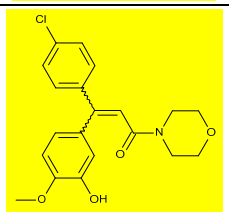
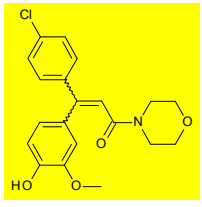
Matrix	RRR [mg/kg]	2% HCl [mg/kg]	Pepsin [mg/kg]	Cellulase [mg/kg]	6N HCl [mg/kg]	Final [mg/kg]
Plant back interval: 30 DAT						
Radish tops	0.03	0.01	<0.01	<0.01	NA	<0.01
Wheat straw	0.06	0.03	<0.01	<0.01	0.02	<0.01
Plant back interval: 60 DAT						
Wheat straw	0.05	0.02	NA	<0.01	0.01	0.010
Plant back interval: 181 DAT						
Wheat grain	0.02	<0.01	<0.01	NA	NA	<0.01
Plant back interval: 274 DAT						
Radish roots	0.04	0.01	<0.01	<0.01	0.01	<0.01
Lettuce	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
Soya bean seed	0.02	<0.01	0.01	NA	NA	<0.01
Soya bean straw	0.03	0.01	<0.01	<0.01	<0.01	<0.01
Plant back interval: 394 DAT						
Wheat straw	0.02	<0.01	NA	NA	<0.01	<0.01

NA Not applied

2. Identification and quantification of extractable residues in rotational crops

Dimethomorph was extensively metabolized into a multitude of minor components in the crops studied and accounted for approximately <0.01–0.01 mg/kg in harvested wheat straw, soya bean, lettuce and radish tops and roots. The *p*-desmethyl derivative of the parent compound (M550F007, Z69) and its glucose conjugate (M550F002, CL 411266), accounted for <0.01 mg/kg and <0.01-0.04 mg/kg in all crop samples, respectively. All other components of the residue were <0.01 mg/kg.
The identified components are summarized in **Error! Reference source not found.**.

Table 6.6.1-9: Summary of identified metabolites in rotational crops (maximal concentration for a specific matrix and plant back interval)

Metabolite	Structure	Wheat	Lettuce	Soya bean	Radishes
		[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)
Dimethomorph (BAS 550 F) Sum of isomers		<0.01 (17.5)	0.01 (11)	<0.01 (4.0)	0.01 (20)
M550F001 (CL-199322)		trace levels	trace levels	trace levels	trace levels
M550F002 (CL-411266)		0.04 (23.3)	0.02 (36)	<0.01 (<2)	0.01 (15.7)
M550F006 (CL-900987)		ND	ND	ND	trace levels
M550F007 (CL-900986)		<0.01 (3.33)	<0.01 -	<0.01 (<2)	<0.01 -

ND Not detected

3. Proposed metabolic pathway

For succeeding crops, the proposed metabolic pathway is shown in Figure 6.2.1-2:.
The main route of metabolic transformation is the demethylation of the parent compound, resulting in metabolites M550F007 (and M550F006, in trace amounts). Subsequent conjugation results in its glucoside M550F002 (and M550F001, in trace amounts).

4. Storage stability

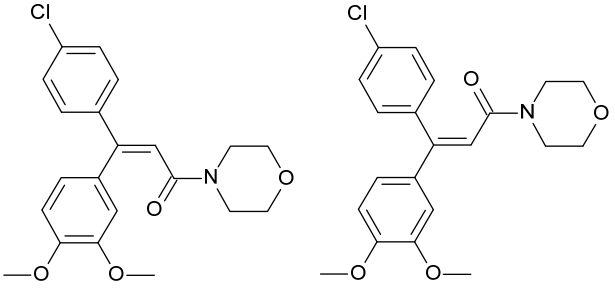
The study was initiated 17 June 1996 and completed 21 January 1999. The experimental phase was started on 26 June 1996 and completed 28 August 1998 and consequently exceeds 6 months of storage interval. However, storage stability data for parent in all types of matrices demonstrate stability of dimethomorph for at least 24 months. Since the structures of the main metabolites M550F002 and M550F007 are very similar to dimethomorph, it can be deduced that the storage stability results of parent can be extrapolated also to them.

III. CONCLUSION

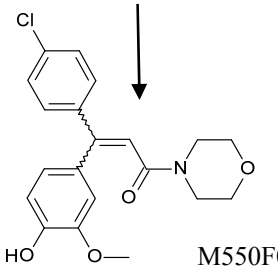
There is no accumulation of dimethomorph or its degradation products in the parts of plants used for human food or animal feed consumption. In the case of root vegetables (radish), leafy vegetables (lettuce), pulses/oilseeds (soya bean) and wheat straw, the concentration of parent was <0.01 mg/kg at mid-maturity and at harvest. The levels of individual metabolites present were below 0.01 mg/kg. Besides the parent compound, the *p*-desmethyl metabolite M550F007 and its glucoside M550F002 were identified.

Based on these results, the following metabolic pathway was proposed for dimethomorph in rotational crops:

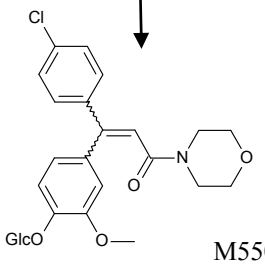
Metabolic pathway of dimethomorph in rotational crops (Chlorophenyl label study)



Dimethomorph



M550F007
(Z69, CL900986)



M550F002
(CL411266)

The following study provides information on the nature and magnitude of residues in rotational crops of dimethomorph radiolabeled in the morpholine moiety.
The main purpose of the study was to address the possible uptake and formation of free morpholine in follow crops. Thus, a special study design was chosen with only one plant back interval (30d), fast growing follow crops and harvest of immature stages.

Report:	CA 6.6.1/3 Rabe U.,Bellwon P., 2015 a Confined rotational crop study with 14C-Dimethomorph 2015/1000561
Guidelines:	EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1850: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), EPA 860.1000: EPA Residue Chemistry Test Guidelines, OECD 502 Metabolism in Rotational Crops (January 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Morpholine-2,3-^{14}C-labeled dimethomorph (BAS 550 F, Reg. No. 247723)
Dimethomorph (Reg. No. 247723)
^{14}C-morpholine (Reg. No. 4451403)
Morpholine (Reg. No. 4451403)

Description: Ratio of (E)- / (Z)- Isomers:
44:56 (labeled dimethomorph)
43.5:56.5 (unlabeled dimethomorph)

Lot/Batch #: 858-0201 (labeled dimethomorph)
AC9978-68A (unlabeled dimethomorph)
130412 (labeled morpholine)

Purity:
Radiochemical purity: 99% (dimethomorph)
99% (morpholine)
Chemical purity: 97.6% (labeled dimethomorph)
97.6% (unlabeled dimethomorph)
Specific activity: 5.57 MBq/mg (labeled dimethomorph)
55 mCi/mmol (labeled morpholine)

CAS#: 110488-70-5 (dimethomorph)
10024-89-2 (morpholine)

Stability of test compound: Not necessary since storage <6 months

3. Test Commodity:

Crop:	Lettuce Radish Wheat
Type:	Leafy vegetables Root and tuber vegetables Cereals
Variety:	Lettuce: Hardy Radish: Eiszapfen Wheat: Thasos
Botanical name:	*Lactuca sativa* *Raphanus sativus* L *Triticum aestivum*
Crop parts(s) or processed commodity:	Lettuce (leaves) Radish (leaves and roots) Wheat (forage)
Sample size:	Not reported

Table 6.6.1-10: Soil physicochemical properties

Soil series	Soil type	pH	OM[2] %	Sand %	Silt %	Clay %	Max. water holding capacity g/100 g dry soil	CEC[1] cmol/kg
Not specified	Sandy loam*	7.3**	2.8	77*	13.6*	9.4*	25.9	9.6

1 Cation exchange capacity
2 Organic matter; organic carbon 1.2%
* USDA scheme
** $CaCl_2$; pH in water 7.3

B. STUDY DESIGN AND METHODS

The present study was conducted to assess the degradation and fate of ^{14}C-morpholine labeled dimethomorph in rotational crops and to address the potential formation and uptake of free morpholine. Thus, a limited study design was chosen, involving only one plant back interval and harvest at an immature stage (harvested approximately 40 days after planting). Therefore, the study does not fulfil the complete requirements of an OECD 502 study. In detail, the following topics were addressed:

- to determine the amount of total radioactive residues of ^{14}C-dimethomorph in different matrices of lettuce, radish and wheat as rotational crops and in the soil
- to investigate the translocation of ^{14}C-dimethomorph and / or its degradation products from the soil into the plant parts after 29 days of soil aging (plant back interval)
- to investigate the occurrence of free M550F021 (morpholine) in rotated crops

The study was conducted during the period 2014 to 2015 in the BASF test facility Limburgerhof, Germany.

1. Test procedure

A confined rotational crop study was conducted with ^{14}C-morpholine labeled BAS 550 F (dimethomorph). The active substance in the DC formulation BAS 550 AB F was applied to bare soil (sandy loam) in plastic containers at a nominal application rate of 750 g a.s./ha per application using an automatic spray track system. ^{14}C-labeled dimethomorph was applied in a two stage spray application on a single day (within 30 minutes). After application, the soil was aged for 29 days (simulating an emergency plant back). After soil aging and simulated ploughing three crops were sowed, namely lettuce, radish and wheat. The maintenance of the growing crops was performed in accordance with normal agricultural practice. Soil samples were taken after ploughing. Samples not analyzed immediately were stored in a freezer set to maintain -18°C until taken for analysis.

2. Description of analytical procedures

Radioanalysis: For the determination of TRR combusted plant subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly.

Extraction: For the determination of the extractable radioactive residues (ERR), the homogenized plant material was extracted three times with methanol and two times with water. The residual radioactive residues (RRR) after solvent extraction was completely combusted and analyzed by LSC. Extracts with a sufficient level of radioactivity were analyzed by HPLC in order to identify, characterize and quantify labeled components. The total radioactive residues (TRR) were obtained by calculating the sum of ERR and RRR values (TRR calculated) and additionally by combustion of sample aliquots (TRR combusted). Quantification throughout the study was based on the TRR calculated.

Characterization: All extracts were analyzed by radio-HPLC. The residual radioactive residues (RRR) were not further investigated considering the early stage of plant development and resulting immaturity of the storage organs. Consequently, further investigation of the radioactive residue will not lead to a representative assessment of the actual situation at commercial harvest (maturity of crops). Furthermore, low absolute levels were determined for radish leaf and root.

Identification of metabolites: The peak assignment of BAS 550 F and M550F021 (morpholine) was primarily based on comparison of the retention times of the components with the ^{14}C-signals of the HPLC analyses performed with the respective reference items. The peaks for *(E)*- and *(Z)*-dimethomorph were assigned based on chromatograms obtained in another study. M550F021 (morpholine) was additionally assigned on the basis of co-chromatography experiments. All other metabolites were assigned based on retention time comparison of the ^{14}C signal from radio-HPLC and HPLC MS/MS analyses within the present study and a grape metabolism study (see chapter 6.2, report 6.2.1/1). Additionally, co-chromatography experiments were successfully performed on the purified methanol extract from wheat forage with the methanol extract from grape.

II. RESULTS AND DISCUSSION

TRR in plant matrices

For the representative leafy crop, lettuce, the calculated TRR in leaves was determined in plants sowed 29 days after soil treatment with ^{14}C-dimethomorph. The residue level accounted for 0.132 mg/kg. For the representative root crop, radish, the calculated TRR was 0.063 mg/kg and 0.014 mg/kg in leaves and roots, respectively, while the calculated TRR was 0.229 mg/kg for wheat forage, the representative cereal crop.

Table 6.6.1-11: Total radioactive residues in crops after treatment with ^{14}C-morpholine labeled dimethomorph

Matrix	TRR combusted [mg/kg]	TRR calculated[1] [mg/kg]
Plant back interval: 29 DAT (41 DAP)		
Lettuce leaf	0.141	0.132
Radish leaf	0.063	0.063
Radish root	0.013	0.014
Wheat forage	0.247	0.229

1 TRR was calculated as the sum of ERR (extraction with methanol and water) + RRR
DAT Days after treatment
DAP Days after planting

TRR in soil

For all soil samples, the TRR was determined by direct combustion analysis of subsamples. Measurements were carried out after soil aging and ploughing (29 DAT). Additionally, soil was sampled after harvest of the individual crops (70 DAT). The residue concentration in the soil accounted for 0.321 mg/kg after aging and ploughing (29 DAT) and was 0.399 mg/kg for lettuce, 0.255 mg/kg for radish and 0.249 mg/kg for wheat soil (70 DAT).

Table 6.6.1-12: Total radioactive residues in soil after treatment with ^{14}C-morpholine labeled dimethomorph

Matrix	TRR determined by direct combustion [mg/kg]
After ploughing	
29 DAT	0.321
After harvest of crops (70 DAT)	
Lettuce (41 DAP)	0.399
Radish (41 DAP)	0.255
Wheat (41 DAP)	0.249

1. Extraction and characterization of residues in rotational crops

The extractability of radioactive residues from rotational crops (plant back interval 29 DAT) is summarized in Table 6.6.1-16. The ERR accounted for at least 54.1% TRR for lettuce leaf and radish root and was equal or above 71.8% TRR for radish leaf and wheat forage. The majority was extracted with methanol for all crops (equal or above 46.6% TRR) and minor amounts were extracted with water ranging from 2.9 to 7.5% TRR. The radioactive residues after solvent extraction (RRR) accounted for 45.9% TRR (0.060 mg/kg) for lettuce leaf, 25.3% TRR (0.016 mg/kg) for radish leaf, 43.7% TRR (0.006 mg/kg) for radish root as well as 28.2% TRR (0.064 mg/kg) for wheat forage. These non-extractable radioactive residues were not further investigated considering the immature stage of plant development and consequently the missing of fully developed storage organs. Therefore, the investigation of the radioactive residue will not represent realistically the actual situation during commercial usage. Furthermore, low absolute levels were determined for radish leaf and root.

Table 6.6.1-13: Extractability of radioactive residues in rotational crop samples (morpholine label)

Matrix	TRR calculated	Methanol extract		Water extract		ERR		RRR	
	[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Lettuce leaf	0.132	0.061	46.6	0.010	7.5	0.071	54.1	0.060	45.9
Radish leaf	0.063	0.045	70.8	0.003	4.0	0.047	74.7	0.016	25.3
Radish root	0.014	0.007	52.1	0.001	4.2	0.008	56.3	0.006	43.7
Wheat forage	0.229	0.158	69.0	0.007	2.9	0.164	71.8	0.064	28.2

2. Identification and quantification of extractable residues in rotational crops

The peak assignment was based on co-chromatography experiments and retention time comparison.
In all matrices, the parent compound dimethomorph and M550F002 were identified as the main peaks, except for radish root, where M550F002 was not detected. Dimethomorph accounted for up to 27.4% TRR, whereby the *(Z)*-isomer of dimethomorph was mainly present in the parent fraction. The metabolite M550F002 accounted for up to 24.6% TRR (sum of peaks at 36.2 min and 39.2 min). M550F021 (morpholine) and other metabolites (M550F007, M550F017 and M550F018) were present only in low amounts ranging from 0.6% TRR to 6.0% TRR. In general, both the *(E)*- and *(Z)*-isomer of the parent compound dimethomorph were detected, whereby the *(Z)*-isomer was predominant. For lettuce leaf, radish leaf and wheat forage, the *(E)*:*(Z)* ratio was approximately 11:89, 14:86 and 6:94, respectively. In the matrix radish root only the *(Z)*-isomer was recovered in trace amounts.

Table 6.6.1-14: Total identified and characterized radioactive residues in lettuce, radish and wheat matrices (70 DAT)

Metabolite	Crop parts							
	Lettuce leaf		Radish leaf		Radish root		Wheat forage	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residue								
Identified								
(E)-Dimethomorph	0.002	1.2	0.002	3.4	ND	ND	0.004	1.6
(Z)-Dimethomorph	0.013	9.8	0.014	21.4	0.002	11.4	0.059	25.8
M550F002 - 36.2 min	0.005	4.1	0.003	4.2	ND	ND	0.021	9.2
M550F002 - 39.2 min	0.011	8.4	0.005	8.1	ND	ND	0.035	15.4
M550F021 (morpholine)[1]	0.002	1.3	0.004	5.9	<0.001	3.0	0.008	3.3
M550F007	0.001	0.9	<0.001	1.3	<0.001	0.6	0.002	1.0
M550F017	ND	ND	0.004	6.0	ND	ND	0.004	1.8
M550F018	ND	ND	0.002	3.1	<0.001	1.4	0.002	1.0
Total identified from ERR by HPLC	**0.034**	**25.7**	**0.034**	**53.5**	**0.002**	**16.3**	**0.135**	**59.0**
Characterized								
Total characterized from ERR	**0.033**	**24.8**	**0.011**	**18.0**	**0.006**	**43.4**	**0.019**	**8.3**
Total identified and characterized from ERR	**0.066**	**50.5**	**0.045**	**71.4**	**0.008**	**59.8**	**0.154**	**67.2**
Final Residue (Residual Radioactive Residue, RRR)	**0.060**	**45.9**	**0.016**	**25.3**	**0.006**	**43.7**	**0.064**	**28.2**
Grand total	**0.127**	**96.4**	**0.061**	**96.7**	**0.014**	**103.5**	**0.218**	**95.4**

[1] Quantitation of M550F021 (morpholine) was not done with the primary HPLC system since this did not separate morpholine from a polar fraction. A confirmatory HPLC method was used to separate M550F021 (morpholine) and determine the ratio of M550F021 (morpholine) and the residual polar fraction. The individual M550F021 (morpholine) concentration for each matrix was calculated by applying the determined ratio (of each matrix) to the peak amount of the polar fraction. The remaining amount of the polar fraction was considered as characterised in this table.

ND Not detected

Table 6.6.1-15: Summary of identified metabolites in rotational crops

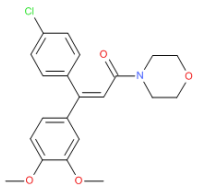
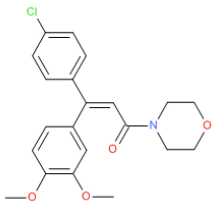
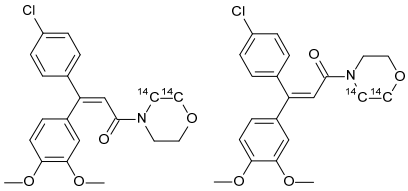
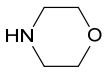
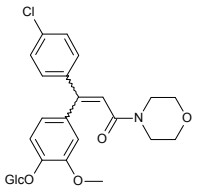
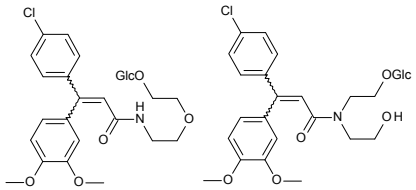
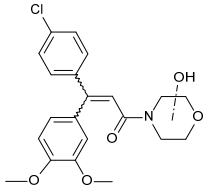
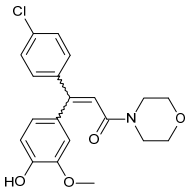
Metabolite	Structure	Lettuce leaf	Radish leaf	Radish root	Wheat forage
		[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)
(E)-Dimethomorph		0.002 (1.2)	0.002 (3.4)	ND	0.004 (1.6)
(Z)-Dimethomorph		0.013 (9.8)	0.014 (21.4)	0.002 (11.4)	0.059 (25.8)
Dimethomorph (BAS 550 F) Sum of isomers		0.015 (11.0)	0.016 (24.9)	0.002 (11.4)	0.063 (27.4)
M550F021 (Morpholine)		0.002 (1.3)	0.004 (5.9)	<0.001 (3.0)	0.008 (3.3)
M550F002 – (Sum of isomers)		0.016 (12.5)	0.008 (12.3)	ND	0.056 (24.6)
M550F017		ND	0.004 (6.0)	ND	0.004 (1.8)
M550F018		ND	0.002 (3.1)	<0.001 (1.4)	0.002 (1.0)

Table 6.6.1-15: Summary of identified metabolites in rotational crops

Metabolite	Structure	Lettuce leaf	Radish leaf	Radish root	Wheat forage
		[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)
M550F007		0.001 (0.9)	<0.001 (1.3)	<0.001 (0.6)	0.002 (1.0)

ND Not detected

3. Proposed metabolic pathway

The proposed metabolic pathway of dimethomorph in rotational crops after application to soil is shown in Figure 6.6.1-2. The main route of metabolic transformation is the demethylation of the parent compound and subsequent conjugation, resulting in metabolites M550F007 and its glucoside M550F002. Hydroxylation of the morpholine ring of the parent compound leads to the formation of isomers of the metabolite M550F018. M550F017 results from oxidative ring opening of the morpholine ring (either at the nitrogen or the oxygen leading to various constitutional isomers) followed by glycosylation. Furthermore, cleavage of the parent molecule leads to generation of M550F021 (morpholine) in low amounts.

4. Storage stability

As the study was performed within 6 months (time interval between sampling to analysis), no storage stability investigations were performed.

III. CONCLUSION

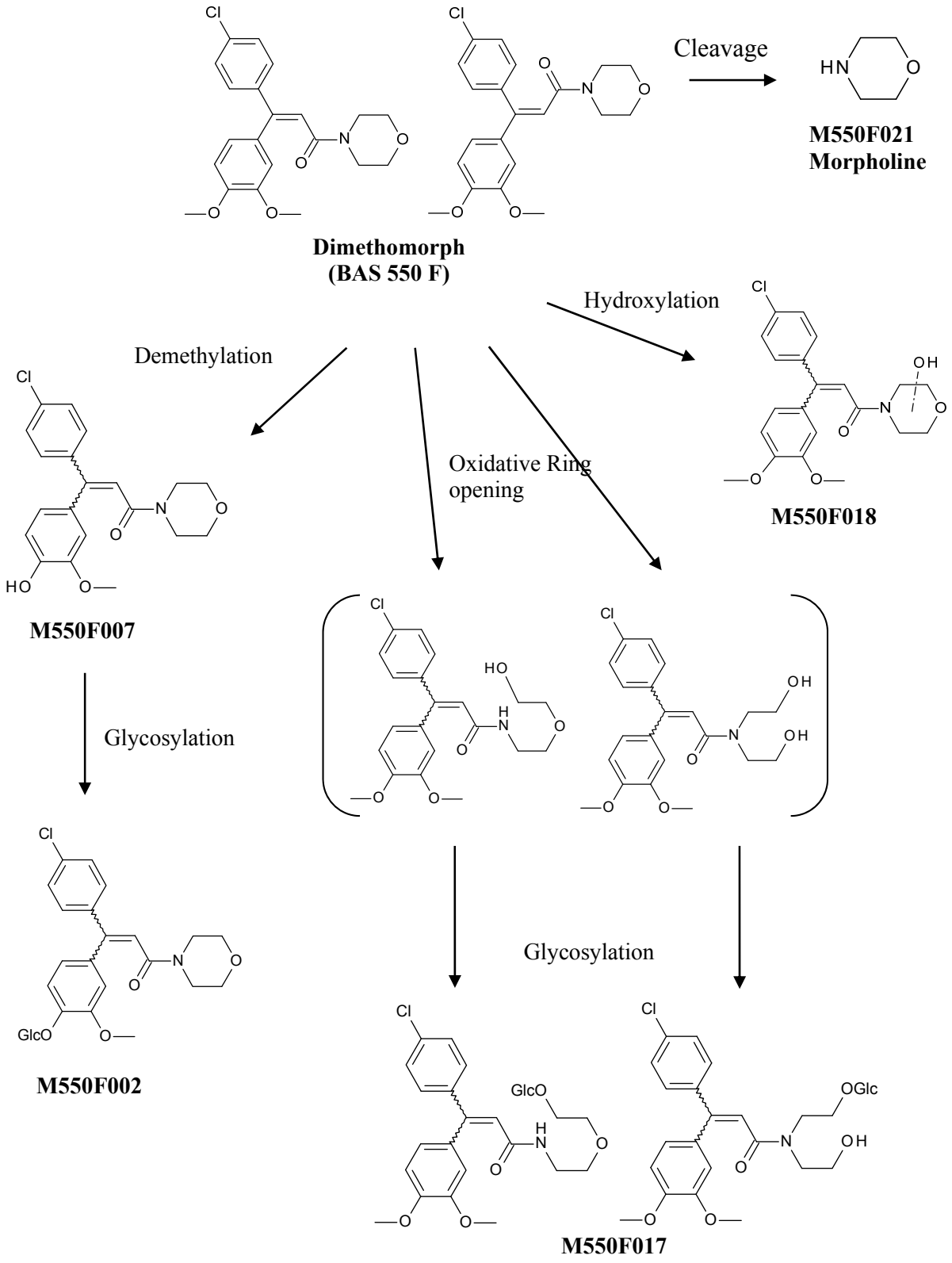
The translocation of ^{14}C-labeled dimethomorph (BAS 550 F) to confined rotational crops yielded radioactive residues concentrations of 0.132 mg/kg in lettuce leaf, 0.063 mg/kg and 0.014 mg/kg in radish leaf and root, respectively, and 0.229 mg/kg in wheat forage. The residue concentration in the soil accounted for 0.321 mg/kg after soil aging and ploughing (29 DAT) and was 0.399 mg/kg for lettuce, 0.255 mg/kg for radish and 0.249 mg/kg for wheat (70 DAT).
Most of the radioactive residues were extracted with methanol for all crops and matrices (46.6 to 70.8% TRR) and only minor amounts of residues were extracted with water (up to 7.5% TRR). The radioactive residues after solvent extraction (RRR) were 45.9% TRR (0.060 mg/kg) for lettuce leaf, 25.3% TRR (0.016 mg/kg) for radish leaf, 43.7% TRR (0.006 mg/kg) for radish root as well as 28.2% TRR (0.064 mg/kg) for wheat forage.
In all matrices, the parent compound dimethomorph and M550F002 were identified as the main peaks, except for radish root, where M550F002 was not detected. Dimethomorph accounted for up to 27.4% TRR, whereby the *(Z)*-isomer of dimethomorph was mainly present in the parent fraction. The metabolite M550F002 accounted for up to 24.6% TRR (sum of peaks at 36.2 min and 39.2 min). M550F021 (morpholine) and other metabolites (M550F007, M550F017 and M550F018) were present only in low amounts ranging from 0.6% TRR to 6.0% TRR.
The main route of metabolic transformation is the demethylation of the parent compound and subsequent conjugation, resulting in metabolites M550F007 and its glucoside M550F002. Free M550F021 (morpholine) generated through cleavage of the parent molecule is only a minor reaction.

Overall Conclusion

The new confined rotational crop study with morpholine ring labeled dimethomorph confirms and enlarges the understanding of the metabolism of dimethomorph in rotational crops as derived from the previous study with the chlorophenyl radiolabel. The major pathway in rotational crops proceeds via *p*-desmethylation and formation of metabolite M550F007 and subsequent glycosylation to result in M550F002. To a lesser extent, also hydroxylation of the morpholine ring as well as opening of the morpholine ring and subsequent glucosylation was observed. The only label specific metabolite occurring in the new study with the morpholine radiolabel was M550F021 (morpholine), the amounts of which were low (<0.001 – 0.008 mg/kg, 1.3-5.9% TRR). Besides that, all other identified metabolites are not label specific. This confirms that the nature of residues in rotational crops is validly and sufficiently described by the already peer reviewed study with the chlorophenyl label.

Taken together all available results it is concluded that the metabolism of dimethomorph in rotational crops proceeds along the same pathways as for primary crops.

Figure 6.6.1-1: Metabolic pathway of dimethomorph (BAS 550 F) in rotational crops



Putative intermediates not found; Glc: Glucose

CA 6.6.2 Magnitude of residues in rotational crops

During the last EU peer review several field studies were evaluated addressing the residue levels in the follow crops carrot, spinach and beans (DK-790-028, DK-790-010) after applications onto potatoes as primary crops.
Dimethomorph residues were generally below the LOQ (0.01 mg/kg) except in some circumstances (for instance, in dry beans or in case of early harvest of carrots or spinach, levels at 0.02 mg/kg). An individual highest residue level of 0.09 mg/kg was found in one spinach sample. The conclusion of the peer review process at Annex I inclusion was that residue levels in follow crops are generally low and are not representing a risk for the consumer. During the EU MRL review according to Art. 12 of Regulation 396/2005, EFSA concluded that the need for a plant-back restriction should be considered at national level before granting an authorization of dimethomorph. At that time a field rotational crop study was already ongoing, which could not be considered for the EU MRL review anymore. This study is available now and summarized below.

Report:	CA 6.6.2/1 Kreke N.,Grall E., 2012 a Residue of BAS 550 F on rotational crops: wheat, carrots, cauliflower, lettuce (seeded)/spinach after one application of BAS 550 01 F to bare soil, 30 and 90 days prior planting/seeding under field conditions in N.- and S.-Europe 2010/2011 2012/1182393
Guidelines:	EEC 96/68, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7524/VI/95 rev. 2 (July 22 1997)
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 550 01 F (WP)
Lot/Batch #: 41064736WO, dimethomorph 500 g/kg nominal
Purity: Not reported
CAS#: 36734-19-7
Development code:
Spiking levels: 0.01-1.0 mg/kg

2. Test Commodity:

Crop: Wheat
Carrot
Cauliflower
Lettuce
Spinach

Type: Cereals
Root and tuber vegetables
Flowering brassica
Leafy vegetables

Variety: Wheat: Chevalier, Claire, Solehio, Badiel
Carrot: Jubila, Trevor, Touchon, Belgrado F1
Cauliflower: Flamenco, Boris, Serac, Skywalker
Lettuce: Edox + Nobellan, Little Gem Pearl, Icaro
Spinach: Viroflay

Botanical name: Wheat: *Triticum aestivum*
Carrot: *Daucus carota* L. ssp. *sativus*
Cauliflower: *Brassica oleracea* var. *botrytis*
Lettuce: *Lactuca sativa*
Spinach: *Spinacia oleracea*

Crop parts: Wheat: whole plant without roots, grain, straw
Carrot: whole plant with root, top, root
Cauliflower: whole plant without roots, inflorescence
Lettuce/spinach: leaves

Sample size: Min. 0.5 kg plant material / 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2010 and 2011 growing seasons, a total of four open field rotational crop trials with wheat, carrot, cauliflower, lettuce and spinach were conducted in Northern and Southern Europe in order to determine the magnitude of residues of dimethomorph (BAS 550 F) in or on raw agricultural commodities (crop and soil).
The test item BAS 550 01 F (WP) was applied once on bare soil on one plot (30 days before sowing/planting) and another plot (90 days before sowing/planting) at a target rate of 3.0 kg/ha, corresponding to 1.5 kg a.s./ha. The application volume of spray solution was 200 L/ha. Actual rates were within 10% of these nominal rates. Two plots served as untreated control.
Each plot consisted of four subplots where wheat, carrots, cauliflower or lettuce/spinach were sowed or planted, respectively. Soil specimens were collected immediately after the application (for application verification), at sowing/planting and at harvest. Crop specimens were collected at BBCH 30-33, 65, 89 (wheat), BBCH 41 and 49 (carrots, cauliflower, lettuce/spinach).
All specimens were generally stored frozen at or below -18°C until analysis for a maximum period of about ten months (299 days) for plant material and 22 months for soil.

2. Description of analytical procedures

BASF method No L0013/01 (575/0) was used for the analysis of dimethomorph. The limit of quantitation (LOQ) was 0.01 mg/kg.
Dimethomorph was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS. The two isomer peaks were determined together.

Table 6.6.2-1: Summary of recoveries for dimethomorph (BAS 550 F)

Crop	Matrix	Fortification level (mg/kg)	Summary recoveries		
			n	Mean (%)	RSD (%)
Wheat	BASF method No L0013/01		Dimethomorph (BAS 550 F)		
	Whole plant without roots	0.01-1.0	3	104	6
	Grain	0.01-1.0	5	94	8
	Straw	0.01-1.0	5	92	4
Carrot	Whole plant with roots	0.01-1.0	5	95	4
	Tops	0.01-1.0	5	85	7
	Roots	0.01-1.0	5	90	5
Cauliflower	Whole plant without roots	0.01-1.0	7	90	3
	Inflorescences	0.01-1.0	7	81	13
Lettuce	Leaves	0.01-1.0	3	93	3
Spinach	Leaves	0.01-1.0	3	100	4

II. RESULTS AND DISCUSSION

A summary of the residues in rotational crops is shown in Table 6.6.2-2. Detailed results can be found in Table 6.6.2-4. At a replant interval of about 30 days, residues of dimethomorph in wheat whole plant without roots ranged between <0.01 and 0.026 mg/kg at BBCH 30-33 and between <0.01 and 0.040 mg/kg at BBCH 65. At harvest (BBCH 89), residues ranged between <0.01 and 0.24 mg/kg in straw. No residues above the LOQ were detected in any of the grain specimens.
In carrot whole plant with roots, residues of dimethomorph ranged from <0.01 to 0.067 mg/kg at BBCH 41. At harvest (BBCH 49), residues ranged between <0.01 and 0.024 mg/kg in tops and between <0.01 and 0.025 mg/kg in roots.
In cauliflower and lettuce or spinach, residues of dimethomorph were below the LOQ in all specimens.

With a replant interval of about 90 days, residues of dimethomorph in wheat whole plant without roots ranged between <0.01 and 0.025 mg/kg at BBCH 30-33 and between <0.01 and 0.029 mg/kg at BBCH 65. At harvest (BBCH 89), residues ranged between <0.01 and 0.23 mg/kg in straw. No residues above the LOQ were detected in any of the grain specimens.
In carrot whole plant with roots, residues of dimethomorph ranged from <0.01 to 0.052 mg/kg at BBCH 41. At harvest (BBCH 49), residues ranged between <0.01 and 0.021 mg/kg in tops and between <0.01 and 0.026 mg/kg in roots.
In cauliflower and lettuce or spinach, residues of dimethomorph were below the LOQ in all specimens.

No residues of dimethomorph above the LOQ were detected in any of the analyzed control specimens.

Table 6.6.2-2: Summary of residues in rotational crops

Crop	Portion analyzed	Growth stage (BBCH)	DAT	Residues (mg/kg)
				BAS 550 F
30±1 days replant interval				
Wheat	Whole plant without roots	30-33	113-244	<0.01-0.026
	Whole plant without roots	65	167-245	<0.01-0.040
	Grain	89	218-318	<0.01
	Straw	89	218-318	<0.01-0.24
Carrot	Whole plant with roots	41	94-132	<0.01-0.067
	Tops	49	123-171	<0.01-0.024
	Roots	49	123-171	<0.01-0.025
Cauliflower	Whole plant without roots	41	88-152	<0.01
	Inflorescence	49	101-178	<0.01
Lettuce/ spinach	Leaves	41	71-106	<0.01
	Leaves	49	86-144	<0.01
90±1 days replant interval				
Wheat	Whole plant without roots	30-33	172-292	<0.01-0.025
	Whole plant without roots	65	228-299	<0.01-0.029
	Grain	89	280-364	<0.01
	Straw	89	280-364	<0.01-0.23
Carrot	Whole plant with roots	41	154-191	<0.01-0.052
	Tops	49	183-230	<0.01-0.021
	Roots	49	183-230	<0.01-0.026
Cauliflower	Whole plant without roots	41	150-212	<0.01
	Inflorescence	49	162-238	<0.01
Lettuce/ spinach	Leaves	41	142-166	<0.01
	Leaves	49	160-204	<0.01

Petri dishes filled with standard soil were placed on the soil of each subplot before application and collected after application. Based on the calculated amounts applied in kg/ha, the application rate of dimethomorph was found to be in the range of the target rate of active substance per hectare (1.5 kg a.s./ha).
In the treated soil samples, residues ranged from 0.17-0.84 mg/kg on the day of the application to <0.01-0.23 mg/kg at harvest. All subplots showed a decrease of the dimethomorph residue in soil between the application on bare soil and crop harvest.

Table 6.6.2-3: Summary of residues in soil

Timing	BAS 550 F residues (mg/kg) in soil with crop growing on subplot			
	Wheat	Carrot	Cauliflower	Lettuce/ spinach
30±1 days replant interval				
0 DALA	0.24-0.51	0.24-0.84	0.34-0.55	0.23-0.33
At sowing/planting	0.18-0.28	0.080-0.27	0.096-0.33	0.12-0.44
At harvest	0.047-0.23	0.019-0.16	0.048-0.18	0.033-0.23
90±1 days replant interval				
0 DALA	0.17-0.47	0.21-0.47	0.28-0.38	0.27-0.49
At sowing/planting	0.097-0.26	0.13-0.25	0.056-0.46	0.091-0.53
At harvest	<0.01-0.12	0.021-0.16	0.014-0.14	0.034-0.23
Overall				
0 DALA	0.17-0.84			
At sowing/planting	0.056-0.53			
At harvest	<0.01-0.23			

III. CONCLUSION

The results of the study show that no residues above the limit of quantitation of dimethomorph are taken up into edible parts of follow crops such as wheat, cauliflower, lettuce and spinach planted/seeded about 30 or 90 days after application of BAS 550 01 F to bare soil. In carrot roots, only low residues of up to 0.026 mg/kg were found.

Table 6.6.2-4: Residues in succeeding crops

Study details		Formulation, Appl. rate (kg a.s./ha)	DAA	Crop	Residues (mg/kg)	
					Matrix	BAS 550 F
Study code:	386589	BAS 550 01 F 1 x 1.5 to bare soil		**30±1 day plant back interval**		
DocID:	2012/1182393		244	Wheat	Whole plant without roots	<0.01
GLP:	Yes		245		Whole plant without roots	<0.01
Year:	2010/2011		318		Grain	<0.01
Trial:	L100443		318		Straw	<0.01
Country:	Germany		96	Carrot	Whole plant with roots	<0.01
			126		Tops	<0.01
			126		Roots	<0.01
			88	Cauliflower	Whole plant without roots	<0.01
			108		Inflorescence	<0.01
			71	Lettuce	Leaves	<0.01
			86		Leaves	<0.01
				90±1 day plant back interval		
			292	Wheat	Whole plant without roots	<0.01
			299		Whole plant without roots	<0.01
			364		Grain	<0.01
			364		Straw	<0.01
			155	Carrot	Whole plant with roots	<0.01
			209		Tops	<0.01
			209		Roots	<0.01
			154	Cauliflower	Whole plant without roots	<0.01
			171		Inflorescence	<0.01
			142	Lettuce	Leaves	<0.01
			160		Leaves	<0.01
Study code:	386589	BAS 550 01 F 1 x 1.5 to bare soil		**30±1 day plant back interval**		
DocID:	2012/1182393		127	Wheat	Whole plant without roots	0.026
GLP:	Yes		167		Whole plant without roots	0.015
Year:	2010/2011		218		Grain	<0.01
Trial:	L100444		218		Straw	0.017
Country:	United Kingdom		113	Carrot	Whole plant with roots	0.032
			146		Tops	<0.01
			146		Roots	0.017
			124	Cauliflower	Whole plant without roots	<0.01
			155		Inflorescence	<0.01
			92	Lettuce	Leaves	<0.01
			107		Leaves	<0.01
				90±2 day plant back interval		
			189	Wheat	Whole plant without roots	0.025
			229		Whole plant without roots	0.017
			280		Grain	<0.01
			280		Straw	0.016
			174	Carrot	Whole plant with roots	0.016
			206		Tops	0.013
			206		Roots	0.018
			182	Cauliflower	Whole plant without roots	<0.01
			214		Inflorescence	<0.01
			152	Lettuce	Leaves	<0.01
			167		Leaves	<0.01

Table 6.6.2-4: Residues in succeeding crops

Study details	Formulation, Appl. rate (kg a.s./ha)	DAA	Crop	Residues (mg/kg)	
				Matrix	BAS 550 F
Study code: 386589 **DocID:** 2012/1182393 **GLP:** Yes **Year:** 2010/2011 **Trial:** L100445 **Country:** France (South)	BAS 550 01 F 1 x 1.5 to bare soil			**30±1 day plant back interval**	
		172	Wheat	Whole plant without roots	<0.01
		195		Whole plant without roots	<0.01
		245		Grain	<0.01
		245		Straw	0.027
		94	Carrot	Whole plant with roots	<0.01
		123		Tops	<0.01
		123		Roots	<0.01
		89	Cauliflower	Whole plant without roots	<0.01
		101		Inflorescence	<0.01
		86	Lettuce	Leaves	<0.01
		99		Leaves	<0.01
				90±2 day plant back interval	
		233	Wheat	Whole plant without roots	<0.01
		256		Whole plant without roots	<0.01
		306		Grain	<0.01
		306		Straw	0.015
		154	Carrot	Whole plant with roots	<0.01
		183		Tops	<0.01
		183		Roots	<0.01
		150	Cauliflower	Whole plant without roots	<0.01
		162		Inflorescence	<0.01
		147	Lettuce	Leaves	<0.01
		160		Leaves	<0.01
Study code: 386589 **DocID:** 2012/1182393 **GLP:** Yes **Year:** 2010/2011 **Trial:** L100446 **Country:** Spain	BAS 550 01 F 1 x 1.5 to bare soil			**30±1 day plant back interval**	
		113	Wheat	Whole plant without roots	<0.01
		169		Whole plant without roots	0.04
		230		Grain	<0.01
		230		Straw	0.24
		132	Carrot	Whole plant with roots	0.067
		171		Tops	0.024
		171		Roots	0.025
		152	Cauliflower	Whole plant without roots	<0.01
		178		Inflorescence	<0.01
		106	Spinach	Leaves	<0.01
		144		Leaves	<0.01
				90±1 day plant back interval	
		172	Wheat	Whole plant without roots	<0.01
		228		Whole plant without roots	0.029
		289		Grain	<0.01
		289		Straw	0.23
		191	Carrot	Whole plant with roots	0.052
		230		Tops	0.021
		230		Roots	0.026
		212	Cauliflower	Whole plant without roots	<0.01
		238		Inflorescence	<0.01
		166	Spinach	Leaves	<0.01
		204		Leaves	<0.01

In order to generate information on the residue levels in rotational crops of other categories (for the purpose of MRL derivation) and also to provide residue data for the most predominant metabolites M550F002 and M550F007, an additional field rotational crop study is ~~currently ongoing and will be~~ available ~~in March 2016~~ under DocID 2015/1241720. This study ~~will~~ covers potatoes, cucurbits and legumes/pulses at a plant back interval of 30 days.

~~**Report:**~~	~~CA 6.6.2/2~~ ~~Meyer M., 2015 a~~ ~~Residue behaviour of Dimethomorph on rotational crops cucumber/zucchini, beans/peas and potato after one application of BAS 550 01 F to bare soil 30 days prior planting under field conditions in Germany, Denmark, Italy and Spain, 2015~~ ~~2015/1241720~~
~~**Guidelines:**~~	~~none~~
~~**GLP:**~~	~~no~~

Report:	CA 6.6.2/2 Meyer M.,Jansen C., 2016 a Residue behaviour of Dimethomorph on rotational crops cucumber/zucchini, beans/peas and potato after one application of BAS 550 01 F to bare soil 30 days prior planting under field conditions in Germany, Denmark, Italy and Spain, 2015 2015/1241720
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EU Regulation Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), OECD 504
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 01 F (WP)
Lot/Batch #:	FRE-001184, dimethomorph 500 g/kg nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	
Spiking levels:	0.005-1.0 mg/kg

2. Test Commodity:

Crop:	Zucchini Peas Beans Potato
Type:	Fruit Legume vegetables Legume vegetables Root and tuber vegetables
Variety:	Zucchini: Diamant F1, Brillante Peas: Bördi, Maxigold Beans: Borlotto, Fasili Potato: Saturna, Sava, Primura, Desiree
Botanical name:	Zucchini: *Cucurbita pepo* Peas: *Pisum sativum* Beans: *Phaseolus vulgaris* Potato: *Solanum tuberosum*
Crop parts:	Zucchini: fruits Peas/beans: rest of plant without roots, pod with seeds, pods without seeds, seeds Potato: tuber
Sample size:	Zucchini: min 2 kg / 12 fruits Peas/beans: rest of plant without roots min. 1 kg / 10 pieces, pods with seeds min 0.5 kg / 25 pieces, pods without seeds min 0.2 kg and seeds min 0.2 kg (BBCH 79) and 1 kg (BBCH 89) Potato: min 2 kg / 12 tubers

B. STUDY DESIGN

1. Test procedure

During the 2015 growing season, a total of four open field rotational crop trials with zucchini, peas/beans and potato were conducted in Northern and Southern Europe in order to determine the magnitude of residues of dimethomorph (BAS 550 F) in rotational crops.
Each trial consisted of one untreated control plot and one treated plot, where the test item BAS 550 01 F (WP) was applied once on bare soil (30±1 days before sowing/planting) at a target rate of 3.0 kg/ha, corresponding to 1.5 kg a.s./ha. The application volume of the spray solution was 200 L/ha. Actual rates were within 5% of these nominal rates.
Each plot consisted of three subplots, where zucchini, peas/beans or potato were sowed or planted, respectively. Soil specimens were collected immediately after the application (for application verification), at sowing/planting and at harvest. Crop specimens were collected at BBCH 49 (potato), BBCH 79 (bean/pea) and BBCH 89 (bean/pea, zucchini).
All specimens were generally stored frozen at or below 18°C until analysis for a maximum period of about seven months (217 days) for plant material, except for trial L150167 and L150170, where the temperature raised to a maximum of -11.5°C for a short term.

2. Description of analytical procedures

BASF method No L0013/02 was used for the analysis of dimethomorph, and method No L0013/03 for metabolites M550F001, M550F002, M550F006 and M550F007. The limit of quantitation (LOQ) was 0.005 mg/kg for the *E*- and *Z*-isomer of dimethomorph and 0.01 mg/kg for each metabolite.
For analysis of dimethomorph (method No L0013/02), plant matrices were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned under alkaline conditions against cyclohexane.
For analysis of the metabolites (method No L0013/03), plant matrices were extracted as described above. An aliquot of the extract was centrifuged and partitioned against dichloromethane.
The final determination was performed by HPLC-MS/MS.

Table 6.6.2-5: Summary of recoveries for dimethomorph (BAS 550 F)and its metabolites

Crop	Matrix	Fortification level (mg/kg)	Summary recoveries								
			n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0013/02 and L0013/03#			***E*-dimethomorph (BAS 550 F)**			***Z*-dimethomorph (BAS 550 F)**			**M550F001**		
Zucchini	Fruit[1]	0.005-0.5 / 0.01-1.0#	6	88.1	1.6	6	91.6	2.3	6	82.3	6.0
Peas	Rest of plant*	0.005-0.5 / 0.01-1.0#	6	79.3	6.4	6	81.2	6.9	6	81.5	14
	Pods with seeds	0.005-0.5 / 0.01-1.0#	6	82.5	4.1	6	86.8	2.4	6	78.9	3.4
	Pods without seeds	0.005-0.5 / 0.01-1.0#	6	83.1	4.8	6	87.3	2.5	6	78.1	4.4
	Seeds	0.005-0.5 / 0.01-1.0#	6	78.9	11	6	80.9	10	6	75.5	5.4
Beans	Rest of plant*	0.005-0.5 / 0.01-1.0#	6	80.8	7.7	6	86.2	5.2	6	78.7	9.4
	Pods with seeds	0.005-0.5 / 0.01-1.0#	6	99.5	8.1	6	105	8.0	6	74.3	11
	Pods without seeds	0.005-0.5 / 0.01-1.0#	6	96.1	10	6	103	9.7	6	73.3	12
	Seeds[1]	0.005-0.5 / 0.01-1.0#	6	84.7	2.5	6	89.6	2.5	6	77.9	7.0
Potato	Tuber	0.005-0.5 / 0.01-1.0#	6	83.7	6.5	6	89.4	7.5	6	77.8	6.1
BASF method No L0013/03			**M550F002**			**M550F006**			**M550F007**		
Zucchini	Fruit[1]	0.01-1.0	6	88.7	3.5	6	100	4.1	6	97.2	6.0
Peas	Rest of plant*	0.01-1.0	6	87.9	9.6	6	101	3.3	6	91.0	9.6
	Pods with seeds	0.01-1.0	6	80.8	5.6	6	98.5	3.3	6	90.0	6.0
	Pods without seeds	0.01-1.0	6	79.3	8.3	6	95.1	2.2	6	93.1	4.9
	Seeds	0.01-1.0	6	75.0	6.4	6	95.7	10	6	80.9	11
Beans	Rest of plant*	0.01-1.0	6	84.6	6.6	6	103	3.5	6	98.5	2.9
	Pods with seeds	0.01-1.0	6	81.1	9.3	6	97.1	2.8	6	91.6	8.3
	Pods without seeds	0.01-1.0	6	80.6	8.0	6	92.6	5.0	6	89.9	7.0
	Seeds[1]	0.01-1.0	6	86.7	5.0	6	109.0	1.4	6	105.0	1.9
Potato	Tuber	0.01-1.0	6	83.8	3.2	6	98.8	1.7	6	96.1	1.4

* Without roots
# For metabolite M550F001
1 With blank subtraction for the metabolites

II. RESULTS AND DISCUSSION

A summary of the residues in rotational crops is shown in Table 6.6.2-6 and Table 6.6.2-7. Detailed results can be found in Table 6.6.2-8 and Table 6.6.2-9. In general, residues of dimethomorph (isomers and metabolites) in the investigated crops were below the LOQ (0.005 mg/kg for isomers of dimethomorph and 0.010 mg/kg for metabolites of dimethomorph) at the replant interval of approximately 30 days. Exceptions were observed for the *E*- and *Z*-isomer of dimethomorph for peas (rest of plant without roots) with 0.016 mg/kg (BBCH 79) and 0.083 mg/kg (BBCH 89) and potato (tuber) with <0.010-0.018 (BBCH 49).
No residues of dimethomorph above the LOQ were detected in any of the analyzed control specimens, except for one sample of beans seeds.

Table 6.6.2-6: Summary of residues of E- and Z-dimethomorph in rotational crops after replant interval of 30/31 days

Crop	Portion analyzed	Growth stage (BBCH)	DALA[1]	Residues (mg/kg)		
				E-dimethomorph (BAS 550 F)	*Z*-dimethomorph (BAS 550 F)	Sum[2] of dimethomorph (BAS 550 F)
Zucchini	Fruit	89	64-87	<0.005	<0.005	<0.010
Peas	Rest of plant w/o roots	79	108	<0.005	0.011	0.016
		89	148	0.020	0.063	0.083
	Pods with seeds	79	108	<0.005	<0.005	<0.010
	Pods without seeds	79	108	<0.005	<0.005	<0.010
	Seeds	79	108	<0.005	<0.005	<0.010
		89	148	<0.005	<0.005	<0.010
Beans	Rest of plant w/o roots	79	105-120	<0.005	<0.005	<0.010
		89	118-147	<0.005	<0.005	<0.010
	Pods with seeds	79	105-120	<0.005	<0.005	<0.010
	Pods without seeds	79	105-120	<0.005	<0.005	<0.010
	Seeds	79	105	<0.005	<0.005	<0.010
		89	118-147	<0.005	<0.005	<0.010
Potato	Tuber	49	108-124	<0.005	<0.005-0.013	<0.010-0.018

1 Days after last application
2 For the calculation of the sum, values below the LOQ were set to the LOQ (0.005 mg/kg)

Table 6.6.2-7: Summary of residues of dimethomorph metabolites in rotational crops after replant interval of 30/31 days

Crop	Portion analyzed	Growth stage (BBCH)	DALA[1]	Residues expressed as parent equivalent (mg/kg)[2]			
				M550F001	M550F002	M550F006	M550F007
Zucchini	Fruit	89	64-87	<0.007	<0.007	<0.010	<0.010
Peas	Rest of plant w/o roots	79	108	<0.007	<0.007	<0.010	<0.010
		89	148	<0.007	<0.007	<0.010	<0.010
	Pods with seeds	79	108	<0.007	<0.007	<0.010	<0.010
	Pods without seeds	79	108	<0.007	<0.007	<0.010	<0.010
	Seeds	79	108	<0.007	<0.007	<0.010	<0.010
		89	148	<0.007	<0.007	<0.010	<0.010
Beans	Rest of plant w/o roots	79	105-120	<0.007	<0.007	<0.010	<0.010
		89	118-147	<0.007	<0.007	<0.010	<0.010
	Pods with seeds	79	105-120	<0.007	<0.007	<0.010	<0.010
	Pods without seeds	79	105-120	<0.007	<0.007	<0.010	<0.010
	Seeds	79	105	<0.007	<0.007	<0.010	<0.010
		89	118-147	<0.007	<0.007	<0.010	<0.010
Potato	Tuber	49	108-124	<0.007	<0.007	<0.010	<0.010

1 Days after last application
2 Conversion factor is 0.724 for metabolites M550F001 and M550F002 and 1.038 for metabolites M550F006 and M550F007

III. CONCLUSION

The results of the study show that no residues of dimethomorph above the limit of quantitation are taken up into edible parts of fruiting vegetables (such as zucchini) or legumes/pulses (peas/beans) when grown in crop rotation approximately 30 days after application of BAS 550 01 F to bare soil. For potato as follow crop, on very low residues of up to 0.018 mg/kg were observed.

Table 6.6.2-8: Residues of dimethomorph in succeeding crops

Study details	Formulation, Appl. rate[1] (kg a.s./ha)	DALA[2]	Crop	Residues (mg/kg)			
				Matrix	*E*-isomer	*Z*-isomer	Sum[3]
30±1 day plant back interval							
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150167 **Country:** Germany	BAS 550 01 F 1 x 1.5 to bare soil	69	Zucchini	Fruit	<0.005	<0.005	<0.010
		124	Potato	Tuber	<0.005	<0.005	<0.010
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150168 **Country:** Denmark	BAS 550 01 F 1 x 1.5 to bare soil	87	Zucchini	Fruit	<0.005	<0.005	<0.010
		108	Peas	Rest of plant w/o roots	<0.005	0.011	0.016
		148			0.020	0.063	0.083
		108		Pods with seeds	<0.005	<0.005	<0.010
		108		Pods without seeds	<0.005	<0.005	<0.010
		108		Seeds	<0.005	<0.005	<0.010
		148		Seeds	<0.005	<0.005	<0.010
		108	Potato	Tuber	<0.005	0.011	0.016
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150169 **Country:** Italy	BAS 550 01 F 1 x 1.5 to bare soil	73	Zucchini	Fruit	<0.005	<0.005	<0.010
		120	Beans	Rest of plant w/o roots	<0.005	<0.005	<0.010
		147			<0.005	<0.005	<0.010
		120		Pods with seeds	<0.005	<0.005	<0.010
		120		Pods without seeds	<0.005	<0.005	<0.010
		120		Seeds	0.011[4]	0.043[4]	0.054[4]
		147		Seeds	<0.005	<0.005	<0.010
		120	Potato	Tuber	<0.005	0.013	0.018
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150170 **Country:** Spain	BAS 550 01 F 1 x 1.5 to bare soil	64	Zucchini	Fruit	<0.005	<0.005	<0.010
		105	Beans	Rest of plant w/o roots	<0.005	<0.005	<0.010
		118			<0.005	<0.005	<0.010
		105		Pods with seeds	<0.005	<0.005	<0.010
		105		Pods without seeds	<0.005	<0.005	<0.010
		105		Seeds	<0.005	<0.005	<0.010
		118		Seeds	<0.005	<0.005	<0.010
		113	Potato	Tuber	<0.005	<0.005	<0.010

1 Actual application rates varied by 5% at most
2 Days after last application
3 Sum of *E*- and *Z*-dimethomorph. For calculation purposes, residues below the LOQ are set on the LOQ.
4 Residue results considered as not plausible since no residues were detected neither in the corresponding "pods with seeds" sample nor in the dry seeds sample collected at BBCH 89; thus the residue results were not considered for further evaluations and are not reflected in the summary tables

Table 6.6.2-9: Residues of dimethomorph metabolites in succeeding crops

Study details	Formulation, Appl. rate[1] (kg a.s./ha)	DALA[2]	Crop	Residues expressed as parent equivalent (mg/kg) [3]				
				Matrix	M550 F001	M550 F002	M550 F006	M550 F007
30±1 day plant back interval								
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150167 **Country:** Germany	BAS 550 01 F 1 x 1.5 to bare soil	69	Zucchini	Fruit	<0.007	<0.007	<0.010	<0.010
		124	Potato	Tuber	<0.007	<0.007	<0.010	<0.010
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150168 **Country:** Denmark	BAS 550 01 F 1 x 1.5 to bare soil	87	Zucchini	Fruit	<0.007	<0.007	<0.010	<0.010
		108	Peas	Rest of plant w/o roots	<0.007	<0.007	<0.010	<0.010
		148			<0.007	<0.007	<0.010	<0.010
		108		Pods with seeds	<0.007	<0.007	<0.010	<0.010
		108		Pods without seeds	<0.007	<0.007	<0.010	<0.010
		108		Seeds	<0.007	<0.007	<0.010	<0.010
		148			<0.007	<0.007	<0.010	<0.010
		108	Potato	Tuber	<0.007	<0.007	<0.010	<0.010
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150169 **Country:** Italy	BAS 550 01 F 1 x 1.5 to bare soil	73	Zucchini	Fruit	<0.007	<0.007	<0.010	<0.010
		120	Beans	Rest of plant w/o roots	<0.007	<0.007	<0.010	<0.010
		147			<0.007	<0.007	<0.010	<0.010
		120		Pods with seeds	<0.007	<0.007	<0.010	<0.010
		120		Pods without seeds	<0.007	<0.007	<0.010	<0.010
		120		Seeds	<0.007	<0.007	<0.010	<0.010
		147			<0.007	<0.007	<0.010	<0.010
		120	Potato	Tuber	<0.007	<0.007	<0.010	<0.010
Study code: 472888 **DocID:** 2015/1241720 **GLP:** Yes **Year:** 2015 **Trial:** L150170 **Country:** Spain	BAS 550 01 F 1 x 1.5 to bare soil	64	Zucchini	Fruit	<0.007	<0.007	<0.010	<0.010
		105	Beans	Rest of plant w/o roots	<0.007	<0.007	<0.010	<0.010
		118			<0.007	<0.007	<0.010	<0.010
		105		Pods with seeds	<0.007	<0.007	<0.010	<0.010
		105		Pods without seeds	<0.007	<0.007	<0.010	<0.010
		105		Seeds	<0.007	<0.007	<0.010	<0.010
		118			<0.007	<0.007	<0.010	<0.010
		113	Potato	Tuber	<0.007	<0.007	<0.010	<0.010

* Without roots
1 Actual application rates varied by 5% at most
2 Days after last application
3 Conversion factor of 0.724 (for M550F001 and M550F002) and 1.038 (for M550F006 and M550F007)

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The residue definitions currently established in the EU and supported in future are compiled in Table 6.7.1-1. A detailed justification for BASF's proposal is provided in this section and in M-CA 6.9. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently it includes considerations for all crops with established EU MRLs and is not limited to the representative uses in grapes, lettuce and strawberries.

Table 6.7.1-1: Residue definitions - dimethomorph

End-Point	**Active substance: Dimethomorph**	
	EU agreed endpoints (EFSA conclusion on the peer review of dimethomorph; EFSA Scientific Report (2006) 82, 1-69)	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Dimethomorph (sum of isomers)	Sum of parent compound dimethomorph plus M550F002 and M550F007, expressed as parent equivalents
Residue definition in plant matrices for monitoring	Dimethomorph (sum of isomers)	Dimethomorph (sum of isomers)
Conversion factors between residue definitions	Not necessary	Lettuce: no valid conversion factor can be derived from metabolism (generation of field data ongoing) Grape: 1.21 (based on metabolism study; generation of field data ongoing) Strawberry: 1.33 (based on field residue data)
Residue definition in animal matrices for risk assessment	Dimethomorph (for poultry and milk this is to be considered as a default residue definition)	Animal tissues and eggs: Sum of dimethomorph plus metabolites M550F006 and M550F007, expressed as parent equivalents Milk: sum of parent compound plus metabolite M550F008, expressed as parent equivalents
Residue definition in animal matrices for monitoring	Dimethomorph (for poultry and milk this is to be considered as a default residue definition)	Dimethomorph,(sum of isomers)
Conversion factors between residue definitions (animal)	Not necessary	Bovine liver: 4 (based on cow feeding study) Bovine kidney:4 (based on cow feeding study) Meat, fat, milk, egg, poultry tissues: no valid conversion factor can be derived since all residues <LOQ

For deriving appropriate residue definitions for monitoring and risk assessment purposes the principles described in the following document were considered:

- OECD GUIDANCE DOCUMENT ON THE DEFINITION OF RESIDUE (as revised in 2009), SERIES ON TESTING AND ASSESSMENT No. 63 and SERIES ON PESTICIDES No. 31 (ENV/JM/MONO(2009)30)
- EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07): 2799

The first document covers both aspects whereas the purpose of the PPR Scientific Opinion is limited to the residue definition for risk assessment purposes. The corresponding EU guidance document is in preparation and will be available in Q1/2016.

Plant Matrices

For proposing a suitable residue definition in plant matrices, multiple investigations were performed:

- Crop metabolism studies
- Confined rotational crop studies
- Hydrolysis studies at exaggerated temperatures simulating processing

Plant metabolism studies were carried out in three crops belonging to three different crop categories:

For fruits:	grapes
For root and tuber vegetables:	potatoes
For leafy vegetables:	lettuce

In addition, investigations on the metabolism of dimethomorph in tomato (green plant parts) have been performed in a hydroponic system in order to investigate the translocation and metabolism after uptake via roots.

The metabolic pathways of dimethomorph in plants of three different crop groups (fruits, leafy vegetables, root & tuber vegetables) show a common pattern with regard to the nature of residue. Metabolism of dimethomorph in plants is not extensive after foliar spray application. The unchanged parent is the quantitatively most predominant component of the total residue. Besides that the following metabolic steps were observed both in the plant and in the confined rotational crop studies:

5) Demethylation at the dimethoxyphenyl ring, resulting in a hydroxyl group, yielding metabolites M550F006 (Z67) and M550F007 (Z69). These metabolites undergo subsequent Phase II-reactions by being conjugated with glucose (M550F002 and M550F001).

6) Hydroxylation of the morpholine ring (M550F018)

7) Opening of the morpholine ring, followed by glucose conjugation (M550F017), and its step-wise degradation (M550F009, M550F011, M550F012)

8) Cleavage/hydrolysis (M550F003) and release of the intact morpholine ring (M550F021).

As mentioned above, the metabolic pathway in rotational crops proceeds along the same steps.

In almost all samples investigated from metabolism studies, the parent molecule forms by far the predominant residue followed by the metabolite M550F002. All other metabolites are present in lower amounts, but also not consistently in all crops or commodities.

The studies serving as a basis to describe the metabolic fate of dimethomorph after uptake via the roots (tomato hydroponic study, confined rotational crop studies) in support of the drip/drench use pattern for strawberries show the same qualitative metabolic steps as upon foliar spray application, only differing in the quantities. It seems that the metabolic steps 1 and 3 are more pronounced after root uptake, leading to higher ratios of these metabolites compared to the parent residues. In the confined rotational crop study, metabolite M550F002 was by far the most predominant of all metabolites. In the tomato hydroponic study, metabolite M550F007 was identified as the most prevalent metabolite after acid hydrolysis, showing that it is present in a conjugated form, most probably as its glucoside M550F002.

To simulate processing, a hydrolysis study was performed at exaggerated temperatures. Radiolabelled dimethomorph was incubated under the conditions considered as representative for boiling, baking, brewing, sterilization and pasteurization. It was found to be stable under all test conditions applied. Thus, from processing the only relevant residue to be considered is unchanged parent compound.

Residue definition for monitoring purposes

According to the OECD Guidance Document, the residue definition for tolerance/MRL enforcement purposes should focus on those analytes which would indicate a possible misuse of the pesticide and which can be easily detected /measured by a broad base of national laboratories (use of a multi-residue method). The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, a monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of dimethomorph and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the studies available, the following residue definition is proposed for monitoring purposes in plant commodities (including process fractions thereof) covering foliar spray and drip/drench use:

Dimethomorph, parent only (sum of isomers)

Residue definition for data generation / risk assessment purposes

The derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk needs to be considered.

In general, two factors must be addressed:

- **Potential for exposure** to the metabolite/degradate in the human diet
- **Relative toxicity** of the metabolite/degradate as compared to the parent

Metabolites/degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment. The OECD guidance document provides a first hint on how an indicative risk assessment can be performed if metabolites are not readily available as reference substances. For such cases, the document recommends to calculate metabolite/parent ratios from the metabolism studies and to apply these ratios in a second step to the parent residue level being measured during e.g. supervised field trials. The approach is described much more in detail in the EFSA Scientific Opinion 2799 (2012) which also includes the concept of the threshold of toxicological concern (TTC) as screening tool for pesticide metabolites. Main "purpose" of the TTC concept is to check whether there is negligible exposure.

In order to evaluate the metabolite exposure and to propose a suitable residue definition for risk assessment purposes, the dimethomorph metabolites found in metabolism studies were assigned to five different groups (for details see M-CA 6.9) based on similarity of structure and metabolic transformation steps:

- Group 0: Cleavage (free morpholine)
- Group 1A: Demethylation and conjugation
- Group 1B: Hydroxylation of morpholine ring
- Group 2: Morpholine ring opening and modification (opening at the oxygen atom)
- Group 3: Morpholine ring opening and modification (opening at the nitrogen atom)
- Group 4: Complete loss of morpholine ring (by degradation, free nitrogen)
- Group 5: Complete loss of morpholine ring (by cleavage, no free nitrogen)

The dietary exposure for each metabolite was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk. The assessments were limited to those crops from which a contribution to the dietary risk could be expected.

As either no or only limited residue data from field trials is available for the different metabolites all assessments are based on metabolite/parent ratios derived from metabolism studies. The relevant chronic and acute exposure assessments for plant commodities are summarized in more detail in M-CA 6.9. The acute and chronic assessments are based on suitable endpoints, either on the reference values from the parent compound where justified by structural similarity and weight of evidence approach or on metabolite specific reference values for morpholine (M550F021) and M550F003 (a justification of the selected endpoints can be found in chapter M-CA 5.8). The individual contributions of the metabolites to the dietary risk are summarized in the tables below.

Table 6.7.1-2: Contribution of dimethomorph plant metabolites to chronic dietary risk

Group	Metabolite	ADI used (mg/kg bw/d)	ADI utilization (%)
0	**M550F021**	0.05 (substance specific reference value)	0.3
1A	**M550F002**	0.05 (parent reference value)	2.2
1A	**M550F006**	0.05 (parent reference value)	0.0
1A	**M550F007**	0.05 (parent reference value)	0.7
1B	**M550F018**	0.05 (parent reference value)	0.7
2	**M550F017 (0ne isomer)**	0.05 (parent reference value)	0.7
3	**M550F017 (Other isomer)**	0.05 (parent reference value)	0.7
5	**M550F003**	0.1 (substance specific reference value)	0.0

Table 6.7.1-3: Contribution of dimethomorph plant metabolites to acute dietary risk

Group	Metabolite	ARfD used (mg/kg bw/d)	ARfD utilization (%)
0	**M550F021**	0.75 (substance specific reference value)	1.9 (celery)
1A	**M550F002**	0.6 (parent reference value)	6.1 (table grapes)
1A	**M550F006**	0.6 (parent reference value)	0.4 (celery)
1A	**M550F007**	0.6 (parent reference value)	1.1 (table grapes)
1B	**M550F018**	0.6 (parent reference value)	2.6 (celery)
2	**M550F017 (One isomer)**	0.6 (parent reference value)	7.2 (celery)
3	**M550F017 (Other isomer)**	0.6 (parent reference value)	7.2 (celery)
5	**M550F003**	No ARfD, no acute RA necessary	-

The data show that the contributions of dimethomorph metabolites in plants to the dietary risk are small under unrealistic worst case assumptions for most metabolites. The only metabolite contributing somewhat higher amounts to the dietary risk is M550F002. Since this is a glucose conjugate, which might easily be cleaved in the gastro-intestinal tract, it makes sense to also consider the exposure to its aglycon M500F007.

Following an indicative assessment based on an in-depth analysis of all metabolism, residue and processing fraction studies, the residue definition shown below is proposed for risk assessment in plant commodities (including process fractions thereof) covering foliar spray and drip/drench use:

Sum of dimethomorph and M550F002 and M550F007, expressed as parent equivalents

Animal matrices

For deriving a suitable residue definition in animal matrices, the following studies were performed:

- Metabolism studies in goats (ruminants)
- Metabolism study in hens

For both species already peer reviewed and new studies (including the radiolabel in the morpholine moiety) are available and were all considered for the proposal of a residue definition.

Generally, the metabolism of dimethomorph follows a common pathway in the two different livestock species, which is comparable to the one observed in rats. The same metabolic conversion steps as in plant matrices were observed in the relevant studies:

The metabolic pathways in goats and hens proceed along the following main routes:

5) Demethylation of the two methoxy groups of the dimethoxyphenyl ring to produce M550F006, M550F007 andM550F016. These metabolites were confirmed to be further conjugated in Phase II reactions to form the respective glucuronides M550F015, M550F013, and M550F029.

6) Hydroxylation of the morpholine ring yielding M550F018, M550F028, and M550F074/M550F076 (latter ones observed in poultry only).

7) Opening and stepwise degradation of the morpholine ring producing metabolites M550F009 (WL 376084), M550F011 (CUR 7216), M550F008 (CUR 7117), M550F028 (other isomer), M550F030, M550F031, M550F053, and M550F012 in both species. In poultry additionally M550F005 (Z43), M550F022, M550F033, M550F069, M550F038, M550F091, M550F062, M550F035, and M550F049 were observed. All these metabolites represent parent-like structures with the morpholine ring opened at either the oxygen or nitrogen atom, oxidized (hydroxyl or carboxy groups) and degraded down to only the nitrogen group, all this in combination with demethylation of the dimethoxy ring and glucuronidation.

8) Cleavage and release of the intact morpholine ring (M550F021).

In the majority of the investigated samples (goat: milk / cream / meat / fat / liver / kidney; poultry: fat / eggs), the parent molecule forms a predominant part of the residue, followed by metabolites M550F007, M550F006, M550F008 and M550F011. In milk and cream, the metabolite M550F008 (CUR 7117) occurred in higher amounts than parent. The investigations with the radiolabel in the morpholine moiety showed for most matrices a significant portion of radioactivity with high polarity, which could despite many different attempts not be identified unambiguously. The most probable hypothesis is that the morpholine moiety is degraded into C2 fragments which are then incorporated into endogenous biosynthetic pathways, producing in the end small endogenous molecules of high polarity (e.g. glycolic acid, 2-amino ethanol, oxo-acetic acid). It could be shown that free morpholine is one among several components in this polar fraction.
All other metabolites are present in significantly lower amounts, but also not consistently in all livestock commodities.

Residue definition for monitoring purposes

For the residue definition in animal commodities the same criteria apply as for plants. The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of dimethomorph and its metabolites in food of animal origin, there is only one component which meets most of the criteria listed in the OECD guidance document. Based on the studies available where unchanged dimethomorph was detected in food items being highly consumed (milk, meat, fat, eggs, liver), the following residue definition is proposed for monitoring purposes in animal commodities:

Dimethomorph, parent only (sum of isomers)

Residue definition for data generation / risk assessment purposes

As in plants, the derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk need to be considered.

In order to propose a suitable residue definition for risk assessment purposes, the dimethomorph metabolites found in the livestock metabolism studies were grouped, following the same selection principles as for plant metabolites. For metabolites that occur in both plant and animal matrices, the additional contribution via feed items was included in the assessment.

- Group 0: Cleavage (free morpholine)
- Group 1A: Demethylation and conjugation
- Group 1B: Hydroxylation of morpholine ring
- Group 2: Morpholine ring opening and modification (opening at the oxygen atom)
- Group 3: Morpholine ring opening and modification (opening at the nitrogen atom)
- Group 4: Complete loss of morpholine ring (by degradation, free nitrogen)

The dietary exposure to each metabolite was assessed separately for identifying the contributions of the livestock metabolites to the total dietary risk. For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue study in cows

Residue data from magnitude of residue studies are available for metabolite M550F006, M550F007 and M550F008. For the rest of the metabolites, residue levels were extrapolated from the metabolism studies based on the estimated maximum worst case feed burden since in some cases no reasonable metabolite/parent ratio could be derived and also since parent residue levels in the cow feeding study were always below the LOQ. The relevant chronic and acute exposure assessments for livestock commodities are summarized more in detail in section 6.9. The assessments are based on suitable endpoints, either on the reference values from the parent compound where justified by structural similarity and weight of evidence approach or on metabolite specific reference values for morpholine (M550F021) (for details refer to M-CA 5.8). The individual contributions of the livestock metabolites to the dietary risk are summarized in the tables below.

Table 6.7.1-4: Contribution of dimethomorph livestock metabolites to chronic dietary risk

Group	Metabolite	ADI used (mg/kg bw/d)	ADI utilization (%)
0	**M550F021**	0.05 (substance specific reference value)	0.3
1A	**M550F006**	0.05 (parent reference value)	1.6
1A	**M550F007**	0.05 (parent reference value)	1.6
1A	**M550F013**	0.05 (parent reference value)	0.0
1A	**M550F015**	0.05 (parent reference value)	0.0
1A	**M550F016**	0.05 (parent reference value)	0.0
1A	**M550F029**	0.05 (parent reference value)	0.0
1B	**M550F018**	0.05 (parent reference value)	0.2
1B	**M550F028 (one isomer)**	0.05 (parent reference value)	0.0
1B	**M550F074**	0.05 (parent reference value)	0.0
1B	**M550F076**	0.05 (parent reference value)	0.0
2	**M550F009**	0.05 (parent reference value)	0.0
2	**M550F028 (Other isomer)**	0.05 (parent reference value)	0.0
2	**M550F035**	0.05 (parent reference value)	0.0
2	**M550F053**	0.05 (parent reference value)	0.2
3	**M550F008**	0.05 (parent reference value)	0.8
3	**M550F011**	0.05 (parent reference value)	0.4
3	**M550F022**	0.05 (parent reference value)	0.0
3	**M550F030**	0.05 (parent reference value)	0.0
3	**M550F031**	0.05 (parent reference value)	0.4
3	**M550F033**	0.05 (parent reference value)	0.0
3	**M550F049**	0.05 (parent reference value)	0.0
3	**M550F069**	0.05 (parent reference value)	0.0
4	**M550F012**	0.05 (parent reference value)	0.0
4	**M550F038**	0.05 (parent reference value)	0.0
4	**M550F062**	0.05 (parent reference value)	0.0
4	**M550F091**	0.05 (parent reference value)	0.0

Table 6.7.1-5: Contribution of dimethomorph livestock metabolites to acute dietary risk

Group	Metabolite	ARfD used (mg/kg bw/d)	ARfD utilization (%)
0	**M550F021**	0.75 (substance specific reference value)	0.1 (Milk and cream)
1A	**M550F006**	0.6 (parent reference value)	0.4 (Milk and cream)
1A	**M550F007**	0.6 (parent reference value)	0.4 (Milk and cream)
1A	**M550F013**	0.6 (parent reference value)	0.0 (Bovine liver)
1A	**M550F015**	0.6 (parent reference value)	0.0 (Bovine liver)
1A	**M550F016**	0.6 (parent reference value)	0.0 (Bovine liver)
1A	**M550F029**	0.6 (parent reference value)	0.0 (Bovine liver)
1B	**M550F018**	0.6 (parent reference value)	0.1 (Milk and cream)
1B	**M550F028 (one isomer)**	0.6 (parent reference value)	0.0 (Bovine liver)
1B	**M550F074**	0.6 (parent reference value)	0.0 (No contribution)
1B	**M550F076**	0.6 (parent reference value)	0.0 (No contribution)
2	**M550F009**	0.6 (parent reference value)	0.0 (Bovine liver)
2	**M550F028 (Other isomer)**	0.6 (parent reference value)	0.0 (Bovine liver)
2	**M550F035**	0.6 (parent reference value)	0.0 (No contribution)
2	**M550F053**	0.6 (parent reference value)	0.0 (Milk and cream)
3	**M550F008**	0.6 (parent reference value)	0.2 (Milk and cream)
3	**M550F011**	0.6 (parent reference value)	0.1 (Milk and cream)
3	**M550F022**	0.6 (parent reference value)	0.0 (Bird's eggs)
3	**M550F030**	0.6 (parent reference value)	0.0 (Bovine liver)
3	**M550F031**	0.6 (parent reference value)	0.1 (Milk and cream)
3	**M550F033**	0.6 (parent reference value)	0.0 (Bird's eggs)
3	**M550F049**	0.6 (parent reference value)	0.0 (No contribution)
3	**M550F069**	0.6 (parent reference value)	0.0 (No contribution)
4	**M550F012**	0.6 (parent reference value)	0.0 (Bovine liver)
4	**M550F038**	0.6 (parent reference value)	0.0 (Bird's eggs)
4	**M550F062**	0.6 (parent reference value)	0.0 (No contribution)
4	**M550F091**	0.6 (parent reference value)	0.0 (Bird's eggs)

The data show that contributions of the individual metabolites to the dietary risk are small even under unrealistic worst case assumptions used in the assessment. The metabolites with the highest contributions are M550F006, M550F007 and M550F008. The proposal below is based on the indicative exposure assessment and on the metabolism studies available wherein metabolites M550F007 (liver, kidney), M550F006 (eggs) and M550F008 (milk) formed a considerable part of the residue in edible animal matrices.

Accordingly, the following residue definitions are proposed for risk assessment in animal commodities:

For tissues and eggs: Sum of parent compound dimethomorph plus metabolites M550F006 and M550F007, expressed as parent equivalents
For milk: Sum of parent compound dimethomorph plus metabolite M550F008, expressed as parent equivalents

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The EU MRLs for dimethomorph shown in the following table are published in Commission Regulation (EU) No 51/2014 and entered into force on January 20, 2014. A review of the EU MRLs according to Art. 12 of Regulation (EU) No 396/2005 was accomplished in 2011, based still on the "old EU method" for MRL calculation, not yet considering the OECD MRL calculator. In addition to the MRLs listed below, there are currently MRL applications ongoing with the following proposed MRLs:

- Papaya 1.5 mg/kg
- Cauliflower 0.6 mg/kg
- Flowering brassica-others 0.6 mg/kg
- Spinach 1.5 mg/kg
- Globe artichokes 3 mg/kg

Table 6.7.2-1: Current EU MRLs for the uses of dimethomorph

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph (sum of isomers)
100000	FRUITS, FRESH or FROZEN; TREE NUTS	
0110000	. Citrus fruits	
0110010	. Grapefruits	0.01*
0110020	. Oranges	0.8
0110030	. Lemons	0.01*
0110040	. Limes	0.01*
0110050	. Mandarins	0.01*
0110990	. Others	0.01*
0120000	. Tree nuts	0.02*
0130000	. Pome fruits	0.01*
0140000	. Stone fruits	0.01*
0150000	. Berries and small fruits	
0151000	. (a) grapes	3
0152000	. (b) strawberries	0.7
0153000	. (c) cane fruits	
0153010	. Blackberries	0.05 (ft)
0153020	. Dewberries	0.01*
0153030	. Raspberries (red and yellow)	0.05 (ft)
0153990	. Others	0.01*
0154000	. (d) other small fruits and berries	0.01*
0160000	. Miscellaneous fruits	0.01*
200000	VEGETABLES, FRESH or FROZEN	0.01*
210000	Root and tuber vegetables	0.01*
0211000	. (a) potatoes	0.05
0212000	. (b) tropical root and tuber vegetables	0.01*
0213000	. (c) other root and tuber vegetables except sugar beets	0.01*
0213080	Radishes	1.5
220000	Bulb vegetables	0.01*

Table 6.7.2-1: Current EU MRLs for the uses of dimethomorph

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph (sum of isomers)
0220010	. Garlic	0.6
0220020	. Onions	0.6
0220030	. Shallots	0.6
0220040	. Spring onions/green onions and Welsh onions	0.2
0220990	. Others	0.15
230000	Fruiting vegetables	
0231000	. (a) solanacea	1
0232000	. (b) cucurbits with edible peel	0.5
0233000	. (c) cucurbits with inedible peel	0.5
0234000	. (d) sweet corn	0.01*
0239000	. (e) other fruiting vegetables	0.01*
240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	
0241000	. (a) flowering brassica	
0241010	. Broccoli	5
0241020	. Cauliflowers	0.05
0241990	. Others	0.01*
0242000	. (b) head brassica	
0242010	. Brussels sprouts	0.01*
0242020	. Head cabbages	6
0242990	. Others	0.01*
0243000	. (c) leafy brassica	3
0244000	. (d) kohlrabies	0.02
250000	Leaf vegetables, herbs and edible flowers	
251000	(a) lettuces and salad plants	
0251010	. Lamb's lettuces/corn salads	10
0251020	. Lettuces	15
0251030	. Escaroles/broad-leaved endives	6
0251040	. Cresses and other sprouts and shoots	10
0251050	. Land cresses	10
0251060	. Roman rocket/rucola	10
0251070	. Red mustards	10
0251080	. Baby leaf crops (including brassica species)	10
0251990	. Others	10
0252000	. (b) spinaches and similar leaves	
0252010	. Spinaches	1
0252020	. Purslanes	0.01*
0252030	. Chards/beet leaves	1
0252990	. Others	0.01*
0253000	. (c) grape leaves and similar species	0.01*
0254000	. (d) watercresses	0.01*
0255000	. (e) witloofs/Belgian endives	0.05
0256000	. (f) herbs and edible flowers	10
260000	Legume vegetables	
0260010	. Beans (with pods)	0.01*
0260020	. Beans (without pods)	0.04
0260030	. Peas (with pods)	0.01*
0260040	. Peas (without pods)	0.1

Table 6.7.2-1: Current EU MRLs for the uses of dimethomorph

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph (sum of isomers)
0260050	. Lentils	0.01*
0260990	. Others	0.01*
270000	Stem vegetables	
0270010	. Asparagus	0.01*
0270020	. Cardoons	0.01*
0270030	. Celeries	15
0270040	. Florence fennels	0.01*
0270050	. Globe artichokes	2
0270060	. Leeks	1.5
0270070	. Rhubarbs	0.01*
0270080	. Bamboo shoots	0.01*
0270090	. Palm hearts	0.01*
0270990	. Others	0.01*
280000	Fungi, mosses and lichens	0.01*
290000	Algae and prokaryotes organisms	0.01*
300000	PULSES, DRY	0.01*
400000	OILSEEDS AND OIL FRUITS	0.02*
500000	CEREALS	0.01*
600000	TEAS, COFFEE, HERBAL INFUSION, COCOA AND CAROBS	0.05*
700000	HOPS	80
800000	SPICES	
0810000	. Seed spices	
0810010	. Anise/aniseed	30
0810020	. Black caraway/black cumin	30
0810030	. Celery	30
0810040	. Coriander	30
0810050	. Cumin	30
0810060	. Dill	30
0810070	. Fennel	30
0810080	. Fenugreek	30
0810090	. Nutmeg	0.05*
0810990	. Others	30
0820000	. Fruit spices	
0820010	. Allspice/pimento	0.05*
0820020	. Sichuan pepper	0.05*
0820030	. Caraway	30
0820040	. Cardamom	0.05*
0820050	. Juniper berry	0.05*
0820060	. Peppercorn (black, green and white)	0.05*
0820070	. Vanilla	0.05*
0820080	. Tamarind	0.05*
0820990	. Others	0.05*
0830000	. Bark spices	0.05*
0840000	. Root and rhizome spices[#]	0.05*
0850000	. Bud spices	0.05*
0860000	. Flower pistil spices	0.05*
0870000	. Aril spices	0.05*
900000	SUGAR PLANTS	0.01*

Table 6.7.2-1: Current EU MRLs for the uses of dimethomorph

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph (sum of isomers)
1000000	PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	0.01*
1040000	Honey and other apiculture products	0.05*
1050000	Amphibians and reptiles	0.01*
1060000	Terrestrial invertebrate animals	0.01*
1070000	Wild terrestrial vertebrate animals	0.01*

* Indicates lower limit of analytical determination

MRL for horseradish is the one established for horseradish in the root and tuber group (0.01* mg/kg)

Plant Matrices

For dimethomorph in plant and animal matrices EU MRLs are established as laid down in Reg. (EU) No 51/2014. In order to support the renewal of approval for dimethomorph, additional residue trials are presented for the representative uses (grapes, strawberries, lettuce).
In the chapters below, these residue data are evaluated (using the OECD calculator) and compared with the currently established MRLs (most of them based on the previous calculation method). As is shown, the different methods for MRL calculation produce different MRL proposals even when using the same data basis (e.g. for grapes).

Target crops

Strawberries

Drip/Drench Field use

Seven field trials in strawberries were conducted in the EU North in the period from 1994 to 2004. Formulations BAS 550 01 F (500g/kg WP) or SBO 8801 (500g/kg WP), a formulation equivalent to BAS 550 01 F, were applied once at rate of 0.05 g dimethomorph/plant ±25% at or shortly after planting/transplanting (no fruits present). Another four field trials are available from EU North conducted in 2015 where formulation BAS 550 01 F was applied 3 times at a rate of 0.05 g dimethomorph/plant. The first two applications were made in autumn and the third application was carried out in spring similar to the above mentioned trials. It is known that the residue levels for dimethomorph are triggered by the last application, thus it is believed that these new residue trials are suitable to support the GAP.

EU North field drip/drench (n=11)

<0.01 (3x), 0.01 (3x), 0.02 (3x), 0.05, 0.058 mg/kg

Drip/Drench Protected use

Eight protected trials in strawberries were conducted in EU North in the period from 2004 to 2007. Formulation BAS 550 01 F (WP) was applied once at rate of 0.05 g dimethomorph/plant ±25% or 0.125 g dimethomorph/plant at or shortly after planting/transplanting.

In the first four trials, two different application rates were tested (0.0625 g a.s./plant and 0.125 g a.s./plant). It was found that the residue levels were in the same range for both application rates, thus the second set of four trials which were carried out with the application rate of 0.125 g a.s./plant are deemed suitable to support the intended GAP for strawberries in greenhouse. Furthermore, all residues resulting from the higher application rate are still below the highest residue of the trials treated according to cGAP. Thus, inclusion of the residues values of the trials with the higher dose rate into the MRL calculation do not lead to a higher MRL.

EU North protected drip/drench (n=8):

0.02, 0.03 (3x), 0.18, 0.21, 0.26, 0.3 mg/kg

Spray field use

Eight trials for foliar spray use in strawberries were carried out in EU North between 1994 and 2009. Formulations BAS 550 01 F (500g/kg WP) or SBO 8801 (500g/kg WP), a formulation equivalent to BAS 550 01 F, were applied once at rate of 1.5 kg dimethomorph/ha shortly after planting/transplanting (no fruits present) or between BBCH 61-81 (targeting a PHI of 35 days).

EU North spray field use (n=8):

<0.01 (5x), 0.012, 0.013 (2x) mg/kg

Table 6.7.2-2: MRL calculation for dimethomorph in strawberries

	Dimethomorph (sum of isomers; mg/kg)		
	North field spray	**North field drip/drench**	**Protected drip/drench**
STMR	0.010	0.010	0.105
HR	0.013	0.058	0.300
OECD	**0.02**	**0.09**	**0.6**

The current **EU MRL** of **0.7 mg/kg** is covering the residues resulting from drip/drench or spray application of BAS 550 01 F according to the intended GAP. Therefore no adjustment of the MRL is needed.

Code number 0152000 (strawberries): 0.7 mg/kg

Lettuce

Eight greenhouse trials on open leaf lettuce varieties were conducted in the EU (6 in the EU North, 2 in the EU South) in 2013 according to the critical GAP. Formulation BAS 550 01 F (WP) was applied twice at a rate of 0.18 kg dimethomorph/ha. Residues of lettuce samples harvested at the intended PHI of 13-14 days were taken into account for MRL derivation.
Since two of these trials were conducted in lamb's lettuce and rucola, which the RMS did not accept as representative of lettuce, 8 additional greenhouse trials in lettuce (closed head and open leaf lettuce) are submitted in this dossier update. The trials were carried out with the WG formulation Acrobat and applied twice at a rate of 0.18 kg dimethomorph/ha. The residue levels from these trials at the intended PHI of 14 days were included in the updated MRL calculation.

EU greenhouse (n=~~8~~ 14):
<0.020, 0.029, ~~0.053, 0.14~~, 0.17, 0.28, 0.31, 0.37, 0.44, 0.46, 0.56, 0.65, 0.74, 0.78 2.1, 2.2 mg/kg

Table 6.7.2-3: MRL calculation for dimethomorph in open leaf lettuce

	Dimethomorph (sum of isomers; mg/kg)
	Greenhouse
STMR	~~0.5~~ 0.45
HR	2.2
Proposed MRL (OECD calculator)	**~~5.0~~ 4.0**

The residues in lettuce treated with BAS 550 01 F according to the intended GAP are well covered by the current **EU MRL** of **15 mg/kg in lettuce**. Therefore no adjustment of the MRL is required.

Code number 0251020 (lettuce): 15 mg/kg

Grapes

For the representative use of BAS 550 02 F (150 g/L DC) in grapes, 16 trials performed in the Northern and Southern EU during 2000 and 2001 are available. The trials were conducted with the formulation BAS 550 09 F, which is equivalent to the representative formulation BAS 550 02 F (minor change). The formulation was applied 5 times at a rate of 300 g dimethomorph/ha, thus supporting the cGAP of the representative formulation in grapes within ±25%. These trials were all peer reviewed in the previous EU Annex I process.

Overview of residue trials with BAS 550 09 F (already peer reviewed)

Crop	Formulation	Applied rate (kg a.s./ha)	PHI (days)	Residues* (mg/kg)	Reference
Grapes	BAS 550 09 F (DC)	5 x 0.300	28-29	0.38	DK-713-082
				0.50	DK-713-083
				0.42	DK-713-084
				4.60$^{\Delta}$	DK-713-085
				2.28	DK-713-087
				0.36	DK-713-088
				0.24 1.30 1.08 0.62 1.65 0.38 0.38 0.51 0.44 0.21	DK-713-089

* Residues at PHI. If higher residues were found at later harvest times these are indicated

Δ Trial was run under extreme drought weather conditions which yielded unusually small berries. The consequently higher ratio of skin area to fruit volume might explain the exceptionally high residues. Residue value was considered an outlier and was not considered for MRL calculation already in the previous peer review.

Already peer reviewed trials:

EU North (n=8): 0.38 (3x), 0.50, 0.62, 1.08, 1.3, 1.65 mg/kg

EU South (n=7): 0.21, 0.24, 0.36, 0.42, 0.44, 0.51, 2.28 mg/kg

The respective MRL calculation with these values in the last peer review were performed according to the standard EU methods valid at the time of evaluation (R_{max} and R_{ber}) and resulted in the currently established EU MRL of 3 mg/kg. An MRL calculation using the OECD calculator based on the identical data set would result in an MRL proposal of 4 mg/kg (see Table 6.7.2-4).

Table 6.7.2-4: MRL calculation for dimethomorph in grapes based on the residue data from the last peer review

	Dimethomorph (sum of isomers; mg/kg)	
	North	**South**
STMR	0.56	0.42
HR	1.65	2.28
OECD rounded MRL	**3**	**4**

More recent residues data are available for grapes. Four trials have been conducted in grapes (2 in EU North and 2 in EU South) in 2007. Formulations BAS 550 01 F (WP) or BAS 551 11 F (WG) were applied five times at a rate of 0.3 or 0.27 kg dimethomorph/ha on side plots. Since both application patterns support also the intended cGAP for the representative use of BAS 550 02 F in grapes within ±25%, the trials can be included into the MRL calculation. Always the higher residue of both plots was considered.
Another 4 trials (2 in EU North and 2 in EU South) were performed with BAS 550 01 F (WP) in 2011. The applied rate was also 5 x 0.3 kg dimethomorph/ha, thus supporting the intended use in grapes within ±25%. The residue results at a PHI of 28-29 days are listed below:

New trials:
EU North (n=4): 0.48, 0.57, 1.09, 1.96 mg/kg
EU South (n=4): 0.27, 0.64, 1.22, 1.85 mg/kg

Since according to SANCO 7525/VI/95, rev. 9, March 2011, WP and WG formulations usually produce the same residue levels like liquid formulations (EC, SC), the residue values from these trials with BAS 550 01 F should adequately describe the residue behaviour of the liquid formulation BAS 550 02 F in grapes as well.

Most recently, 4N and 4S trials were conducted in grapes with the representative formulation BAS 550 02 F (in order to provide data on metabolites M550F002 and M550F007). In the South trials, the formulation was applied five times at a rate of 0.225 kg dimethomorph/ha according to the cGAP of the representative use in grapes (within ±25%), while in the North trials only two applications at 0.225 kg/ha were carried out (compliant with the product GAP in France for this formulation)
The residue results at a PHI of 27-29 days are listed below (data from EU North not further considered in the MRL calculation since only two applications carried out):

New trials with BAS 550 02 F:
EU North (n=4): 0.33, 0.51, 0.53, 0.62 mg/kg
EU South (n=4): 0.059, 0.38, 0.90, 2.6 mg/kg

~~**Table 6.7.2-5: MRL calculation for dimethomorph in grapes with values from the last peer review and the new residue data**~~

	~~**Dimethomorph (sum of isomers; mg/kg)**~~	
	~~**North**~~	~~**South**~~
~~STMR~~	~~0.595~~	~~0.44~~
~~HR~~	~~1.96~~	~~2.28~~
~~**OECD**~~	~~**3**~~	~~**4**~~

Table 6.7.2-5: MRL calculation for dimethomorph in grapes with values from the last peer review and all new residue data

	Dimethomorph (sum of isomers; mg/kg)	
	North	**South**
STMR	0.595	0.44
HR	1.96	2.6
OECD	**3**	**4**

MRL calculations using the OECD calculator either using only the already peer reviewed residue data or a combination of the peer reviewed and more recent residue data result in both cases in a rounded MRL of 4 mg/kg for EU South. However, the highest individual residue value is well below the current EU MRL of 3 mg/kg.

It is proposed to **raise the EU MRL** for Dimethomorph in **table and wine grapes** to **4 mg/kg**.

Code number 0151000 (grapes, table and wine): 4 mg/kg

~~A respective MRL application will be submitted together with the dossier update in March 2016.~~

A respective MRL application is included in this dossier update.

Succeeding crops

Based on the results of the ^{14}C-succeeding crop study, field trials were performed in order to get a more realistic picture of the residue levels in succeeding crops.

In 2010/2011 field trials with succeeding crops were conducted in the Northern and Southern EU (in total 4 trials for carrot, cauliflower, wheat, and lettuce/spinach). BAS 550 01 F (WP formulation) was applied to bare soil once at a rate of 1.5 kg a.s./ha, matching the maximum intended seasonal application rate according to the GAP. After two different plant back intervals (30 and 90 days) the succeeding crops were planted. Crop specimens were collected at BBCH 30-33, 65, 89 (wheat whole plant, grain, straw), BBCH 41 and 49 (carrots whole plant, root, tops; cauliflower whole plant and inflorescence, lettuce/spinach leaves).
A second field rotational crop study is ~~currently still ongoing,~~ now available covering cucurbits, legumes/pulses and potatoes as follow crops at a PBI of 30 days. ~~This study will be available until March 2016.~~

No clear guidance on the establishment of MRLs for succeeding crops exists up to now, however, trials were conducted in accordance with the requirements concerning the geographical distribution, and can serve as basis for deriving MRL values for rotated crops in the groups of leafy, root and tuber vegetables and cereals, potatoes, legumes/pulses and fruiting vegetables. For the time being, any proposed extrapolations are based on the extrapolations suggested in Table 5 of SANCO 7525, rev.9 for seed treatment uses.

Root and tuber vegetables, bulb vegetables, stem vegetables, sugar beet

In four field rotational crop trials, conducted in 2010/2011 (2 N, 2 S), at the PBI of 30 days, the most relevant PBI for the agricultural practice, the following residues of the parent compound (mg/kg BAS 550 F) were found in carrot roots at BBCH 49:

Northern Europe (n=2): <0.01, 0.017

Southern Europe (n=2): <0.01, 0.025

Futhermore, a large number of residue trials in potato as target crop (as a second major crop in the category root&tuber) are available from foliar spray use, in total 7S and 34N trials according to the EU cGAP (see EFSA Reasoned opinion on the MRL review acc. to Art. 12; EFSA Journal 2011;9(8):2348):

Northern Europe (n=34): <0.01 (13x), <0.02 (18x), 0.02 (2x), 0.04

Southern Europe (n=7): <0.05 (7x)

~~It is believed that the residues from the target use cover the residues arising from crop rotation, however to verify this, another four field rotational crop trials are ongoing (with potato as follow crop) and will be submitted in Q1/2016.~~

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials from North and South were considered together.

Table 6.7.2-6: MRL calculation for dimethomorph in root and tuber vegetables (crop rotation)

	Dimethomorph (sum of isomers; mg/kg)
STMR	0.02
HR	0.05
OECD rounded MRL	**0.08**

Following the guidance in SANCO 7525/VI/95, rev. 9, March 2011 as a basis for MRL setting in the rotational crop, it is suggested to extrapolate from carrot roots and one other major crop (potatoes) in the root and tuber group to the whole group of other root and tuber vegetables as well to stem vegetables (except those commodities that do not yet have a higher MRL covering a target use) and sugar beet. An extrapolation to bulb vegetables is not necessary since all crops in this group have sufficiently high MRLs already from target uses.

In conclusion, it is proposed to establish the following **EU MRLs** for **dimethomorph**:

Code number 0213000 (other root and tuber vegetables, except code 0213080 radishes): 0.08 mg/kg

Code number 0270000 (stem vegetables, except code 0270030 celery, code 0270050 globe artichokes and code 0270060 leeks): 0.08 mg/kg

Code number 0900010 (sugar beets): 0.08 mg/kg

to cover possible residues from crop rotation.

~~Possible residues in potatoes from crop rotation will be investigated in the new field rotational crop study which is currently ongoing and will be available in March 2016. Suitable MRL applications for the above mentioned MRL proposals will be included in the dossier update in March 2016 in order to be able to consider the new data as well.~~

Potatoes

In four field rotational crop trials, conducted in 2015 (2 N, 2 S), at the PBI of 30 days, the most relevant PBI for the agricultural practice, the following residues of the parent compound (mg/kg BAS 550 F) were found in potato tubers at BBCH 49:

Northern Europe (n=2): <0.01, 0.016

Southern Europe (n=2): <0.01, 0.018

The results show that the residues in rotated potato tubers are in the same range as from the target use in potatoes. The MRL proposal calculated from only the rotational crop field trials would be 0.03 mg/kg and would be covered by the current EU MRL of 0.05 mg/kg for potatoes.

Table 6.7.2-7: MRL calculation for dimethomorph in potatoes (crop rotation only)

	Dimethomorph (sum of isomers; mg/kg)
STMR	0.013
HR	0.018
OECD rounded MRL	**0.03**

A second MRL calculation is provided, which additionally considers the residue values from the large number of residue trials in potato as target crop from foliar spray use (7S and 34N trials according to the EU cGAP, see EFSA Reasoned opinion on the MRL review acc. to Art. 12; EFSA Journal 2011;9(8):2348) since the residues are in the same range and thus the basis for an MRL derivation is more robust :

Northern Europe (n=34): <0.01 (13x), <0.02 (18x), 0.02 (2x), 0.04

Southern Europe (n=7): <0.05 (7x)

Table 6.7.2-8: MRL calculation for dimethomorph in potatoes (crop rotation and target use)

	Dimethomorph (sum of isomers; mg/kg)
STMR	0.02
HR	0.05
OECD rounded MRL	**0.08**

In conclusion, it is proposed to **raise the EU MRL** for Dimethomorph in **potatoes** to **0.08 mg/kg**.

Code number 0211000 (potato): 0.08 mg/kg

Leaf vegetables, fresh herbs and brassica vegetables

For leafy and brassica vegetables, 3 trials are available in lettuce, 1 trial in spinach and 4 trials in cauliflower from the field rotational crop study from 2010/2011. The following residues of dimethomorph (mg/kg) were found at BBCH 41 at a PBI of 30 days:

Northern Europe (n=2): leafy <0.01(2x)
Brassica <0.01(2x)

Southern Europe (n=2): leafy <0.01(2x)
Brassica <0.01(2x)

The results show that no residues in leaf vegetables and fresh herbs as well as brassica vegetables are expected when grown in crop rotation. Furthermore, for the group of leafy vegetables, fresh herbs as well as for many brassica vegetables, sufficiently high MRLs are already established to cover the target use in these crops. Thus, no further modification of the MRLs in these crop groups are necessary.

Cereal grains and oilseeds group

For cereal grains, 4 trials are available in wheat from the field rotational crop study from 2010/2011. The following residues of dimethomorph (mg/kg) were found at BBCH 89 at a PBI of 30 days:

Northern Europe (n=2): <0.01 (2x)

Southern Europe (n=2): <0.01 (2x)

The results show that no residues of dimethomorph were observed in wheat grains grown in crop rotation. It is proposed to extrapolate this to all cereal grains and oilseeds. Since no residues are expected, no further modification of the MRLs in these crop groups are necessary.

Cereal straw and forage (feed items)

The 4 field rotational crop trials in wheat have shown that residues of dimethomorph can occur in cereal straw and cereal forage. The following residues (in mg/kg) were found in forage (whole plant sampling at either BBCH 30-33 or BBCH65, whatever residue was higher) and in straw at BBCH 89 at a PBI of 30 days:

Northern Europe (n=2): forage <0.01, 0.026
straw <0.01, 0.017

Southern Europe (n=2): forage <0.01, 0.04
straw 0.027, 0.24

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials from North and South were considered together.

Table 6.7.2-9: MRL calculation for dimethomorph in cereal forage and straw (crop rotation)

	Dimethomorph (sum of isomers; mg/kg)	
	Forage	**Straw**
STMR	0.018	0.022
HR	0.04	0.24
OECD	**0.08**	**0.6**

In conclusion, it is proposed to establish the following pseudo MRLs for dimethomorph:

Cereal forage: 0.08 mg/kg
Cereal straw: 0.60 mg/kg

to cover residues in cereal feed items arising from crop rotation.

Legumes/pulses

~~As mentioned above, a field rotational crop study is currently ongoing to investigate possible residues in legumes/pulses grown in crop rotation. The results will be available until March 2016 and an MRL application will be submitted if any MRLs should be necessary for this group.~~

For investigating the residues in rotated legumes and pulses at a PBI of 30 days, three field trials are available, one Northern trial in peas and two Southern trials in beans. In all trials, samples of pods with seeds, pods without seeds (BBCH 79) and seeds (BBCH 79 and 89) were taken. The samples taken at BBCH 79 are considered to be representative for fresh legume vegetables while the seed samples taken at BBCH 89 are representative for pulses.

The following residues of the parent compound (mg/kg BAS 550 F) were found:

Northern Europe (n=1):	pods with seeds (BBCH 79) <0.01 pods without seeds (BBCH 79) <0.01 seeds (BBCH 79) <0.01 seeds (BBCH 89) <0.01
Southern Europe (n=2):	pods with seeds (BBCH 79) <0.01 (2x) pods without seeds (BBCH 79) <0.01 (2x) seeds (BBCH 79) <0.01 seeds (BBCH 89) <0.01 (2x)

The results show that no residues of dimethomorph are to be expected in legumes and pulses grown in crop rotation. Thus, the current EU MRLs are sufficient and do not need to be increased.

~~**Cucurbits vegetables**~~

~~As mentioned above, a field rotational crop study is currently ongoing to investigate possible residues in cucurbits grown in crop rotation. The results will be available until March 2016 and an MRL application will be submitted if any MRLs should be necessary for this group.~~

Fruiting vegetables

Cucurbits vegetables

In four field rotational crop trials conducted in 2015 (2 N, 2 S) at the PBI of 30 days the following residues of the parent compound (mg/kg BAS 550 F) were found in zucchini at BBCH 89:

Northern Europe (n=2): <0.01, <0.01

Southern Europe (n=2): <0.01, <0.01

The results show that no residues of dimethomorph are to be expected in cucurbits grown in crop rotation. No increase of the existing EU MRL of 0.5 mg/kg in cucurbits (edible and inedible peel) is necessary.

Solanacea

No specific information on the residues in ~~fruiting vegetables~~ solanacea from crop rotation are available. For many crops of this group, there are target uses registered in the EU and consequently a sufficiently high MRL of 1 mg/kg is established for the entire group. All observed dimethomorph residues in other rotated crops were at such a low level and especially in cucurbits (as representative crop for fruiting vegetables) no residues from crop rotation were observed. Thus BASF considers any residues in fruiting vegetables grown in crop rotation as covered by the MRLs established for the target use on these crops.

Animal matrices

Estimation of residues in livestock feed

The EU feed intake was estimated for different livestock species according to the OECD feedstuff table as laid down in the OECD Guidance document on Residues in Livestock (ENV/JM/MONO(2013)8, version of Sep 4th, 2013). Two calculations are presented:

(1) Considering all EU registered uses as well as residues from rotated crops
(2) Considering only the feed items derived from the representative crops in this AIR3 dossier (grapes, strawberry, lettuce) plus residues from rotated crops

Generally, from each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.

The evaluation is based on the following formula:

$$\text{Uptake [mg/animal/day]} = \frac{\text{Total intake of dry matter [kg/d/animal] x Diet factor [\%] x residue in item [mg/kg]}}{\text{Dry matter in item [\%]}}$$

The table below summarizes the residue values used for the dietary burden calculations. For Scenario 1 all listed feed items were considered, for scenario 2 only the feed items highlighted in blue were considered.
For the purpose of MRL derivation, a maximum feed burden for parent residues was estimated since the residue definition for MRL setting in both plant and animal matrices is parent only.
For the purpose of deriving input values for animal matrices in the acute and chronic risk assessment, a maximum and median feed intake was calculated considering residues of parent, M550F002 and M550F007 in feed items, according to the residue definition for risk assessment in plants. These calculations can be found in chapter M-CA 6.9.

Table 6.7.2-10: Input values of dimethomorph for the calculation of the maximum and median dietary burden

Commodity	STMR/ $STMR_P$ [mg/kg]	HR / HR_P [mg/kg]	Origin
Forages			
Barley forage/silage	0.018	0.040	Cereal whole plant at PBI 30d from rotational crop study 2012/1182393
Barley straw/hay	0.022	0.24	cereal straw at PBI 30d from rotational crop study 2012/1182393
Kale leaves	0.38	1.73	from EU registered minor use (see EFSA Journal 2012;10(7):2845; RO on MRL application)
Oat forage/silage	0.018	0.040	Cereal whole plant at PBI 30d from rotational crop study 2012/1182393
Oat hay/straw	0.022	0.24	cereal straw at PBI 30d from rotational crop study 2012/1182393
Rye forage/silage	0.018	0.040	Cereal whole plant at PBI 30d from rotational crop study 2012/1182393
Rye straw	0.022	0.24	cereal straw at PBI 30d from rotational crop study 2012/1182393
Triticale forage/silage	0.018	0.040	Cereal whole plant at PBI 30d from rotational crop study 2012/1182393
Triticale hay/straw	0.022	0.24	cereal straw at PBI 30d from rotational crop study 2012/1182393
Wheat forage/silage	0.018	0.040	Cereal whole plant at PBI 30d from rotational crop study 2012/1182393
Wheat hay/straw	0.022	0.24	cereal straw at PBI 30d from rotational crop study 2012/1182393
Root and tubers			
Carrot culls	0.014	0.026	Carrot roots at PBI 90d from rotational crop study 2012/1182393
Potato culls	0.05	0.05	HR/STMR potatoes (see EFSA Journal 2011;9(8):2348)
Cereal grains/crop seeds			
Barley/Oat/Rye/Triticale/Wheat grain	0.010	NA	Cereal grain at PBI 30d from rotational crop study 2012/1182393
By-products			
Citrus dried pulp	0.040	NA	STMR of orange 0.19 (see EFSA Journal 2011;9(8):2348) x median PF for dried pulp of 0.21
Grape pomace, wet	1.55	NA	STMR of grapes 0.595 x median PF for pomace 2.6 (see EFSA Journal 2011;9(8):2348)
Potato process waste	0.125	NA	STMR potatoes 0.05 (see EFSA Journal 2011;9(8):2348) x EFSA default PF for pomace of 2.5
Rape meal	0.04	NA	STMR oilseed rape 0.02 (see EFSA Journal 2011;9(8):2348) x EFSA default PF of 2
Tomato wet pomace	0.675	NA	STMR of tomato 0.27 (see EFSA Journal 2011;9(8):2348) x EFSA default PF for pomace of 2.5

Table 6.7.2-11: Estimated maximum OECD EU dietary burden of dimethomorph residues for cattle (all uses, scenario 1)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Beef cattle		Dairy cattle	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Kale	15	1.73	20	0.055	20	0.089
Root and tubers						
Potato culls	20	0.05	30	0.002	30	0.003
Cereal grain/crop seeds						
Barley/rye grain	88	0.01	10	0.000	20	0.000
By-products						
Potato process waste	12	0.125	40	0.010	30	0.012
Dietary burden:	mg/kg bw/day		0.067		0.104	
	mg/kg total feed (DM)		2.80		2.70	

Table 6.7.2-12: Estimated maximum OECD EU dietary burden of dimethomorph residues for sheep (all uses, scenario 1)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Ram/Ewe		Lamb	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Kale	15	1.73	10	0.038	10	0.049
Root and tubers						
Potato culls	20	0.05	30	0.003	20	0.002
Cereal grain/crop seeds						
Barley grain	88	0.01	20	0.000	50	0.000
By-products						
Potato process waste	12	0.125	40	0.014	20	0.009
Dietary burden:	mg/kg bw/day		0.055		0.060	
	mg/kg total feed (DM)		1.65		1.42	

Table 6.7.2-13: Estimated maximum OECD EU dietary burden of dimethomorph residues for swine (all uses, scenario 1)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Breeding		Finishing	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Kale	15	1.73	10	0.027	-	-
Root and tubers						
Potato culls	20	0.05	50	0.003	50	0.004
Cereal grain/crop seeds						
Barley grain	88	0.01	20	0.000	30	0.000
By-products						
Potato process waste	12	0.125	20	0.005	-	-
Rape meal	88	0.04			20	0.000
Dietary burden:	mg/kg bw/day		0.034		0.004	
	mg/kg total feed (DM)		1.49		0.138	

Table 6.7.2-14: Estimated maximum OECD EU dietary burden of dimethomorph residues for poultry (all uses, scenario 1)

Commodity	Dry matter content (%)	Residue level (HR/ STMRp)	Broiler		Layer		Turkey	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages								
Wheat straw	88	0.24	-	-	10	0.002	-	-
Root and tubers								
Potato culls	20	0.05	10	0.002	10	0.002	20	0.004
Cereal grain/crop seeds								
Barley/rye grain	88	0.01	70	0.001	80	0.001	60	0.000
By-products								
Rape meal	88	0.04			-	-	20	0.001
Dietary burden:	mg/kg bw/day		0.002		0.004		0.005	
	mg/kg total feed (DM)		0.033		0.061		0.066	

Table 6.7.2-15: Estimated maximum OECD EU dietary burden of dimethomorph residues for cattle (representative uses, scenario 2)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Beef cattle		Dairy cattle	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Barley straw	89	0.24	30	0.002	30	0.0031
Root and tubers						
Carrot culls	12	0.026	15	0.001	15	0.0013
Cereal grain/crop seeds						
Barley grain	88	0.01	55	0.000	40	0.0002
Dietary burden:		mg/kg bw/day	0.003		0.005	
		mg/kg total feed (DM)	0.12		0.12	

Table 6.7.2-16: Estimated maximum OECD EU dietary burden of dimethomorph residues for sheep (representative uses, scenario 2)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Ram/Ewe		Lamb	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Barley straw	89	0.24	60	0.005	60	0.007
Root and tubers						
Carrot culls	12	0.026	20	0.001	20	0.002
Cereal grain/crop seeds						
Barley grain	88	0.01	20	0.000	20	0.000
Dietary burden:		mg/kg bw/day	0.007		0.009	
		mg/kg total feed (DM)	0.21		0.21	

Table 6.7.2-17: Estimated maximum OECD EU dietary burden of dimethomorph residues for swine (representative uses, scenario 2)

Commodity	Dry matter content (%)	Residue level (HR/STMRp)	Breeding		Finishing	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages						
Triticale hay	88	0.24	20	0.001	-	-
Root and tubers						
Carrot culls	12	0.026	25	0.001	25	0.002
Cereal grain/crop seeds						
Barley grain	88	0.01	55	0.000	75	0.000
Dietary burden:		mg/kg bw/day	0.003		0.002	
		mg/kg total feed (DM)	0.11		0.06	

Table 6.7.2-18: Estimated maximum OECD EU dietary burden of dimethomorph residues for poultry (representative uses, scenario 2)

Commodity	Dry matter content (%)	Residue level (HR/ STMRp)	Broiler		Layer		Turkey	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Forages								
Wheat hay	88	0.24	-	-	10	0.002	-	-
Root and tubers								
Carrot culls	12	0.026	10	0.002	10	0.002	10	0.004
Cereal grain/crop seeds								
Barley/rye grain	88	0.01	70	0.001	80	0.001	60	0.000
Dietary burden:	mg/kg bw/day			0.002		0.004		0.002
	mg/kg total feed (DM)			0.03		0.06		0.03

For completeness sake, also a median dietary burden for livestock has been calculated based on the residues of dimethomorph parent, considering all registered uses. This is presented in Table 6.7.2-19.

Table 6.7.2-19: Estimated median OECD EU dietary burden of dimethomorph for all livestock species

Species	Median feed intake	
	mg/kg bw/d	mg/kg total feed (DM)
Beef cattle	0.024	1.00
Dairy cattle	0.034	0.90
Ram/Ewe	0.025	0.75
Lamb	0.022	0.52
Swine breeding	0.014	0.59
Swine finishing	0.004	0.14
Broiler	0.002	0.03
Layer	0.003	0.04
Turkey	0.005	0.07

For MRL derivation the estimated maximum feed intakes according to scenario 1 (all EU registered uses) are considered as most relevant.
Thus, the doses to be used when estimating the maximum residues in products of animal origin are for

Dairy cattle	0.104 mg/kg bw/d
Beef cattle	0.067 mg/kg bw/d
Ram/Ewe	0.055 mg/kg bw/d
Lamb	0.060 mg/kg bw/d
Swine breeding	0.034 mg/kg bw/d
Swine finishing	0.004 mg/kg bw/d
Broiler	0.002 mg/kg bw/d
Layer	0.004 mg/kg bw/d
Turkey	0.005 mg/kg bw/d

It should be noted that the doses assume that the diet completely consists of plant material which had been treated with dimethomorph.

Cattle (sheep, goat, horse) tissues
In the livestock feeding study, dairy cows were fed at dose levels of 50 (1x), 150 (3x) and 500 (10x) mg dimethomorph/animal/day, which correspond to actual doses of 0.095, 0.279 and 0.961 mg/kg bw/day. For derivation of MRLs in tissues, the dietary burden for beef cattle is relevant since it is the highest among the meat ruminants. Maximum feed intakes of ram/ewe and lamb are slightly lower. The maximum dietary intake of beef cattle of 0.067 mg/kgbw/d is below the lowest dose level of the cow feeding study, but within 30%. The feedburden of dairy cattle nearly exactly matches the lowest level of the study.
In tissues of the lowest dose group (B, 1x), no residues of dimethomorph above the LOQ (<0.01 mg/kg) were detected in muscle, fat, liver and kidney. Residues of metabolite M550F007 were below the LOQ of 0.01 mg/kg for muscle and fat, in kidney and liver low residues of 0.01-0.02 mg/kg were observed, respectively. No residues of metabolite M550F006 were detected. According to the currently established and proposed residue definition for enforcement of parent dimethomorph only, the current EU MRLs of 0.01 mg/kg in cattle, sheep, goat, and horse tissues are sufficient.

For risk assessment purposes, a residue definition of parent+M550F006+M550F007 is proposed. According to this residue definition, the combined LOQs would be <0.03 mg/kg. Since for muscle and fat no residues of parent as well as of any metabolite was detected, no valid conversion factor can be derived for these matrices since the derivation of a factor based on all residues below the LOQ would be a gross overestimation. In liver and kidney, residues of M550F007 were found at individual highest residue level of 0.02 mg/kg. Residues of parent and M550F006 were below the LOQ. Since in this case, a real residue value was measured for one metabolite, a conversion factor of 4 can be derived, which is still an overestimation since parent and M550F006 were considered at the level of the LOQ:.

Milk and milk products

The relevant feed intake for dairy cattle of 0.107 mg/kg bw/d nearly exactly matches the lowest dose level of the cow feeding study. In milk and processed milk (skim milk, cream, acid whey) of the lowest dose group (B; 1x), no residues of parent dimethomorph above the limit of quantitation (<0.01 mg/kg) were found. Also no residues of the metabolites M550F006/M550F007 (<0.02 mg/kg) and M550F008 (<0.01 mg/kg) were observed in milk and processed milk samples from this dose group.

According to the currently established and proposed residue definition for enforcement of parent dimethomorph only, the current EU MRLs of 0.01 mg/kg in milk and milk products are sufficient.

When considering the proposed residue definition for risk assessment comprising the sum of parent plus metabolite M550F008, the combined LOQs would be <0.02 mg/kg. However, since a summing of LOQs would be a gross overestimation as all observed residue levels were below the LOQ, no valid conversion factor can be derived for milk.

Pig products

For breeding and finishing swine, the maximum dietary feedburden is below the lowest dose level. In tissues of the lowest dose group, no residues of dimethomorph were seen (<0.01 mg/kg). Also, at this intake no residues of M550F006 and M550F007 above the LOQ would be expected for any tissues.
Thus, the currently established EU MRL of 0.01 mg/kg for pig products is sufficient. A conversion factor of 3 from enforcement to risk assessment is proposed for pig products.

Poultry products

The maximum feed intakes are very low for all poultry species, with the feed intake of 0.005 mg/kg bw/d for turkey being the highest and thus the relevant one for deriving MRLs in bird's tissues. For derivation of MRLs for bird's eggs, the estimated maximum feed intake for the EU layer of 0.004 mg/kg bw/d is relevant.

No feeding study in laying hens is available, as justified in chapter 6.4.1. From the results of the metabolism study in laying hens it can be extrapolated that no residues of dimethomorph and/or metabolites M550F006 and M550F007 above the LOQ of 0.01 mg/kg can be expected in any poultry products, even when the complete TRR is used for extrapolation. When considering only the components of the proposed residue definition (comprising the sum of parent plus metabolites M550F006 and M550F007), extrapolated residues at a realistic worst case feed intake would even be lower.

The currently established EU MRL of 0.01 mg/kg for poultry products and bird's eggs is therefore sufficient.
Since no residue of any metabolite is expected in any poultry product (based on the extrapolations from the metabolism study in poultry), no valid conversion factor can be derived for these matrices.

Based on the above considerations it is proposed to **maintain** an **EU MRL** of **0.01 mg/kg** for dimethomorph **in all animal products, including milk and birds eggs.**

Code number 1000000 (products of animal origin-terrestrial animals): 0.01* mg/kg

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The EU MRLs listed in Table 6.7.2-1 and in this entire chapter include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been carefully evaluated by EFSA. Parts of them are resulting from the adoption of CODEX MRLs (CXLs). Dimethomorph and its crops have been assessed by JMPR (2007, 2013).

CA 6.8 Proposed safety intervals

CA 6.8.1 Pre-harvest interval (in days) for each relevant crop

Grapes	28 d
Strawberries	F*
Lettuce	14 d

* The pre-harvest interval(s) for the envisaged area(s) of application is/are covered by the application conditions and/or the growing period remaining between the envisaged application and use (e.g. harvest); it is not necessary to indicate a pre-harvest interval in days (F).

CA 6.8.2 Re-entry period (in days) for livestock, to areas to be grazed

Because dimethomorph is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

CA 6.8.3 Re-entry period for man to crops, buildings or spaces treated

The result of the risk assessment indicates that re-entry of fields with treated crops is possible after the spray solution has completely dried up. Re-entry assessments are given for the representative uses in the supplemental product dossiers (M-CP 7.2).

CA 6.8.4 Withholding period (in days) for animals feedingstuffs

Due to the favorable residue behavior, no withholding period needs to be considered for livestock. Possible residues in animal matrices arising from feedstuffs derived from the intended uses of dimethomorph (including possible residues in cereals as follow crops) are covered by the currently established EU MRLs for dimethomorph in animal products.

CA 6.8.5 Waiting period between last application and sowing or planting

This is not relevant here since a pre-emergence use is not intended and applications of dimethomorph formulations are directed to the crop/transplants only.

CA 6.8.6 Waiting periods between application and handling treated products

This is not relevant here since a post-harvest treatment is not intended.

CA 6.8.7 Waiting period before sowing/planting succeeding crops

No waiting period needs to be considered for phytotoxicity. For crop rotation of root crops, a waiting period of 30 days is recommended to ensure that possible residues in root crops are below the proposed MRL. For other crops, no waiting period is necessary.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Dietary risk assessments for dimethomorph according to the proposed residue definition

Assessments of the potential chronic and acute dietary consumer risk due to exposure to residues of dimethomorph were performed using the EFSA model for chronic and acute risk assessment - rev. 2_0 (Model PRIMo). The EFSA model was used since it considers all the different diets in the EU and all consumer groups.

The toxicological endpoints used in the risk assessments, the ADI and ARfD, are summarized in the table below. They apply for the parent substance dimethomorph as well as for the metabolites included into the proposed residue definition, which are M550F002 and M550F007 in plants and M550F006, M550F007 and M550F008 in animal matrices. These metabolites are structurally very similar to the parent compound as they result from simple demethylation and subsequent conjugation or ring-opening and modification (in case of M550F008). Also, due to their occurrence in significant amounts in the rat metabolism, they are toxicologically covered and application of the parent reference values is thus considered justified.

Table 6.9-1: Toxicological endpoints for proposed residue definition

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.05 mg/kg bw/d	1 year dog study	100	EFSA Conclusion (EFSA Scientific Report (2006) 82, 1-69, Conclusion on the peer review of dimethomorph)
Acute Reference Dose (ARfD)	0.6 mg/kg bw	Developmental toxicity study in rats	100	

Derivation of input values for the chronic and acute dietary risk assessment- *Plant matrices*

For the assessment the sum of the respective HR or STMR values plus metabolite levels were used. The HR and STMR values of parent dimethomorph are summarized in Table 6.9-2. For deriving appropriate residue values for M550F002 and M550F007, due to the lack of field data for most crops, a metabolite/parent ratio was derived from plant metabolism studies and was applied to the HR or STMR values of dimethomorph as listed in Table 6.9-2. If no STMR/HR levels are available, the MRL is used. The applied metabolite/parent ratios are summarized in Table 6.9-3.
The generation of additional field residue data for metabolites M550F002 and M550F007 in lettuce and grapes, as provided in the dossier update of March 2016, provides now reliable conversion factors for lettuce (to be extrapolated to brassica, stem vegetables) and for grapes (to be extrapolated to all fruits and fruiting vegetables). However, in all cases the input values derived from the STMR/HR x conversion factor are lower than the input values derived from using the metabolite/parent ratios from the metabolism studies. Thus, the originally presented dietary risk assessment using metabolite/parent ratios represents a worst case scenario and an update of the IEDI calculation is not considered necessary.

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
100000	FRUITS, FRESH or FROZEN; TREE NUTS				
0110000	. Citrus fruits				
0110010	. Grapefruits	0.01*			
0110020	. Oranges	0.8	0.19	0.61	EFSA RO Art. 12
0110030	. Lemons	0.01*			
0110040	. Limes	0.01*			
0110050	. Mandarins	0.01*			
0110990	. Others	0.01*			
0120000	. Tree nuts	0.02*			
0130000	. Pome fruits	0.01*			
0140000	. Stone fruits	0.01*			
0150000	. Berries and small fruits				
0151000	· (a) grapes	3/4	0.6	2.3	Representative use (data from this dossier)
0152000	. (b) strawberries	0.7	0.13	0.51	EFSA RO Art. 12 (data from the representative use in this dossier are lower)
0153000	. (c) cane fruits				
0153010	. Blackberries	0.05 (ft)			
0153020	. Dewberries	0.01*			
0153030	. Raspberries (red and yellow)	0.05 (ft)			
0153990	. Others	0.01*			
0154000	. (d) other small fruits and berries	0.01*			
0160000	. Miscellaneous fruits	0.01*			
163040	. Papayas	0.01/1.5	0.35	0.67	Import tolerance application ongoing at EFSA

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
200000	VEGETABLES, FRESH or FROZEN	0.01*			
210000	Root and tuber vegetables	0.01*			
0211000	. (a) potatoes	0.05			
0212000	. (b) tropical root and tuber vegetables	0.01*			
0213000	. (c) other root and tuber vegetables except sugar beets	0.08	0.02	0.05	Proposed in MCA 6.7
0213080	Radishes	1.5	0.27	0.63	EFSA RO Art. 12
220000	Bulb vegetables	0.01*			
0220010	. Garlic	0.6	0.2	0.4	EFSA RO, EFSA Journal 2012;10(7):2845
0220020	. Onions	0.6	0.2	0.4	
0220030	. Shallots	0.6	0.2	0.4	
0220040	. Spring onions/green onions and Welsh onions	0.2/9	2.3	6.6	New CXL, adoption as EU MRL foreseen
0220990	. Others	0.15	0.05	0.1	EFSA RO Art. 12
230000	Fruiting vegetables				
0231000	. (a) solanacea	1	0.27	0.74	EFSA RO Art. 12
0232000	. (b) cucurbits with edible peel	0.5	0.21	0.39	EFSA RO Art. 12
0233000	. (c) cucurbits with inedible peel	0.5	0.06	0.29	EFSA RO Art. 12
0234000	. (d) sweet corn	0.01*			
0239000	. (e) other fruiting vegetables	0.01*			
240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)				
0241000	. (a) flowering brassica				
0241010	. Broccoli	5	1.3	2.62	EFSA RO, EFSA Journal 2012;10(7):2845
0241020	. Cauliflowers	0.05/ 0.6	0.045	0.3	MRL application ongoing at EFSA
0241990	. Others	0.01*/0.6			
0242000	. (b) head brassica				
0242010	. Brussels sprouts	0.01*			

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
0242020	. Head cabbages	6	1.2	4.61	EFSA RO, EFSA Journal 2012;10(7):2845
0242990	. Others	0.01*			
0243000	. (c) leafy brassica	3	0.38	1.73	EFSA RO, EFSA Journal 2012;10(7):2845
0244000	. (d) kohlrabies	0.02*			
250000	Leaf vegetables, herbs and edible flowers				
251000	(a) lettuces and salad plants				
0251010	. Lamb's lettuces/corn salads	10	1.27	7.06	EFSA RO Art. 12
0251020	. Lettuces	15	3.48	10.7	EFSA RO, EFSA Journal 2012;10(7):2845
0251030	. Escaroles/broad-leaved endives	6	0.37	3.16	
0251040	. Cresses and other sprouts and shoots	10	1.17	7.2	
0251050	. Land cresses	10	1.17	7.2	
0251060	. Roman rocket/rucola	10	1.27	7.06	EFSA RO Art. 12
0251070	. Red mustards	10	1.27	7.06	EFSA RO Art. 12
0251080	. Baby leaf crops (including brassica species)	10	1.27	7.06	EFSA RO Art. 12
0251990	. Others	10	1.27	7.06	EFSA RO Art. 12
0252000	. (b) spinaches and similar leaves				
0252010	. Spinaches	1/1.5	0.27	1.22	MRL application ongoing at EFSA
0252020	. Purslanes	0.01*			
0252030	. Chards/beet leaves	1	0.017	0.47	EFSA RO EFSA Journal 2011;9(11):2437
0252990	. Others	0.01*			
0253000	. (c) grape leaves and similar species	0.01*			
0254000	. (d) watercresses	0.01*			
0255000	. (e) witloofs/Belgian endives	0.05	0.03	0.03	EFSA RO Art. 12
0256000	. (f) herbs and edible flowers	10	1.27	7.06	EFSA RO Art. 12

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
260000	Legume vegetables				
0260010	. Beans (with pods)	0.01*			
0260020	. Beans (without pods)	0.04	0.02	0.02	EFSA RO Art. 12
0260030	. Peas (with pods)	0.01*			
0260040	. Peas (without pods)	0.1	0.01	0.07	EFSA RO Art. 12
0260050	. Lentils	0.01*			
0260990	. Others	0.01*			
270000	Stem vegetables	0.08	0.02	0.05	Proposed in MCA 6.7
0270030	. Celeries	15	2.48	8.82	EFSA RO, EFSA Journal 2012;10(7):2845
0270050	. Globe artichokes	2/3	0.26	1.14	EFSA RO Art. 12; MRL application ongoing at EFSA
0270060	. Leeks	1.5	0.19	0.69	EFSA RO Art. 12
280000	Fungi, mosses and lichens	0.01*			
290000	Algae and prokaryotes organisms	0.01*			
300000	PULSES, DRY	0.01*			
400000	OILSEEDS AND OIL FRUITS	0.02*			
500000	CEREALS	0.01*			
600000	TEAS, COFFEE, HERBAL INFUSION, COCOA AND CAROBS	0.05*			
700000	HOPS	80	20	42	EFSA RO Art. 12
800000	SPICES				
0810000	. Seed spices				

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
0810010	. Anise/aniseed	30	1.35	12.9	EFSA RO EFSA Journal 2013;11(2):3126
0810020	. Black caraway/black cumin	30	1.35	12.9	
0810030	. Celery	30	1.35	12.9	
0810040	. Coriander	30	1.35	12.9	
0810050	. Cumin	30	1.35	12.9	
0810060	. Dill	30	1.35	12.9	
0810070	. Fennel	30	1.35	12.9	
0810080	. Fenugreek	30	1.35	12.9	
0810090	. Nutmeg	0.05*			
0810990	. Others	30	1.35	12.9	EFSA RO EFSA Journal 2013;11(2):3126
0820000	. Fruit spices				
0820010	. Allspice/pimento	0.05*			
0820020	. Sichuan pepper	0.05*			
0820030	. Caraway	30	1.35	12.9	EFSA RO EFSA Journal 2013;11(2):3126
0820040	. Cardamom	0.05*			
0820050	. Juniper berry	0.05*			
0820060	. Peppercorn (black, green and white)	0.05*			
0820070	. Vanilla	0.05*			
0820080	. Tamarind	0.05*			
0820990	. Others	0.05*			
0830000	. Bark spices	0.05*			
0840000	. Root and rhizome spices[#]	0.05*			
0850000	. Bud spices	0.05*			
0860000	. Flower pistil spices	0.05*			
0870000	. Aril spices	0.05*			

Table 6.9-2: EU MRLs and respective STMRs and HRs for dimethomorph used in dietary risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	MRL (established/proposed)	STMR	HR	Origin
900000	SUGAR PLANTS	0.08	0.02	0.05	Proposed in MCA 6.7
1000000	PRODUCTS OF ANIMAL ORIGIN - TERRESTRIAL ANIMALS	0.01*			
1040000	Honey and other apiculture products	0.05*			
1050000	Amphibians and reptiles	0.01*			
1060000	Terrestrial invertebrate animals	0.01*			
1070000	Wild terrestrial vertebrate animals	0.01*			

* Indicates lower limit of analytical determination
# MRL for horseradish is the one established for horseradish in the root and tuber group (0.01* mg/kg)

Table 6.9-3: Metabolite to parent ratios derived from plant metabolism studies for M550F002 and M550F007

Matrix	M550F002	M550F007	Comment
Grape, fruits	0.228	0.034	Grape metabolism
Citrus fruit	0.228	0.034	Extrapolation from grapes
Tree nuts	0.228	0.034	Extrapolation from grapes
Pome fruit	0.228	0.034	Extrapolation from grapes
Stone fruit	0.228	0.034	Extrapolation from grapes
Berries	0.228	0.034	Extrapolation from grapes
Small fruit	0.228	0.034	Extrapolation from grapes
Grapes	0.228	0.034	Extrapolation from grapes
Fruiting vegetables	0.228	0.034	Extrapolation from grapes
Banana	0.228	0.034	Extrapolation from grapes
Persimmon	0.228	0.034	Extrapolation from grapes
Grape leaves	0.060	0.012	Grape metabolism
Brassica vegetables	0.060	0.012	Extrapolation from grape leaves
Potatoes	-	0.1017	Potato metabolism
Leaf vegetables	0.060	0.012	Extrapolation from grape leaves (not found in lettuce metabolism)
Stem vegetables	0.060	0.012	Extrapolation from grape leaves
Hops	0.060	0.012	Extrapolation from grape leaves
Strawberry	STMR/HRx1.33		Conversion factor derived from field data (see M-CA 6.3.1/4)

Derivation of input values for the chronic and acute dietary risk assessment- *Animal matrices*

Since the residue definition for risk assessment in plants considers residues of parent and the metabolites M550F002 and M550F007, consequently for the derivation of suitable input levels for animal matrices to the dietary risk assessment, a livestock feed burden based on the sum of residues of parent dimethomorph plus M550F002 plus M550F007 has to be estimated according to the OECD feedstuff table as laid down in the OECD Guidance document on Residues in Livestock (ENV/JM/MONO(2013)8, version of Sep 4th, 2013).

For deriving appropriate input values for M550F002 and M550F007 in relevant feed items, due to the lack of field data, a metabolite/parent ratio was derived from plant metabolism studies where feasible. This metabolite/parent ratio (M/P ratio) has then been applied to the HR or STMR of dimethomorph as measured in field residue trials or field rotational crop studies (as described in chapter 6.7, table 6.7.2-8).
Table 6.9-4 shows the input values as well as their origin and extrapolations.
In order to allow an indicative risk assessment based on the metabolism and rotational crop data, potential extrapolations to other crop groups have been considered to derive input values for all relevant feed items and thus derive a worst case feed burden estimation.

For the feed item potato culls, the value for M550F002 was extrapolated from radish roots from the rotational crop study since in the potato metabolism study only M550F007 was determined, but not M550F002.
In case of residues in relevant feed items stemming from crop rotation (cereal forage/straw, carrots) metabolite levels from the confined rotational crop studies have been normalized to the application rate of the cGAP and used as HR/STMR values in parent equivalents. This approach was selected as often in the matrices the parent residues were below LOQ and thus did not allow the derivation of a reliable metabolite/parent ratio.
For the input level for wheat grain, no residue data for the metabolites are available since wheat grain residues were not further characterized in the confined rotational crops study due to being so low. A mere summing up of the LOQs for all three components would be a gross overestimation and is considered highly unrealistic. However, as grain is an important feed item, cereal grain will be accounted for with 0.01 mg/kg as sum of parent, M550F002 and M550F007 as a worst-case assumption.
For the target use in oilseed rape, no suitable extrapolation from available metabolism studies can be made. Thus, extrapolation from soybean from the confined rotational crop studies was considered. However, residues in soybean have not been characterized due to being very low. Therefore, no suitable metabolite input values could be derived for oilseed rape, but since this is a seed treatment use with residues of parent below LOQ, the contribution of metabolite residues is considered negligible.

Table 6.9-4: Input values for the calculation of the maximum and median dietary burden

Matrix	Extrapolation to	Parent	M550F002		M550F007		Input value max/med feed burden [2]	Origin of metabolite data
		HR/ STMR	M/P ratio	mg/kg	M/P ratio	mg/kg	Sum	
Wheat forage	Barley, oat, rye, triticale forage +silage	0.04/ 0.018		0.056		0.002	**0.098/ 0.076**	Confined rotcrop study
Wheat straw	Barley, oat, rye, triticale straw + hay	0.24/ 0.022		0.036		0.0091	**0.286/ 0.068**	Confined rotcrop study
Grape leaves	Kale	1.73/ 0.38	0.0596	0.1031/ 0.0226	0.0124	0.0215/ 0.0047	**1.855/ 0.407**	M/P ratio of grape leaves from grape metabolism
Grapes	Wet pomace	0.595	0.2280	0.1357	0.0339	0.0202	**0.751**	M/P ratio of grape fruits from grape metabolism
	Citrus dried pulp	0.19	0.2280	0.0433	0.0339	0.0064	**0.240**	
	Tomato wet pomace	0.27	0.2280	0.0616	0.0339	0.0092	**0.341**	
Radish	Carrot	0.026/ 0.014		0.0091		0.001	**0.036/ 0.024**	Confined rotcrop study
Potato (peel)	Culls Process waste	0.05/ 0.05		0.0091[1]	0.1017	0.0051	**0.064**	Metabolism and confined rotcrop study
Soya bean	Rape meal	0.02		nd		nd	**0.020**	Confined rotcrop study
Wheat grain	Barley, oat, rye, triticale	<0.01		<0.01		<0.01	**0.01**	Rotational crop

1 Value extrapolated from rotational crop study of radish root, as M550F002 was not determined in the potato metabolism study.

2 The input values for grape pomace, citrus dried pulp, tomato wet pomace, potato process waste and rape meal were further multiplied by the processing factors as specified in Table 6.7.2-8 (see M-CA 6.7)

nd Not determined, as values in straw and forage of soy bean were already so low, grains were not analyzed.

With these input values a maximum and median feed intake was estimated. The results are shown in Table 6.9-5.

Table 6.9-5: Estimated maximum and median OECD EU dietary intake for livestock species considering the residues according to the proposed residue definition for risk assessment in plant commodities (dimethomorph + M550F002 + M550F007)

Species	Maximum feed intake		Median feed intake	
	mg/kg bw/d	mg/kg total feed (DM)	mg/kg bw/d	mg/kg total feed (DM)
Beef cattle	0.074	3.10	0.028	1.18
Dairy cattle	0.114	2.97	0.040	1.04
Ram/Ewe	0.062	1.87	0.030	0.90
Lamb	0.067	1.57	0.026	0.61
Swine breeding	0.038	1.67	0.016	0.70
Swine finishing	0.005	0.17	0.005	0.17
Broiler	0.003	0.04	0.003	0.04
Layer	0.0055	0.08	0.005	0.07
Turkey	0.006	0.08	0.006	0.08

Based on these feed intakes the following input values (HR and STMR) for animal matrices in the dietary exposure assessment have been derived. For meat animals, the highest feed intake is with beef cattle. Both the median and maximum feed intake of beef cattle is below or close to the lowest dose level of the cattle feeding study. Thus, for deriving STMR values the group mean residue levels of dimethomorph and M550F006 and M550F007 of the 1x dose group are relevant while for deriving a HR value the highest individual residue level of the 1x dose group is relevant.

For deriving input levels for milk and cream, the dietary burden for dairy cattle is relevant. The maximum feed intake for dairy cattle is slightly above the 1x dose level of the cattle feeding study while the median dietary burden is well below the 1x dose level. For both the 1x and the 3x dose level, no residues of parent and M550F008 have been observed.

For poultry products and bird's eggs, input values were derived by extrapolation from the metabolism study. No residues of dimethomorph and/or metabolites M550F006 and M550F007 above the LOQ of 0.01 mg/kg can be expected in any poultry products, even when the complete TRR is used for extrapolation. Since extrapolation from the complete TRR results in residues below 0.01 mg/kg, this was used as a worst case input value, the summing up of LOQs was considered to result in unrealistically high levels and was thus not done here.

Table 6.9-6: Input values for animal products in the dietary risk assessment

Commodity	Derived HR	Derived STMR	Origin
Meat (swine, bovine, sheep, goat, horse, other farm animals)	<0.03	<0.03	Sum of LOQ for parent +M550F006+M550F007, no residues observed at 1 x dose level
Liver /Kidney /Edible offal (swine, bovine, sheep, goat, horse, other farm animals)	0.036	<0.03	Sum of residues for parent +M550F006 (both <0.01 each) and observed residues of M550F007 at 1x dose level, normalized to max or med feed intake of beef cattle
Fat (swine, bovine, sheep, goats, horse, other farm animals)	<0.03	<0.03	Sum of LOQ for parent +M550F006+M550F007, no residues observed at 1 x dose level
Poultry	<0.01	<0.01	Extrapolation from TRR in poultry tissues from metabolism study
Milk and cream	<0.02	<0.02	Sum of LOQ for parent +M550F008, no residues observed at 1x or 3 x dose level
Bird's eggs	<0.01	<0.01	Extrapolation from TRR in poultry eggs from metabolism study

Table 6.9-7 summarizes the input values used for the chronic and acute dietary risk assessment.

Table 6.9-7: Input values used for risk assessment according to the proposed residue definition in plant and animal matrices

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph[1] (sum of parent plus metabolites) STMR	Dimethomorph[1] (sum of parent plus metabolites) HR
100000	FRUITS, FRESH or FROZEN; TREE NUTS		
0110000	. Citrus fruits		
0110010	. Grapefruits	0.01	0.01
0110020	. Oranges	0.24	0.77
0110030	. Lemons	0.01	0.01
0110040	. Limes	0.01	0.01
0110050	. Mandarins	0.01	0.01
0110990	. Others	0.01	0.01
0120000	. Tree nuts	0.03	0.03
0130000	. Pome fruits	0.01	0.01
0140000	. Stone fruits	0.01	0.01
0150000	. Berries and small fruits		
0151000	. (a) grapes	0.75	2.90
0152000	. (b) strawberries	0.17	0.68
0153000	. (c) cane fruits		
0153010	. Blackberries	0.06	0.06
0153020	. Dewberries	0.01	0.01
0153030	. Raspberries (red and yellow)	0.06	0.06
0153990	. Others	0.01	0.01
0154000	. (d) other small fruits and berries	0.01	0.01
0160000	. Miscellaneous fruits		
0161000	. (a) edible peel	0.01	0.01
0162000	. (b) inedible peel, small	0.01	0.01
0163000	. (c) inedible peel, large		
0163010	. Avocados	0.01	0.01
0163020	. Bananas	0.01	0.01
0163030	. Mangoes	0.01	0.01
0163040	. Papayas	0.35	0.67
0163050	. Granate apples/pomegranates	0.01	0.01
0163060	. Cherimoyas	0.01	0.01
0163070	. Guavas	0.01	0.01
0163080	. Pineapples	0.01	0.01
0163090	. Breadfruits	0.01	0.01
0163100	. Durians	0.01	0.01
0163110	. Soursops/guanabanas	0.01	0.01
0163990	. Others (2)	0.01	0.01
200000	VEGETABLES, FRESH or FROZEN		
210000	Root and tuber vegetables		
0211000	. (a) potatoes	0.055	0.055
0212000	. (b) tropical root and tuber vegetables	0.01	0.01
0213000	. (c) other root and tuber vegetables except sugar beets	0.02	0.05
0213080	Radishes	0.27	0.63

Table 6.9-7: Input values used for risk assessment according to the proposed residue definition in plant and animal matrices

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph[1] (sum of parent plus metabolites) STMR	Dimethomorph[1] (sum of parent plus metabolites) HR
220000	Bulb vegetables		
0220010	. Garlic	0.20	0.40
0220020	. Onions	0.20	0.40
0220030	. Shallots	0.20	0.40
0220040	. Spring onions/green onions and Welsh onions	2.30	6.60
0220990	. Others	0.05	0.10
230000	Fruiting vegetables		
0231000	. (a) solanacea	0.34	0.93
0232000	. (b) cucurbits with edible peel	0.27	0.49
0233000	. (c) cucurbits with inedible peel	0.08	0.37
0234000	. (d) sweet corn	0.01	0.01
0239000	. (e) other fruiting vegetables	0.01	0.01
240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)		
0241000	. (a) flowering brassica		
0241010	. Broccoli	1.39	2.81
0241020	. Cauliflowers	0.05	0.32
0241990	. Others	0.05	0.32
0242000	. (b) head brassica		
0242010	. Brussels sprouts	0.01	0.01
0242020	. Head cabbages	1.29	4.94
0242990	. Others	0.01	0.01
0243000	. (c) leafy brassica	0.41	1.85
0244000	. (d) kohlrabies	0.02	0.02
250000	Leaf vegetables, herbs and edible flowers		
251000	(a) lettuces and salad plants		
0251010	. Lamb's lettuces/corn salads	1.36	7.57
0251020	. Lettuces	3.73	11.47
0251030	. Escaroles/broad-leaved endives	0.40	3.39
0251040	. Cresses and other sprouts and shoots	1.25	7.72
0251050	. Land cresses	1.25	7.72
0251060	. Roman rocket/rucola	1.36	7.57
0251070	. Red mustards	1.36	7.57
0251080	. Baby leaf crops (including brassica species)	1.36	7.57
0251990	. Others	1.36	7.57
0252000	. (b) spinaches and similar leaves		
0252010	. Spinaches	0.29	1.31
0252020	. Purslanes	0.01	0.01
0252030	. Chards/beet leaves	0.02	0.50
0252990	. Others	0.01	0.01
0253000	. (c) grape leaves and similar species	0.01	0.01
0254000	. (d) watercresses	0.01	0.01

Table 6.9-7: Input values used for risk assessment according to the proposed residue definition in plant and animal matrices

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph[1] (sum of parent plus metabolites) STMR	Dimethomorph[1] (sum of parent plus metabolites) HR
0255000	. (e) witloofs/Belgian endives	0.03	0.03
0256000	. (f) herbs and edible flowers	1.36	7.57
260000	Legume vegetables		
0260010	. Beans (with pods)	0.01	0.01
0260020	. Beans (without pods)	0.02	0.02
0260030	. Peas (with pods)	0.01	0.01
0260040	. Peas (without pods)	0.01	0.07
0260050	. Lentils	0.01	0.01
0260990	. Others	0.01	0.01
270000	Stem vegetables		
0270010	. Asparagus	0.02	0.05
0270020	. Cardoons	0.02	0.05
0270030	. Celeries	2.66	9.46
0270040	. Florence fennels	0.02	0.05
0270050	. Globe artichokes	0.28	1.22
0270060	. Leeks	0.20	0.74
0270070	. Rhubarbs	0.02	0.05
0270080	. Bamboo shoots	0.02	0.05
0270090	. Palm hearts	0.02	0.05
0270990	. Others	0.02	0.05
280000	Fungi, mosses and lichens	0.01	0.01
290000	Algae and prokaryotes organisms	0.01	0.01
300000	PULSES, DRY	0.01	0.01
400000	OILSEEDS AND OIL FRUITS	0.02	0.02
500000	CEREALS	0.01	0.01
600000	TEAS, COFFEE, HERBAL INFUSION, COCOA AND CAROBS	0.05	0.05
700000	HOPS	21.44	45.03
800000	SPICES		
0810000	. Seed spices		
0810010	. Anise/aniseed	1.35	12.90
0810020	. Black caraway/black cumin	1.35	12.90
0810030	. Celery	1.35	12.90
0810040	. Coriander	1.35	12.90
0810050	. Cumin	1.35	12.90
0810060	. Dill	1.35	12.90
0810070	. Fennel	1.35	12.90
0810080	. Fenugreek	1.35	12.90
0810090	. Nutmeg	0.05	0.05
0810990	. Others	1.35	12.90
0820000	. Fruit spices		
0820010	. Allspice/pimento	0.05	0.05
0820020	. Sichuan pepper	0.05	0.05
0820030	. Caraway	1.35	12.90
0820040	. Cardamom	0.05	0.05

Table 6.9-7: Input values used for risk assessment according to the proposed residue definition in plant and animal matrices

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph[1] (sum of parent plus metabolites) STMR	Dimethomorph[1] (sum of parent plus metabolites) HR
0820050	. Juniper berry	0.05	0.05
0820060	. Peppercorn (black, green and white)	0.05	0.05
0820070	. Vanilla	0.05	0.05
0820080	. Tamarind	0.05	0.05
0820990	. Others	0.05	0.05
0830000	. Bark spices	0.05	0.05
0840000	. Root and rhizome spices[3]	0.05	0.05
0850000	. Bud spices	0.05	0.05
0860000	. Flower pistil spices	0.05	0.05
0870000	. Aril spices	0.05	0.05
900000	SUGAR PLANTS	0.02	0.05
1000000	PRODUCTS OF ANIMAL ORIGIN - TERRESTRIAL ANIMALS		
1011000	. (a) swine		
1011010	. Muscle	0.03	0.03
1011020	. Fat tissue	0.03	0.03
1011030	. Liver	0.03	0.04
1011040	. Kidney	0.03	0.04
1011050	. Edible offals (other than liver and kidney)	0.03	0.04
1011990	. Others	0.03	0.03
1012000	. (b) bovine		
1011010	. Muscle	0.03	0.03
1011020	. Fat tissue	0.03	0.03
1011030	. Liver	0.03	0.04
1011040	. Kidney	0.03	0.04
1011050	. Edible offals (other than liver and kidney)	0.03	0.04
1011990	. Others	0.03	0.03
1013000	. (c) sheep		
1013010	. Muscle	0.03	0.03
1013020	. Fat tissue	0.03	0.03
1013030	. Liver	0.03	0.04
1013040	. Kidney	0.03	0.04
1013050	. Edible offals (other than liver and kidney)	0.03	0.04
1013990	. Others	0.03	0.03
1014000	. d) goat		
1014010	. Muscle	0.03	0.03
1014020	. Fat tissue	0.03	0.03
1014030	. Liver	0.03	0.04
1014040	. Kidney	0.03	0.04
1014050	. Edible offals (other than liver and kidney)	0.03	0.04
1014990	. Others	0.03	0.03
1015000	. (e) equine		

Table 6.9-7: Input values used for risk assessment according to the proposed residue definition in plant and animal matrices

Code number	Groups and examples of individual products to which the MRLs apply	Dimethomorph[1] (sum of parent plus metabolites) STMR	Dimethomorph[1] (sum of parent plus metabolites) HR
1015010	. Muscle	0.03	0.03
1015020	. Fat tissue	0.03	0.03
1015030	. Liver	0.03	0.04
1015040	. Kidney	0.03	0.04
1015050	. Edible offals (other than liver and kidney)	0.03	0.04
1015990	. Others	0.03	0.03
1016000	. (f) poultry		
1016010	. Muscle	0.01	0.01
1016020	. Fat tissue	0.01	0.01
1016030	. Liver	0.01	0.01
1016040	. Kidney	0.01	0.01
1016050	. Edible offals (other than liver and kidney)	0.01	0.01
1016990	. Others	0.01	0.01
1017000	. (g) other farmed terrestrial animals		
1017010	. Muscle	0.03	0.03
1017020	. Fat tissue	0.03	0.03
1017030	. Liver	0.03	0.04
1017040	. Kidney	0.03	0.04
1017050	. Edible offals (other than liver and kidney)	0.03	0.04
1017990	. Others	0.03	0.03
1020000	. Milk	0.02	0.02
1030000	. Birds eggs	0.01	0.01
1040000	Honey and other apiculture products	0.05	0.05
1050000	Amphibians and reptiles	0.01	0.01
1060000	Terrestrial invertebrate animals	0.01	0.01
1070000	Wild terrestrial vertebrate animals	0.01	0.01

1 Sum of STMR/HR plus M550F002 and M550F007 (in parent equivalents)
Plants: STMR/HR$_{dimetomorph}$+M550F002+ M550F007 in parent equivalents;
Animals: dimethomorph+M550F006+M550F007 for tissues and dimethomorph+M550F008 for milk.
Metabolite levels were calculated from plant (grape and lettuce)/livestock metabolism studies and extrapolated according to OECD 501, Annex 1 (see also TTC paper submitted with this dossier).

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI and IEDI calculation

In a first step a TMDI calculation was performed with the current EFSA model (version 2) using the MRLs as listed in Table 6.9-2. The risk assessment was conducted considering all current established MRLs as well the proposed MRLs for papaya, flowering brassica, spinach and globe artichokes, for which MRL applications are ongoing at EFSA. For grapes, the MRL of 4 mg/kg as proposed in this dossier was considered as well as for spring onions the new Codex MRL of 9 mg/kg, which will likely be adopted as EU MRL.
The summary of the chronic assessment is presented in Table 6.9-8. The ADI utilization ranges from 8 to 50% ADI. The diet with the highest TDMI is the “WHO cluster diet B” with 49.5% ADI. For this diet the highest contributors are table and wine grapes with 17.1% of the ADI. The diet with the second highest TDMI is the “French all population” with 44.7% ADI, for which again table and wine grapes are the highest contributor.

In a second step, a chronic exposure assessment was performed using all crops and the residues according to the residue definition proposed for plant and animal matrices (see Table 6.9-7).

The summary of the calculation using the EFSA model is presented in Table 6.9-9. For the assessment, the ADI of 0.05 mg/kg bw/day was used. According to the EFSA model the IEDI has been simultaneously calculated for adults, children, toddlers and infants (different age groups), vegetarian and elderly in different EU countries.
With the current EFSA model the chronic risk assessment ranges from 2.8 to 12.5% of the ADI (see Table 6.9-9). The diet with the highest IEDI is "WHO cluster diet B" with 12.5% of the ADI. For this diet, the highest contributors are wine grapes with 2.7% of the ADI. The diet with the second highest IEDI is “NL child” with 9.3% of the ADI, in which oranges are the major contributors with 1.5% of the ADI.

The ADI utilization is for both calculations significantly below 100% and thus a further refinement of the chronic risk assessment is not necessary. According to the presented TMDI and IEDI calculations a chronic intake of dimethomorph residues is unlikely to present a public health concern.

Table 6.9-8: TMDI calculation based on established and proposed EU MRLs for dimethomorph as listed in Table 6.9-2

Dimethomorph			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,05	ARfD (mg/kg bw):	0,6
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
8	50
No of diets exceeding ADI:	—

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
49,5	WHO Cluster diet B	17,1	Table and wine grapes	10,8	Lettuce	7,8	Solanacea	
44,7	FR all population	32,9	Table and wine grapes	3,8	Other lettuce and other salad plants	2,8	Lettuce	
32,0	NL child	6,1	Table and wine grapes	5,0	Oranges	3,8	Head cabbage	
29,8	DE child	10,2	Table and wine grapes	6,1	Oranges	2,5	Solanacea	
29,8	WHO cluster diet E	14,3	Table and wine grapes	3,1	Head cabbage	2,8	Lettuce	
29,8	IE adult	9,3	Table and wine grapes	3,8	Celery	2,6	Lettuce	
28,8	WHO regional European diet	11,3	Lettuce	4,4	Head cabbage	3,1	Table and wine grapes	
26,8	PT General population	22,1	Table and wine grapes	2,2	Solanacea	1,0	Oranges	
26,2	ES adult	16,1	Lettuce	3,7	Table and wine grapes	2,1	Oranges	
25,1	WHO Cluster diet F	9,0	Lettuce	5,9	Table and wine grapes	3,3	Head cabbage	
22,7	IT adult	11,3	Lettuce	3,2	Other lettuce and other salad plants	2,7	Solanacea	
22,4	NL general	6,9	Table and wine grapes	3,6	Lettuce	2,5	Head cabbage	
21,6	ES child	12,5	Lettuce	3,5	Oranges	2,2	Solanacea	
20,2	FR toddler	4,0	Broccoli	3,2	Oranges	2,2	Leek	
19,7	UK vegetarian	7,1	Table and wine grapes	4,2	Lettuce	1,5	Solanacea	
19,2	SE general population 90th percentile	7,5	Head cabbage	2,0	Solanacea	1,8	Table and wine grapes	
19,0	IT kids/toddler	8,7	Lettuce	3,2	Solanacea	2,2	Other lettuce and other salad	
18,7	UK Adult	9,1	Table and wine grapes	3,5	Lettuce	1,0	HOPS (dried),	
17,9	WHO cluster diet D	4,4	Table and wine grapes	2,3	Solanacea	2,2	Leafy brassica	
15,7	DK adult	11,8	Table and wine grapes	1,1	Solanacea	0,7	Head cabbage	
12,9	UK Toddler	3,2	Oranges	2,2	Table and wine grapes	1,2	Solanacea	
12,7	DK child	4,2	Lettuce	1,6	Cucurbits - edible peel	1,5	Solanacea	
11,1	FR infant	3,0	Broccoli	1,5	Oranges	1,3	Spinach	
11,0	PL general population	4,4	Head cabbage	2,6	Table and wine grapes	2,0	Solanacea	
9,7	FI adult	2,6	Table and wine grapes	2,3	Lettuce	1,6	Oranges	
9,3	LT adult	4,8	Head cabbage	1,9	Lettuce	1,3	Solanacea	
7,8	UK Infant	2,1	Oranges	1,2	Head cabbage	0,8	PRODUCTS OF ANIMAL ORIGIN	

Table 6.9-9: IEDI calculation with input values for plant and animal matrices (dimethomorph plus relevant metabolites) as listed in Table 6.9-7

Dimethomorph			
Status of the active substance:	Approved	Code no.	
LOQ (mg/kg bw):	0,01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,05	ARfD (mg/kg bw):	0,6
Source of ADI:	EFSA	Source of ARfD:	EFSA
Year of evaluation:	2006	Year of evaluation:	2006

Prepare workbook for refined calculations

Undo refined calculations

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	3 12
No of diets exceeding ADI:	—

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
12,5	WHO Cluster diet B	2,7	Wine grapes	2,7	Lettuce	2,7	Solanacea	0,3
9,3	NL child	1,5	Oranges	1,2	Milk and cream,	1,1	Table grapes	0,2
9,0	FR all population	6,0	Wine grapes	0,7	Lettuce	0,5	Other lettuce and other salad	0,1
8,3	DE child	1,9	Table grapes	1,8	Oranges	0,9	Solanacea	0,1
7,8	IE adult	1,9	Wine grapes	0,7	Celery	0,6	Lettuce	0,3
7,7	WHO regional European diet	2,8	Lettuce	0,9	Head cabbage	0,9	Solanacea	0,1
7.1	FR toddler	1.6	Milk and cream.	1.1	Broccoli	1.0	Oranges	0.1
7,0	WHO cluster diet E	2,4	Wine grapes	0,7	Lettuce	0,7	Head cabbage	0,2
7,0	ES adult	4,0	Lettuce	0,7	Solanacea	0,6	Wine grapes	0,1
6,7	WHO Cluster diet F	2,2	Lettuce	0,9	Wine grapes	0,7	Head cabbage	0,1
6,7	ES child	3,1	Lettuce	1,0	Oranges	0,8	Solanacea	0,1
6,2	PT General population	3,7	Wine grapes	0,7	Solanacea	0,6	Potatoes	0,1
5,6	NL general	0,9	Wine grapes	0,9	Lettuce	0,7	Oranges	0,1
5,5	IT adult	2,8	Lettuce	0,9	Solanacea	0,4	Other lettuce and other salad	0,1
5,4	UK Toddler	0,9	Oranges	0,9	SUGAR PLANTS	0,8	Milk and cream,	0,1
5,3	SE general population 90th percentile	1,6	Head cabbage	0,7	Solanacea	0,5	Milk and cream,	0,1
5,1	UK vegetarian	1,2	Wine grapes	1,1	Lettuce	0,5	Solanacea	0,1
4,9	IT kids/toddler	2,2	Lettuce	1,1	Solanacea	0,3	Other lettuce and other salad	0,2
4,7	WHO cluster diet D	0,8	Solanacea	0,5	Wine grapes	0,5	Head cabbage	0,2
4,7	UK Adult	1,6	Wine grapes	0,9	Lettuce	0,3	Solanacea	0,1
4,5	DK child	1,1	Lettuce	0,9	Cucurbits - edible peel	0,5	Solanacea	0,2
4,4	FR infant	1,0	Milk and cream,	0,8	Broccoli	0,5	Potatoes	0,1
4,2	UK Infant	1,5	Milk and cream,	0,6	Oranges	0,4	SUGAR PLANTS	0,1
3,7	DK adult	2,1	Wine grapes	0,4	Solanacea	0,2	Milk and cream,	0,1
3,0	PL general population	0,9	Head cabbage	0,7	Solanacea	0,5	Table grapes	0,0
3,0	LT adult	1,0	Head cabbage	0,5	Lettuce	0,5	Solanacea	0,1
2,8	FI adult	0,6	Lettuce	0,5	Oranges	0,5	Wine grapes	0,0

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

IESTI calculation

An acute exposure assessment was performed using all crops and the residues according to the residue definition proposed for plants; the sum of HR plus M550F002 and M550F007, expressed as parent equivalents, and for animal matrices (sum of parent dimethomorph plus M550F006 and M550F007 for tissues and dimethomorph plus M550F008 for milk) as indicated in Table 6.9-7. Metabolite levels were derived from metabolism studies in plant and livestock as explained above and the results were extrapolated to the crop groups indicated in OECD guideline document 501. The summary of the calculation using the EFSA model is presented in Table 6.9-10.

The ARfD utilization was highest for consumption of celery of children with 72.3% followed by 51.4% ARfD for lettuce. Highest ARfD utilization for adults is by celery and head cabbage consumption (36.3 and 26.1%, respectively).

According to the presented IESTI calculation an acute intake of dimethomorph residues is unlikely to present a public health concern.

Table 6.9-10: IESTI calculation with input values for plant and animal matrices (dimethomorph plus relevant metabolites) listed in Table 6.9-7

Acute risk assessment /children	Acute risk assessment / adults / general population

The acute risk assessment is based on the ARfD.

For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.

In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.

In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.

Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.

Unprocessed commodities

No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
72.3	Celery	9.45570913323	72.3	Celery	9.45570913323	36.3	Celery	9.45570913323	26.8	Celery	9.45570913323
51.4	Lettuce	11.4712117602	31.7	Table grapes	2.90247962861	26.1	Head cabbage	4.94226973970	15.7	Head cabbage	4.94226973970
49.4	Scarole (broad-leaf	3.38775973481	30.9	Lettuce	11.4712117602	21.0	Lettuce	11.4712117602	15.4	Table grapes	2.90247962861
43.4	Head cabbage	4.94226973970	29.6	Scarole (broad-leaf	3.38775973481	15.4	Table grapes	2.90247962861	12.6	Lettuce	11.4712117602
31.7	Table grapes	2.90247962861	26.0	Head cabbage	4.94226973970	11.5	Wine grapes	2.90247962861	11.5	Wine grapes	2.90247962861
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
No of critical MRLs (IESTI 1)		0	---			**No of critical MRLs (IESTI 2)**		0	---		

Processed commodities

No of commodities for which ARfD/ADI is exceeded:		---	**No of commodities for which ARfD/ADI is exceeded:**		---
		***)			***)
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)
#ZAHL!	Grape juice	#ZAHL!	#ZAHL!	Wine	#ZAHL!
15.9	Orange juice	0.76978807541 4085 / -	1.9	Orange juice	0.76978807541 4085 / -
6.4	Tomato juice	0.93384127181	1.3	Tomato (preserved-	0.93384127181
2.7	Carrot, juice	0.05 / -	0.3	Raisins	2.90247962861
0.4	Wine	2.90247962861	0.2	Apple juice	0.01261947664
0.0	0	0.00	0.0	0	0.00
0.0	0	0.00			0.00
0.0	0	0.00			0.00
0.0	0	0.00			0.00
0.0	0	0.00			0.00
		0			0

Dietary risk assessments for dimethomorph metabolites that are not covered by the proposed residue definition

The main purpose of the information presented below is to support the establishment of a robust residue definition for risk assessment purposes. The data being summarized is going far beyond the scope of this dossier which supports the uses in grapes, strawberries and lettuce. In the assessment the contributions to the dietary risk of all crops that are currently registered in the EU (including import tolerances) were taken into account.

In order to keep this chapter more concise, details on the performed exposure assessment as well as on the derivation of the relevant exposure data for the metabolites are described in a separate report which is summarized in the following.

Report:	CA 6.9/1 Seiferlein M.,Bohner M., 2015 a Dimethomorph (BAS 550 F): Evaluation of the relevance of Dimethomorph metabolites in dietary risk assessment 2015/1253634
Guidelines:	none
GLP:	no

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 550 F (dimethomorph, Reg No.247723)
Description: not relevant
Lot/Batch #: not relevant
Purity: not relevant
CAS#: 110488-70-5
Development code: not applicable
Spiking levels: not relevant

3. **Test Commodity:**
Crop: not relevant
Type: not relevant
Variety: not relevant
Botanical name: not relevant
Crop parts(s) or processed commodity: not relevant
Sample size: not relevant

B. STUDY DESIGN AND METHODS

In order to assess the contribution of plant and livestock metabolites to the chronic dietary risk, the metabolites identified in the plant and livestock metabolism studies were grouped into the following groups based on structural similarity and similar metabolic steps:

- Group 0: Cleavage (free morpholin)
- Group 1A: Demethylation and conjugation
- Group 1B: Hydroxylation of morpholine ring
- Group 2: Morpholine ring opening and modification (opening at the oxygen atom)
- Group 3: Morpholine ring opening and modification (opening at the nitrogen atom)
- Group 4: Complete loss of morpholine ring (free nitrogen)
- Group 5: Complete loss of morpholine ring (cleavage, no free nitrogen)

Table 6.9-11: Grouping of dimethomorph plant and livestock metabolites and their endpoints used for risk assessment

Group No	Name	Metabolites	Proposed reference values	
			ADI (Chronic)	ARfD (Acute)
0	Cleavage	M550F021 (Morpholine)	0.05	0.75
1A	Demethylation and conjugation	M550F002, M550F006, M550F007, M550F013, M550F015, M550F016, M550F029	0.05	0.6
1B	Hydroxylation of morpholine ring	M550F018, M550F028 (one isomer), M550F074, M550F076	0.05	0.6
2	Morpholine ring opening and modification (opening at the oxygen atom)	M550F009, M550F017 (one isomer), M550F028 (other isomer), M550F035, M550F053	0.05	0.6
3	Morpholine ring opening and modification (opening at the nitrogen atom)	M550F008, M550F011, M550F017 (other isomer), M550F022, M550F030, M550F031, M550F033, M550F049, M550F069,	0.05	0.6
4	Complete loss of morpholine ring (by degradation, free nitrogen)	M550F012, M550F038, M550F062, M550F091	0.05	0.6
5	Complete loss of morpholine ring (by cleavage, no free nitrogen)	M550F003	0.05	No ARfD

A justification for the proposed reference values can be found in chapter M-CA 5.8 of this dossier.

The input data for the exposure assessment were derived for the individual groups as follows:

Groups 0, 1B, 2, 4, and 5:
Input values were derived from plant metabolism and confined rotational crop and livestock metabolism studies. From plant metabolism studies, metabolite/parent ratios were calculated and applied to parent STMR/HR values. Suitable extrapolations to other crop groups were made. Residue levels from rotational crops were taken directly and normalized to the cGAP if necessary. Residue levels in animal tissues were taken from the metabolism studies and normalized to the maximum dietary burden for livestock. The contribution via feed items into animal tissues was additionally considered by estimating feed intakes for each metabolite and considering the transference to animal tissues by the excretion factor as observed in the livestock metabolism studies.

Group 1A:
Generally the same strategy as described for the other groups was used. For metabolites M550F002 and M550F007 in plants, additionally field residue data was available for strawberries, which was used to derive a metabolite/parent ratio. This ratio from field trials was compared to the ratio derived for grapes from the metabolism studies and the higher one of both was used in order to capture a worst case. For the livestock metabolites M550F006 and M550F007 in this group, residue data from the cattle feeding study (from the 1x dose level) was used.

Group 3:
Generally the same strategy as described for the other groups was used. For the livestock metabolite M550F008 in this group, residue data from the cattle feeding study (from the 1x dose level) was used.

II. RESULTS AND DISCUSSION

A. Chronic risk assessment

Group 0 – Cleavage (Free morpholine)

Group 0 – Metabolite M550F021

With the current EFSA model the chronic risk assessment shows a maximum ADI exhaustion of 0.6%. For the contribution of plant matrices, the diet with the highest TMDI is "WHO cluster diet B" with 0.3% of ADI. For this diet, the highest contributors is lettuce with 0.1% of ADI.
For the contribution of animal matrices, the diet with the highest TMDI is "FR toddler" with 0.3% of ADI. For this diet, the highest contributors is milk and cream with 0.3% of ADI.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
0	M550F021	0.05	Plant Rot crop Animal Feed item Overall	0.3 0.0 0.3 0.0 0.6

This calculation, based on metabolite/parent ratios or metabolite levels from the metabolism studies, shows already a negligible exposure. Comparing the input data of this calculation with the now available field residue data for morpholine in the representative crops strawberry, grapes and lettuce shows that the above shown original calculation already covered a worst case and that the realistic consumer exposure might even be a bit lower.

Group 1A – Demethylation and conjugation

Group 1A -M550F002

With the current EFSA model the chronic risk assessment shows a maximum ADI exhaustion of 2.2%. For the contribution of plant matrices (target use), the diet with the highest TMDI is "WHO cluster diet B" with 1.5% of ADI. For this diet, the highest contributors are wine grapes with 0.5% of ADI.

For the contribution of rotational crops, the diet with the highest TMDI is "UK toddler" with 0.7% of ADI. For this diet, the highest contributors sugar beet (root) with 0.6% of ADI.

Group 1A -M550F006

With the current EFSA model the chronic risk assessment shows a maximum ADI exhaustion of 1.6%. The entire contribution is via animal matrices. The diet with the highest TMDI is "FR toddler" with 1.6% of ADI. For this diet, the highest contributors is milk and cream with 1.6% of ADI.

Group 1A -M550F007

With the current EFSA model the chronic risk assessment shows a maximum ADI exhaustion of 2.3%. For the contribution of plant matrices (target use), the diet with the highest TMDI is "WHO cluster diet B" with 0.2% of ADI. For this diet, the highest contributors are wine grapes with 0.1% of ADI.

For the contribution of rotational crops, the diet with the highest TMDI is "UK toddler" with 0.5% of ADI. For this diet, the highest contributors sugar beet (root) with 0.4% of ADI.

For the contribution of animal products, the diet with the highest TMDI is "FR toddler" with 1.6% of ADI. For this diet, the highest contributors is milk and cream with 1.6% of ADI.

Group 1A -M550F013, M550F015, M550F016, M550F029

With the current EFSA model the chronic risk assessment for all these livestock metabolites is 0.0% of ADI. The diet with the highest TMDI is "IE adult" with 0.0% of ADI. For this diet, the highest contributors is sheep liver with 0.0% of ADI.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
1A	M550F002	0.05	Plant Rot crop Feed item Overall	1.5 0.7 0.0 2.2
	M550F006		Plant Animal Overall	0.0 1.6 1.6
	M550F007		Plant Rot crop Animal Feed item Overall	0.2 0.5 1.6 0.0 2.3
	M550F013		Animal	0.0
	M550F015		Animal	0.0
	M550F016		Animal	0.0
	M550F029		Animal	0.0

Group 1 B – Hydroxylation of morpholine ring

Group 1B -M550F018

With the current EFSA model the chronic risk assessment shows a maximum ADI exhaustion of 0.9% .For the contribution of plant matrices, the diet with the highest TMDI is "WHO cluster diet B" with 0.6% of ADI. For this diet, the highest contributors are wine grapes with 0.2% of ADI.
For the contribution of rotational crops, the diet with the highest TMDI is "UK toddler" with 0.1% of ADI. For this diet, the highest contributors sugar beet (root) with 0.0% of ADI.
For the contribution of animal products the diet with the highest TMDI is "FR toddler" with 0.2% of ADI. For this diet, the highest contributors is milk and cream with 0.2% of ADI.

Group 1B -M550F028, M550F074, M550F076

With the current EFSA model the chronic risk assessment for all these livestock metabolites is 0.0% of ADI. The diet with the highest TMDI for M550F028 is "IE adult" with sheep liver as the highest contributor with 0.0% of ADI.
For metabolites M550F074 and M550F076, using the toxicological endpoints of the group 1B for the contribution of animal matrices the chronic risk assessment is 0.0% of ADI. The diet with the highest TMDI is "NL child" with 0.0% of ADI. For this diet, the highest contributors is poultry liver with 0.0% of ADI. The same outcome is observed using the genotoxic trigger of 0.0025 µg/kg as toxicological endpoint for these two metabolites.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
1B	M550F018	0.05	Plant Rot crop Animal Feed item Overall	0.6 0.1 0.2 0.0 0.9
	M550F028		Animal	0.0
	M550F074		Animal	0.0
	M550F076		Animal	0.0

Group 2 – Morpholine ring opening and modification (opening at the oxygen atom)

Group 2 -M550F009, M550F028, M550F035
With the current EFSA model the chronic risk assessment for all these livestock metabolites is 0.0% of ADI. The diet with the highest TMDI is either "IE adult" with sheep liver as the highest contributor with 0.0% of ADI or "NL child" with poultry liver as the highest contributor with 0.0% of ADI.

Group 2 -M550F017
With the current EFSA model the chronic risk assessment for this plant metabolite shows a maximum exhaustion of 0.7% of ADI. The diet with the highest TMDI is "WHO cluster diet B" with 0.7% of ADI. For this diet, the highest contributors is lettuce with 0.3% of ADI.

Group 2 -M550F053
With the current EFSA model the chronic risk assessment for this livestock metabolite shows a maximum exhaustion of 0.2% of ADI. The diet with the highest TMDI is "FR toddler" with 0.2% of ADI. For this diet, the highest contributors are milk and cream with 0.2% of ADI.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
2	M550F009	0.05	Animal	0.0
	M550F017		Plant Feed item Overall	0.7 0.0 0.7
	M550F028		Animal	0.0
	M550F035		Animal	0.0
	M550F053		Animal	0.2

Group 3 – Morpholine ring opening and modification (opening at the nitrogen atom)

Group 3 -M550F008
With the current EFSA model the chronic risk assessment for this livestock metabolite shows a maximum exhaustion of 0.8% of ADI. The diet with the highest TMDI is "FR toddler" with 0.8% of ADI. For this diet, the highest contributors are milk and cream with 0.8% of ADI.

Group 3 -M550F011
With the current EFSA model the chronic risk assessment for this livestock metabolite shows a maximum exhaustion of 0.4% of ADI. The diet with the highest TMDI is "FR toddler" with 0.4% of ADI. For this diet, the highest contributors are milk and cream with 0.4% of ADI.

Group 3 -M550F017
With the current EFSA model the chronic risk assessment for this plant metabolite shows a maximum exhaustion of 0.7% of ADI. The diet with the highest TMDI is "WHO cluster diet B" with 0.7% of ADI. For this diet, the highest contributors is lettuce with 0.3% of ADI.

Group 3 -M550F031
With the current EFSA model the chronic risk assessment for this livestock metabolite shows a maximum exhaustion of 0.4% of ADI. The diet with the highest TMDI is "FR toddler" with 0.4% of ADI. For this diet, the highest contributors are milk and cream with 0.4% of ADI.

Group 3 -M550F022, M550F030, M550F033, M550F049
With the current EFSA model the chronic risk assessment for all these livestock metabolites is 0.0% of ADI. The diets with the highest TMDI is either "UK infant" with bird's eggs with 0.0% of ADI, "IE adult" with sheep liver as the highest contributor with 0.0% of ADI or "NL child" with poultry liver as the highest contributor with 0.0% of ADI.

Group 3 -M550F069
Using the toxicological endpoints of the group 1B for the contribution of animal matrices the chronic risk assessment is 0.0% of ADI. The diet with the highest TMDI is "NL child" with 0.0% of ADI. For this diet, the highest contributors is poultry liver with 0.0% of ADI. The same outcome is observed using the genotoxic trigger of 0.0025 µg/kg as toxicological endpoint.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
3	M550F008	0.05	Animal	0.8
	M550F011		Animal	0.4
	M550F017		Plant Feed item Overall	0.7 0.0 0.7
	M550F022		Animal	0.0
	M550F030		Animal	0.0
	M550F031		Animal	0.4
	M550F033		Animal	0.0
	M550F049		Animal	0.0
	M550F069		Animal	0.0

Group 4 - Complete loss of morpholine ring (by degradation, free nitrogen)

Group 4 -M550F012, M550F038, M550F091
With the current EFSA model the chronic risk assessment for all these livestock metabolites is 0.0% of ADI. The diets with the highest TMDI is either "UK infant" with milk and cream or bird's eggs with 0.0% of ADI as the highest contributor.

Group 4 -M550F062
Using the toxicological endpoints of the group 1B for the contribution of animal matrices the chronic risk assessment is 0.0% of ADI. The diet with the highest TMDI is "NL child" with 0.0% of ADI. For this diet, the highest contributors is poultry liver with 0.0% of ADI. The same outcome is observed using the genotoxic trigger of 0.0025 µg/kg as toxicological endpoint.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
4	M550F012	0.05	Animal	0.0
	M550F038		Animal	0.0
	M550F062		Animal	0.0
	M550F091		Animal	0.0

Group 5 - Complete loss of morpholine ring (by cleavage, no free nitrogen)

Group 5 -M550F003
With the current EFSA model the chronic risk assessment for this plant metabolite is 0.0% of ADI. The diet with the highest TMDI is "WHO regional European diet" with the highest contributor being lettuce with 0.0% of ADI.

Metabolite group	Metabolites	ADI used [mg/kg bw/day]	Contribution	ADI utilization [%]
5	M550F003	0.1	Plant	0.0

B. Acute risk assessment

Group 0 – Cleavage (Free morpholine)

Group 0 – Metabolite M550F021
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for celery (1.9 %), followed by lettuce (1.3%) and scarole (1.3 %).
Residues of M550F021 from rotational crops resulted in ARfD utilizations of 0% and thus did not contribute to the acute dietary risk.
Concerning the contribution via animal products, highest ARfD utilization resulted from cattle milk and cream (0.1%) followed by goat milk and cream (0.0%).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
0	M550F021	0.75	Plant Rot crop Animal Feed item Overall	1.9 0.0 0.1 0.0 2.0

Group 1A – Demethylation and conjugation

Group 1A -M550F002

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for table grapes (5.7%), followed by celery (4.0%) and oranges (3.1 %).
In the acute risk assessment for the contribution of rotational crops, the highest ARfD utilization was observed for scarole (0.4%), followed by potatoes (0.3%) and kale (all 0.3%).

Group 1A -M550F006

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for celery (0.4%), followed by lettuce (0.3%) and scarole (0.2%).
Concerning the contribution via animal products, highest ARfD utilization resulted from cattle milk and cream (0.4%) followed by goat milk and cream (0.1%).

Group 1A -M550F007

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for table grapes (0.9%), followed by oranges (0.5%) and peppers (0.3 %). In the acute risk assessment for the contribution of rotational crops, the highest ARfD utilization was observed for potatoes (0.2%), followed by scarole (0.1%) and kale (0.1%).
Concerning the contribution via animal products, highest ARfD utilization resulted from cattle milk and cream (0.4%) followed by goat milk and cream (0.1%).

Group 1A -M550F013, M550F015, M550F016, M550F029

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization for all these livestock metabolites were for bovine liver, followed by bovine kidney and swine kidney or liver (all 0.0%).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
1A	M550F002	0.6	Plant Rot crop Feed item Overall	5.7 0.4 0.0 6.1
	M550F006		Plant Animal Overall	0.4 0.4 0.8
	M550F007		Plant Rot crop Animal Feed item Overall	0.9 0.2 0.4 0.0 1.5
	M550F013		Animal	0.0
	M550F015		Animal	0.0
	M550F016		Animal	0.0
	M550F029		Animal	0.0

Group 1 B – Hydroxylation of morpholine ring

Group 1B -M550F018

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed was observed for celery (2.6 %), followed by table grapes (2.1%) and lettuce (1.9 %). The contribution via animal products showed a highest ARfD utilization for cattle milk and cream (0.1%) followed by goat milk and cream and bovine liver (all 0.0%).

Group 1B -M550F028, M550F074, M550F076

With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization for M550F028 was for bovine liver, swine liver and bird's eggs (all 0.0%). Metabolites M550F074 and M550F076 had no contribution at all independent of the toxicological endpoints used (either parent reference value or genotox trigger of 0.0025 µg/kg).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
1B	M550F018	0.6	Plant Rot crop Animal Feed item Overall	2.6 0.0 0.1 0.0 2.7
	M550F028		Animal	0.0
	M550F074		Animal	0.0
	M550F076		Animal	0.0

Group 2 – Morpholine ring opening and modification (opening at the oxygen atom)

Group 2 -M550F009, M550F028, M550F035, M550F053
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization for M550F009 was for bovine liver, swine liver and milk and cream (all 0.0%). For M550F028, highest ARfD utilization was for bovine liver, swine liver and bird's eggs (all 0.0%).
Metabolite M550F035 had no contribution at all and for M550F053 the highest ARfD utilization was for milk and cream (0.0%).

Group 2 -M550F017
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for celery (7.2 %), followed by lettuce (5.1%) and scarole (4.9 %).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
2	M550F009	0.6	Animal	0.0
	M550F017		Plant Feed item Overall	7.2 0.0 7.2
	M550F028		Animal	0.0
	M550F035		Animal	0.0
	M550F053		Animal	0.0

Group 3 – Morpholine ring opening and modification (opening at the nitrogen atom)

Group 3 -M550F008
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for cattle milk and cream (0.2%) followed by goat milk and cream and bovine liver (0.0%).

Group 3 -M550F011
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for milk and cream (0.1%) followed by bovine liver (0.0%).

Group 3 -M550F017
With the current EFSA model, using the HR for the acute risk assessment, highest ARfD utilization was observed for celery (7.2 %), followed by lettuce (5.1%) and scarole (4.9 %).

Group 3 -M550F022, M550F030, M550F033, M550F049
With the current EFSA model, using the HR for the acute risk assessment, for all these livestock metabolites a highest ARfD of 0% was estimated. M550F022 and M550F033 had only an ARfD utilization for bird's eggs (0.0%). M550F030 had the highest ARfD utilization for bovine liver (0.0%) followed by swine liver (0.0%). M550F049 showed no ARfD utilization at all.

Group 3 -M550F069
Contribution of animal products had no highest ARfD utilization independent of toxicological endpoints used (either parent reference value or genotox trigger of 0.0025 µg/kg).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
3	M550F008	0.6	Animal	0.2
	M550F011		Animal	0.1
	M550F017		Plant Feed item Overall	7.2 0.0 7.2
	M550F022		Animal	0
	M550F030		Animal	0
	M550F031		Animal	0.1
	M550F033		Animal	0
	M550F049		Animal	0
	M550F069		Animal	0

Group 4 - Complete loss of morpholine ring (by degradation, free nitrogen)

Group 4 -M550F012, M550F038, M550F091
With the current EFSA model, using the HR for the acute risk assessment, for all these livestock metabolites an ARfD utilization of 0% was estimated. M550F012 had the only ARfD utilization for bovine liver, followed by milk and cream and swine liver (all 0.0%). M550F038 and M550F091 had the only ARfD utilization for bird's eggs (0.0%).

Group 4 - M550F062
Contribution of animal products had no highest ARfD utilization for this metabolite independent of toxicological endpoints used (either parent reference value or genotox trigger of 0.0025 µg/kg).

Metabolite group	Metabolites	ARfD used [mg/kg bw/day]	Contribution	ARfD utilization [%]
4	M550F012	0.6	Animal	0.0
	M550F038		Animal	0.0
	M550F062		Animal	0.0
	M550F091		Animal	0.0

Group 5 - Complete loss of morpholine ring (by cleavage, no free nitrogen)

Group 5 -M550F003
No acute risk assessment is necessary as no ARfD has been allocated for this metabolite.

III. CONCLUSION

Dimethomorph follows a common pathway in crops and livestock. In general, the following metabolic key steps were found in all commodities investigated.

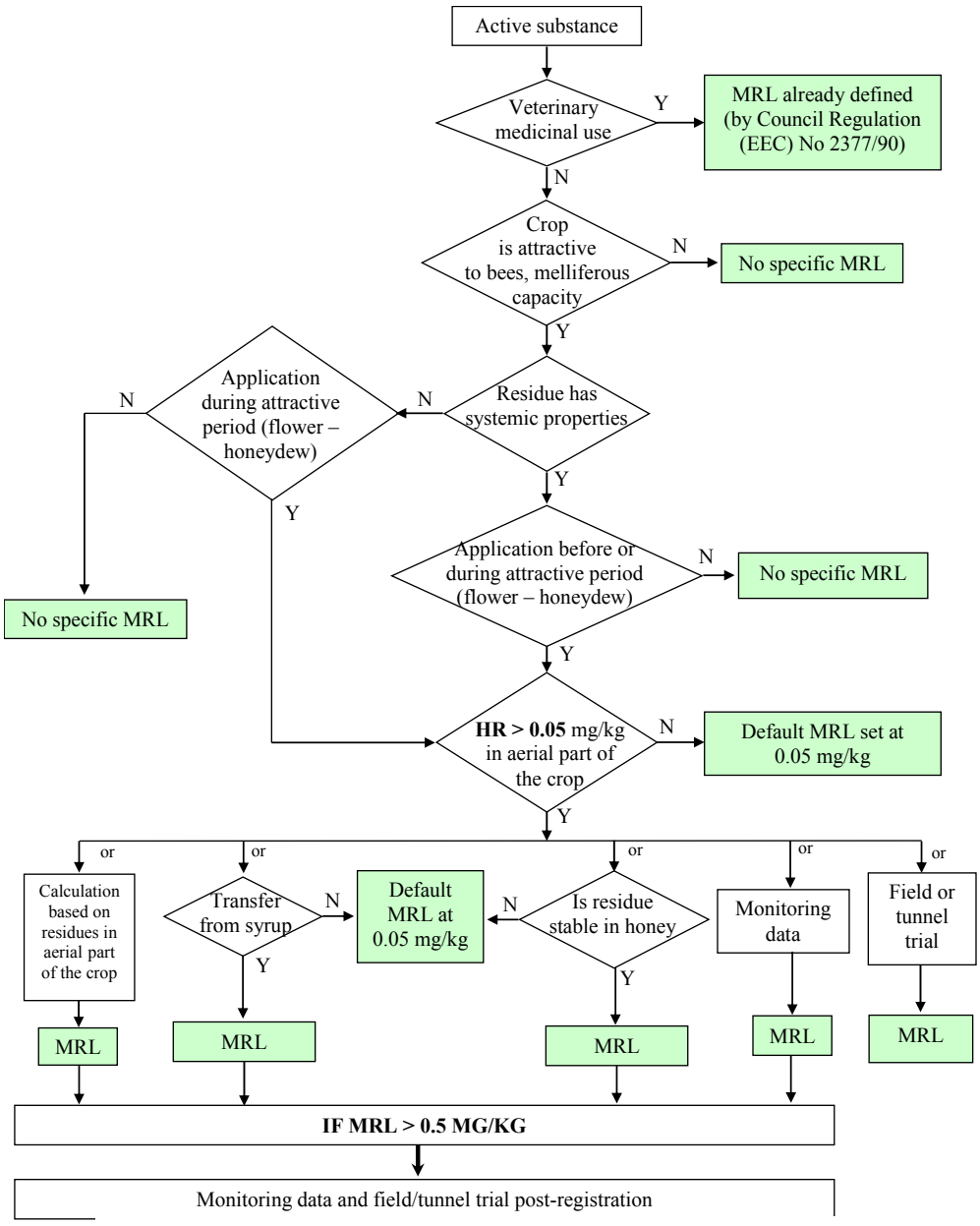
- Demethylation at the dimethoxyphenyl ring and subsequent conjugation
- Hydroxylation of the morpholine ring
- Opening of the morpholine ring and further modifications (degradation, conjugation)
- Cleavage/hydrolysis and release of the intact morpholine ring

For performing indicative assessments, the metabolites were grouped according to similarity of structures and metabolic steps. The acute and chronic dietary exposure was estimated based on all available data (combination of metabolism information and data from residue field trials / feeding studies). The exposure estimates applying worst case assumptions did not indicate any dietary concern, neither chronic nor acute for any population subgroup. The calculation of the % ADI or ARfD utilizations resulted in values far below 100% and – even more important - clearly below the respective utilizations of the parent molecule. No significant contribution to the chronic or acute exposure can be expected from any plant or livestock metabolite. Nevertheless, also based on the overall picture gained from the metabolism studies, the plant and livestock metabolites showing the highest contributions (M550F002, M550F006, M550F007, M550F008) were included into the residue definitions for risk assessment.

CA 6.10 Other studies

CA 6.10.1 Effect on the residue level in pollen and bee products

The need to address this data requirement was discussed with the RMS since up to now no final guidance document is available. It was agreed to follow the decision making scheme for setting MRLs in honey as contained in the working document SANCO 11105/2009 rev. 0 from December 2009 (see Appendix II).



MRL : Maximal Residue Level – HR : Highest Residue

The representative uses in this dossier are grapes, lettuce and strawberries. Of these crops, grapes and strawberries could be relevant for bees and honey production. While in the working document SANCO 11105/2009 rev. 0 for both grapes and strawberries the presence of nectar/honeydew (i.e. melliferous properties) are mentioned, in the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees (see EFSA Journal 2013;11(7):3295) the attractiveness of grapes for honeybees is only mentioned for pollen, while for nectar the attractiveness is low. For strawberries however, both documents state an attractiveness to honeybees for nectar and the presence of nectar/honeydew, i.e. melliferous capacity. So, as a conclusion strawberry can be seen as a crop attractive to bees and having melliferous capacities. The next step is the question on systemic properties of the active ingredient. Dimethomorph is known to have acropetal systemicity, i.e. residues can be transported from the root upwards to the leaves while no transport occurs in the other direction (no transport from leaves into the roots). This means that it cannot be excluded that dimethomorph residues are transported from leaves (after spray application) or from roots (after drip application) to upper parts of the plant. According to the proposed GAP, application is intended at growth stage BBCH 15-42, thus at the stages of leaf unfolding and development of stolons and young plants. Inflorescence emergence starts at BBCH 55 and full flowering is around BBCH 65. Thus, the application is made well before development of flowers. Following the decision making scheme, the next step is the question on residue levels in aerial parts of the respective crop. Since no information on the residue levels in flowers and leaves from strawberries treated according to the proposed GAP were available, it was decided to generate as a first step this information and perform residue trials and measure residues in leaves and inflorescences.

The following study provides this information about residue levels in infloresences of strawberries that may be used for estimating an MRL for dimethomorph residues in honey.
Since the study was also intended to serve as a field residue trial study, fruits were sampled as well and all samples were analyzed for residues of parent dimethomorph and metabolites M550F002 and M550F007. The study was conducted in order to respect a PHI of 35 days (for serving as a valid residue study), thus the timepoint of last application was as late as BBCH 55-61 (inflorescences already present) with the crop varieties used in the study. However, the use pattern as it is actually practiced by the farmer and defined by the label has a much earlier application timing until BBCH 42 latest (one application directly after transplanting, no inflorescences present). A combination of this application timing with a PHI of 35 days is only the case with certain fast-developing varieties. It can thus be concluded that in practice the intended use pattern in strawberries will have applications earlier than in this study, definitely before flowering and thus the residue values generated in the study below do represent a worst case, which will not be reached by common practice of the intended use pattern.

Report:	CA 6.10.1/1 Plier S., 2015 a Study on the residue behaviour of Dimethomorph (BAS 550 F) in strawberries after treatment with BAS 550 01 F under field conditions in Northern Europe, 2014/2015 2015/1000641
Guidelines:	EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009), EC 1107/2009 (14 June 2011), EEC 7029/VI/95 rev. 5 (July 22 1997)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 550 01 F (WP)
Lot/Batch #:	FRE-001045, FRE-001184 (dimethomorph 500 g/kg, nominal)
Purity:	Not relevant
CAS#:	110488-70-5 (dimethomorph)
Development code:	
Spiking levels:	0.005-250 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and small fruit
Variety:	Clery, Polka, Ostara, Christine
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Leaves, inflorescences, fruits
Sample size:	Min. 0.2 kg

B. STUDY DESIGN

1. Test procedure

In 2014/15, 4 field trials were conducted with strawberries in Northern Europe (Germany, The Netherlands, Belgium and United Kingdom). Formulation BAS 550 01 F was applied three times as root drench applications at individual rates of 0.05 g a.s./plant in 100 mL water. The first application was directly after planting, the second was 27-28 days thereafter, and the third was 35-36 days before harvest.
On a separate plot, the third application was a spray application of 1.5 kg a.s./ha in a spray volume of 400 L/ha instead. Data related to this plot are not summarized any further as they are not relevant here.
For the drench plot there were four samplings. Sampling 1 was immediately before the last application (leaves), sampling 2 was at growth stage BBCH 65 (leaves and inflorescences) and sampling 3 was 35-36 days after the last application (DALA; fruits). A fourth sampling was only needed in one trial because the growth stage BBCH 87/89 was not yet reached at sampling 3.
Samples were stored deep frozen from harvest until analysis for a maximum of 209 days.

Table 6.10.1-1: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (g a.s./pant^2)	Water volume (mL/plant)	Target date/ Timing
2014/15	4	3	F	BAS 550 01 F (WP)	Dimethomorph	0.05	100	4. Directly after planting 5. 28 days after 1st appl. 6. 35 DBH

DBH Days before harvest

2. Description of analytical procedures

All specimens were analyzed for dimethomorph *E*-isomer and *Z*-isomer using BASF method No L0013/02 and for metabolites M550F002 and M550F007 using BASF method No L0013/03. Both methods have a limit of quantitation (LOQ) of 0.01 mg/kg for each metabolite. For BASF method No L0013/02, 0.01 mg/kg is the sum of both isomers (LOQ 0.005 mg/kg for each isomer). The limit of detection (LOD) is 0.001 mg/kg for each isomer and 0.0025 mg/kg for each metabolite.

Principle of BASF Method No. L0013/02

Dimethomorph *E*- and *Z*-Isomer are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination is performed by HPLC-MS/MS.

Principle of BASF Method No. L0013/03

M550F002 and M550F007 are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned twice against dichloromethane. The final determination is performed by HPLC-MS/MS.

The results of procedural recovery experiments are summarized in the following table:

Table 6.10.1-2: Summary of recoveries for dimethomorph in strawberry

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Method No L0013/02		***E*-BAS 550 F**			***Z*-BAS 550 F**		
Leaves	0.005-250	14	92.0	9.0	14	94.9	13
Inflorescences	0.005-50	4	91.2	3.1	4	82.9	3.7
Fruit	0.005-0.5	6	96.4	5.3	6	96.3	16
Method No L0013/03		**M550F002**			**M550F007**		
Leaves	0.01-50	9	78.5	6.7	9	103	4.7
Inflorescences	0.01-10	4	77.2	9.2	4	103	9.9
Fruit	0.01-0.1	8	74.1	2.9	8	89.6	2.3

II. RESULTS AND DISCUSSION

A summary of residues is presented in Table 6.10.1-3 and Table 6.10.1-4. Details are shown in Table 6.10.1-5 and Table 6.10.1-6.

Dimethomorph *E*- and *Z*-isomers
In inflorescences specimens the sum of residues of dimethomorph *E*- and *Z*-isomers ranged between 0.033-2.0 mg/kg.
In fruits specimens the sum of residues of dimethomorph *E*- and *Z*-isomers ranged between <0.01-0.058 mg/kg at harvest (growth stage BBCH 87-89).
No residues of dimethomorph *E*- and *Z*-isomers above the LOQ were found in any control specimen, except for one inflorescences sample with 0.10 mg/kg and one leaf sample with 0.36 mg/kg.

Residues of M550F002 and M550F007
In inflorescences specimens the residues of M550F002 (expressed as parent equivalent) ranged between <0.007-0.020 mg/kg and the residues of M550F007 (expressed as parent equivalent) ranged between <0.01-0.018 mg/kg.
In fruits specimens the residues of M550F002 (expressed as parent equivalent) ranged between <0.007-0.008 mg/kg at growth stage BBCH 87-89 and the residues of M550F007 (expressed as parent equivalent) were <0.01 mg/kg at growth stage BBCH 87-89.
No residues of M550F002 and M550F007 above the LOQ were found in any control specimen.

Table 6.10.1-3: Summary of dimethomorph residues in strawberry after drench application of BAS 550 01 F

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	E-BAS 550 F	Z-BAS 550 F	Total BAS 550 F
Strawberry (drench; field)	2014/15	BAS 550 01 F 3 x 0.05 g a.s./plant	0	55-61	Leaves	0.010-0.40	0.057-1.5	0.066-1.9
			4-9	65	Leaves	0.008-1.2	0.057-3.4	0.065-4.5
			4-9	65	Inflorescences	0.011-1.0	0.022-1.0	0.033-2.0
			36	81-85	Fruits	0.006	0.062	0.069
			35/42	87-89	Fruits	<0.005-0.014	<0.005-0.050	<0.01-0.058

1 Days after last application

Table 6.10.1-4: Summary of dimethomorph metabolite residues in strawberry after drench application of BAS 550 01 F

Crop	Year	Application	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)		
					Matrix	M550F002[2]	M550F007[3]
Strawberry (drench; field)	2014/15	BAS 550 01 F 3 x 0.05 g a.s./plant	-0	55-61	Leaves	0.12-0.28	<0.01-0.020
			4-9	65	Leaves	0.075-0.36	<0.01-0.028
			4-9	65	Inflorescences	<0.007-0.020	<0.01-0.018
			36	81-85	Fruits	0.008	<0.01
			35/42	87-89	Fruits	<0.007-0.008	<0.01

1 Days after last application
2 Expressed as parent equivalent; conversion factor 0.723654
3 Expressed as parent equivalent; conversion factor 1.03752

III. CONCLUSION

Residues of dimethomorph in inflorescence specimens were in the range of 0.033-2.0 mg/kg. Residues of M550F002 ranged from <0.007-0.02 mg/kg, and M550F007 residues were between <0.01 and 0.018 mg/kg.

Table 6.10.1-5: Residues of dimethomorph (BAS 550 F) in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)			
							Matrix	*E*-BAS 550 F	*Z*-BAS 550 F	Total BAS 550 F
Study code:	425661	Strawberry	Germany	BAS 550 01 F	61	-0	Leaves	0.40	1.5	1.9
Doc ID:	2015/1000641			3 x 0.05		7	Inflorescences	1.0	0.97	2.0
Trial No:	L140723					7	Leaves	1.2	3.4	4.5
GLP:	yes					36	Fruits	0.0062	0.062[3]	0.069
Year:	2014/15					42	Fruits	0.014	0.036	0.050
Study code:	425661	Strawberry	The Netherlands	BAS 550 01 F	55	-0	Leaves	0.0097	0.057	0.066
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	0.074	0.13[3]	0.20
Trial No:	L140724					9	Leaves	0.0078	0.057	0.065
GLP:	yes					35	Fruits	<0.005	<0.005	<0.01
Year:	2014/15									
Study code:	425661	Strawberry	Belgium	BAS 550 01 F	55	-0	Leaves	0.10	0.33	0.43
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	0.011	0.022	0.033
Trial No:	L140725					9	Leaves	0.044	0.17[3]	0.21
GLP:	yes					35	Fruits	<0.005	<0.005	<0.01
Year:	2014/15									
Study code:	425661	Strawberry	United Kingdom	BAS 550 01 F	61	-0	Leaves	0.082	0.36	0.44
Doc ID:	2015/1000641			3 x 0.05		4	Inflorescences	0.52[4]	1.0[5]	1.5[6]
Trial No:	L140726					4	Leaves	0.64[7]	1.6[8]	2.2[9]
GLP:	yes					35	Fruits	0.0079	0.050	0.058
Year:	2014/15									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two injections
4 A residue of 0.035 mg/kg was found in the untreated control sample
5 A residue of 0.066 mg/kg was found in the untreated control sample
6 A residue of 0.10 mg/kg was found in the untreated control sample
7 A residue of 0.12 mg/kg was found in the untreated control sample
8 A residue of 0.24 mg/kg was found in the untreated control sample
9 A residue of 0.36 mg/kg was found in the untreated control sample

Table 6.10.1-6: Residues of dimethomorph metabolites in strawberry after root drench application in Northern Europe

Trial details		Crop	Country	Formulation Application rate[0] (g a.s./plant)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)		
							Matrix	M550F002[4]	M550F007[5]
Study code:	425661	Strawberry	Germany	BAS 550 01 F	61	-0	Leaves	0.28	0.020
Doc ID:	2015/1000641			3 x 0.05		7	Inflorescences	0.015	<0.01
Trial No:	L140723					7	Leaves	0.22	0.021
GLP:	yes					36	Fruits	0.0084	<0.01
Year:	2014/15					42	Fruits	<0.007	<0.01
Study code:	425661	Strawberry	The Netherlands	BAS 550 01 F	55	-0	Leaves	0.12	<0.01
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	<0.007	<0.01
Trial No:	L140724					9	Leaves	0.085[3]	<0.01
GLP:	yes					35	Fruits	<0.007	<0.01
Year:	2014/15								
Study code:	425661	Strawberry	Belgium	BAS 550 01 F	55	-0	Leaves	0.13	<0.01
Doc ID:	2015/1000641			3 x 0.05		9	Inflorescences	<0.007	<0.01
Trial No:	L140725					9	Leaves	0.075	<0.01
GLP:	yes					35	Fruits	<0.007	<0.01
Year:	2014/15								
Study code:	425661	Strawberry	United Kingdom	BAS 550 01 F	61	-0	Leaves	0.20	0.011
Doc ID:	2015/1000641			3 x 0.05		4	Inflorescences	0.020	0.018
Trial No:	L140726					4	Leaves	0.36	0.028
GLP:	yes					35	Fruits	0.008	<0.01
Year:	2014/15								

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two injections
4 Expressed as parent equivalent; conversion factor 0.723654
5 Expressed as parent equivalent; conversion factor 1.03752

Estimation of a maximum residue level in honey
As proposed in the working document SANCO 11105/2009 rev. 0 from December 2009, residue levels in honey can be roughly estimated based on the residue data for parent dimethomorph (sum of isomers) in inflorescences.

The observed residues were

0.033, 0.20, 1.5, 2.0 mg/kg

Based on these residue values and using the OECD MRL calculator, an **MRL proposal of 5 mg/kg** would result. This value would clearly represent a gross overestimate of dimethomorph residues in honey for two reasons: first due to the unrealistically late application in the study (later than practiced) and second also due to not considering the transfer from flowers to honey. No reliable data are available for the determination of such a transfer factor for dimethomorph. However, it should be mentioned that honey from flowers is always a blend and would not be consisting purely of nectar coming from strawberries inflorescences. Also, up to now no dimethomorph residues were ever occurring in any monitoring honey samples. Respective relevant hits in the literature search which dealt with investigation of honey samples for pesticide residues also confirm this finding, no residues of dimethomorph were observed in honey.

Dimethomorph

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

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e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
31/03/2016	MCA Section 7, Assessment regarding the POP/PBT/vPvB properties of a.s. dimethomorph CA 7.1.2 Rate of degradation in soil CA 7.2.1 Route and rate of degradation in aquatic systems	MCA Section 7 Version 1 BASF DocID 2016/1000211
31/05/2017	Due to a mistake in the report, Doc ID 2014/1183299 is replaced by 2017/1021008	MCA Section 7 Version 2 BASF DocID 2016/1103874

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Dimethomorph (BAS 550 F), a fungicide for use in various crops (e.g. grapes, lettuce and strawberries), is registered in Europe for many years. It was fully reviewed under Directive 91/414/EEC and is included in Annex I by Commission Directive No 2007/25/EC. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 July 2018 by Implementing Regulation (EU) No 2015/404 of 11 March 2015, amending Implementing Regulation (EU) No 430/2011.

All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the DAR (2004) plus addendum (2006), in the EFSA conclusion on dimethomorph (2006) and in SANCO/1004/06-rev. 3 (EU Review Report of November 2006).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective environmental fate chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for environmental fate no summaries of public literature data on dimethomorph are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

The second source of data was unpublished reports which were made available by BASF SE for review. Most of these reports were of recent studies conducted according to guidelines and conducted under good laboratory practice with quality assurance and quality control. All relevant information on these studies is given in detail in the following chapters.

Based on new information and new guidance available the following areas have been re-evaluated in detail:

- New evaluations of previously submitted data considering new scientific and technical developments (kinetic evaluations).
- New metabolism and degradation studies (aerobic and anaerobic metabolism studies) to supply information on a third label of dimethomorph.
- Support of existing information by further reports on aerobic degradation in laboratory and field as well as adsorption/desorption

In each relevant compartment, the P criterion of the PBT assessment was verified. According to Section 3.7.2.1. of Annex II of EC Regulation 1107/2009, an active substance, safener or synergist fulfils the persistence criterion where:

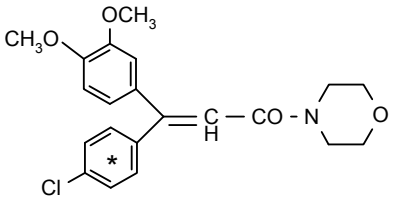
- The half-life in marine water is higher than 60 days,
- The half-life in fresh or estuarine water is higher than 40 days,
- The half-life in marine sediment is higher than 180 days,
- The half-life in fresh or estuarine water sediment is higher than 120 days, or
- The half-life in soil is higher than 120 days

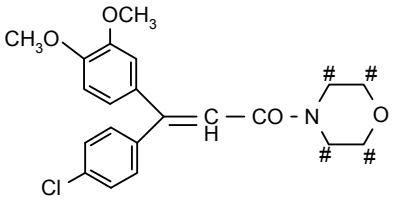
CA 7.1 Fate and behaviour in soil

Studies on the route of degradation were performed using the morpholine-^{14}C or chlorophenyl^{14}C labeled dimethomorph. Additional studies using a third label (dimethoxyphenyl-^{14}C labeled dimethomorph) are introduced in this supplemental dossier for the Annex I Renewal process.

Kinetic re-evaluation of laboratory and field information derives endpoints in line with new scientific and technical developments

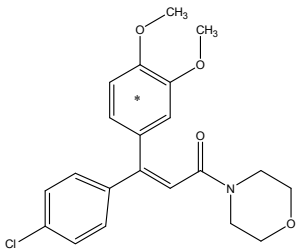
The fate and behaviour of dimethomorph in the environment (points 7.1 and 7.2) were investigated using the chlorophenyl- and morpholine-labeled compounds.





* denotes uniformly labeled ^{14}C-phenyl ring (chlorophenyl)
# denotes ^{14}C-label (morpholine)

Additionally, studies with the dimethoxyphenyl-^{14}C label are introduced during Annex I renewal process.



* denotes position of ^{14}C-label (dimethoxyphenyl)

CA 7.1.1 Route of degradation in soil

A brief summary of the degradation of dimethomorph in soil as evaluated during the Annex I listing process is provided below.

The route of degradation in soil of dimethomorph was overall investigated with two different radio-labels (^{14}C-chlorophenyl- and ^{14}C-morpholine-label). For Annex I Renewal process, additional studies on a third label (dimethoxyphenyl-^{14}C label) were performed and are summarized below.

Dimethomorph appears in two isomer forms (E and Z-isomer). By definition, dimethomorph comprises of a racemic mixture of the E-isomer and Z-isomer. At the end of the studies, the ratio of E:Z usually shifts to approximately 20:80 (mainly due to different degradation rates of the two isomers and transformation of E to Z under the influence of light). Overall, the behaviour of dimethomorph in soil is therefore best described by considering the sum of both isomers (isomer mixture).

Most of the information on the route of degradation is derived from studies already peer-reviewed during the previous Annex I inclusion process. Nevertheless, some new studies are described below, which were conducted in order to confirm or complement the knowledge of dimethomorph degradation in soil.

No major metabolites were found for dimethomorph.

CA 7.1.1.1 Aerobic degradation

The five studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

The degradation of dimethomorph showed microbial metabolism to be the primary route of dissipation.

No aerobic soil metabolism degradates were identified other than small amounts of $^{14}CO_2$ (maximum of 31% AR after 120 days). As dimethomorph degraded, most of the radioactivity was not extracted from soil (accounting for 18 to 52% AR after 120 days).

One study was added to supplement already existing information on the morpholine and chlorophenyl labelled dimethomorph (CA 7.1.1.1/1).

A new aerobic soil metabolism study with dimethomorph was performed with an additional third label to the substance in the dimethoxyphenyl ring of the structure (CA 7.1.1.1/2). The study was conducted to verify that no metabolites above 5% were found during the previous Annex I process and to confirm the behavior of the substance.

Report: CA 7.1.1.1/1
Fang C., 2003 a
Aerobic soil metabolism of 14C-BAS 550 F (Dimethomorph)
2003/5000477
Guidelines: EPA Subdivision N, Series 162-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The aerobic soil metabolism of BAS 550 F was conducted using [^{14}C-2, 3, 5, 6-morpholine]-BAS 550 F with a radiochemical purity of 99.3% and a specific activity of 18.95 µCi mg^{-1} and [^{14}C-p-chlorophenyl]-BAS 550 F with a radiochemical purity of 98.9% and a specific activity of 44.0 µCi mg^{-1}. An Idaho loamy sand (Idaho, USA) was used and the study lasted 361 days. BAS 550 F was applied at the rate of approximately 1.5 mg a.i. kg^{-1} soil, equivalent to approximately 1.7 kg a.i ha^{-1} per year.

The material balance of the morpholine label ranged from 96.0 – 102.2% of the applied radioactivity (% AR), while material balance of the p-chlorophenyl label ranged from 99.7 – 109.6% AR.

This study demonstrated that BAS 550 F degraded and mineralized in soil under aerobic conditions with a DT_{50} of 70-80 days. $^{14}CO_2$ was the major transformation product and accounted for approximately 23% AR for both morpholine and p-chlorophenyl labels, at the end of the study. Several minor polar metabolites were observed, but <6% (polar fraction consisting of several components). Hence no relevant metabolites could be found during the study duration of 361 days.

I. MATERIAL AND METHODS

A. MATERIALS

Table 7.1.1.1-1: Test substances

[^{14}C-2, 3, 5, 6-morpholine]-BAS 550 F	
Company reference number:	BAS 550 F, dimethomorph
Chemical nomenclature (IUPAC):	(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine
CAS number:	E isomer 113210-97-2; Z isomer 113210-98-3
Lot number:	AC 12694-26
Chemical purity:	99.5%
Site of radiocarbon labeling:	^{14}C-2, 3, 5, 6-morpholine
Radiochemical purity:	99.3%
Specific activity:	18.95 µCi mg^{-1}
Molecular weight:	387 g mol^{-1}
Solubility:	in water at 20 °C and pH 7: E Isomer 13 mg L^{-1}; Z isomer 6 mg L^{-1}
[^{14}C-U-p-chlorophenyl] BAS 550 F	
Company reference number:	BAS 550 F, dimethomorph
Chemical nomenclature (IUPAC):	(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine
CAS number:	E isomer 113210-97-2; Z isomer 113210-98-3
Lot number:	AC 10011-58
Chemical purity:	98.8%
Site of radiocarbon labeling:	^{14}C-U-labeled on p-chlorophenyl ring
Radiochemical purity:	98.9%
Specific activity:	44.0 µCi mg^{-1}
Molecular weight:	387 g mol^{-1}
Solubility:	in water at 20 °C and pH 7: E Isomer 13 mg L^{-1}; Z isomer 6 mg L^{-1}

Table 7.1.1.1-2: Reference substances

Compound Name or Code	**Lot No.**	**%Purity**	**Isomer Ratio (E:Z)**
Dimethomorph (BAS 550 F)	AC 9978-68A	97.6	44:56
Dimethomorph (E Isomer)	AC 11187-92	98.9	-
Para-desmethyl-dimethomorph (CL 900986)	AC 10538-99	92.0	44:56

Details of soil properties are shown in Table 7.1.1.1-3.

Table 7.1.1.1-3: Physico-chemical characteristics of the test soil

Sample Number	Percent recovered	Corrected recovery in stored sample *
Geographic Location	Payette, ID, USA	NA
Soil Taxonomic Classification (USDA)	Coarse-loamy. Mixed, superactive, mesic Aridic Haploxerolls	USDA-NRCS Soil Survey Division
Soil Series	Cashmere	USDA-NRCS Soil Survey Division
Soil Mapping Unit	Latitude: N44° 07.226' Longitude: W116° 53.280'	USDA-NRCS Soil Survey Division
Texture Class	Loamy Sand	USDA-NRCS Soil Survey Division
Sand Silt Clay	80% 14% 6%	Hydrometer method (NUC.02.06)
pH	6.8	1:1 Soil: Water suspension (NUC.02.05)
Organic Matter	1.1%	Walkley-Black (NUT.02.09)
Organic Carbon	0.65%	By calculation (Org. Matter/1.724)
Soil Biomass	Initial: 89.6 µg/g dry soil Middle: 77.0 µg/g dry soil Final: 67.3 µg/g dry soil	Fumigation Extration Method (Reference 7)
Soil Microbial Plate Counts	CFU/g dry soil (actinomycetes, fungi, bacteria) Initial: 573000, 2360, 560000 Middle: 643000, 12700, 1800000 Final: 284000, 11900, 1780000	Determined by enumerating soil organisms by the plate count method
Cation Exchange Capacity (CEC)	11 meq/100 g	Determined by summing the cations with hydrogen (NUT.02.03)
Field Moisture Capacity at 1/3 bar	10.3%	Water remaining when saturated soil was placed under 1/3 bar pressure
Field Moisture Capacity at 15 bar	5.6%	Water remaining when saturated soil was placed under 15 bar pressure (NUC.02.013)

B. STUDY DESIGN

Soil used was an Idaho loamy sand from Payette, Idaho. This soil was representative of the intended use areas. After collection, the soil was characterized at AGVISE Laboratories, Northwood, ND. (Table 7.1.1.1-2). Soil microbial biomass and plate counts were determined at AGVISE at the beginning of the study, at 6 months, and at the end of the study. The soil (0-4 cm layer) was freshly collected from the town of Payette, ID. After collection, it was transported to the laboratory of the test facility in a plastic bag with headspace. After removing the vegetation, larger soil fauna and stones, the soil was passed through a 2-mm sieve. The soil was used immediately after the sieving (Table 7.1.1.1-4). Prior to dosing, the moisture of the soil was adjusted to approximately 75% of the moisture level at 1/3 bar by adding an appropriate amount of deionized water. Aliquots of the sieved soil were dried in an oven (105 °C) until the final weights remained constant. The soil moisture content was calculated based on the moist and dry soil weights.

Table 7.1.1.1-4: Experimental design

Parameter		Description
Duration of the test		361 days
Soil condition		Fresh
Soil sample weight		50 g (dry weight basis)/replicate
Test concentrations	mg a.i. kg^{-1} soil	~1.5
	kg a.i. ha^{-1}	~1.7
Control conditions (if used)		Soil without test substance applied for biomass determination
Number of replications	Treatments	2
Test apparatus		Air flow-through system with 250-mL Nalgene bottles as test vessels
Traps for CO_2 and organic volatiles		Two 1.0 N NaOH traps for CO_2 and one ethylene glycol for organic volatiles per label
Test material application	Identity of solvent	Acetonitrile
	Volume of dosing solution used/treatment	0.2 mL
	Application method	Pipette dropwise onto surface of soil and mix by manually shaking the test vessels
	Evaporation of application solvent	Yes, open to atmosphere
Indication of test material adsorbing to walls of test apparatus		Dosing solution was in acetonitrile and there was no material adsorbing to the walls
Experimental conditions	Temperature (°C)	27 ± 1 °C
	Moisture content	75% of 1/3 bar moisture content
	Moisture maintenance method	Weighing the test vessels and add water if moisture was lost
	Continuous darkness (Yes/No):	Yes
Other details		NA

Application Procedures:

An aliquot of 200 µL of [^{14}C-morpholine]- or [^{14}C-p-chlorophenyl]-BAS 550 F application solution was applied randomly to each of the appropriate soil samples. The application solutions were applied drop-wise to each test vessel on the soil surface using a pipettor. After dosing, each test vessel was gently shaken by hand to incorporate the test substance into the soil, and was opened to the atmosphere to evaporate the solvent. Aliquots of the application solution were assayed by LSC before and after dosing. The average concentrations of BAS 550 F in soil were approximately 1.64 and 1.54 mg kg^{-1} soil in samples treated with [^{14}C-morpholine]- and [^{14}C-p-chlorophenyl]-BAS 550 F application solutions, respectively.

Sample Preparation and Processing:

For the early sampling points (DAT 0 to 60), the soil extraction procedures were continuously modified because of the rapid bound residue formation after DAT 0. In a first step, soil samples were extracted with acetonitrile (2-3 times) by shaking for 1 hour. If the total recovery from acetonitrile extraction was <90% TAR, the post-extracted soil palettes were extracted with methanol and/or methanol/water mixtures (70/30 or 20/80, v/v). Sonication was applied to enhance the release of radioactivity from soil residues. Approximately 100 mL of solvent was used to extract 50 g of soil for each extraction. After centrifugation, the supernatants were assayed by LSC and then concentrated on a N-Evap (or rotary evaporator) before HPLC analysis. For the DAT 32 samples, the methanol/water (20/80, v/v) extract was separately concentrated by a rotary evaporator and then combined with other concentrated extracts for HPLC analysis. For the DAT 60 samples, the methanol/water (20/80, v/v) extract was also separately concentrated by a rotary evaporator but was not combined with other extracts for HPLC analysis because this extraction procedure was considered to be too harsh. The concentrated extracts from the methanol/water (20/80, v/v) extraction of the DAT 60 samples were separately analyzed by HPLC. The post-extracted soil palettes were then air-dried, and the non-extractable residue (NER) was determined by combustion analysis.

From Day 90 to 361, the same soil extraction procedure was used for all soil samples. The soil samples were extracted twice with acetonitrile and followed by methanol/water (70/30, v/v) extraction (2 times) and methanol/water (70/30, v/v) with sonication (one time). All samples were analyzed as soon as they were processed.

To characterize the NER, one of the DAT 60 post-extracted soil residue samples from each label (Replicate 2) were further extracted with 0.5 N NaOH and fractionated into fulvic and humic fractions by acid precipitation before combustion of the humin fraction. At the end of the study, the post-extracted soil residues (air-dried) of DAT 361 were extracted with 0.5 N NaOH and fractionated into fulvic, humic and humin fractions.

II. RESULTS AND DISCUSSION

The microbial biomass did not change significantly during the incubation period. The CFU of actinomycetes decreased 50% at the end of the incubation while the population of fungi and bacteria did not change significantly.

Material balance

The total recoveries of radioactivity from soils treated with [^{14}C-morpholine]- and [^{14}C-p-chlorophenyl]-BAS 550 F are summarized in Table 7.1.1.1-5 and Table 7.1.1.1-6.

For the morpholine label, the total radioactivity recoveries ranged from 96.02 to 102.22% AR. The corresponding values from the p-chlorpphenyl label ranged from 99.66 to 109.55% AR.

For the morpholine label, the total extractable radioactivity from the soil decreased from approximately 95% AR at 0-time to 38% AR at the end of the study while the non-extractable residue increased from approximately 5% AR at Day 0 to 38% AR at the end of the study (Table 7.1.1.1-5). The fulvic, humic, and humin in the bound residues (DAT 361) accounted for approximately 37, 9, and 54% of the total (or 14, 4 and 20% AR), respectively (Table 7.1.1.1-7). After the 1-year incubation, approximately 23% of AR had been collected in the NaOH traps (identified as $^{14}CO_2$). The volatile organic compounds collected in the ethylene glycol trap accounted for approximately 0.01% AR at study conclusion.

For the p-chlorophenyl label, the total extractable radioactivity of the soil decreased from approximately 95% AR at DAT0 to 38%AR at the end of the study while the non-extractable residue increased from approximately 6% AR at DAT0 to 42% AR at the end of the study (Table 7.1.1.1-6). The fulvic, humic, and humin in the bound residues accounted for approximately 36, 10, and 54% of the total (or 15, 4 and 23% AR), respectively (Table 7.1.1.1-7). After one year of incubation, approximately 23% of AR had been collected in the NaOH traps (identified as 14CO2). The volatile organic compounds collected in the ethylene glycol trap accounted for less than 0.01% AR at study conclusion.

Table 7.1.1.1-5: Material balance of radioactivity from [^{14}C-morpholine]-BAS 550 F (expressed as percent of applied radioactivity, % AR)

Sampling Time	Rep No.	Volatiles		ExtractableRadioactivity					Total Solvent Extractable	0.5N NaOH	Bound	Total
		$^{14}CO_2$	VOC*	100% ACN	100% MeOH	70% MeOH	70% MeOH	20% MeOH				
Day0	1	ns	ns	95.13	ns	ns	ns	ns	95.13	ns	4.80	99.93
	2	ns	ns	95.33	ns	ns	ns	ns	95.33	ns	4.36	99.69
	Mean	**ns**	**ns**	**95.23**	**ns**	**ns**	**ns**	**ns**	**95.23**	**ns**	**4.58**	**99.81**
Day7	1	2.36	0.00	66.54	6.48	ns	ns	ns	73.02	ns	22.08	97.46
	2	2.36	0.00	67.50	6.58	ns	ns	ns	74.08	ns	22.53	98.97
	Mean	**2.36**	**0.00**	**67.02**	**6.53**	**ns**	**ns**	**ns**	**73.55**	**ns**	**22.31**	**98.22**
Day14	1	3.82	0.01	56.12	8.03	ns	ns	ns	64.16	ns	29.03	97.02
	2	3.82	0.01	56.35	8.53	ns	ns	ns	64.88	ns	30.43	99.14
	Mean	**3.82**	**0.01**	**56.23**	**8.28**	**ns**	**ns**	**ns**	**64.52**	**ns**	**29.73**	**98.08**
Day32	1	6.62	0.01	44.23	5.93	ns	5.93	8.94	65.03	ns	24.74	96.40
	2	6.62	0.01	44.66	6.71	ns	7.77	8.08	67.23	ns	26.08	99.94
	Mean	**6.62**	**0.01**	**44.44**	**6.32**	**ns**	**6.85**	**8.51**	**66.13**	**ns**	**25.41**	**98.17**
Day60	1	9.64	0.01	37.46	ns	12.79	2.22	6.53	59.00	ns	27.98	96.63
	2	9.64	0.01	39.14	ns	11.63	2.03	ns	52.80	19.45	15.01	96.91
	Mean	**9.64**	**0.01**	**38.30**	**ns**	**12.21**	**2.13**	**na**	**55.90**	**na**	**21.49**	**96.77**
Day90	1	12.37	0.01	34.21	ns	10.29	2.79	ns	47.29	ns	39.36	99.03
	2	12.37	0.01	35.72	ns	10.20	2.39	ns	48.32	ns	41.52	102.22
	Mean	**12.37**	**0.01**	**34.97**	**ns**	**10.25**	**2.59**	**ns**	**47.81**	**ns**	**40.44**	**100.63**
Day 120	1	14.29	0.01	31.77	ns	10.73	2.07	ns	44.57	ns	38.75	97.62
	2	14.29	0.01	32.86	ns	11.20	1.96	ns	46.02	ns	37.83	98.16
	Mean	**14.29**	**0.01**	**32.32**	**ns**	**10.97**	**2.01**	**ns**	**45.30**	**ns**	**38.29**	**97.89**
Day 180	1	16.89	0.01	29.10	ns	10.90	3.14	ns	43.15	ns	35.96	96.02
	2	16.89	0.01	30.64	ns	10.49	3.31	ns	44.44	ns	34.72	96.06
	Mean	**16.89**	**0.01**	**29.87**	**ns**	**10.69**	**3.23**	**ns**	**43.79**	**ns**	**35.34**	**96.04**
Day 270	1	20.01	0.01	29.12	ns	11.00	1.61	ns	41.73	ns	34.90	96.65
	2	20.01	0.01	29.00	ns	10.31	1.35	ns	40.66	ns	36.60	97.28
	Mean	**20.01**	**0.01**	**29.06**	**ns**	**10.66**	**1.48**	**ns**	**41.19**	**ns**	**35.75**	**96.96**
Day 361	1	23.42	0.01	26.73	ns	9.15	2.29	ns	38.18	ns	37.74	99.35
	2	23.42	0.01	27.32	ns	8.16	2.23	ns	37.71	ns	38.36	99.50
	Mean	**23.42**	**0.01**	**27.03**	**ns**	**8.66**	**2.26**	**ns**	**37.95**	**ns**	**38.05**	**99.43**

ns – no sample
na – not applicable
* volatile organic compound

Table 7.1.1.1-6: Material balance of radioactivity from [^{14}C-p-chlorophenyl]-BAS 550 F (expressed as Percent of Applied Radioactivity, % AR)

Sampling Time	Rep No.	Volatiles		ExtractableRadioactivity					Total Solvent Extractable	0.5N NaOH	Bound	Total
		$^{14}CO_2$	VOC	100% ACN	100% MeOH	70% MeOH	70% MeOH	20% MeOH				
Day0	1	ns	ns	96.10	ns	ns	ns	ns	96.10	ns	5.87	101.96
	2	ns	ns	94.54	ns	ns	ns	ns	94.54	ns	5.72	100.26
	Mean	**ns**	**ns**	**95.32**	**ns**	**ns**	**ns**	**ns**	**95.32**	**ns**	**5.79**	**101.11**
Day7	1	2.91	nd	69.41	7.16	ns	ns	ns	76.57	ns	23.26	102.74
	2	2.91	nd	68.83	7.03	ns	ns	ns	75.86	ns	24.00	102.77
	Mean	**2.91**	**nd**	**69.12**	**7.10**	**ns**	**ns**	**ns**	**76.22**	**ns**	**23.63**	**102.76**
Day14	1	5.22	0.00	56.65	9.25	ns	ns	ns	65.90	ns	32.67	103.80
	2	5.22	0.00	58.85	10.34	ns	ns	ns	69.18	ns	35.14	109.55
	Mean	**5.22**	**0.00**	**57.75**	**9.79**	**ns**	**-**	**-**	**67.54**	**ns**	**33.91**	**106.68**
Day32	1	8.47	0.00	44.73	7.62	ns	8.81	6.65	67.81	ns	27.82	104.10
	2	8.47	0.00	45.86	8.06	ns	8.16	7.77	69.86	ns	27.51	105.84
	Mean	**8.47**	**0.00**	**45.30**	**7.84**	**ns**	**8.49**	**7.21**	**68.83**	**ns**	**27.66**	**104.97**
Day60	1	11.61	0.00	38.31	ns	11.49	2.16	6.22	58.18	ns	31.77	101.56
	2	11.61	0.00	40.44	ns	11.11	2.04	ns	53.59	19.96	18.04	103.20
	Mean	**11.61**	**0.00**	**39.38**	**ns**	**11.30**	**2.10**	**na**	**55.88**	**na**	**24.90**	**102.38**
Day90	1	13.72	0.00	35.86	ns	9.98	2.33	ns	48.18	ns	41.87	103.77
	2	13.72	0.00	37.16	ns	9.22	2.40	ns	48.78	ns	37.15	99.66
	Mean	**13.72**	**0.00**	**36.51**	**ns**	**9.60**	**2.37**	**ns**	**48.48**	**ns**	**39.51**	**101.71**
Day 120	1	15.12	0.00	33.97	ns	9.78	1.95	ns	45.69	ns	40.85	101.67
	2	15.12	0.00	35.01	ns	10.43	1.84	ns	47.28	ns	41.50	103.90
	Mean	**15.12**	**0.00**	**34.49**	**ns**	**10.10**	**1.89**	**ns**	**46.49**	**ns**	**41.17**	**102.79**
Day 180	1	17.24	0.00	31.45	ns	11.91	2.32	ns	45.67	ns	37.53	100.44
	2	17.24	0.00	31.37	ns	10.91	2.65	ns	44.93	ns	38.51	100.68
	Mean	**17.24**	**0.00**	**31.41**	**ns**	**11.41**	**2.48**	**ns**	**45.30**	**ns**	**38.02**	**100.56**
Day 270	1	19.86	0.00	29.90	ns	10.54	1.46	ns	41.90	ns	38.43	100.19
	2	19.86	0.00	31.02	ns	10.43	1.44	ns	42.90	ns	40.31	103.07
	Mean	**19.86**	**0.00**	**30.46**	**ns**	**10.49**	**1.45**	**ns**	**42.40**	**ns**	**39.37**	**101.63**
Day 361	1	22.65	0.00	27.19	ns	8.26	2.63	ns	38.08	ns	40.23	100.97
	2	22.65	0.00	27.19	ns	8.00	2.92	ns	38.11	ns	43.17	103.93
	Mean	**22.65**	**0.00**	**27.19**	**ns**	**8.13**	**2.78**	**ns**	**38.10**	**ns**	**41.70**	**102.45**

nd – not detected
ns – no sample
na – not applicable
* volatile organic compound

Table 7.1.1.1-7: Characterization of bound residues (DAT361 samples)

Label	Rep No.	Expression	Bound Residues	Fulvic	Humic	Humin
Morpholine	1	% NER	100.00	38.14	9.44	52.42
	2	% NER	100.00	35.88	9.46	54.66
	Mean		**100.00**	**37.01**	**9.45**	**53.54**
	1	% AR	37.74	14.39	3.56	19.78
	2	% AR	38.36	13.76	3.63	20.96
	Mean		**38.05**	**14.08**	**3.60**	**20.37**
p-Chlorophenyl	1	% NER	100.00	35.64	10.57	53.79
	2	% NER	100.00	35.47	10.23	54.30
	Mean		**100.00**	**35.56**	**10.40**	**54.04**
	1	% AR	40.23	14.34	4.25	21.64
	2	% AR	43.17	15.31	4.42	23.44
	Mean		**41.70**	**14.83**	**4.33**	**22.54**

Distribution and composition of residues:

The amount of BAS 550 F and its metabolites recovered at each time point is shown in Table 7.1.1.1-8 and Table 7.1.1.1-9 as percent of the applied radioactivity.

Table 7.1.1.1-8: Biotransformation of [^{14}C-morpholine]-BAS 550 F (expressed as % AR) in Idaho Loamy Sand under aerobic conditions

%AR applied dimethomorph and metabolites										
DAT	**Sample**	**Parent BAS 550 F E-isomer**	**Parent BAS 550 F Z-isomer**	**Parent BAS 550 F (sum of E and Z)**	**Polar**	**Others[A]**	**CO_2**	**VOC[B]**	**NER[C]**	**Total**
0	Rep 1	42.00	52.62	94.62	0.00	0.51	-	-	4.80	99.93
0	Rep 2	42.64	51.52	94.17	0.00	1.15	-	-	4.36	99.69
Mean	**-**	**42.32**	**52.07**	**94.39**	**0.00**	**0.83**	**ns**	**ns**	**4.58**	**99.81**
7	Rep 1	25.98	47.04	73.02	0.00	0.00	-	-	22.08	97.46
7	Rep 2	27.11	46.98	74.08	0.00	0.00	-	-	22.53	98.97
Mean	**-**	**26.54**	**47.01**	**73.55**	**0.00**	**0.00**	**2.36**	**0.01**	**22.31**	**98.22**
14	Rep 1	19.32	44.83	64.16	0.00	0.00	-	-	29.03	97.02
14	Rep 2	17.76	47.12	64.88	0.00	0.00	-	-	30.43	99.14
Mean	**-**	**18.54**	**45.98**	**64.52**	**0.00**	**0.00**	**3.82**	**0.01**	**29.73**	**98.08**
32	Rep 1	8.97	52.76	61.73	2.65	0.65	-	-	24.74	96.40
32	Rep 2	9.94	51.64	61.58	**5.65**	0.00	-	-	26.08	99.93
Mean	**-**	**9.45**	**52.20**	**61.65**	**4.15**	**0.33**	**6.62**	**0.01**	**25.41**	**98.16**
60	Rep 1	5.18	41.42	46.60	4.91	0.96	-	-	34.51*	96.63
60	Rep 2	4.49	43.34	47.83	4.44	0.53	-	-	34.46**	96.90
Mean	**-**	**4.83**	**42.38**	**47.21**	**4.68**	**0.75**	**9.64**	**0.01**	**34.48**	**96.77**
90	Rep 1	2.68	39.82	42.50	3.54	1.26	-	-	39.36	99.03
90	Rep 2	2.85	40.80	43.64	2.28	2.39	-	-	41.52	102.22
Mean	**-**	**2.76**	**40.31**	**43.07**	**2.91**	**1.82**	**12.37**	**0.01**	**39.36**	**100.63**
120	Rep 1	1.70	37.89	39.59	3.33	1.64	-	-	38.75	97.62
120	Rep 2	1.43	38.25	39.68	3.99	2.35	-	-	37.83	98.16
Mean	**-**	**1.56**	**38.07**	**39.64**	**3.66**	**1.99**	**14.29**	**0.01**	**38.29**	**97.89**
180	Rep 1	1.71	36.08	37.78	4.13	1.23	-	-	35.96	96.02
180	Rep 2	1.42	36.31	37.73	2.79	3.92	-	-	34.72	96.06
Mean	**-**	**1.56**	**36.19**	**37.75**	**3.46**	**2.58**	**16.89**	**0.01**	**35.34**	**96.04**
270	Rep 1	0.51	36.04	36.56	4.14	1.04	-	-	34.90	96.65
270	Rep 2	0.44	35.73	36.17	4.23	0.26	-	-	36.60	97.28
Mean	**-**	**0.48**	**35.89**	**36.36**	**4.18**	**0.65**	**20.01**	**0.01**	**35.75**	**96.96**
361	Rep 1	0.40	30.15	30.55	2.86	4.77	-	-	37.74	99.35
361	Rep 2	0.46	31.76	32.22	2.28	3.21	-	-	38.36	99.50
Mean	**-**	**0.43**	**30.96**	**31.39**	**2.57**	**3.99**	**23.42**	**0.01**	**38.05**	**99.43**

A multiple components
B volatile organic compounds
C non extractable residue
ns no sample
* Includes 6.53% AR from MeOH/Water (20/80, v/v) extractions that was not combined with other extracts for metabolite profiling.
** Includes 19.45% AR from 0.5N NaOH extraction.

Table 7.1.1.1-9: Biotransformation of [^{14}C-p-chlorophenyl]-BAS 550 F (expressed as % AR) in Idaho Loamy Sand under aerobic conditions

%AR applied dimethomorph and metabolites										
DAT	**Sample**	**Parent BAS 550 F E-isomer**	**Parent BAS 550 F Z-isomer**	**Parent BAS 550 F (sum of E and Z)**	**Polar**	**Others[A]**	**CO_2**	**VOC[B]**	**NER[C]**	**Total**
0	Rep 1	40.27	51.95	92.22	0.00	3.88	-	-	5.87	101.96
0	Rep 2	39.28	52.88	92.16	0.00	2.39	-	-	5.72	100.26
Mean	**-**	**39.77**	**52.41**	**92.19**	**0.00**	**3.14**	**ns**	**ns**	**5.79**	**101.11**
7	Rep 1	25.55	49.80	75.34	0.00	1.23	-	-	23.26	102.74
7	Rep 2	26.33	47.33	73.66	0.00	2.20	-	-	24.00	102.77
Mean	**-**	**25.94**	**48.56**	**74.50**	**0.00**	**1.72**	**2.91**	**0.00**	**23.63**	**102.75**
14	Rep 1	14.54	48.83	63.38	0.00	2.52	-	-	32.67	103.80
14	Rep 2	18.45	49.83	68.28	0.00	0.91	-	-	35.14	109.55
Mean	**-**	**16.50**	**49.33**	**65.83**	**0.00**	**1.71**	**5.22**	**0.00**	**33.91**	**106.68**
32	Rep 1	7.93	52.23	60.15	5.26	2.41	-	-	27.82	104.10
32	Rep 2	10.14	53.95	64.10	4.14	1.62	-	-	27.51	105.83
Mean	**-**	**9.03**	**53.09**	**62.13**	**4.70**	**2.02**	**8.47**	**0.00**	**27.66**	**104.97**
60	Rep 1	3.64	40.76	44.41	4.68	2.87	-	-	37.99*	101.56
60	Rep 2	3.87	40.03	43.90	5.42	4.27	-	-	38.00**	103.21
Mean	**-**	**3.76**	**40.40**	**44.15**	**5.05**	**3.57**	**11.61**	**0.00**	**37.99**	**102.38**
90	Rep 1	2.36	40.53	42.89	2.76	2.53	-	-	41.87	103.77
90	Rep 2	2.19	41.50	43.68	2.24	2.86	-	-	37.15	99.66
Mean	**-**	**2.27**	**41.01**	**43.28**	**2.50**	**2.70**	**13.72**	**0.00**	**39.51**	**101.71**
120	Rep 1	1.54	38.66	40.20	3.19	2.31	-	-	40.85	101.67
120	Rep 2	1.38	40.16	41.54	2.37	3.37	-	-	41.50	103.90
Mean	**-**	**1.46**	**39.41**	**40.87**	**2.78**	**2.84**	**15.12**	**0.00**	**41.17**	**102.79**
180	Rep 1	1.41	38.06	39.47	2.62	3.58	-	-	37.53	100.44
180	Rep 2	1.40	37.42	38.81	2.66	3.46	-	-	38.51	100.68
Mean	**-**	**1.40**	**37.74**	**39.14**	**2.64**	**3.52**	**17.24**	**0.00**	**38.02**	**100.56**
270	Rep 1	0.38	37.57	37.94	3.96	0.00	-	-	38.43	100.19
270	Rep 2	0.77	38.10	38.87	4.03	0.00	-	-	40.31	103.07
Mean	**-**	**0.57**	**37.84**	**38.41**	**3.99**	**0.00**	**19.86**	**0.00**	**39.37**	**101.63**
361	Rep 1	0.56	34.77	35.33	1.45	1.30	-	-	40.23	100.97
361	Rep 2	0.50	34.73	35.23	1.06	1.81	-	-	43.17	103.93
Mean	**-**	**0.53**	**34.75**	**35.28**	**1.26**	**1.56**	**22.65**	**0.00**	**41.70**	**102.45**

A multiple components
B volatile organic compounds
C non extractable residue
ns no sample
* Includes 6.22% AR from MeOH/Water (20/80, v/v) extractions that was not combined with other extracts for metabolite profiling.
** Includes 19.96% AR from 0.5N NaOH extraction.

For the morpholine label, approximately 94% AR was recovered as BAS 550 F (total isomers) at DAT0; this value decreased to approximately 31% AR by the end of the study (Table 7.1.1.1-8). Meanwhile, the non-extractable bound residue increased from approximately 5% AR at Day 0 to approximately 38% AR at the end of the study. In addition to the parent compound, a polar fraction, which probably contains multiple components, was also observed. This polar fraction accounted for less than 6% AR. During the incubation period, a maximum of 23.42% AR was identified as 14CO2. No significant amount of volatile organic compounds was detected.

For the p-chlorophenyl label, approximately 92% AR was recovered as BAS 550 F (total isomers) at 0-time; this value decreased to approximately 35% AR after 1 year incubation (Table 7.1.1.1-9). Meanwhile, the non-extractable bound residue increased from approximately 6% AR at Day 0 to approximately 42% AR at the end of the study. In addition to the parent compound, a polar fraction, which probably contains multiple components, was also observed. This polar fraction accounted for less than 6% AR. During the incubation period, a maximum of 22.65% AR was identified as $^{14}CO_2$. No significant amount of volatile organic compounds was detected.

The fulvic acid fraction of each DAT361 sample was profiled by HPLC. In addition to the parent compound, some polar components were also extracted from the residue by 0.5 N NaOH solution. For some HPLC chromatograms in this study, the retention time of the parent compound shifted slightly from time to time due to different injection volumes or different sample matrix. However, in each HPLC analysis, the stock solutions of [^{14}C-morpholine]-, [^{14}C-p-chlorophenyl]-BAS 550 F, and BAS 550 F cold standards were always analyzed at the same time with all other samples. The slight shift in the retention time for those samples does not affect the integrity of the study.

Identification and characterization of transformation products

At the beginning of the study, the E/Z isomer ratios in soil extracts were approximately 45:55 and 42:55 for samples treated with [^{14}C-morpholine]- and [^{14}C-p-chlorophenyl]-BAS 550 F, respectively. However, the corresponding ratios at the end of the study decreased to approximately 1:87 and 1:89, suggesting that there was a conversion of the E to the Z isomer or the E isomer was degraded faster than the Z isomer in soil under aerobic conditions in the dark.

To confirm the major peak observed in the soil extract was ^{14}C-BAS 550 F, a LC/MS method was used to analyze the DAT270 samples and BAS 550 F analytical standard solutions (both E/Z mixture and E Isomer). The retention times of the target peaks in the soil extracts matched that of the analytical standard. The results from LC/MS analyses also revealed that the target peaks observed in the soil extracts had a molecular ion of m/z=388 and a major product ion of m/z=301, indicating that the predominant peak in the radiochromatograms was ^{14}C-BAS 550 F. Because the minor polar fraction eluted near the void volume and probably contains multiple components, their identities were not further investigated by LC/MS. The polar fraction accounted for < 6% AR (but only single replicates exceeded 5%, without repetition).

Kinetics of parent compound degradation

Based on the Gustafson-Holden model (FOMC), DT_{50} values of BAS 550 F in soil were calculated to be approximately 70 and 80 days for morpholine and p-chlorophenyl labels, respectively. The DT_{90} of BAS 550 F in the soil was longer than 361 days for both labels. The kinetic data of BAS 550 F degradation in soil are summarized in Table 7.1.1.1-10.

Table 7.1.1.1-10: Kinetic evaluation of both dimethomorph labels

^{14}C-label position	Kinetic model	First-order DT_{50} (days)	First-order DT_{90} (days)	R^2
Morpholine	FOMC	69.6	> 361	0.983
Chlorophenyl	FOMC	80.2	> 361	0.962

Degradation pathway of BAS 550 F in soil under aerobic conditions

Under aerobic conditions, BAS 550 F degraded slowly in Payette loamy sand. $^{14}CO_2$ was the principal degradation product detected and accounted for approximately 23% of the total applied radioactivity in each label. Low levels of polar material, accounting for less than 6% AR in total (single peaks) and were also extracted from soil during the study. Non-extractable residues accounted for 5 to 6% AR at the beginning of the study, rapidly increased to 22 to 24% AR at DAT7 and further increased to 38 to 42% AR at the end of the study.

III. CONCLUSION

BAS 550 F slowly degraded in soil with DT_{50} ranging from 70-80 days and a DT_{90} of more than 361 days in Idaho loamy sand. Since significant amounts of $^{14}CO_2$ were produced, it is postulated that BAS 550 F formed bound residue in soil where it was degraded by soil microorganisms. The structure of BAS 550 F is similar to the simple building blocks of humic and fulvic acids and it was probably mineralized in soil through the similar microbial processes for the degradation of natural soil components.

Report: CA 7.1.1.1/2
Keenan D.,Brusky M., 2014 a
[14C]Dimethomorph: Aerobic soil metabolism study
2014/7001068
Guidelines: EPA 835.4100, OECD 307
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The objectives of this study were (1) to determine the rate of degradation of the systemic fungicide BAS 550 F, dimethomorph (E + Z isomer), under aerobic conditions at 20 ± 2ºC in a single soil type, (2) to determine the nature and quantities of degradation products formed, and (3) to determine the estimates of the disappearance times for DT_{50} and DT_{90} of the BAS 550 F and metabolites.

The average amount of radioactivity as dimethomorph (E + Z isomer) in the extractable fraction decreased from 98.7% AR at Day 0 to 44.8% AR at day 120. Only minor degradation occurred into two known metabolites (Para-desmethyl-dimethomorph and Meta-desmethyl-dimethomorph). All other degradates were observed at < 1.0 %AR.

This study demonstrated that dimethomorph was degraded at a moderate rate under the aerobic, microbially active, soil conditions employed in this study. A very minor degradation product of dimethomorph was released as radioactive carbon dioxide. Trace amounts < 0.1% of the applied radioactivity (AR) was detected in the 1 M NaOH and ethylene glycol volatile organic traps. Analysis of soil extracts by HPLC showed that the main but minor metabolism products consisted of Para-desmethyl-dimethomorph and Meta-desmethyl-dimethomorph.

The double first-order in parallel (DFOP) model was selected to kinetically model the data (excluding the 120 day data points), as it provided the best visual and statistical fit as compared with the single first-order (SFO) and first-order multi-compartment (FOMC) kinetic models. The resulting DT_{50} and DT_{90} values for dimethomorph (E + Z isomers) were 71.0 days and 329 days, respectively. The DT_{90} should be interpreted with caution, as it exceeds the 120 day study duration. Kinetic analyses of the individual isomers (E or Z) indicated that the E isomer degraded more rapidly under aerobic conditions than the Z isomer. The DT_{50} and DT_{90} of the E isomer were 21.8 and 72.5 days, respectively. The DT_{50} and DT_{90} of the Z isomer were 150 and 500 days, respectively. Both the DT_{50} and DT_{90} for the Z isomer should be interpreted with caution, as they exceed the 120 day study duration.

I. MATERIAL AND METHODS

A. MATERIALS

Approximately 3.9 mCi of [^{14}C]Dimethomorph (E + Z isomer) was received at Ricerca Biosciences in 3.7 g solution of acetonitrile.

Table 7.1.1.1-11: Test Substance

[$_{14}$C]BAS 550 F (E + Z isomer)	
Common Name	[^{14}C]BAS 550 F or [^{14}C]Dimethomorph
Chemical Name	(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
CAS Number	110488-70-5
Equivalent Mass	387.86
Molecular Formula	C21H22ClNO4
Lot Number	1069-0101
Stated Radiochemical Purity	98.8%
Specific Activity	450084 dpm/µg

Table 7.1.1.1-12: Reference Substances

Non-labeled BAS 550 F (E + Z isomer)	
Common Name	[^{14}C]BAS 550 F or [^{14}C]Dimethomorph
Chemical Name	(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
CAS Number	110488-70-5
Reg Number	247723
Average Molecular Weight	387.86
Molecular Formula	C21H22ClNO4
Lot Number	AC9978-68A
Stated Radiochemical Purity	97.6%
(E,Z) Meta-desmethyl-dimethomorph	
Common Name	M550F006 or Meta-desmethyl-dimethomorph
Chemical Name	(*E*,*Z*)-3-(4-chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-1-morpholin-4-yl)prop-2-en-1-one
Reg Number	4060806
Average Molecular Weight	373.84
Molecular Formula	C20H20ClNO4
Lot Number	L83-10
Stated Radiochemical Purity	94.8%
(E,Z) Para-desmethyl-dimethomorph	
Common Name	M550F007 or Para-desmethyl-dimethomorph
Chemical Name	(E,Z)-3-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-1-morpholin-4-yl)prop-2-en-1-one
Reg Number	4060805
Equivalent Mass	373.84
Molecular Formula	C20H20ClNO4
Lot Number	L83-12
Stated Radiochemical Purity	90.3%

Details of soil properties are shown in Table 7.1.1.1-13.

Table 7.1.1.1-13: Physico-chemical characteristics of the test soil

Soil	**LUFA 5M**
Sampling Site	Germany/Rheinland-Pfalz/Mechtersheim
Other name	EFS-418
Percent Sand [%]	54
Percent Silt [%]	29
Percent Clay [%]	17
USDA Textural Class	Sandy Loam
Bulk Density (disturbed) [g/cc]	1.31
Cation exchange capacity [meq/100 g]	10.2
Maximum Water Holding Capacity [gm /100 gm]	27.8
% Moisture at 2.0 pF Units	14.5
% Moisture at 1/3 bar	12.0
% Moisture at 2.5 pF Units	12.0
% Organic matter	1.6
pH in 1:1 soil: water ratio	7.7
pH in 0.01M $CaCl_2$	7.4
Calcium [% / ppm]	80.6 / 1640
Magnesium [% / ppm]	5.4 / 66
Sodium [% / ppm]	0.4 / 10
Potassium [% / ppm]	4.9 / 195
Hydrogen [% / ppm]	8.7 / 9

B. STUDY DESIGN

The soil used was LUFA 5M collected from typical agricultural areas in Germany. After collection, the soil was characterized at AGVISE Laboratories, Northwood, ND. (Table 7.1.1.1-13). Soil microbial biomass and plate counts were determined at AGVISE at the beginning of the study, at 6 months, and at the end of the study. After collection, soil was transported to the laboratory. After removing the vegetation, larger soil fauna and stones, the soil was passed through a 2-mm sieve. The soil was used immediately after the sieving. Prior to dosing, the moisture of the soil was adjusted to pF2 by adding an appropriate amount of deionized water. Aliquots of the sieved soil were dried in an oven (110 °C) until the final weights remained constant. The soil moisture content was calculated based on the moist and dry soil weights.

The experimental design for the aerobic soil metabolism system is presented in the following table.

Table 7.1.1.1-14: Experimental Design

Parameter		Description
Duration of the test		120 days following acclimation
Soil sample weight		50g (dry weight basis)
Test concentration (µg a.i. per g of soil)		1.2
Traps for CO_2 and organic volatiles		Ethylene glycol and two 1 N NaOH traps
Test material	Identity of solvent	Acetonitrile
	Volume of test solution used for treatment	25 µL
	Application method	50 µL Hamilton syringe
Experimental conditions	Temperature	20 ± 2 °C
	Continuous darkness	Yes
Sampling intervals/ Number of Samples		Duplicate samples on Days 0, 3, 7, 14, 30, 59, 91, and 120
Collection of CO_2 and volatile organics		All traps collected and replenished at each time point.

Application Procedures

An aliquot of 25 µL of [^{14}C]-BAS 550 F application solution was applied randomly to each of the appropriate soil samples. After dosing, each test vessel was gently shaken by hand to incorporate the test substance into the soil. Aliquots of the application solution were assayed by LSC before and after dosing. The average concentrations of BAS 550 F in soil were approximately 1.2 mg kg^{-1} soil in samples treated with [^{14}C]-BAS 550 F application solutions, respectively.

Sample Preparation and Processing

In a first step, soil samples were extracted with acetonitrile by shaking for 1 hour. The post-extracted soil palettes were extracted with (2) acetonitrile/water (80/20), (3) acetonitrile/water (70/30) and methanol/water mixtures (80/20). Approximately 100 mL of solvent was used to extract 50 g of soil for each extraction. After centrifugation, the supernatants were assayed by LSC and concentrated before HPLC analysis. The post-extracted soil palettes were air-dried, and the non-extractable residue (NER) was determined by combustion analysis.

To characterize the NER (Day 30 and 120), soil residue samples were further extracted with 0.5 N NaOH and fractionated into fulvic and humic fractions by acid precipitation before combustion of the humin fraction.

II. RESULTS AND DISCUSSION

The test substance was applied to individual aerobic test systems at a target rate of 1.2 µg/g. This target application rate was calculated based on a 50 g dry-soil weight.

The treated soil samples were allowed to age aerobically for specific time intervals. At each time interval, selected samples were analyzed for degradation products. The evolution of volatile radioactive degradates was also monitored throughout the course of the study.

The Limit of Detection (LOD) for a given analytical method was calculated from two times the background response in the vicinity of the analyte response.

Distribution of the applied radioactivity in the test system, that is, amount in soil, amount unextracted and amount volatile, was determined by LSC analysis of triplicate subsamples. Random treatment of the soil samples and analysis of replicates reduced any systematic error in the treatment procedure. No other methods were employed to control experimental bias.

Table 7.1.1.1-15: Distribution of Radioactivity and Mass Balance in the German Soil System (EFS-418) Treated with [14C]Dimethomorph as Percent of the Total Applied Radioactivity

Day	Sample [%]	Extractable [%]	Bound [%]	$^{14}CO_2$ [%]	VOC [%]	Total Recovery [%]
Day 0	101	99.01	0.51	na	na	99.51
Day 0	102	98.32	0.46	na	na	98.78
Mean	**-**	**98.66**	**0.49**	**na**	**na**	**99.15**
Day 3	103	95.56	3.46	0.00	0.00	99.02
Day 3	104	96.42	3.68	0.00	0.00	100.10
Mean	**-**	**95.99**	**3.57**	**0.00**	**0.00**	**99.56**
Day 7	105	90.72	6.41	0.00	0.00	97.13
Day 7	106	89.08	6.18	0.00	0.00	95.27
Mean	**-**	**89.90**	**6.30**	**0.00**	**0.00**	**96.20**
Day 14	107	82.41	12.33	0.92	0.00	95.66
Day 14	108	83.04	11.45	0.92	0.00	95.41
Mean	**-**	**82.72**	**11.89**	**0.92**	**0.00**	**95.54**
Day 30	109	63.77	30.07	0.96	0.00	94.81
Day 30	110	70.57	24.11	0.96	0.00	95.64
Mean	**-**	**67.17**	**27.09**	**0.96**	**0.00**	**95.22**
Day 59	111	57.48	33.22	0.96	0.00	91.66
Day 59	112	53.98	35.89	0.96	0.00	90.84
Mean	**-**	**55.73**	**34.56**	**0.96**	**0.00**	**91.25**
Day 91	113	46.91	42.48	0.96	0.00	90.35
Day 91	114	42.67	47.68	0.96	0.00	91.31
Mean	**-**	**44.79**	**45.08**	**0.96**	**0.00**	**90.83**
Day 120	115	41.70	44.73	0.97	0.00	87.40
Day 120	116	47.80	47.58	0.97	0.00	96.35
Mean		**44.75**	**46.15**	**0.97**	**0.00**	**91.87**

Table 7.1.1.1-16: Distribution of Radioactivity and Mass Balance in the German Soil System (EFS-418) Treated with [14C]Dimethomorph as mg kg^{-1}

Day	Sample	Applied ppm	Extractable ppm	Bound ppm	$^{14}CO_2$ ppm	VOC ppm	Total Recovery ppm
Day 0	101	1.241	1.229	0.006	na	na	1.235
Day 0	102	1.245	1.224	0.006	na	na	1.230
Mean	**-**	**1.243**	**1.227**	**0.006**	**na**	**na**	**1.233**
Day 3	103	1.235	1.180	0.041	0.000	0.000	1.221
Day 3	104	1.240	1.195	0.044	0.000	0.000	1.239
Mean	**-**	**1.238**	**1.188**	**0.042**	**0.000**	**0.000**	**1.230**
Day 7	105	1.236	1.122	0.072	0.000	0.000	1.194
Day 7	106	1.244	1.109	0.069	0.000	0.000	1.177
Mean	**-**	**1.240**	**1.115**	**0.070**	**0.000**	**0.000**	**1.185**
Day 14	107	1.241	1.023	0.126	0.001	0.000	1. 149
Day 14	108	1.239	1.029	0.118	0.001	0.000	1.147
Mean	**-**	**1.240**	**1.026**	**0.122**	**0.001**	**0.000**	**1.148**
Day 30	109	1.244	0.793	0.239	0.002	0.000	1.032
Day 30	110	1.245	0.879	0.212	0.002	0.000	1.090
Mean	**-**	**1.245**	**0.836**	**0.225**	**0.002**	**0.000**	**1.061**
Day 59	111	1.243	0.715	0.237	0.002	0.000	0.952
Day 59	112	1.239	0.669	0.240	0.002	0.000	0.909
Mean	**-**	**1.241**	**0.692**	**0.239**	**0.002**	**0.000**	**0.931**
Day 91	113	1.244	0.584	0.248	0.002	0.000	0.832
Day 91	114	1.245	0.531	0.253	0.002	0.000	0.784
Mean	**-**	**1.245**	**0.557**	**0.251**	**0.002**	**0.000**	**0.808**
Day 120	115	1.239	0.517	0.231	0.002	0.000	0.748
Day 120	116	1.244	0.595	0.283	0.003	0.000	0.878
Mean		**1.241**	**0.556**	**0.257**	**0.002**	**0.000**	**0.813**

Table 7.1.1.1-17: Distribution of Dimethomorph and Metabolites in the Soil Extractable Fraction of the German Soil System (LUFA 5M) Treated with [^{14}C]Dimethomorph as Percent of the Total Applied Radioactivity

Percent Applied Dimethomorph and Metabolites												
Day	**Sample**	**% Para-desmethyl 30.8 min**	**% Meta-desmethyl 31.8 min**	**E isomer 33 min**	**Z isomer 34 min**	**isomer ratio**	**Total**	**22 min**	**35 min**	**36 min**	**38 min**	**Total**
Day 0	101	1.20	nd	35.95	61.50	3/5	97.45	nd	nd	nd	0.36	99.01
Day 0	102	1.27	nd	34.64	61.72	5/9	96.35	nd	nd	0.31	0.37	98.31
Mean	**-**	**1.23**	**nd**	**35.29**	**61.61**	**4/7**	**96.90**	**nd**	**nd**	**0.16**	**0.37**	**98.66**
Day 3	103	1.04	nd	32.52	61.76	1/2	94.28	nd	nd	nd	0.24	95.56
Day 3	104	1.25	nd	32.01	63.15	1/2	95.16	nd	nd	nd	nd	96.42
Mean	**-**	**1.15**	**nd**	**32.27**	**62.46**	**1/2**	**94.72**	**nd**	**nd**	**nd**	**0.12**	**95.99**
Day 7	105	1.26	nd	29.79	59.67	1/2	89.47	nd	nd	nd	nd	90.73
Day 7	106	1.34	nd	31.11	56.64	5/9	87.75	nd	nd	nd	nd	89.08
Mean	**-**	**1.30**	**nd**	**30.45**	**58.16**	**1/2**	**88.61**	**nd**	**nd**	**nd**	**nd**	**89.90**
Day 14	107	3.02	nd	21.33	57.16	3/8	78.49	nd	0.49	0.40	nd	82.40
Day 14	108	1.64	nd	24.01	56.64	3/7	80.65	nd	0.37	0.39	nd	83.04
Mean	**-**	**2.33**	**nd**	**22.67**	**56.90**	**2/5**	**79.57**	**nd**	**0.43**	**0.40**	**nd**	**82.72**
Day 30	109	1.36	nd	10.67	51.74	1/5	62.41	nd	nd	nd	nd	63.77
Day 30	110	1.33	nd	14.44	54.80	1/4	69.24	nd	nd	nd	nd	70.57
Mean	**-**	**1.35**	**nd**	**12.55**	**53.27**	**1/4**	**65.82**	**nd**	**nd**	**nd**	**nd**	**67.17**
Day 59	111	0.91	nd	5.94	50.63	1/9	56.56	nd	nd	nd	nd	57.48
Day 59	112	0.73	nd	5.99	47.26	1/8	53.25	nd	nd	nd	nd	53.98
Mean	**-**	**0.82**	**nd**	**5.96**	**48.94**	**1/8**	**54.91**	**nd**	**nd**	**nd**	**nd**	**55.73**

Table 7.1.1.1-17: Distribution of Dimethomorph and Metabolites in the Soil Extractable Fraction of the German Soil System (LUFA 5M) Treated with [^{14}C]Dimethomorph as Percent of the Total Applied Radioactivity

Day 91	113	0.97	0.58	2.92	41.71	3/43	44.63	0.73	nd	nd	nd	46.91
Day 91	114	0.77	nd	3.29	37.35	8/91	40.64	nd	1.25	nd	nd	42.67
Mean	**-**	**0.87**	**0.29**	**3.10**	**39.53**	**4/51**	**42.64**	**0.36**	**0.63**	**nd**	**nd**	**44.79**
Day 120	115	nd	nd	2.07	39.63	1/19	41.70	nd	nd	nd	nd	41.70
Day 120	116	nd	nd	1.96	45.84	3/70	47.80	nd	nd	nd	nd	47.80
Mean		**nd**	**nd**	**2.02**	**42.73**	**1/21**	**44.75**	**nd**	**nd**	**nd**	**nd**	**44.75**

Table 7.1.1.1-18: Distribution of Dimethomorph and Metabolites in the Soil Extractable Fraction of the German Soil System (LUFA 5M) Treated with [^{14}C]Dimethomorph as mg kg^{-1}

Percent Applied Dimethomorph and Metabolites											
Day	Sample	Applied ppm	Para-desmethyl 30.8 min	Meta-desmethyl 31.8 min	Dimethomorph (ppm)		22 min	35 min	36 min	38 min	Total
					E isomer 33 min	Z isomer 34 min					
Day 0	101	1.241	0.01	nd	0.45	0.76	nd	nd	nd	0.00	1.22
Day 0	102	1.245	0.02	nd	0.43	0.77	nd	nd	0.00	0.00	1.22
Mean	**-**	**1.243**	**0.02**	**nd**	**0.44**	**0.77**	**nd**	**nd**	**0.00**	**0.00**	**1.22**
Day 3	103	1.235	0.01	nd	0.40	0.76	nd	nd	nd	0.00	1.18
Day 3	104	1.240	0.02	nd	0.40	0.78	nd	nd	nd	nd	1.20
Mean	**-**	**1.238**	**0.01**	**nd**	**0.40**	**0.77**	**nd**	**nd**	**nd**	**0.00**	**1.19**
Day 7	105	1.236	0.02	nd	0.37	0.74	nd	nd	nd	nd	1.12
Day 7	106	1.244	0.02	nd	0.39	0.70	nd	nd	nd	nd	1 .11
Mean	**-**	**1.240**	**0.02**	**nd**	**0.38**	**0.72**	**nd**	**nd**	**nd**	**nd**	**1.12**
Day 14	107	1.241	0.04	nd	0.26	0.71	nd	0.00	0.00	nd	1.01
Day 14	108	1.239	0.02	nd	0.30	0.70	nd	0.00	0.00	nd	1.02
Mean	**-**	**1.240**	**0.03**	**nd**	**0.28**	**0.71**	**nd**	**0.00**	**0.00**	**nd**	**1.02**
Day 30	109	1.244	0.02	nd	0.13	0.64	nd	nd	nd	nd	0.79
Day 30	110	1.245	0.02	nd	0.18	0.68	nd	nd	nd	nd	0.88
Mean	**-**	**1.245**	**0.02**	**nd**	**0.16**	**0.66**	**nd**	**nd**	**nd**	**nd**	**0.84**
Day 59	111	1.243	0.01	nd	0.07	0.63	nd	nd	nd	nd	0.71
Day 59	112	1.239	0.01	nd	0.07	0.59	nd	nd	nd	nd	0.67
Mean	**-**	**1.241**	**0.01**	**nd**	**0.07**	**0.61**	**nd**	**nd**	**nd**	**nd**	**0.69**
Day 91	113	1.244	0.01	0.01	0.04	0.52	0.00	nd	nd	nd	0.57
Day 91	114	1.245	0.01	nd	0.04	0.47	nd	0.00	nd	nd	0.52
Mean	**-**	**1.245**	**0.01**	**0.00**	**0.04**	**0.49**	**0.00**	**0.00**	**nd**	**nd**	**0.55**

Table 7.1.1.1-18: Distribution of Dimethomorph and Metabolites in the Soil Extractable Fraction of the German Soil System (LUFA 5M) Treated with [^{14}C]Dimethomorph as mg kg^{-1}

Day 120	115	1.239	nd	nd	0.03	0.49	nd	nd	nd	nd	0.52
Day 120	116	1.244	nd	nd	0.02	0.57	nd	nd	nd	nd	0.59
Mean		**1.241**	**nd**	**nd**	**0.03**	**0.53**	**nd**	**nd**	**nd**	**nd**	**0.56**

Determination of rate of dissipation

The DT_{50} and DT_{90} of dimethomorph (E + Z isomers) under aerobic soil conditions were estimated by fitting the data to the appropriate kinetic model using the full data set (through 120 DAT) and excluding the 120 sampling time (due to no observed degradation beyond 91 DAT). The results are summarized in the following table. The double first-order in parallel (DFOP) model was selected to model the data (excluding the 120 day sampling time), as it provided the best visual and statistical fit as compared with the single first-order (SFO) and first-order multi-compartment (FOMC) kinetic models. The resulting DT_{50} and DT_{90} values for dimethomorph (E + Z isomers) were 71.0 days and 329 days, respectively. The DT_{90} should be interpreted with caution, as it exceeds the 120 day study duration.

Table 7.1.1.1-19: DT_{50} and DT_{90} values of dimethomorph (E + Z isomers) under aerobic soil conditions in LUFS 5M

Step in FOCUS flowchart	Model	χ^2 error [%]	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Complete data set						
Run SFO, FOMC Models	SFO	5.61	k: < 0.01	Poor	86.6	288
	FOMC	2.35	α,β: < 0.01	Acceptable	76.6	2671
⇨ SFO fit visually poor, but statistically acceptable. FOMC visually and statistically acceptable. However, lack of degradation between 91 and 120 days could be attributed to reported decrease in microbial biomass during study. Therefore, remove 120 day data points, and repeat fitting procedure through 91 days.						
Data excluding 120 day data points						
Run SFO, FOMC Models	SFO	3.18	k: < 0.01	Poor	72.2	240
	FOMC	1.33	α,β: < 0.01	Acceptable	70.2	1084
⇨ SFO fit visually poor, but statistically acceptable. FOMC visually and statistically acceptable. Therefore, run DFOP to compare biphasic model.						
Run DFOP	DFOP	1.31	k_{fast} < 0.05 k_{slow} < 0.01 g < 0.05	Acceptable	71.0	329
⇨ DFOP visually and statistically acceptable, with lowest χ^2error.						
⇨ **Conclusion: Use DFOP endpoints for BAS 550 F (E + Z isomers).**						

Kinetic analyses were also determined for each individual isomer. Results indicated a more rapid degradation of the dimethomorph E isomer versus the Z isomer under aerobic conditions. The DT_{50} and DT_{90} (SFO kinetics) of the E isomer were 21.8 and 72.5 days, respectively. The DT_{50} and DT_{90} (SFO kinetics) of the Z isomer were 150 and 500 days, respectively. Both the DT_{50} and DT_{90} of the dimethomorph Z isomer should be interpreted with caution, as they exceed the 120 day study duration.

Table 7.1.1.1-20: DT_{50} and DT_{90} values of dimethomorph (E isomer) under aerobic soil conditions LUFA 5M

Step in FOCUS flowchart	Model	χ^2 error [%]	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO, FOMC models	SFO	3.75	k: << 0.01	Acceptable	21.8	72.5
	FOMC	4.63	α,β> 0.05	Acceptable	20.1	67.0
⇨ SFO fit visually and statistically acceptable. FOMC visually acceptable, but χ^2 (FOMC) > χ^2 (SFO).						
⇨ **Conclusion: Use SFO endpoints for BAS 550 F (E isomer).**						

Table 7.1.1.1-21: DT_{50} and DT_{90} values of dimethomorph (Z isomer) under aerobic soil conditions in LUFA 5M

Step in FOCUS flowchart	Model	χ^2 error [%]	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO, FOMC models	SFO	1.83	k: << 0.01	Acceptable	150	500
	FOMC	2.09	α,β:> 0.05	Acceptable	142	479
⇨ SFO fit visually and statistically acceptable. FOMC visually acceptable, but χ^2 (FOMC) > χ^2 (SFO).						
⇨ **Conclusion: Use SFO endpoints for BAS 550 F (Z isomer).**						

Dimethomorph was metabolized in the soil to Meta-desmethyl-dimethomorph and Para-desmethyl-dimethomorph. These compounds reached maximum averages of 2.33% AR at Day 14 for Para-desmethyl-dimethomorph and 0.29% at Day 91 for Meta-desmethyl-dimethomorph. Other minor degradates (<1.0%) were observed over the course of the study.

III. CONCLUSION

This study demonstrated that dimethomorph was degraded at a moderate rate under the aerobic, microbially active, soil conditions employed in this study. Very minor degradation product of dimethomorph was released as radioactive carbon dioxide. Trace amounts <0.1 % of the applied radioactivity (AR) was detected in the 1 M NaOH and ethylene glycol volatile organic traps. Analysis of soil extracts by HPLC showed that the main but minor metabolism products consisted of Para-desmethyl and Meta-desmethyl-dimethomorph.

Report:	CA 7.1.1.1/3 Keenan D.,Brusky M., 2014 b [14C]Dimethomorph: Aerobic soil metabolism study 2014/7002299
Guidelines:	EPA 835.4100, OECD 307
GLP:	yes (certified by United States Environmental Protection Agency)

This report is an amendment of the study summarized above [see KCA 7.1.1.1/2 2014/7001068]. The amendment contains no further information, but a change of the internal study ID.

CA 7.1.1.2 Anaerobic degradation

The three studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

Degradation under anaerobic conditions was faster than under aerobic conditions, however in some studies not complete due to the duration of the studies (30 and 60 days). The degradation pathway involved demethylation of the dimethoxy groups on the phenyl ring and formation of non-extractable soil-residues. No change in E:Z ratio was observed in two of the anaerobic studies, a shift to 23:77 (E:Z) occurred in a third study, however the ratio of the two isomers approached again at later stages.

One new anaerobic soil metabolism study with dimethomorph was performed with an additional third label to the substance in the dimethoxyphenyl ring of the structure. The originally intended study (BASF DocID 2014/7001069) could not establish anoxic conditions and is replaced by the following study by Ta (2014) with BASF DocID 2014/7002658.

Report:	CA 7.1.1.2/1 Ta C., 2015 a Anaerobic soil metabolism of 14C-BAS 550 F 2014/7002658
Guidelines:	OECD 307 (2002), EPA 835.4200, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The metabolism of BAS 550 F under anaerobic conditions was conducted in a sandy loam from Germany (LUFA 5M). The soil was treated with BAS 550 F using radiolabeled BAS 550 F. The radiochemical purity of [dimethoxyphenyl-U-^{14}C]-BAS 550 F was 98.8%. [Dimethoxyphenyl-U-^{14}C]-BAS 550 F was applied at a rate of 1.2 µg a.i./g dry soil, which corresponds to the maximum proposed maximum field application rate of 300 g a.i./ha (assuming that the test substance is distributed into 2.5 cm depth of soil and a bulk density of soil of 1.0 g/cm^3). Additional samples were dosed at approximately 5-times the maximum application rate in order to allow for the identification and quantitation of the parent and metabolites.

Soil equivalent to 50 g of dry weight was placed in tared test vessels and connected to a flow-through test system. Soil samples were dosed with the [dimethoxyphenyl-U-^{14}C]-BAS 550 F. The soil moisture levels were adjusted to approximately 50% of the soils' maximum water holding capacity prior to the application of the test solutions and were maintained throughout the incubation period by adding HPLC water if necessary. Each vessel received a constant air-flow (during aerobic phase) or nitrogen gas (during anaerobic phase) gently downward over the soil. Samples were analyzed during both the aerobic and anaerobic phases of the experiment. For the aerobic phase, the soil was maintained aerobically in the dark at 20 ± 2°C until 30 days had passed. The anaerobic phase was initiated by adding an aliquot of nitrogen-purged water to each sample container. The soil was maintained anaerobically in the dark at 20 ± 2°C for 111 days post-flooding. Air or N_2 leaving the system was passed through an aqueous solution of NaOH (1N) in order to collect any volatiles produced.

During the aerobic phase of the experiment, duplicate samples (rep1 and rep2) were analyzed at 0, 7, 14, and 30 days after treatment (DAT). After the anaerobic phase of the experiment was initiated, duplicate samples (rep1 and rep2) were analyzed at 3, 7, 14, 31, 45, 60, 90, and 111 days after flooding. The soil samples were extracted with solvent. Aliquots were analyzed by LSC in triplicate. The amount of CO_2 and other volatiles produced was determined at each sampling time (excluding 0 DAT). The traps were assayed directly by adding aliquots of the trapping solutions into scintillation cocktail and counting by liquid scintillation counting. The solutions in the traps were replaced at each sampling interval.

The mean total radioactivity recovered (material balance) from with [dimethoxyphenyl-U-^{14}C]-BAS 550 F ranged from 91.40 to 100.04% of the TAR. The mean solvent extracted radioactive residues (ERR) from the soil decreased from approximately 96.48% TAR at 0 DAT to approximately 59.81% TAR at 141 DAT. The mean total non-extractable [^{14}C]-residues (NER) increased from approximately 1.19% TAR at 0 DAT to 27.65% TAR at 141 DAT. The mean total recovered volatile radioactivity at 7 DAT was 3.98% TAR and reached a maximum of 8.50% TAR at 141 DAT.

For the 30 DAT (or 0 days after flooding (DAF)) and 141 DAT (or 111 DAF) samples, the NER were further characterized for their fulvic, humic, and humin content. For the 30 DAT samples, the NER was determined to be 36.38% TAR. This was further characterized for fulvic (mean 8.56% TAR), humic (mean 15.01% TAR), and humin (mean 12.81% TAR) content. At 141 DAT, the NER content decreased to 27.65% TAR. The fulvic (mean 6.68% TAR), humic (mean 11.82% TAR), and humin (mean 9.23% TAR) components were also determined.

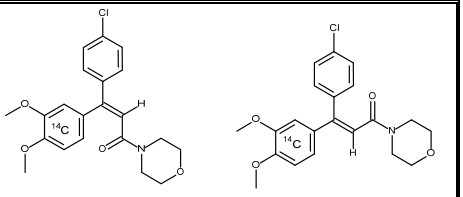
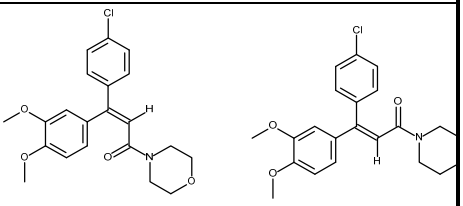
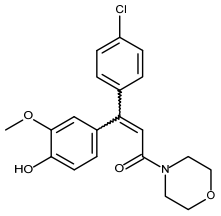
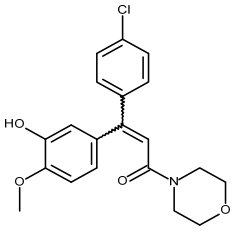
This study demonstrated that BAS 550 F degraded slowly in soil under anaerobic conditions. The kinetic analysis were performed, however due to the lack of degradation over the course of the study and poor statistical estimation of model parameters, no endpoints were determined.

I. MATERIAL AND METHODS

A. MATERIALS

The test substance dimethoxyphenyl-U-^{14}C]-BAS 550 F was provided by BASF SE.

Table 7.1.1.2-1: Test and reference substances

Common Name	Chemical Name	Details	Structure
Dimethomorph BAS 550 F Reg.No. 247723 CAS No. 110488-70-5	(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine	Batch No. 1069-0101 Chemical Purity: 92.7% Radio Purity: 98.8% Mol.Wt.: 387.86 Formula: $C_{21}H_{22}ClNO_4$ Label: dimethoxyphenyl-U-^{14}C	
Dimethomorph BAS 550 F Reg.No. 247723 CAS No. 110488-70-5 Internal Code: M550F000	(*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine	Batch No. AC9978-68A Chemical Purity: 97.6% Mol.Wt.: 387.86 Formula: $C_{21}H_{22}ClNO_4$	
Internal Code: M550F006 Reg.No. 4060806	(*E*,*Z*)-3-(4-chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one	Batch No. L83-10 Chemical Purity: 94.8% Mol.Wt.: 373.84 Formula: $C_{20}H_{20}ClNO_4$	
Internal Code: M550F007 Reg.No. 4060805	(*E*,*Z*)-3-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one	Batch No. L83-12 Chemical Purity: 90.3% Mol.Wt.: 373.84 Formula: $C_{20}H_{20}ClNO_4$	

Details of soil properties are shown in Table 7.1.1.2-2.

Table 7.1.1.2-2: Physico-chemical characteristics of test soils

Soil	LUFA 5M Soil
USDA Texture Class	sandy loam
Sand (%)	62.6
	27.3
Clay (%)	10.2
pH (water)	8.0
pH ($CaCl_2$)	7.2
Total Organic Matter (%)	1.55[a]
Total Organic Carbon (%)	0.90
Soil Biomass-0 DAT (µg/g dry soil)	408.0
Soil Biomass- 30 DAT (µgC/g dry soil)	337.5
Cation Exchange Capacity (meq/100 g)	10.9
Maximum Water Holding Capacity (g/100 g dry soil)	28.9
Bulk Density (g/L)	1235

The soil used in this study was a sandy loam (LUFA 5M) from Germany (USDA soil class). The soil is representative of the intended use areas.

The soil sample was collected from its respective field (top 20 cm layer) and was sieved through a 2 mm sieve in order to remove any vegetation, larger soil fauna, and stones and stored in the refrigerator until use, but for no longer than 30 days.

Prior to dosing, the moisture of the soil was adjusted to approximately 40-60% of the MWHC by adding the appropriate amount of deionized water. The percent soil moisture was determined as the difference between the weights of fresh soil and dried soil (after drying in an oven) divided by dried weights.

During the aerobic phase of the study, the moisture content was checked at approximately 2-week intervals.

Soil microbial biomass was determined at the beginning of the study and at 30 DAT.

B. STUDY DESIGN

The test vessels were connected to a flow-through system and incubated at 20 ± 2°C in the dark. Throughout the aerobic portion of the study, soil samples were checked periodically to determine the soil moisture content. The soil moisture was maintained at approximately 50% of the moisture level by periodically adding reagent water to the soil samples, as necessary.

During the anaerobic phase of the study (after 30 days), degassed water (100 mL) was added to all the samples. The samples were put back into the incubator (20 ± 2°C) and the flow-through lines were switched from air to nitrogen. Samples were removed at 3, 7, 14, 31, 45, 60, 90 and 111 DAF. Duplicate samples from each soil were processed for analyses.

Table 7.1.1.2-3: Experimental Design

Parameter		Description
Duration of the test aerobic phase (pre-flooding) anaerobic phase (post-flooding)		 30 days 90 days
Soil condition		Approximately 40-60% of maximum water holding capacity
Soil sample weight		50 g (dry weight basis)/replicate
Test concentrations	µg a.i./g dry soil	1.2
	g a.i./ha	300
	lb a.i./A	0.27
Application solution concentration	dimethoxyphenyl-U-^{14}C labeled material	0.54 mg/mL
Control conditions		Soil without test substance applied for biomass determination
Number of replications		2
Test apparatus		Air flow-through test system
Traps for CO_2 and organic volatiles		Kinetic samples: Two 1N NaOH trap High dose samples: One 1N NaOH trap
Test material application	Identity of solvent	Acetonitrile
	Volume of test solution used/treatment	100 µL/50 g of soil
	Application method	Pipette dropwise onto surface of soil and mix by manually shaking the test vessels
	Evaporation of application solvent	Yes, open to atmosphere with air flow
Experimental conditions	Temperature (°C)	20 ± 2°C
	Moisture content	Approximately 40-60% of maximum water holding capacity
	Moisture maintenance method	Addition of water if weight indicated moisture loss
	Continuous darkness (Yes/No):	Yes

Application Procedures

Approximately 50 g of soil (dry weight basis) was added to the individual test vessels. If necessary, the moisture content of each sample was adjusted to approximately 50% of the MWHC by the addition of water. The soil samples were then allowed to acclimate in a chamber at 20 ± 2°C for 7 days.

The test vessels were connected to a flow-through system in which each vessel received a constant air-flow (during aerobic phase) or nitrogen gas (during anaerobic phase) gently downward over the soil. The test vessels were incubated in the dark at 20 ± 2°C in a constant temperature chamber. Test bottles were capped with 2-hole rubber stoppers holding the inlet and outlet tubes and were connected to each other with Tygon® tubing from the outlet of one bottle to the inlet of the next. The test bottles (kinetic samples) were arranged in a series with one series per replicate. Samples dosed at a higher rate (5-times) and those used for microbial biomass determination were connected in a similar but separate series.

During the aerobic phase, before air was allowed to enter the test vessels, it was bubbled through an aqueous solution of sodium hydroxide (1N), which served to remove carbon dioxide from the air and to moisten it. The intention of this procedure was to minimize the drying of the soil samples and to prevent the saturation of the sodium hydroxide in the traps with CO2 during the incubation period. Air leaving the test vessels was passed through traps containing NaOH to capture volatiles that formed during the incubation period. During the anaerobic phase, air was replaced by nitrogen gas.

Sample preparation and processing

The test vessels were connected to a flow-through system and incubated at 20 ± 2°C in the dark. Throughout the aerobic portion of the study, soil samples were checked periodically to determine the soil moisture content. The soil moisture was maintained at approximately 50% of the moisture level by periodically adding reagent water to the soil samples, as necessary.

During the anaerobic phase of the study (after 30 days), degassed water (100 mL) was added to all the samples. The samples were put back into the incubator (20 ± 2°C) and the flow-through lines were switched from air to nitrogen. Samples were removed at 3, 7, 14, 31, 45, 60, 90, and 111 DAF. Duplicate samples from each soil were processed for analyses (rep1 and rep2).

During the anaerobic phase of the study, samples for each soil were removed at intervals of 0, 7, 14, and 30 DAT. At each sampling interval, the redox potential and dissolved oxygen will be measured immediately after the samples are removed from the incubator. The redox potential will be measured with the probe inserted into both the soil and water layers and also with the probe close to the soil/water interface. The pH will be measured for both the water and soil.

The soil samples (50 g dry weight basis) were extracted with 100 mL of each of the following solvents in the following order: ACN, ACN:water (8:2), ACN:water (7:3), and MeOH:water (8:2) by shaking for 30 minutes at 300 revolutions per minute followed by centrifugation for 15 minutes at 3000 rpm. The supernatant was decanted into a graduated cylinder, and the volume was adjusted to 100 mL with the same solvent used for the extraction. Aliquots of the pooled extracts were analyzed by LSC. A portion of the pooled samples were concentrated by removing an aliquot (10 mL or 15 mL) and evaporating to near dryness (nitrogen evaporator). The volume was next adjusted to 1 mL by the addition of ACN:water (1:1) and an aliquot was analyzed by LSC. The samples were next centrifuged (5 minutes) and an aliquot was analyzed by HPLC.

After the last extraction, the soil residues were allowed to dry, and their weights were recorded. The soil residues were homogenized and the radioactivity in each soil residue sample was quantitated by combustion (LSC).

For each sampling time point, with the exception of 0-DAT, the traps were collected and brought to a final volume of 100 mL by the addition of water. An aliquot was removed from the trap solutions and analyzed by LSC. The traps were replenished with fresh 1N NaOH (50 mL for each series).

For the anaerobic phase of the study (post-flooding), the soil samples were first centrifuged and the water was decanted into an amber bottle and ACN (100 mL) was added. Samples were then treated to the same extraction procedure outlined above. The water layer was pooled with the rest of the extracts.

Table 7.1.1.2-4: Sampling Details

Parameter		Description
Sampling intervals	Aerobic phase	0, 7, 14, and 30 DAT.
	Anaerobic phase	3, 7, 14, 31, 45, 60, 90, and 111 DAF
Soil sampling procedures		Two samples from each label were collected from the test system at each sampling point
Collection of CO_2		The NaOH traps were collected at each time of sampling and renewed with fresh solutions
Moisture content		Soil moisture was ~ 50% of maximum water holding capacity during aerobic incubation
Sterility checks (if applicable)		NA
Redox potential / Other		3, 7, 14, 31, 45, 60, 90, and 111 DAF

Bound residue analysis: after the extractions were completed, the soils were air-dried, and the amount of non-extractable residues (NER) was determined by oxidative combustion analysis. At selected sampling intervals (30 DAT and 111 DAF), the extracted soil samples were further extracted with 0.5N NaOH solution to characterize the bound residues. A soil sample , was transferred to a Nalgene bottle and 50 mL of 0.5N NaOH was added. The mixture was shaken for 7 hours. After centrifugation (15 min), the supernatant was decanted into a clean bottle and the volume was adjusted to 50 mL by the addition of water (HPLC grade). An aliquot was analyzed by LSC (3×0.5 mL). A second extraction was performed by adding 50 mL of 0.5N NaOH to each sample followed by shaking on the mechanical shaker for overnight (300 revolutions per minute). Samples were centrifuged (30 minutes), the supernatant was decanted and the volume was brought to 50 mL by the addition of water (HPLC grade). Three aliquots were analyzed by LSC. A third extraction was performed by adding 50 mL of 0.5N NaOH to each sample followed by shaking on the mechanical shaker. After centrifugation, the supernatant was decanted and the volume was brought to 50 mL by the addition of water (HPLC grade). Three aliquots were analyzed by LSC. A fourth and final extraction (water wash) was performed by adding 25 mL of HPLC grade water to each sample followed by shaking on the mechanical shaker for 30 minutes. After centrifugation, the supernatant was decanted into a graduated cylinder and the volume was adjusted to 26 mL by the addition of water (HPLC grade). Three aliquots were analyzed by LSC.

The NaOH and water extracts were pooled together and fractionated into fulvic and humic components by adjusting the pH of the solutions to 1-2 by the addition of concentrated HCl and allowing for precipitation in the refrigerator overnight. The supernatant (fulvic acid) was separated from the humic acid (precipitate) by centrifugation. The supernatant was decanted, the volume was adjusted to 180 mL by the addition of water and three aliquots were analyzed by LSC. The supernatant was extracted with ethyl acetate in a separatory funnel, and three aliquots of each extract were analyzed by LSC. The volume of each ethyl acetate extract was recorded. The ethyl acetate extracts were pooled, concentrated to dryness (rotary evaporator) and dissolved in ACN:water (1:1) for HPLC analysis. Three aliquots from the aqueous phase were analyzed by LSC. The humic acids remaining in the centrifuge bottle were dissolved in 0.5N NaOH (70 mL) and analyzed by LSC.

Table 7.1.1.2-5: Characterization of Bound Residues in [dimethoxyphenyl-U-^{14}C]-BAS 550 F Treated LUFA 5M Soil (expressed as % TAR mg kg^{-1})

DAT	Rep	NER	Soil Extract					Fulvic	Humic Acid	Humins[a]	% Recovery
			1	2	3	4	Total				
0 DAF	1	36.07	7.91	10.27	4.05	0.94	23.17	8.20	14.87	12.90	99.74
	2	36.70	8.30	11.00	3.72	0.95	23.97	8.92	15.16	12.73	100.27
	mean	**36.38**	**8.10**	**10.63**	**3.89**	**0.95**	**23.57**	**8.56**	**15.01**	**12.81**	**100.00**
111 DAF	1	25.86	4.97	8.02	3.39	0.89	17.27	6.28	11.06	8.60	100.28
	2	29.44	5.96	9.24	3.39	0.99	19.59	7.08	12.59	9.85	100.30
	mean	**27.65**	**5.47**	**8.63**	**3.39**	**0.94**	**18.43**	**6.68**	**11.82**	**9.23**	**100.29**

NER: Non-Extractable Residues (by combustion)
Extract 1 – 3 : 0.5N NaOH (50 mL)
Extract 4 : Water (25 mL)
% Recovery = 100 × (Fulvic Acids + Humic Acids + Humins) / NER
[a] Calculated by subtracting the NER from the extract total.

Statistical methods

The statistical methods used were means and coefficients of determination (R^2) calculated by Excel software. The data presented in the Tables and Appendices were also calculated using Excel. Minor differences between values found in the Tables and in the Appendices may arise because of the method of rounding used in the spreadsheet.

Methods to control bias

For control of bias, replicate sampling and/or multiple aliquots of sample will be used for LSC analysis with the average and relative standard deviation determined for the results. The coefficient of variation of the triplicate LSC measurements should be less than or equal to 10. If the DPM values are below 150, a coefficient of variation value of greater than 10 may be deemed acceptable by the study director.

Definitions of Quantitation Limits (LOD/LOQ)

The detection limit of the LSC is set to twice the highest background radioactivity. The LOD is thus 0.101% TAR. The limit of quantification (LOQ) is set to three times the background or 0.151% TAR.

The values from the 90 DAF replicate 1 were used to determine the LOD and LOQ for the HPLC analyses. The values used are summarized in the table below. LOQ is defined as twice the LOD. Hence, LOD was 0.091% TAR and LOQ as 0.183% TAR.

Storage stability

At the end of the experimental period, the original dosing solutions were analyzed by HPLC. The results indicate that the test samples were stable during the experimental period.

Determination of degradation kinetics

Kinetic analyses were performed in accordance with the guidance of the FOCUS kinetics workgroup (FOCUS, 2006). All analyses were conducted by non-linear regression methods utilizing KinGUII (v. 2.2014.224.1704). The iteratively reweighted least squares (IRLS) method was selected to optimize model parameters, and the program then estimated parameter standard deviations, determined parameter confidence via the t-test, calculated correlation coefficients (R^2) and χ error percentage, and calculated DT_{50} and DT_{90} endpoints. The error tolerance and maximum iterations were set to 0.000001 and 100, respectively. Both time-series and residual plots were created by KinGUII for visual data assessment, while Microsoft Excel (Microsoft, 2013) was used for table development.

II. RESULTS AND DISCUSSION

Material balance

LUFA 5M soil treated with [dimethoxyphenyl-U-14C]-BAS 550 F: The mean total radioactivity recovered (material balance) from with [dimethoxyphenyl-U-14C]-BAS 550 F ranged from 91.40 to 100.04% of the TAR. The mean solvent extracted radioactive residues (ERR) from the soil decreased from approximately 96.48% TAR at 0 DAT to approximately 59.81% TAR at 141 DAT. The mean total non-extractable [14C]-residues (NER) increased from approximately 1.19% TAR at 0 DAT to 27.65% TAR at 141 DAT. The mean total recovered volatile radioactivity at 7 DAT was 3.98% TAR and reached a maximum of 8.50% TAR at 141 DAT.

For the 30 DAT (or 0 DAF) and 141 DAT (or 111 DAF) samples, the NER were further characterized for their fulvic, humic, and humin content. For the 30 DAT samples, the NER was determined to be 36.38%. This was further characterized for fulvic (mean 8.56%), humic (mean 15.01%), and humin (mean 12.81%) content. At 141 DAT, the NER content decreased to 27.65%. The fulvic (mean 6.68%), humic (mean 11.82%), and humin (mean 9.23%) components were also determined.

Table 7.1.1.2-6: Material balance of [dimethoxyphenyl-U-^{14}C]-BAS 550 F in LUFA 5M Soil (%TAR)

DAT	DAF	Extract					ERR	NER	Trap NaOH	Material balance
		Water	1	2	3	4				
0 rep1	NA	NA	81.36	14.39	2.63	0.44	98.81	1.19	NA	100.00
0 rep2	NA	NA	77.67	13.53	2.55	0.40	94.15	1.20	NA	95.35
0 mean	**NA**	**NA**	**79.51**	**13.96**	**2.59**	**0.42**	**96.48**	**1.19**	**NA**	**97.67**
7 rep1	NA	NA	52.39	11.95	3.36	1.29	68.99	20.25	3.98	93.22
7 rep2	NA	NA	55.23	12.18	3.38	1.29	72.08	17.37	3.98	93.44
7 mean	**NA**	**NA**	**53.81**	**12.07**	**3.37**	**1.29**	**70.53**	**18.81**	**3.98**	**93.33**
14 rep1	NA	NA	49.82	12.92	3.64	1.12	67.50	21.42	5.91	94.83
14 rep2	NA	NA	57.47	13.65	3.70	1.06	75.88	15.88	5.91	97.67
14 mean	**NA**	**NA**	**53.64**	**13.29**	**3.67**	**1.09**	**71.69**	**18.65**	**5.91**	**96.25**
30 rep1	0 rep 1	NA	38.70	9.74	3.15	1.19	52.78	36.07	7.39	96.24
30 rep2	0 rep 2	NA	36.87	9.92	3.21	1.20	51.20	36.70	7.39	95.29
30 mean	**0 mean**	**NA**	**37.78**	**9.83**	**3.18**	**1.19**	**51.99**	**36.38**	**7.39**	**95.77**
33 rep1	3 rep1	2.82[a]	39.39	8.09	2.85	1.03	54.18	29.52	7.42	91.12
33 rep2	3 rep2	4.02[a]	36.82	8.17	2.89	1.32	53.22	31.04	7.42	91.68
33 mean	**3 mean**	**3.42[a]**	**38.10**	**8.13**	**2.87**	**1.17**	**53.70**	**30.28**	**7.42**	**91.40**
37 rep1	7 rep1	8.95	36.14	8.77	2.95	1.09	57.90	28.37	7.44	93.71
37 rep2	7 rep2	10.43	39.29	9.34	2.94	1.10	63.10	22.23	7.44	92.77
37 mean	**7 mean**	**9.69**	**37.71**	**9.06**	**2.94**	**1.09**	**60.50**	**25.30**	**7.44**	**93.24**
44 rep1	14 rep1	7.51	50.23	9.24	2.90	1.27	71.15	22.22	7.49	100.87
44 rep2	14 rep2	13.86	39.45	7.99	2.63	1.17	65.10	26.62	7.49	99.22
44 mean	**14 mean**	**10.69**	**44.84**	**8.61**	**2.76**	**1.22**	**68.12**	**24.42**	**7.49**	**100.04**
61 rep1	31 rep1	12.12	37.05	8.66	2.52	1.27	61.62	28.69	7.69	98.00
61 rep2	31 rep2	13.52	38.03	8.98	2.44	1.21	64.18	25.60	7.69	97.47
61 mean	**31 mean**	**12.82**	**37.54**	**8.82**	**2.48**	**1.24**	**62.90**	**27.14**	**7.69**	**97.73**
75 rep1	45 rep1	13.10	42.05	8.64	2.37	0.94	67.11	23.34	7.85	98.29
75 rep2	45 rep2	14.23	41.48	8.73	2.36	0.95	67.76	23.92	7.85	99.53
75 mean	**45 mean**	**13.67**	**41.77**	**8.68**	**2.37**	**0.95**	**67.43**	**23.63**	**7.85**	**98.91**
90 rep1	60 rep1	9.15	39.31	9.52	2.82	1.34	62.14	25.83	8.07	96.03
90 rep2	60 rep2	12.71	34.36	8.75	2.66	1.37	59.84	26.86	8.07	94.77
90 mean	**60 mean**	**10.93**	**36.83**	**9.13**	**2.74**	**1.36**	**60.99**	**26.34**	**8.07**	**95.40**

Table 7.1.1.2-6: Material balance of [dimethoxyphenyl-U-^{14}C]-BAS 550 F in LUFA 5M Soil (%TAR)

120 rep1	90 rep1	11.10	37.44	9.36	2.78	1.36	62.03	27.79	8.29	98.10
120 rep2	90 rep2	14.40	33.17	8.69	2.66	1.37	60.29	28.03	8.29	96.61
120 mean	**90 mean**	**12.75**	**35.30**	**9.02**	**2.72**	**1.36**	**61.16**	**27.91**	**8.29**	**97.35**
141 rep1	111 rep1	12.61	39.04	7.51	2.39	1.50	63.05	25.86	8.50	97.41
141 rep2	111 rep2	12.18	33.04	7.41	2.48	1.46	56.56	29.44	8.50	94.50
141 mean	**111 mean**	**12.39**	**36.04**	**7.46**	**2.44**	**1.48**	**59.81**	**27.65**	**8.50**	**95.96**

Extract 1 ACN
Extract 2 ACN:water (8:2)
Extract 3 ACN:water (7:3)
Extract 4 Methanol water (8:2)
DAT days after treatment
DAF days after flooding
ERR extractable radioactive tesidues
NER non extractable residues (by combustion)
NA not applicable (no sample analyzed)
LOQ limit of quantification (calculated as 0.151% TAR)
[a] The water was not analyzed separately by LSC, however the radioactivity in the pooled samples (water + soil extractions) were measured. Using these values, the radioactivity in the water can be calculated by subtracting the soil extractions

Table 7.1.1.2-7: HPLC quantitation of [dimethoxyphenyl-U-^{14}C]-BAS 550 F in LUFA 5M extract

DAT	DAF	26.4-26.6	M550 F07 (E) 26.7-26.9	M550 F06 (E) 27.0-27.3	M550 F07 (Z) 28.0-28.3	M550F 06 (Z) 28.7-28.8	29.0-29.1	BAS 550F (*E*) 29.2-29.8	BAS 550F (*Z*) 29.8-30.3	33.5-33.7	Others
	t_R (min)										
0 rep1	NA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	37.41	58.47	2.94	< LOQ
0 rep2	NA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	35.76	55.49	2.90	< LOQ
0 mean	**NA**	**< LOQ**	**< LOQ**	**< LOQ**	**< LOQ**	**< LOQ**	**< LOQ**	**36.58**	**56.98**	**2.92**	**< LOQ**
7 rep1	NA	< LOQ	< LOQ	1.46	< LOQ	< LOQ	< LOQ	22.49	44.33	0.70	< LOQ
7 rep2	NA	< LOQ	< LOQ	0.75	< LOQ	< LOQ	< LOQ	21.98	48.73	0.62	< LOQ
7 mean	**NA**	**< LOQ**	**< LOQ**	**1.11**	**< LOQ**	**< LOQ**	**< LOQ**	**22.23**	**46.53**	**0.66**	**< LOQ**
14 rep1	NA	< LOQ	0.97	< LOQ	< LOQ	< LOQ	0.89	19.36	46.08	< LOQ	0.21
14 rep2	NA	< LOQ	1.07	< LOQ	< LOQ	< LOQ	0.30	23.44	50.10	0.96	< LOQ
14 mean	**NA**	**< LOQ**	**1.02**	**< LOQ**	**< LOQ**	**< LOQ**	**0.60**	**21.40**	**48.09**	**0.48**	**0.10**
30 rep1	0 rep 1	1.04	< LOQ	0.81	< LOQ	< LOQ	< LOQ	9.10	40.90	< LOQ	0.93
30 rep2	0 rep 2	< LOQ	0.89	0.50	0.37	< LOQ	< LOQ	10.21	39.22	< LOQ	< LOQ
30 mean	**0 mean**	**0.52**	**0.45**	**0.66**	**0.19**	**< LOQ**	**< LOQ**	**9.66**	**40.06**	**< LOQ**	**0.47**
33 rep1	3 rep1	< LOQ	1.29[a]	< LOQ	1.52	< LOQ	< LOQ	10.52	40.57	< LOQ	0.29
33 rep2	3 rep2	< LOQ	0.56	0.62	1.10	< LOQ	1.02	10.86	39.05	< LOQ	< LOQ
33 mean	**3 mean**	**< LOQ**	**0.93**	**0.31**	**1.31**	**< LOQ**	**0.51**	**10.69**	**39.81**	**< LOQ**	**0.14**
37 rep1	7 rep1	< LOQ	0.90	< LOQ	2.42	< LOQ	0.72	11.42	39.83	< LOQ	2.61
37 rep2	7 rep2	< LOQ	1.58	0.93	0.93	< LOQ	< LOQ	16.27	41.31	< LOQ	2.08
37 mean	**7 mean**	**< LOQ**	**1.24**	**0.46**	**1.68**	**< LOQ**	**0.36**	**13.85**	**40.57**	**< LOQ**	**2.35**
44 rep1	14 rep1	< LOQ	1.19	0.85	0.95	0.41	< LOQ	18.23	44.06	< LOQ	5.46
44 rep2	14 rep2	< LOQ	0.87	0.88	1.32	1.11	< LOQ	15.40	42.83	< LOQ	2.68
44 mean	**14 mean**	**< LOQ**	**1.03**	**0.86**	**1.13**	**0.76**	**< LOQ**	**16.82**	**43.44**	**< LOQ**	**4.07**
61 rep1	31 rep1	1.12	< LOQ	1.69[b]	2.06	0.39	< LOQ	12.34	40.53	< LOQ	3.49
61 rep2	31 rep2	0.67	0.58	0.65	1.74	< LOQ	< LOQ	14.12	42.20	< LOQ	4.21
61 mean	**31 mean**	**0.90**	**0.29**	**1.17**	**1.90**	**0.19**	**< LOQ**	**13.23**	**41.37**	**< LOQ**	**3.85**
75 rep1	45 rep1	0.94	0.54	1.48[a]	2.42	0.66	< LOQ	15.07	42.78	< LOQ	3.25
75 rep2	45 rep2	1.17	0.64	0.85	2.11	< LOQ	< LOQ	14.45	44.48	< LOQ	3.67
75 mean	**45 mean**	**1.06**	**0.59**	**1.17**	**2.26**	**0.33**	**< LOQ**	**14.76**	**43.63**	**< LOQ**	**3.46**
90 rep1	60 rep1	1.08	1.17	0.98	2.27	0.48	< LOQ	12.53	40.52	< LOQ	2.75
90 rep2	60 rep2	1.07	0.72	0.84	1.72	0.55	< LOQ	11.69	40.60	< LOQ	2.66
90 mean	**60 mean**	**1.07**	**0.95**	**0.91**	**2.00**	**0.51**	**< LOQ**	**12.11**	**40.56**	**< LOQ**	**2.70**
120 rep1	90 rep1	0.91	< LOQ	1.56	2.06	0.12	< LOQ	12.43	39.13	< LOQ	4.50
120 rep2	90 rep2	0.70	0.64	1.02	1.47	0.51	< LOQ	10.69	38.76	< LOQ	6.22
120 mean	**90 mean**	**0.81**	**0.32**	**1.29**	**1.76**	**0.32**	**< LOQ**	**11.56**	**38.94**	**< LOQ**	**5.36**

141 rep1	111 rep1	1.23	< LOQ	0.96	1.22	0.76	< LOQ	11.72	40.56	< LOQ	6.62
141 rep2	111 rep2	1.47	0.40	0.56	1.19	0.62	< LOQ	8.96	35.68	0.52	6.85
141 mean	**111 mean**	**1.35**	**0.20**	**0.76**	**1.21**	**0.69**	**< LOQ**	**10.34**	**38.12**	**0.26**	**6.74**

NA not applicable (sample not analyzed)
LOQ 0.18% TAR
DAT days after treatment
DAF days after flooding
Arithmetic mean values may be reported as a value less than the stated LOQ value. Individual replicates reported as <LOQ have been treated as a zero value in the calculation of arithmetic mean values.
[a] Two close peaks were summed.
[b] Three close peaks were summed

Identification/characterization of transformation products

MS analysis of 26.9 and 27.7 (M550F007)

Mass Spectrometric Analysis of Metabolite at ~26.9 and ~27.7 min. (LC/MS method) or 26.7-26.9 min and 28-28.3 min (HPLC kinetic method), called M550F007. This component is detected in the LC/MS work at ~26.9 and ~27.7 minutes (LC/MS method) or 26.7-26.9 minutes and 28-28.3 minutes (HPLC kinetic method).

A standard of a known compound, Reg. No. 4060805 or M550F007, was analyzed. Based on its known structure, the two functional groups (methoxyphenyl ring and chlorophenyl ring, which are directly attached with the doubly bonded carbon) may not be able to rotate freely. Therefore, it is reasonable to predict that this compound has stereoisomers. From the MS observation of this standard, two peaks, with same m/z = 374, eluting at ~26.9 and ~27.7 minutes could be discerned.

The MS spectrum for the methoxyphenyl ring labeled sample contains peaks with a distinctive ^{14}C isotope pattern. The isotope intensity ratio [MH:(M+2)H:(M+4)H] is unique and roughly 0.6:1.0:0.9. The *m/z* = 374 peak is the $[M+H]^+$ or MH^+ species and shows this pattern. The MS/MS product ion spectrum was collected at two different collision energies (CID @10 and CID @30). Although the product ion intensity for *m/z* = 374 is weak, there are still a few product ions available to partially reveal its structure. Product ion *m/z* = 287 is thought to be formed from the parent ion (*m/z* = 374) by losing a morpholine unit (C_4H_9NO, *m* = 87). It may lose one methyl unit (CH_3, *m* = 15) to form product ion *m/z* = 272. Product ion *m/z* = 272 may lose a hydroxyl unit (OH, *m* = 17) to form product ion *m/z* = 255. The chlorine unit (Cl, *m* = 35) may be further cleaved to form product ion *m/z* = 220. Product ion *m/z* = 199 ($C_{13}H_8Cl^+$) is thought to be formed by combining a chlorophenol (6 carbon atoms) unit with a methylbenzene (7 carbon atoms) unit, based on the carbon allocation. Other product ions, like *m/z* = 114, are thought to represent the morpholine-4-carbaldehyde ion or its substructures. How product ion *m/z* = 151 forms is not clearly understood, however based on its elemental composition ($C_8H_7O_3^+$), it is thought to be the rearrangement product incorporating a methoxyphenyl moiety. Fortunately, the above-mentioned standard (Reg. No. 4060805 or M550F007) has been analyzed and has matching MS and MS/MS characteristics. This standard is unlabeled and has MS and MS/MS data comparable to the metabolite observed in the sample.

MS analysis of 27.2 and 27.9 (M550F006)

Mass Spectrometric analysis of metabolite at ~27.2 and ~27.9 min. (LC/MS method) or 27-27.3 min and 28.7-28.8 min (HPLC kinetic method), called M550F006. This component is detected in the LC/MS work at ~27.2 and ~27.9 minutes LC/MS method) or 27-27.3 minutes and 28.7-28.8 minutes (HPLC kinetic method).

A standard of the known compound Reg. No. 4060806, or M550F006, was analyzed. Based on their known structures, compounds M550F006 and M550F007 are structural isomers of one another. In M550F006, the para position of the phenyl ring is occupied by a methoxyl group and a hydroxyl group occupies the phenyl ring's meta position. In M550F007, those positions are switched. As for M550F007, this compound should have stereoisomers. From the MS observation of this standard, two peaks with same m/z = 374 eluted at ~27.2 and ~27.9 minutes.

Peak m/z = 374 and its isotope clusters emerged as the most interesting peaks after background subtraction at those RTs. MS/MS product ion spectra were collected at CID @10 and CID @30. The MS/MS intensity for products of peak m/z = 374 at RT ~27.2 minutes were quite weak in intensity. At RT ~27.7 minutes, a clearer product ion spectrum was achieved, allowing the following interpretation to be made. The MS/MS production ion spectra of M550F006 and M550F007 were similar to each other. Product ions *m/z* = 287, *m/z* = 255, *m/z* = 199, *m/z* = 151 and *m/z* = 114 have already been discussed. A product ion corresponding to morpholine (*m/z* = 88) was also observed. Product ion *m/z* = 139 is likely to be the rearrangement product incorporating a chlorophenyl moiety. Nevertheless, the M550F006 standard was available and has been analyzed. The MS and MS/MS characteristics support the existence of M550F006 in the above-mentioned samples.

MS analysis of 29.1 and 29.6 (BAS 550 F)

Mass Spectrometric Analysis of Parent at ~29.1 and ~29.6 min. (LC/MS method) or 29.2-29.8 min and 29.8-30.3 min (HPLC kinetic method): BAS 550 F / M550F000 (dimethomorph):

This component is detected by at ~29.1 and ~29.6 minutes (LC/MS method) or 29.2-29.8 minutes and 29.8-30.3 minutes (HPLC kinetic method). It is the dominant component based on the β-RAM chromatogram.

A standard of the known compound, BAS 550 F or M550F000 or dimethomorph, was analyzed. It has a similar structure to M550F006 and M550F007. The LC/MS data confirmed the existence of stereoisomers.

Peak m/z = 388 stood out in the MS spectrum at ~29.1 and ~29.6 minutes. Unique isotope patterns were also observed in the zoom MS spectra which indicate the compounds are 14C radio-labeled. In the MS/MS spectra, peak m/z = 301 corresponds to the product ion for which the morpholine ring is cleaved from the parent ion (m/z = 388). Product ion m/z = 301 may further lose a CO (m = 28) unit to form product ion m/z = 273. Then, a methoxyl (CH_3O, *m* = 31) unit may dissociate to form product ion *m/z* = 242. Product ion *m/z* = 114 is thought to be the morpholine-4-carbaldehyde ion. How product ion *m/z* = 165 arose is not clearly understood. Based on its elemental composition ($C_9H_9O_3^+$), it's thought to be a rearrangement product incorporating a 1,2-dimethoxybenzene moiety. As for M550F006, product ion *m/z* = 139 is most likely the rearrangement product incorporating a chlorophenyl moiety. Regardless, the above-mentioned standard (Reg. No. 247723 or M550F000) has been analyzed and has matching MS and MS/MS characteristics. This standard is unlabeled and has MS and MS/MS data comparable to the metabolites observed in the sample.

Summary MS analysis

Neither metabolite exceeded 5% TAR in sequential measurements.

Kinetic analysis of data

The DT_{50} values for BAS 550 H were calculated.

Only parent kinetics were analyzed due to the low levels of metabolite formation (maximum reported value of 2.42% TAR).

Half-life calculation

Table 7.1.1.2-8: Kinetic evaluation

Step in FOCUS flowchart	Model	p (t-test)	χ^2 error [%]	r^2	DT_{50} [d]	DT_{90} [d]
Run SFO, FOMC Models	SFO	k: > 0.05	5.35	0.10	1070	3554
	FOMC	NA [a]	6.03	-- [b]	--	--
⇨ No discernible degradation under anaerobic conditions. Parameter estimates for SFO were not statistically significant. Therefore, DT_{50} and DT_{90} estimates should not be used.						

[a] NA: Not applicable. Since α and β are not rate constants, t-test results and parameter significance were not considered
[b] '--': Not estimated by KinGUII

The DT_{50} values could not be used because the parameter estimates for SFO were not statistically significant.

III. CONCLUSION

BAS 550 F degraded very slowly in soil under anaerobic conditions. The calculated DT_{50} values could not be used because the parameter estimates for SFO were not statistically significant. No single metabolite exceeded 3% TAR at any of the time points. Some of the metabolites were identified as M550F07 (E and Z), M550F06 (E and Z), and M550F06 (Z). Due to the low level of degradation over the study period and poor statistical estimation of model parameters, no endpoints were determined.

CA 7.1.1.3 Soil photolysis

No new soil photolysis study was performed with dimethomorph. The studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

Dimethomorph degraded slowly by irradiation in soil. Two minor unidentified photolysis products (each <4%) of the applied radioactivity were found in the study after 15 days of continuous irradiation.

CA 7.1.2 Rate of degradation in soil

While endpoints did not change for several studies, new data was generated for the aerobic and anaerobic soil metabolism of dimethomorph.

The field DT_{50} values were significantly different from the laboratory values (EFSA $DegT_{50}$ endpoint selector) and were used for the derivation of modelling endpoints exclusively.

Overall degradation of dimethomorph is best described by degradation behaviour of the isomer mixture (sum of both isomers) in field, since rates and ratios of the isomers can be considered concurrently. According to the PBT assessment, laboratory and field DT_{50} values are all under the cut-off value of 120 days.

Supplementary laboratory and field studies address the degradation behaviour of dimethomorph in soil when applied with a third label.

CA 7.1.2.1 Laboratory studies

The already peer-reviewed laboratory aerobic soil studies are considered still valid.

No new laboratory soil studies to determine the rate of degradation are presented in this chapter, but the aerobic soil metabolism study reported above contains additional information on the degradation rate of dimethomorph [KCA 7.1.1.1/2 2014/7001068] which will be considered for endpoint derivation.

Existing dimethomorph degradation rates were re-calculated using the experimental data of the already peer-reviewd studies. Kinetic parameters were analyzed according to current FOCUS guidance.

Summary tables of all obtained laboratory soil degradation values for dimethomorph can be found at the end of the following chapter (Table 7.1.2.1.1-8, Table 7.1.2.1.1-9 and Table 7.1.2.1.1-10).

CA 7.1.2.1.1 Aerobic degradation of the active substance

Report: CA 7.1.2.1.1/1
Maleri M., 2015 d
Kinetic evaluation of the aerobic soil degradation studies for BAS 550 F - Dimethomorph according to FOCUS
~~2014/1183299~~
2017/1021008

Guidelines: FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 2.0 (December 2014)

GLP: no

Executive Summary

The degradation behavior of BAS 550 F– dimethomorph in soil under aerobic conditions has been investigated in five laboratory degradation studies in a total of five soils.

The purpose of this evaluation was to analyze the degradation kinetics observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics in order to derive degradation parameters as triggers for additional work (best-fit endpoints) and degradation parameters for environmental fate models (modeling endpoints). The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics.

The appropriate models were selected based on visual and statistical assessment. For the evaluated study, the visual assessment and goodness-of-fit statistics of the respective models indicate plausible fit. Therefore, the resulting endpoints can be considered reliable.

Normalization of $DegT_{50}$ values suitable for modeling to reference temperature and reference moisture was performed where necessary.

I. MATERIAL AND METHODS

A. MATERIALS

The kinetic evaluation was based on the findings of five laboratory aerobic soil degradation studies (already peer-reviewed during previous EU registration) with five typical agricultural soils (three sandy loam, one loamy sand and one silty clay loam) which are briefly described below. Characteristics of the test soils are summarized in in the table below.

Table 7.1.2.1.1-1: Soil characteristics

Study	**1990/7000078***	**1998/7000145***	**1998/7000149***	**1990/7000076***	**2002/7004446***
Origin	Woodstock, UK	New Jersey, USA	Nieder-Ingelheim, DE	LUFA 2.2, DE	Inveresk Soil Code S371, UK
Textural class	Silty clay loam	Sandy loam	Sandy loam	Loamy sand	Sandy loam
Particle size distribution [%] sand silt clay	 10 71 19	 62 [a] 25 [a] 13 [a]	 60 [b] 17 [b] 23 [b]	 77 [c] 15 [c] 8 [c]	 65 [a] 28 [a] 7 [a]
Organic C [%]	1.6	1.5	1.86	4.3 [d]	1.3
Microbial biomass [mg C 100 g^{-1} dry soil] initial final	 27.0 19.4	 - -	 - -	 34.9 23.0	 17.7 18.9
CEC [meq 100 g^{-1}]	17.8	9.1	12.5	16.3	8.6
pH [-]	5.8	6.8	7.0	5.7 ($CaCl_2$)	5.7 [e]

* Study already peer-reviewed during the previous Annex I inclusion

[a] According to USDA classification

[b] In this case: sand >0.02 mm, silt 0.002 to 0.02 mm, clay <0.002 mm

[c] According to DIN classification

[d] Humus (otherwise 2500 mg/100 g soil dm)

[e] 1M KCl

B. STUDY DESIGN

Kinetic modeling strategy:

Kinetic evaluation was performed in order to derive:

i) Degradation parameters as triggers for additional work (best-fit endpoints)
ii) Degradation parameters for environmental fate models (modeling endpoints)

For the test substance dimethomorph, the appropriate kinetic model for deriving trigger and modeling endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [FOCUS Kinetics v2.0 (2006)]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluation:

For the data sets, the kinetic models proposed by the FOCUS Kinetics guidance document [FOCUS Kinetics v2.0 (2006)] were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the double-first-order in parallel model (DFOP) were applied.

The appropriateness of a distinct kinetic model to describe soil degradation can be tested with the following checks recommended by FOCUS [FOCUS Kinetics v2.0 (2006), chapter 6.3.1]:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed (Equation 6-2 in FOCUS Kinetics v2.0 (2006))
- t-test to evaluate whether estimated degradation parameters differ from zero (Equation 6-3 in FOCUS Kinetics v2.0 (2006))

The visual fit is categorized as follows:

- Poor fit = the fit does not follow the pattern of the measured residues, not acceptable to derive persistence and modeling endpoints;
- Acceptable fit = the fit mainly follows the pattern of the measured residues with small deviations, acceptable to derive modeling endpoints, acceptable to derive trigger endpoints on a case-by-case basis;
- Good fit = the fit follows the pattern of the measured residues well, residuals are randomly scattered around zero, acceptable to derive trigger and modeling endpoints.

A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 - error value is ideally < 15% and the estimated degradation parameters differ from zero as outlined by FOCUS (FOCUS Kinetics v2.0 (2006), chapter 6.3.1).

II. RESULTS AND DISCUSSION

Persistence and modelling endpoints:

The complete parameter estimation for derivation of persistence and modeling endpoints of dimethomorph is presented in Table 7.1.2.1.1-2.

Table 7.1.2.1.1-2: Estimated model parameters for dimethomorph for derivation of trigger (best-fit) and modeling endpoints – isomer mix

Soil	Kinetic model	χ^2	Estimated parameters	Trigger (best-fit) endpoints		Modeling endpoint
				$DegT_{50}$ [d]	$DegT_{90}$ [d]	$DegT_{50}$ [d]
UK soil – silty clay loam	FOMC [a]	3.1	α: 3.227 β: 367.782	88.1	382.9	n.a.
	SFO [b]	4.4	k: 0.0072 d^{-1}	n.a.	n.a.	95.9
US soil – sandy loam	FOMC [a]	6.6	α: 1.839 β: 74.138	34.0	185.3	n.a.
	SFO [b]	8.1	k: 0.0169 d^{-1}	n.a.	n.a.	40.9
DE soil – sandy loam	SFO [ab]	2.5	k: 0.0144 d^{-1}	48.0	159.5	48.0
DE soil – loamy sand	DFOP [a]	1.9	k1: 0.1302 d^{-1} k2: 0.0056 d^{-1} g: 0.29	62.4	347.3	n.a.
	SFO [b]	7.9	k: 0.0089 d^{-1}	n.a.	n.a.	77.5
UK soil – sandy loam	FOMC [a]	7.3	α: 0.822 β: 37.09	49.1	573.9	n.a.
	SFO [b]	3.7	K: 0.011 d^{-1}	n.a.	n.a.	63.1

n.a. Not applicable
[a] Best-fit model for derivation of trigger endpoints
[b] Appropriate for derivation of modeling endpoints

Table 7.1.2.1.1-3: Estimated model parameters for dimethomorph for derivation of trigger (best-fit) and modeling endpoints – E isomer

Soil	Kinetic model	χ^2	Estimated parameters	Trigger (best-fit) endpoints		Modeling endpoint
				$DegT_{50}$ [d]	$DegT_{90}$ [d]	$DegT_{50}$ [d]
US soil – sandy loam	FOMC [a]	11.4	α: 2.122 β: 55.299	21.4	108.4	n.a.
	SFO [b]	12.7	k: 0.0286 d^{-1}	n.a.	n.a.	24.2
DE soil – loamy sand	FOMC [a]	0.5	α: 1.118 β: 31.890	27.4	218.3	n.a.
	SFO [b]	2.3	k: 0.0191 d^{-1}	n.a.	n.a.	36.3
UK soil – sandy loam	SFO [ab]	6.7	k: 0.022 d^{-1}	32.2	107.0	32.2

n.a. Not applicable
[a] Best-fit model for derivation of trigger endpoints
[b] Appropriate for derivation of modeling endpoints

Table 7.1.2.1.1-4: Estimated model parameters for dimethomorph for derivation of trigger (best-fit) and modeling endpoints – Z isomer

Soil	Kinetic model	χ^2	Estimated parameters	Trigger (best-fit) endpoints		Modeling endpoint
				$DegT_{50}$ [d]	$DegT_{90}$ [d]	$DegT_{50}$ [d]
US soil – sandy loam	SFO [ab]	4.0	k: 0.0121 d^{-1}	57.3	190.4	57.3
DE soil – loamy sand	DFOP [a]	**2.3**	k_1: 0.1608 d^{-1} k_2: 0.0031 d^{-1}	182.4	704.2	n.a.
	SFO [b]	3.6	k: 0.0039 d^{-1}	n.a.	n.a.	175.7
UK soil – sandy loam	DFOP [a]	2.1	k_1: 0.2078 d^{-1} k_2: 0.0037 d^{-1}	141.5	579.3	n.a.
	SFO [b]	4.8	k: 0.005 d^{-1}	n.a.	n.a.	144.5

n.a. Not applicable
[a] Best-fit model for derivation of trigger endpoints
[b] Appropriate for derivation of modeling endpoints

Normalization

In the five studies, the $DegT_{50}$ values suitable for modeling were obtained at a soil temperature of 20 to 25°C and a soil moisture of 33% of MWHC, 75% 1/3 bar or 82% 1/3 bar. The $DegT_{50}$ values were normalized to a reference temperature of 20°C and a soil moisture of pF 2 according to FOCUS as outlined in Chapter 2.4.2. Parameters included in the normalization procedure and the resulting modeling $DegT_{50}$ values (isomer mix) of dimethomorph (BAS 550 F) are summarized in Table 7.1.2.1.1-5.

Table 7.1.2.1.1-5: Normalization of dimethomorph SFO-$DegT_{50}$ values to reference conditions – isomer mix

Soil, soil type	Study soil moisture cond.	θ_{act} [g/100g]	θ_{ref} [g/100g]	f_{moist} [-]*	T_{act} [°C]	T_{ref} [°C]	f_{temp}	$DegT_{50act}$ [d]	$DegT_{50ref}$ [d]
UK soil – silty clay loam	82% (1/3 bar)	21.32[A]	30[F]	0.79	22	20	1.21	95.9	91.3
US soil – sandy loam	75% (1/3 bar)	10.58[B]	19[F]	0.66	25	20	1.61	40.9	43.6
DE soil – sandy loam	75% (1/3 bar)	11.25[C]	19[F]	0.69	22	20	1.21	48.0	40.2
DE soil – loamy sand	33% (MWHC)	7.92[D]	14[F]	0.67	20	20	1.00	77.5	52.0
UK soil – sandy loam	45% (MWHC)	12.1[E]	19[F]	0.73	10	20	0.38	63.1	17.9

[A] Measured field capacity in study is 26% (1/3 bar) which was used in calculations; FOCUS value at 1/3 bar for silty clay loam is 27% [FOCUS Generic Guidance v2.2 (2014), Table 2.2].

[B] Measured field capacity in study is 14.1% (1/3 bar) which was used in calculations; FOCUS value at 1/3 bar for sandy loam is 15% [FOCUS Generic Guidance v2.2 (2014), Table 2.2].

[C] Measured field capacity in study is 28.2% (1/3 bar), FOCUS value for sandy loam at 1/3 bar is 15% [FOCUS Generic Guidance v2.2 (2014), Table 2.2] which was used in the calculations.

[D] Measured field capacity in study 48% (MWHC), FOCUS value for loamy sand at MWHC is 24% [FOCUS Generic Guidance v2.2 (2014), Table 2.2] which was used in the calculations.

[E] Measured field capacity in study 44.1% (MWHC), FOCUS value for loamy sand at MWHC is 27% [FOCUS Generic Guidance v2.2 (2014), Table 2.2] which was used in the calculations.

[F] Reference value from FOCUS [FOCUS Generic Guidance v2.2 (2014), Table 2.2] for gravimetric water content at 10 kPa (field capacity).

θ_{act} Actual soil moisture [g / 100 g dry soil]

θ_{ref} Reference soil moisture at field capacity (pF 2) according to FOCUS Generic Guidance v2.2 (2014) (Table 2.2)[g/ 100 g dry soil]

f_{moist} Moisture correction factor [-]

f_{temp} temperature correction factor [-]

$Temp_{act}$ Incubation temperature at study conditions [°C]

$Temp_{ref}$ Incubation temperature at reference conditions [°C]

$DegT_{50act}$ $DegT_{50}$ at study conditions [d]

$DegT_{50ref}$ $DegT_{50}$ at reference conditions [d]

A summary of persistence and modeling endpoints for dimethomorph is given in Table 7.1.2.1.1-6 and Table 7.1.2.1.1-7 (with mixed isomers, where two isomers have been measured).

Table 7.1.2.1.1-6: Persistence endpoints for dimethomorph

Soil	Kinetic model	χ^2	Non-normalized $DegT_{50}$ [d] [a]
UK soil – silty clay loam	FOMC	3.1	88.1
US soil – sandy loam	FOMC	6.6	34.0
DE soil – sandy loam	SFO	2.5	48.0
DE soil –loamy sand	DFOP	1.9	62.4
UK soil – sandy loam	FOMC	3.7	49.1

[a] Actual study conditions

Table 7.1.2.1.1-7: Modeling endpoints for dimethomorph

Soil	Kinetic model	χ^2	Non-normalized $DegT_{50}$ [d] [a]	Normalized $DegT_{50}$ [d] [b]
Silty clay loam, UK	SFO	4.4	95.9	91.3
Sandy loam, USA	SFO	8.1	40.9	43.6
Sandy loam, DE	SFO	2.5	48.0	40.2
Loamy sand, DE	SFO	7.9	77.5	52.0
Sandy loam, UK	SFO	7.3	63.1	17.9

[a] Actual study conditions
[b] Reference conditions: 20°C, pF 2

III. CONCLUSION

The kinetic evaluation showed that with one exception (German soil sandy loam) the bi-phasic models (FOMC, DFOP) provided the best fit to the measured data for dimethomorph incubated while the SFO model was thoroughly appropriate for derivation of modeling endpoints.

For the evaluated studies, the visual assessment and goodness-of-fit statistics of the respective models indicate plausible fit. Therefore, the resulting endpoints can be considered reliable.

Normalization of $DegT_{50}$ values suitable for modeling to reference temperature and reference moisture was performed.

Summary of degradation endpoints for dimethomorph in different soils under aerobic conditions

Table 7.1.2.1.1-8: Summary table on degradation endpoints of dimethomorph obtained in of five peer-reviewed laboratory studies – persistence endpoints

BASF DocID	Soil / Soil type	pH	Org. C [%]	Temp [°C]	Moisture conditions in soil	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error level
1990/7000078*, 2017/1021008	Silty clay loam, UK [A]	5.8[D]	1.6	22	82% (1/3 bar)	88.1 / 382.9	FOMC	3.1
1998/7000145, 2017/1021008	Sandy loam, USA [AB]	6.8[D]	1.5	25	75% (1/3 bar)	34.0 / 185.3	FOMC	6.6
1998/7000149, 2017/1021008	Sandy loam, DE [B]	7.0[D]	1.9	22	75% (1/3 bar)	48.0 / 159.5	SFO	2.5
1990/7000076, 2017/1021008	Loamy sand, DE [B]	5.7[E]	4.3	20	33% (MWHC)	62.4 / 347.3	DFOP	1.9
2002/7004446, 2017/1021008	Sandy loam, UK [A]	5.7[D]	1.3	10	45% (MWHC)	49.1 / 573.9	FOMC	3.7
2014/7001068	Sandy loam, DE [C]	7.4[E]	1.6	20	pF 2.5	71.0 / 329 [+]	DFOP	1.3

* already peer-reviewed during previous Annex I review
** new study [see KCA 7.1.1.1/2]
A morpholine label
B chlorophenyl label
C dimethoxyphenyl label
D water
E $CaCl_2$
MWHC maximum water holding capacity
pF field capacity
[+] endpoints following exclusion of DAT120

Table 7.1.2.1.1-9: Normalization of dimethomorph SFO-DegT$_{50}$ value to reference conditions in BASF DocID 2014/7000168 [KCA 7.1.1.1/2]

Soil, soil type	Study soil moisture cond.	θ_{act} [g/100g]	θ_{ref} [g/100g]	f_{moist} [-]*	T_{act} [°C]	T_{ref} [°C]	f_{temp}	$DegT_{50act}$ [d]	$DegT_{50ref}$ [d]
Sandy loam, DE	pF 2.5	12.0[A]	14.5[A]	0.87	20	20	1	72.2[+]	63.2[+]

[+] SFO endpoint following exclusion of DAT120 was found acceptable as modeling endpoint, FOCUS value at pF2.5 for sandy loam is 15% [FOCUS Generic Guidance v2.2 (2014), Table 2.2].
[A] Measured field capacity in study according to soil certificate
θ_{act} Actual soil moisture [g 100 g^{-1} dry soil]
θ_{ref} Reference soil moisture at field capacity (pF 2) according to FOCUS Generic Guidance v2.2 (2014) (Table 2.2) [g 100 g^{-1} dry soil]
f_{moist} Moisture correction factor [-]
f_{temp} temperature correction factor [-]
$Temp_{act}$ Incubation temperature at study conditions [°C]
$Temp_{ref}$ Incubation temperature at reference conditions [°C]
$DegT_{50act}$ $DegT_{50}$ at study conditions [d]
$DegT_{50ref}$ $DegT_{50}$ at reference conditions [d]

Table 7.1.2.1.1-10: Summary table on degradation endpoints of dimethomorph obtained in laboratory soil studies (modeling endpoints)

BASF DocID	Soil / Soil type	pH	Org. C [%]	Temp [°C]	Moisture conditions in soil	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error level
1990/7000078*, 2017/1021008	Silty clay loam, UK [A]	5.8[D]	1.6	22	82% (1/3 bar)	91.3	SFO	4.4
1998/7000145*, 2017/1021008	Sandy loam, USA [AB]	6.8[D]	1.5	25	75% (1/3 bar)	43.6	SFO	8.1
1998/7000149*, 2017/1021008	Sandy loam, DE [B]	7.0[D]	1.9	22	75% (1/3 bar)	40.2	SFO	2.5
1990/7000076*, 2017/1021008	Loamy sand, DE [B]	5.7[E]	4.3	20	33% (MWHC)	52.0	SFO	7.9
2002/7004446*, 2017/1021008	Sandy loam, UK [A]	5.7[D]	1.3	10	45% (MWHC)	17.9	SFO	7.3
2014/7001068	Sandy loam, DE [C]	7.4[E]	1.6	20	pF 2.5	63.2[+]	SFO	3.2

* already peer-reviewed during previous Annex I review
** new study [see KCA 7.1.1.1/2]
[A] morpholine label
[B] chlorophenyl label
[C] dimethoxyphenyl label
[D] water
[E] $CaCl_2$
MWHC maximum water holding capacity
pF field capacity
[+] endpoint following exclusion of DAT120

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

No relevant metabolites were found in the metabolism studies of dimethomorph analyzed with three labels.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

One new anaerobic soil metabolism studies with dimethomorph was performed with an additional third label to the substance in the dimethoxyphenyl ring of the structure.

The study with BASF DocID 2014/1183300 indicated in the application is obsolete. Information on the kinetics of the anerobic metabolism studies were already included in section 7.1.1.2 on the route of degradation [see KCA 7.1.1.2/1 2014/7002658 and KCA 7.1.1.2/2 2014/7001069].

In both studies, the calculated DT_{50} values could not be used because the parameter estimates were not statistically significant. In 120 days, the original test substance decreased to about half to two third of the original residue level.

Degradation under anaerobic conditions was slow in the two new studies with DT_{50} values longer than 120 days (which can be estimated from the residue levels which were about 50 to 60% after 120 days). No reliable endpoints could be estimated in both studies.

The degradation pathway involved demethylation of the dimethoxy groups on the phenyl ring and formation of non-extractable soil-residues. A change of the E:Z ratio was observed in the two new studies and altogether in three of the five available studies, with a faster degradation/transformation of the E isomer versus the Z isomer (ratio of 1:5 or 1:6 at the end of the study).

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

No relevant metabolites were found in the additional study on anaerobic metabolism in soil.

Therefore, no metabolite needs to be considered for anaerobic degradation rate in soil.

CA 7.1.2.2 Field studies

The six field soil dissipation studies submitted for the previous Annex I listing were peer-reviewed and are considered still valid. Overall, 8 field trials (bare soil) distributed over Europe were performed (5 in Germany, 1 in UK, 1 in France and 1 in Spain) at nominal application rates of 428 to 622 g ha^{-1}. However, the kinetic evaluation of these trials were updated according to the newest guidelines and guidance documents and can be found below [see KCA 7.1.2.2.1/1, BASF DocID 2015/1110621].

Since no DT_{90} values of dimethomorph from field studies exceed one year (neither mixture, nor single isomer values), accumulation of the substance is not expected.

Five field studies (USA) are reported as supplementary data. These studies were not submitted in the previous Annex I process and they are presented to provide supporting information on the behaviour of dimethomorph in the field. A correlating storage stability study not previously submitted, covering the test material in all five field studies is presented as well.

CA 7.1.2.2.1 Soil dissipation studies

Kinetic re-evaluation

Summary tables of all obtained field soil degradation values for dimethomorph can be found at the end of the kinetic summary in Table 7.1.2.2.1-6 (sum of isomers), Table 7.1.2.2.1-7 (E isomer) and Table 7.1.2.2.1-8 (Z isomer).

Report:	CA 7.1.2.2.1/1 Maleri M., 2015 a Kinetic evaluation of European field dissipation studies with BAS 550 F - Dimethomorph: Determination of modeling endpoints according to EFSA (2014) 2015/1110621
Guidelines:	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 2.0 (December 2014)
GLP:	no

Executive Summary

The dissipation behavior of the fungicide BAS 550 F – dimethomorph in soil has been investigated in six field dissipation studies including eight field trials located in Europe. The purpose of this evaluation was to analyze the degradation kinetics of dimethomorph in the eight soils according to the current guidance of the FOCUS workgroup on degradation kinetics and under consideration of the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil for modeling purposes.

Prior to kinetic evaluation, the sampling intervals of the field studies were normalized to reference conditions regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on normalized sampling interval datasets in order to derive degradation parameters that can be used as modeling endpoints. Evaluations were performed for the isomer mix and separately for each of the two isomers of dimethomorph (E- and Z-isomer) when possible.

The initial sharp decline observed for the E-isomer concentrations can partly be attributed to the conversion of the E-isomer to the Z-isomer in the first few days (due to phytolysis), but independent of that process it is concluded that degradation of the E isomer is faster than degradation of the Z isomer. The geometric mean DT_{50} of the E-isomer was 24.0 days and the geometric mean DT_{50} for the Z-isomer was 39.8 days. An overall geometric mean DT_{50} of 35.1 days was calculated for the isomer mix.

I. MATERIAL AND METHODS

Description of the field dissipation studies

The kinetic evaluation was conducted for eight field trials with dimethomorph from six field dissipation studies. The trials were situated in typical regions of agricultural practice in Germany (five study sites), France, Spain and the UK (see Table 7.1.2.2.1-1), covering a range of different soils and climatic conditions. The individual studies are briefly described below.

Table 7.1.2.2.1-1: Summary of field trial details

Trial Code	Location	Application date	Study duration [d]	Application rate [g ha^{-1}]	Sampling intervals [DAT]
CU 88/516	Schwabenheim, Germany	06.06.88	168	428	0, 14, 28, 42, 56, 84, 112, 140, 168
CU 88/517	Malborn, Germany	22.06.88	168	461	0, 14, 28, 42, 56, 84, 112, 140, 168
CUE 3/89	Leibertingen, Germany	02.06.89	194	527	0, 26, 54, 82, 110, 138, 171, 194
CUE 4/89	Schwabenheim, Germany	16.06.89	203	490	0, 28, 56, 84, 112, 140, 181, 203
CUE 90/6	Krögsberg, Germany	24.04.90	224	606	0, 28, 56, 84, 112, 140, 168, 224
OAT/20/01	Stratford upon Avon, UK	20.04.01	210	601	0, 6, 14, 28, 59, 102, 154, 210
FTL/19/01	Merville, France	26.04.01	211	622	0, 7, 15, 28, 65, 103, 147, 211
ALO/24/01	Utrera, Spain	27.04.01	214	613	0, 7, 14, 28, 60, 101, 151, 214

Table 7.1.2.2.1-2: Summary of soil characteristics of field trials (Soil type ISSS)

Parameter	CU 88/516	CU 88/517	CUE 3/89
Soil type (ISSS)	Sandy loam soil	Sandy loam soil	clay soil
Particle size distribution [%]			
% Sand (>0.02 mm)	57.8	45.0	26.6
%Silt (0.002-0.02 mm)	23.7	34.0	17.5
%Clay (<0.002 mm)	18.5	21.0	55.9
OM [%]	2.89	4.65	6.8
pH [-]	7.3	5.2	7.3
MWC [g H_20 100 g^{-1} dry soil]	41	47.1	68.8

Table 7.1.2.2.1-3: Summary of soil characteristics of field trials (soil type DIN)

Parameter	CU 90/6	OAT/20/01	FTL/19/01	ALO/24/01
Soil type (DIN)	loamy sand soil	loamy sand	loamy sand	sand
Particle size distribution [%]				
% Sand (0.063-2 mm)	66.0	52.7	40.3	90.8
%Silt (0.002-0.063 mm)	24.2	32.1	49.0	5.7
%Clay (<0.002 mm)	9.8	14.3	10.8	3.4
OM [%]	1.1	2.55	2.0	0.66
pH [-]	6.6	6.7	6.5	6.5
MWC [g H_20 100 g^{-1} dry soil]	32.2	37.3	44.3	31.9

Time-step normalization approach

Time-step normalization for the standardization of transformation parameters to reference soil temperature (20°C) and moisture (pF2) conditions was carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors (f_{temp} and f_{moist}) identical to those used in most regulatory leaching models. Daily soil moisture and soil temperature values were calculated by FOCUS-PEARL 4.4.4. Based on the model results, daily correction factors for the normalized day length were calculated, and the cumulative time between sampling points was determined and used as input for a standard kinetic evaluation according to FOCUS.

Estimation of actual soil temperature and moisture

Daily soil temperature and moisture was estimated with the simulation model FOCUS-PEARL 4.4.4 using actual soil characteristics and weather data for the different field trials (Table 7.1.2.2.1-1). The soil and climate scenario of each trial site was implemented in FOCUS-PEARL 4.4.4.

Weather data

The required actual weather data (maximum temperature, minimum temperature, precipitation, solar radiation) over the study duration were provided in the original study reports (trials OAT/20/01, FTL/19/01 and ALO/24/01) and completed by data from near-by weather stations, or obtained from official German weather stations (German trial sites). The closest appropriate weather stations providing the required data were considered in all cases. In addition to the period relevant for the normalization procedure, the prepared weather data were extended by one year to allow for a warm-up period in the FOCUS-PEARL 4.4.4 model runs. This was done by replicating the data from the study period to cover the preceding year. Due to the trial duration of less than one year, this warm-up period of the trials in the UK, France and Spain had to be complemented by weather data from additional sources. None of the field trials were irrigated within the study duration.

Soil profile settings

The actual evaporation of the different field trials was estimated within FOCUS-PEARL 4.4.4 using the Makkink approach. For the FOCUS-PEARL simulations, soil profiles of 1 m depth with three horizons were defined for each trial site, considering the soil properties given in Table 7.1.2.2.1-2 and Table 7.1.2.2.1-3. For the subsoil layers, the OM content was calculated by dividing the OM content of the respective upper layer by 2 to account for decreasing OM with increasing soil depth.

The number of numerical compartments (= model output layers) in each soil horizon was always chosen so that each compartment had a depth of 2.5 cm. The lower boundary condition of the simulation profiles was set to 'Free Drainage'. As no trial site was influenced by groundwater, the initial groundwater level was set to 3 m below soil surface for all scenarios.

For description of the hydraulic characteristics, the van Genuchten parameters, which describe the soil-water retention characteristics, were used. The soil hydraulic pedotransfer functions based on the HYPRES database were applied.

Estimated normalized day lengths

Table 7.1.2.2.1-4 shows the field sampling days for the trial locations and the normalized (20 °C and pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-4: Time-step normalized sampling days

CU 88/516		CU 88/517		CUE 3/89		CUE 4/89	
DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}
0	0	0	0	0	0	0	0
14	10.0	14	8.4	26	13.7	28	26.3
28	20.5	28	16.8	54	33.4	56	50.7
42	31.1	42	27.4	82	54.5	84	74.7
56	43.2	56	39.2	110	69.7	112	93.1
84	66.9	84	56.7	138	80.4	140	106.1
112	83.8	112	69.0	171	89.7	181	113.7
140	97.3	140	79.2	194	90.3	203	119.1
168	105.6	168	85.3				
CUE 90/6		**OAT/20/01**		**FTL/19/01**		**ALO/24/01**	
DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}
0	0	0	0	0	0	0	0
28	13.4	6	1.7	7	3.1	7	4.7
56	26.6	14	4.5	15	6.8	14	9.2
84	45.2	28	10.8	28	16.0	28	23.2
112	67.4	59	26.7	65	50.6	60	77.6
140	86.8	102	59.0	103	93.1	101	131.7
168	99.7	154	94.0	147	134.1	151	193.6
224	117.4	210	121.0	211	170.7	214	252.3

Kinetic modeling strategy

The residue data resulting from the data handling procedures were combined with the normalized day length data that were obtained. The resulting data sets for dimethomorph, the E-isomer of dimethomorph and the Z-isomer of dimethomorph were analyzed according to EFSA's recommendations for obtaining $DegT_{50}$ values in soil from field dissipation studies for modeling purposes.

As outlined by EFSA, the Panel proposed the splitting of field dissipation studies into two parts viz. before and after at least 10 mm of rain has fallen since application. The kinetic evaluation of data concentrated on the residues measured after 10 mm of rain has fallen to guarantee that the results of the evaluation describe the degradation in the soil matrix rather than at the soil surface.

The appropriate kinetic model for a dataset was selected in a step-wise approach according to EFSA. The goodness-of-fit was assessed based on visual and statistical assessment, and corresponding $DegT_{50}$ values are reported as modeling endpoints.

Kinetic models included in the evaluations

The set of models which can be employed for kinetic evaluations was described in the reported of the FOCUS workgroup on degradation kinetics [FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration"]: Single-First-Order (SFO), Double first-order in parallel (DFOP), First-order multi-compartment (FOMC) and the Hockey-stick (HS) model. The selection was modified by the EFSA guidance, excluding the FOMC from the calculation of the $DegT_{50}$ for normalized decline curves:

- Single-First-Order (SFO); Box 5-1, p. 51.
- Double first-order in parallel (DFOP); Box 5-4, p. 57.
- Hockey-stick (HS) kinetic: Box 5-3, p. 55.

Software for kinetic evaluation

The software package KinGUI, version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

II. RESULTS AND DISCUSSION

The data sets of the eight trial locations were evaluated for the criterion of 10 mm cumulative rainfall. The evaluation showed that it was possible to perform a kinetic modeling analysis for dimethomorph for all the trial locations, because the number of data points in each of the data sets was still appropriate (five to eight sampling days, Table 7.1.2.2.1-5) after excluding sampling dates before 10 mm of cumulative rainfall. Kinetic analysis for the separate isomers is not appropriate for data sets where only three sampling days remain after 10 mm of cumulative rainfall.

Table 7.1.2.2.1-5: Summary of field trial details

Trial	Location	Day of 10 mm rain (DAT / D_{norm})	Rain * [mm]	Remaining sampling days		
				Dimethomorph	E-isomer	Z-isomer
CU 88/516	Schwabenheim, Germany	3 / 1.6	12.5	8	4	4
CU 88/517	Malborn, Germany	5 / 2.5	22.4	8	4	4
CUE 3/89	Leibertingen, Germany	5 / 1.8	10.0	7	3	3
CUE 4/89	Schwabenheim, Germany	6 / 5.7	10.6	7	4	4
CUE 90/6	Krögsberg, Germany	16 / 7.7	21.8	7	4	4
OAT/20/01	Stratford upon Avon, UK	4 / 1.1	14.6	7	7	7
FTL/19/01	Merville, France	3 / 1.4	13.8	7	6	7
ALO/24/01	Utrera, Spain	9 / 5.9	13.9	5	5	6

* Amount of rain until first considered data point

A summary of the adequate kinetic models and the resulting $DegT_{50}$ values of dimethomorph to be used as modeling endpoints is given in Table 7.1.2.2.1-6. The geometric mean DT_{50} for the isomer mix was 35.1 days.

Table 7.1.2.2.1-6: Summary of endpoints for use in modeling of BAS 550 F - isomer mix

Field trial (Country)	Soil type	Kinetic model	Data points used	Endpoints	
			[-]	Rate k [d^{-1}]	DT_{50} [d]
CU 88/516	sandy loam soil *	SFO	8	0.0206	33.6
CU 88/517	sandy loam soil *	SFO	8	0.0250	27.8
CUE 3/89	clay soil *	SFO 2)	6	0.0255 2)	27.1 2)
CUE 4/89	sandy loam soil *	SFO 4)	7	(0.0153) 4)	(45.2) 4)
CUE 90/6	loamy sand soil **	SFO	7	0.0224	31.0
OAT/20/01	loamy sand **	DFOP 1)	7	0.0091 1)	76.2 1)
FTL/19/01	loamy sand **	SFO	7	0.0220	31.5
ALO/24/01	sand **	- 3)	8	- 3)	- 3)
Geometric mean 5)			**-**	**0.0197**	**35.1**

1) k-rate and DT_{50} derived from slow phase
2) outlier removed
3) no adequate model found
4) qualitative information only (plot treated with BAS 550 F in year before study begin)
5) trial CUE 4/89 excluded
* soil classification based on German classification (DIN)
** soil classification based on ISSS system

Summaries of the separate evaluation of the two isomers of dimethomorph are presented in Table 7.1.2.2.1-7 and Table 7.1.2.2.1-8.

Table 7.1.2.2.1-7: Summary of endpoints for use in modeling of BAS 550 F - E isomer

Field trial (Country)	Soil type	Kinetic model	Data points used	Endpoints	
			[-]	Rate k [d^{-1}]	DT_{50} [d]
CU 88/516	sandy loam soil *	SFO	4	0.0286	24.3
CU 88/517	sandy loam soil *	SFO	4	0.0413	16.8
CUE 3/89	clay soil *	SFO [2)]	3	(0.0529) [2)]	(13.1) [2)]
CUE 4/89	sandy loam soil *	SFO [4)]	4	(0.0149) [4)]	(46.5) [4)]
CUE 90/6	loamy sand soil **	SFO	4	0.0266	26.0
OAT/20/01	loamy sand **	HS [1)]	8	0.0161 [1)]	43.1 [1)]
FTL/19/01	loamy sand **	SFO	6	0.0398	17.4
ALO/24/01	sand **	- [3)]	7	- [3)]	- [3)]
Geometric mean [5)]			**-**	**0.0289**	**24.0**

1) k-rate and DT_{50} derived from slow phase
2) only 3 data points after application of 10 mm rain criterion, leads to overestimation of degradation rate
3) no adequate model found
4) qualitative information only (plot treated with BAS 550 F in year before study begin)
5) trial CUE 3/89 and CUE 4/89 excluded
* soil classification based on German classification (DIN)
** soil classification based on ISSS system

Table 7.1.2.2.1-8: Summary of endpoints for use in modeling of BAS 550 F - Z isomer

Field trial (Country)	Soil type	Kinetic model	Data points used	Endpoints	
			[-]	Rate k [d^{-1}]	DT_{50} [d]
CU 88/516	sandy loam soil *	SFO	4	0.0210	33.0
CU 88/517	sandy loam soil *	SFO	4	0.0222	31.2
CUE 3/89	clay soil *	SFO [2)]	3	(0.0458) [2)]	(15.1) [2)]
CUE 4/89	sandy loam soil *	SFO [4)]	4	(0.0132) [4)]	(52.7) [4)]
CUE 90/6	loamy sand soil **	SFO	4	0.0222	31.2
OAT/20/01	loamy sand **	HS [1)]	8	0.0078 [1)]	88.9 [1)]
FTL/19/01	loamy sand **	SFO	7	0.0197	35.1
ALO/24/01	sand **	- [3)]	8	- [3)]	- [3)]
Geometric mean [5)]			**-**	**0.0174**	**39.8**

1) k-rate and DT_{50} derived from slow phase
2) only 3 data points after application of 10 mm rain criterion, leads to overestimation of degradation rate
3) no adequate model found
4) qualitative information only (plot treated with BAS 550 F in year before study begin)
5) trial CUE 3/89 and CUE 4/89 excluded
* soil classification based on German classification (DIN)
** soil classification based on ISSS system

III. CONCLUSION

A Single First Order (SFO) kinetic model was appropriate to describe the observed degradation behavior of the parent compound and formation and degradation of its metabolites. Statistical valid degradation and formation rates were obtained.

It is known that the E-isomer is partially converted into the Z-isomer under the influence of light (photolysis). As the initial sharp decline observed for the E-isomer concentrations can partly be attributed to the conversion of the E-isomer to the Z-isomer due to soil surface processes under the influence of light, it is concluded that overall, the DT_{50} of the E-isomer is independently shorter than the DT_{50} for the Z-isomer.

The calculations resulted in a geometric mean of 24.0 days for the E-isomer and a geometric mean of 39.8 days for the Z-isomer, with a geometric mean of 35.1 days for the isomer mix (sum of both isomers).

Supplementary reports on field soil dissipation to complement existing information

Report: CA 7.1.2.2.1/2
Leonard R.C., 1997a
CL 336379 (Dimethomorph) - Rate of dissipation of CL 336379 residues in soil after treatment with Acrobat MZ (90/600) WP fungicide applied to bare ground
DK-620-032
Guidelines: Agriculture Canada Trade Memorandum T-1-255
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The dissipation of dimethomorph (CL 336379) in soil under Canadian field conditions was examined in two replicate bare plots at a site in New Glasgow, Prince Edward Island, Canada.

The product Acrobat MZ (90/600) (containing dimethomorph and mancozeb), formulated as WP, was broadcast applied to bare soil in a threefold application (4-8 days interval) at a target rate of approx. 225 g a.i. ha^{-1} in 257-464 liters of spray solution per hectare. Applications took place in July 1996 using a tractor-mounted boom sprayer. Monitoring of each application was performed by taking tank mix samples before and after each application. Results from spray broth analysis for all three applications revealed concentrations between 65 and 76% of the nominal value. Dose verification conducted via application monitors yielded recovery values for the third application of 81% or 97% of the target rate.

The experiment was conducted for 366 days following the third application (377 days total). No tillage or fertilisation was performed during the course of the study and no crops were grown on the trial site. The plots were kept free of vegetation via the application of Roundup. Rainfall was supplemented twice by irrigation.

The half-life of dimethomorph residues in this soil was calculated to be 102 days. The DT_{50} and DT_{90} values were 35 and 316 days, respectively. Apparent soil residues of dimethomorph in untreated soil were always less than 1.5 µg kg^{-1}. The limit of detection was approximately 1 µg kg^{-1}.

The major route of dissipation of dimethomorph under field conditions at the test site could not be determined because samples were not analyzed for transformation products, leaching was not observed (no residues detectable below the 7.5-15 cm soil layer) and volatilization and runoff were not studied.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation): Acrobat MZ (90/600) WP Fungicide
Active substance (a.s.): dimethomorph (BAS 550 F), mancozeb
Type of formulation: WP (wettable powder)
Lot No.: AC 9531-85R
Content of a.s.: 9% dimethomorph, 60% mancozeb

Test sites

The dissipation of dimethomorph in soil under field conditions was investigated at one trial site in New Glasgow, Prince Edward Island, Canada. The site characteristics are presented in Table 7.1.2.2.1-9.

Twenty 45 cm soil cores were taken from the test plot prior to the application of the test substance for the characterization of the test system. Similar depths for each core were composited and labeled A (0-7.5 cm), B (7.5-15 cm), C (15-30 cm) and D (30-45 cm). After analysis by an independent laboratory, soil texture was determined to be a sandy loam (67% sand, 17% silt, 16% clay) in the top 7.5 cm of soil with 4.0% organic matter and a soil pH of 5.7.

Table 7.1.2.2.1-9: Soil characteristics of the trial site

	Depth [cm]			
	0 – 7.5	7.5 - 15	15 - 30	30 - 45
Textural classification	Sandy loam	Sandy loam	Sandy loam	Sandy loam
% sand	67	67	61	61
% silt	17	19	25	25
% clay	16	14	14	14
pH	5.7	5.4	5.7	5.9
organic matter (%)	4.0	3.9	2.4	1.2
CEC (meq/100 g)	10	9.1	7.6	6.7
bulk density - disturbed (g/cm³)	1.03	1.02	1.04	1.14
soil moisture at 1/3 bar (%)	30.1	29.9	29.7	27.9

CEC = cation exchange capacity

The selected trial site represents an area of the country where the product is intended for use as a fungicide on potatoes. It is located in a major potato-growing area of Canada. The test site had a slope of approximately <2%. The depth of the water table was >8 meters.

During the fall of 1995, the field site was plowed with a moldboard plow to a depth of 20 cm. On April 25th, 1996, the test site was disked to a depth of 20 cm. Later in the year, the test site was harrowed to a depth of 15 cm with triple K harrows on June 1st and with C-tine harrows on July 7th.

In the year before the study the pesticide BANVEL 480 (3,6-dichloro-2-methoxybenzoic acid, dicamba) was applied to the test site. No product containing the test item a.i. had been used on the test plots in the last three years.

B. STUDY DESIGN

Experimental conditions

For the analysis of the dissipation behaviour of dimethomorph in soil, two untreated control plots and two treated plots were examined, each with a size of 6 m x 20 m. A buffer of 10 meters was used to separate each of the treated plots from each other as well as from the control plot while the untreated plots were separated by 2 meters.

The product, formulated as a wettable powder, was broadcast applied to bare soil in a threefold application (4-8 days apart) at a target rate of ca. 225 g a.i. ha^{-1} (2.5 kg formulated product per ha) dissolved in 257-464 liters of spray solution per hectare. Applications took place in July 1996 using a tractor-mounted boom sprayer equipped with twelve flat-fan nozzles spaced 50 cm apart. For each application, a spray mixture was prepared. Aliquots of the spray mixtures were taken before and after each application for later analysis. In addition, the dose was verified by means of sampling Petri dishes filled with 50 to 70 g of top soil collected from the treated plot. The petri dishes, without their lids, were buried at ground level in the treated plots (six/plot, randomly placed) prior to application.

The application rate for the third application was verified by analyzing the petri dish samples. The determined application rate was equivalent to 219 and 183 g a.i. ha^{-1} for both treated plots, which is 97% and 81%, respectively, of the nominal amount. Further details of the application are presented in Table 7.1.2.2.1-10 below.

Table 7.1.2.2.1-10: Application parameters

	Application date	Tank mix concentration [µg mL^{-1}]			Application rate per treatment* [g a.i. ha^{-1}]		
		nominal	actual**	% of nominal	nominal	actual	% of nominal
Plot 1	08-July-1996	497	325	65	225	n.a.	-
	15-July-1996	866	584	67		n.a.	-
	19-July-1996	848	646	76		219	97
Plot 2	08-July-1996	497	325	65		n.a.	-
	15-July-1996	866	584	67		n.a.	-
	19-July-1996	848	646	76		183	81

* determined by means of petri dishes filled with soil (average of 6 values)
** average of 4 samples before application and 4 samples after application
n.a. not analyzed

No tillage or fertilization was performed during the course of the study. The plots were kept free of vegetation via two applications of Roundup (glyphosate) (712 g a.i. ha^{-1}). Rainfall was supplemented on two occasions: August 30, 1996 (21 mm) and August 31, 1996 (29 mm). The total water input was at least 21% of the historical average rainfall during the study period at the test site.

Daily weather and rainfall data was collected on-site by a weather station located less than 100 m west of the test site (except from September 12 to October 17 where data was lost) and by a weather station located approximately 21 km from the test site (Environment Canada Weather Station at the Charlottetown Airport). Due to the missing data of the on-site weather station, the Environment Canada weather data was used in this study to document weather conditions. Historical (long-term) weather data on precipitation and average air temperature from at least 10 years (1961-1990) were taken from the same weather station. Actual and historical data (average temperature and precipitation) and irrigation during the study are presented in Table 7.1.2.2.1-11

Table 7.1.2.2.1-11: Actual and historical weather data from the trial site

Month	T_{mean} Air		∑ Precipitation		∑ Irrigation [mm]	Sum of actual precipitation and irrigation	
	normal* [°C]	actual [°C]	normal* [mm]	actual [mm]		[mm]	% of historic precipitation
Jun 96	14.4	15.2	91.1	89.7	-	89.7	98.5
Jul 96	18.4	17.8	81.6	153.9	-	153.9	188.6
Aug 96	18.0	18.6	88.6	8.6	50	58.6	66.1
Sep 96	13.4	13.6	94.1	155.2	-	155.2	164.9
Oct 96	8.0	6.9	111.7	111.5	-	111.5	99.8
Nov 96	2.5	2.0	121.9	90.0	-	90.0	73.8
Dec 96	-4.1	-0.1	133.2	101.9	-	101.9	76.5
Jan 97	-7.7	-7.6	105.3	72.5	-	72.5	68.9
Feb 97	-8.0	-8.4	91.5	79.2	-	79.2	86.6
Mar 97	-8.4	-6.8**	92.2	78.5**	-	78.5**	85.1
Apr 97	2.5	0.4	91.8	65.3**	-	65.3**	71.1
May 97	8.8	8.3	96.8	98.7	-	98.7	102.0
Jun 97	14.4	13.1	91.1	74.1	-	74.1	81.3
Jul 97	18.4	18.8	81.6	17.0	-	17.0	20.8

* normal: 1961 – 1990
** difficult value to read in scan of study report

Sampling

Soil specimens were taken at intervals up to 366 days and down to a maximum soil depth of 45 cm. Immediately after each treatment (0DAT1, 0DAT2 and 0DAT3), fourty soil cores were taken from each of the two treated plots. Twenty soil cores were further collected from the treated plots on the following dates: 3, 7, 14, 28, 60, 91, 123 and 366 days after the third treatment. Soil cores from the control plot were taken at various intervals throughout the course of the study.

Collection of cores from the treated plots was performed by excavating cores 5.7 centimeters in diameter and 15 centimeters deep. Then a 4.8 centimeter wide by 30 centimeter long core (15-45 cm depth) was pulled from the center of the first core. A hand held Concord hydraulic probe with acetate liners was used by the field investigator to collect all soil samples. The top of each tube was capped with a red cap, and the acetate liner with the sample inside was removed from the soil probe.

Details about the sampling are presented in Table 7.1.2.2.1-12.

Table 7.1.2.2.1-12: Details of sampling

Method of sampling		**Random**
Sampling intervals	Application 1	0 days
	Application 2	0 days
	Application 3	0, 3, 7, 14, 28, 60, 91, 123 and 366 days
Method of collection		Cores were taken in two stages, 0-15 cm and 15-45 cm
Number of cores collected per plot		40 (day 0 samples at all applications) or 20 (all other intervals)
Number of segments per core (after sectioning)		4 (0-7.5, 7.5-15, 15-30 and 30-45 cm)
Core diameter		5.7 cm for the 0-15 cm cores and 4.8 cm for the 15-45 cm cores

The soil samples were collected and transferred into a freezer within four hours of the start of sampling and stored at temperatures between -25.9°C to -0.3°C.

For segmentation and homogenization, soil samples were transported to the processing facility. Each soil core was segmented into 7.5 cm increments from the top layer to 15 cm, then in 15 cm increments from 15 to 45 cm. Corresponding segments from control cores or treated cores (20 per replicate) were composited to obtain a single analytical sample for each increment. The soil was allowed to thaw only as much as necessary for homogenization. Each composite sample was passed through a sieve to remove stones and plant material, and was thoroughly mixed. Composited samples were packed as two separate subsamples into appropriately labeled cardboard containers and stored in the freezer. One subsample was retained at the processing facility, the other was shipped to the analytical facility.

Shipment verification specimens (transit stability samples) were not prepared.

Analytical procedure

Samples were analyzed following American Cyanamid method M2656 [*CA 4.1.2/1*]. The final determination of dimethomorph is made by gas chromatography with a nitrogen-phosphorus detector. The limit of quantification (LOQ) of the method is 10 µg kg^{-1} (ppb) and the limit of detection (LOD) is ca. 1 µg kg^{-1} (ppb).

The validity of the analytical method was proven within the present study by analysis of fortified field samples (10-5000 µg kg^{-1}).

Storage stability experiments

The freezer storage stability study is presented below [see KCA 7.1.2.2.1/7, BASF DocID DK 326-026]. Data show that dimethomorph (CL 336379) residues are stable in soil for up to 22 months when stored under frozen conditions.

Calculation of dissipation times

The sum of the positive average apparent residues of dimethomorph are plotted on a logarithmic scale versus the sampling interval (in days) on a linear scale. The commercial program SIGMAPLOT 4.0 for Windows (SPSS, Inc, Chicago, Illinois) was used to generate a best-fit linear equation. The slope of the equation was used to calculate the half-life of dimethomorph.

Besides the DT_{50} and DT_{90} values in soil were determined based on Timme and Frehse kinetics.

II. RESULTS AND DISCUSSION

Spray broth concentration and application verification

The application rate for the third application was verified by randomly placing six petri-dish tops, each containing ca. 50-70 g of top soil, into each treated plot prior to the third application only. Recoveries achieved on extraction and analysis of application monitors were in the range of 145.0 - 271.7 g a.i. ha^{-1} for both treated replicate plots. Mean recovery from both replicate plots was equivalent to 219 and 183 g a.i. ha^{-1}, which is 97% and 81%, respectively, of the nominal amount.

Concentrations of dimethomorph in samples of the tank mix taken before and after each application averaged 65%, 67%, and 76% of the anticipated concentration for the first, second, and third application, respectively (Table 7.1.2.2.1-10). These averages, while lower than expected, do not negatively reflect on the quality of the applications as both soil core results and petri dish soil results indicate that the test substance was applied according to label.

Residues in field soil samples

Untreated soil specimens (control specimens) from two control plots were analyzed for residues of dimethomorph. All samples showed residues less than 1.5 µg kg^{-1} (ppb). The limit of detection of the method was approximately 1 µg kg^{-1} (ppb).
Procedural recovery experiments were performed with field soil specimens fortified at concentration levels of 10-5000 µg kg^{-1}. The procedural recoveries were within the range of 70 – 115%.

Apparent residues of dimethomorph were determined in soil in 7.5 cm increments to a depth of 15 cm, and in 15 cm increments from 15 to 45 cm. All averages were calculated using 5 µg kg^{-1} as a default value for <10 µg kg^{-1}. After averaging the two replicates of each increment at each sampling interval, the sum of all positive averages was calculated to derive the concentration of dimethomorph at each sampling date. Results are presented in Table 7.1.2.2.1-13.

Table 7.1.2.2.1-13: Residues of dimethomorph under field conditions in soil

Sampling Intervals (days)	Concentration (µg kg^{-1})										
	App 1	App 2	App 3								
	0	0	0	3	7	14	28	60	91	123	366
Plot 1											
0-7.5 cm	143	271	669	555	659	466	506	240	152	136	51.0
	166	459	582								
7.5-15 cm	<LOQ	<LOQ	10.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	10.9								
15-30 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
Plot 2											
0-7.5 cm	156	228	937	588	830	575	404	244	188	129	69.0
	162	493	656								
7.5-15 cm	<LOQ	<LOQ	10.0	<LOQ	12.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	10.4	<LOQ	10.9								
15-30 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								

Average apparent soil residues of dimethomorph declined from a high of 712 µg kg^{-1} immediately after the third application to 60 µg kg^{-1} at 366 days after the third treatment (366 DAT3). No average apparent residues of dimethomorph (10 µg kg^{-1} or greater) were found in the 15-30 cm and 30-45 cm layers during the study. The half-life of dimethomorph residues in this soil was calculated to be 102 days.

Dimethomorph dissipated in the soil according to a second order kinetic model and DT_{50} and DT_{90} values of 35 and 316 days, respectively, were calculated.

Since the soil samples were not analyzed for transformation products of dimethomorph, a route of dissipation could not be determined.

Time of storage

The soil samples of the study were frozen for no more than 14 months from the time of sampling to analysis.

III. CONCLUSION

The DT_{50} and DT_{90} values of dimethomorph residues were calculated to be 35 and 316 days, respectively. The major route of dissipation of dimethomorph under field conditions at the test site could not be determined because samples were not analyzed for transformation products, leaching was not observed (no residues detectable below the 7.5-15 cm soil layer) and volatilization and runoff were not studied.

Report:	CA 7.1.2.2.1/3 Leonard R.C., 1998a CL 336379 (Dimethomorph) - Rate of dissipation of CL 336379 residues in soil after treatment with Acrobat MZ (90/600) WP fungicide applied to bare ground DK-620-033
Guidelines:	Agriculture Canada Trade Memorandum T-1-255
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The purpose of this study was to determine soil rate of dissipation of dimethomorph after treatment with ACROBAT MZ (90/600). WP fungicide to soil at the proposed label rate for potatoes. The field portion of the study was conducted in Minto, Manitoba at a test site with a loam soil texture.

ACROBAT TM MZ (90/600) WP was broadcast applied to each treated plot at the rate of 2.5-2.6 kg formulated product per ha (221-236 g a.i. ha^{-1}). Samples of soil were collected from 0.1 days after the first treatment (0.1 DAT1) until 356 days after the third treatment (356 DAT3) The soil samples were analyzed for apparent dimethomorph residues according to Cyanamid method M 2656, which has a validated sensitivity (Limit of Quantitation, LOQ) of 10.0 µg kg^{-1}.

Average apparent soil residues of dimethomorph declined from 809 µg kg^{-1} immediately after the third application (0.1 DAT3) to 64.2 µg kg^{-1} at 356 days after the third treatment (356 DAT3). The "B" layer (7.5 -15 cm) had average apparent residues from immediately after the third treatment (0.1 DAT3) until 13 DAT3. The "C" layer had average apparent residues from immediately after the third treatment (0.1 DAT3) until 3 DAT3. No average apparent residues of dimethomorph (10 µg kg^{-1} or greater) were found in the "D" layer.

The half-life of dimethomorph residues in this soil during the 1997 "growing season" (from July 1997 application until October 1997) was calculated to be 53 days. The DT_{50} and DT_{90} of dimethomorph residues in this soil for all time intervals (0.1 DAT3 - 356 DAT3) was calculated to be 25 and 275 days, respectively. As indicated by the respective correlation coefficients (r^2 = 0.89, r^2 = 0.97), the square root first order model is a more accurate depiction of the presented data.

Average apparent soil residues of dimethomorph in untreated soil were less than 3.81 µg kg^{-1}.

Soil samples were not analyzed for transformation products of dimethomorph and a route of dissipation could not be determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test Substance Characterization

Test item (formulation): Acrobat MZ (90/600) WP Fungicide
Active substance (a.s.): 9% Dimethomorph (BAS 550 F), 60% Mancozeb, 31% inert ingredients
Type of formulation: WP (wettable powder)
Assay of lot number: AC 9531-85R
CAS number (CL 336379) 110488-70-5
Expiration date: 31 Dec 2012

Test Sites

Two untreated control and two treated plots each measured 6 m x 37.5 m. A buffer of 10 m was used to separate each of the two treated and two untreated plots from each other while the untreated plots were separated from the treated plots by 15 m The test site had a slope of approximately <2% The depth of the water table was 6 m. No crops were planted in the test plots for this bare ground study.

Table 7.1.2.2.1-14: Soil characteristics of the trial site

	Depth [cm]			
	0 – 7.5	7.5 - 15	15 - 30	30 - 45
Textural classification	Loam	Loam	Loam	Silt loam
% sand	41	37	39	31
% silt	47	47	45	51
% clay	12	16	16	18
pH	7.7	7.9	8.1	8.2
organic matter (%)	6.5	5.3	3.0	1.8
CEC (meq/100 g)	35.1	43.5	55.6	53.9
bulk density - disturbed (g/cm^3)	1.08	1.07	1.09	1.11
soil moisture at 1/3 bar (%)	35.5	33.3	31.5	29.5

CEC = cation exchange capacity

In 1993 and 1994, the test plots were planted with wheat and treated with tralkoxydim and dichlorprop. In 1995, the test plots were planted with peas and (4-chloro-2-methylphenoxy) acetic acid and 4-(4-chloro-2-methylphenoxy) butanoic acid were applied.

ROUNDUP was applied to the test plots for weed control twice a year in 1996 and 1997 at the rate of 450 g a.i. ha^{-1} per treatment. In 1997, the grower was applying REFINE (containing the plant hormone 1-naphthaleneacetic acid) to an adjacent field and reported that some overspray occurred in the southeast comer of the test plots.

In June 1996, the test site was rototilled to a depth of 15 cm. Fertilizer was not applied to the test site during this trial.

B. STUDY DESIGN

Test Substance Application

For the analysis of the dissipation behaviour of dimethomorph in soil, two untreated control plots and two treated plots were examined, each with a size of 6 m x 37.5 m. A buffer of 10 meters was used to separate each of the treated plots from each other as well as from the control plot while the untreated plots were separated by 15 meters.

The product, formulated as a wettable powder, was broadcast applied to bare soil in a threefold application (4-8 days apart) at a target rate of ca. 221 to 236 g a.i. ha^{-1} (2.5-2.6 kg formulated product ha^{-1}) dissolved in 197-210 liters of spray solution per hectare. Applications took using a tractor-mounted boom sprayer equipped with twelve flat-fan nozzles spaced 50 cm apart. For each application, a spray mixture was prepared. Aliquots of the spray mixtures were taken before and after each application for later analysis. In addition, the dose was verified by means of sampling Petri dishes filled with approximately 50 g of top soil collected from the treated plot. The petri dishes, without their lids, were buried at ground level in the treated plots (six/plot, randomly placed) prior to application.

The application rate for the third application was verified by analyzing the petri dish samples. The determined application rate was equivalent to 232 g a.i. ha^{-1} for both treated plots, which is 103% of the nominal amount.

Using the previously calibrated equipment, dimethomorph was mixed and applied three times as follows:

Table 7.1.2.2.1-15: Application parameters for dimethomorph to test plots

Date	Plot number	Dimethomorph (g)	Spray Solution (L)	Boom Height (cm)	Actual (L ha^{-1})	Rate (kg a.i. ha^{-1})
06/24/96	11	187.58	15000	50	202	227
06/24/96	12	187.58	15000	50	199	223
06/29/96	11	187.53	15000	50	202	227
06/29/96	12	187.53	15000	50	197	221
07/04/96	11	187.52	15000	50	210	236
07/04/96	12	187.52	15000	50	206	231

g a.i. ha^{-1} = (test substance (g) x 9 g a.i. travel time (sec) x 1000 mL L^{-1} x actual L ha^{-1}) / (total spray solution (ml) x 100 test substance (g))

Climatic Conditions

The plots were kept free of vegetation via two applications of Roundup (glyphosate). No supplemental irrigation was necessary during the study.

Daily weather and rainfall data was collected on-site by a weather station located approximately 300 m north of the test site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-16.

Table 7.1.2.2.1-16: Summary of climatic conditions at field trial sites used to investigate the dissipation of dimethomorph

Climatic conditions	T_{mean} Air [°C]		∑ Precipitation [mm]		∑ Irrigation [mm]
Month	actual	normal	actual	normal	
Apr 96	0.2	4.3	15.0	36.4	0
May 96	9.6	11.8	57.7	50.8	0
Jun 96	17.6	17.0	43.1	80.8	0
Jul 96	18.5	19.7	104.4	64.8	0
Aug 96	19.2	18.3	31.0	57.5	0
Sep 96	11.3	12.2	33.3	52.3	0
Oct 96	3.4	5.5	30.1	30.3	0
Nov 96	-12.0	-4.3	24.6	19.7	0
Dec 96	-18.2	-13.1	32.2	19.8	0
Jan 97	-20.5	-16.3	19.6	21.5	0
Feb 97	-11.1	-12.2	4.0	17.0	0
Mar 97	-9.2	-5.0	21.6	24.5	0
Apr 97	1.5	4.3	33.4	36.4	0
May 97	9.6	11.8	21.2	50.8	0
Jun 97	18.6	17.0	92.9	80.8	0

Soil Sampling

Soil specimens were taken at intervals up to 356 days and down to a maximum soil depth of 45 cm. Immediately after each treatment (0DAT1, 0DAT2 and 0DAT3), forty soil cores and six petri dishes containing topsoil were taken from each of the two treated plots. Samples were further collected in the same manner from the treated plots on the following dates: 3, 7, 13, 27, 61, 91, 111 and 356 days after the third treatment.

Prior to application, approximately 50 g of top soil collected from the treated plot was placed into each of 12 separate petri dishes. The petri dishes, without their lids, were buried at ground level in the treated plots (six/plot, randomly placed) and served as application verification samples.

Collection of cores from the treated plots was performed by excavating cores 5.7 cm in diameter and 15 cm deep. A 3.8 cm wide by 30 cm long core (15-45 cm depth) was pulled from the center of the first core. A hand held Concord hydraulic probe with acetate liners was used by the field investigator to collect all soil samples. The top of each tube was capped with a red cap, and the acetate liner with the sample inside was removed from the soil probe.

Details about the sampling are presented in Table 7.1.2.2.1-17.

Table 7.1.2.2.1-17: Details of sampling

Method of sampling		Random
Sampling intervals	Application 1	0 days
	Application 2	0 days
	Application 3	0, 3, 7, 13, 27, 61, 91, 111 and 356 days
Method of collection		Cores were taken in two stages, 0-15 cm and 15-45 cm
Number of cores collected per plot		40 (day 0 samples, all applications) or 20 (all other intervals)
Number of segments per core (after sectioning)		4 (0-7.5, 7.5-15, 15-30 and 30-45 cm)
Length of soil segments		7.5 or 15 cm
Core diameter		5.7 cm for the 0-15 cm cores and 3.8 cm for the 15-45 cm cores

Sample Handling and Freezer Stability

Sample shipments were placed into a walk-in freezer and maintained at approximately -10°C until preparation for analysis. The soil was allowed to thaw only as much as necessary for homogenization. Freezer storage stability studies on dimethomorph residues in soil are in progress according to American Cyanamid protocol DM97PT03. The soil samples from study DM96CN04 were frozen for no more than 15 months from the time of sampling to analysis.

Analytical procedure

Samples were analyzed following American Cyanamid method M2656 [*CA 4.1.2/1*]. The final determination of dimethomorph is made by gas chromatography with a nitrogen-phosphorus detector. The limit of quantification (LOQ) of the method is 10 µg kg^{-1} (ppb) and the limit of detection (LOD) is ca. 1 µg kg^{-1} (ppb).

The validity of the analytical method was proven within the present study by analysis of fortified field samples (10-5000 µg kg^{-1}).

Storage stability experiments

The freezer storage stability study is presented below [see KCA 7.1.2.2.1/7, BASF DocID DK-326-026]. Data show that dimethomorph (CL 336379) residues are stable in soil for up to twenty-two months when stored under frozen conditions.

Calculation of dissipation times

A linear regression (1st order) by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues over the entire study (0.1 DAT3 to 356 DAT3) test period. The equation for this linear regression is shown below.

Log Y= -0.00286X + 2.71 (r^2-correlation coefficient = 0.83)

A better fit of the data was obtained by considering only the data from the 1997 "growing season" (0.1 DAT3 - 111 DAT3). The equation for this linear regression is shown below.

Log Y= -0.005713X +2.80656 (r^2-correlation coefficient = 0.89)

The half-life of dimethomorph residues was calculated from the slope as follows:

Half-life= Log 0.50/slope= -0.301/-0.005713 =53 days

The DT_{50} and DT_{90} values for dimethomorph in this soil for all sampling intervals (0.1 DAT3- 356 DAT3) were determined using Timme and Frehse. The dimethomorph value for 0.1 DAT3 was used as the starting point (100% value) for the calculation of disappearance. Dimethomorph dissipated in the soil according to a square root first order kinetic model.

II. RESULTS AND DISCUSSION

Residues in field soil samples

All untreated soil specimens (control specimens) showed residues less than 3.81 µg kg^{-1}. The limit of detection of the method was approximately 1 µg kg^{-1}.

Apparent residues of dimethomorph were determined in soil in 7.5 cm increments to a depth of 15 cm, and in 15 cm increments from 15 to 45 cm. All averages were calculated using 5 µg kg^{-1} as a default value for <10 µg kg^{-1}. After averaging the two replicates of each increment at each sampling interval, the sum of all positive averages was calculated for the analyte at each sampling interval. Results are presented in Table 7.1.2.2.1-18.

Table 7.1.2.2.1-18: Residues of dimethomorph under field conditions in soil

Sampling Intervals (days)	Concentration (µg kg^{-1})										
	App 1	App 2	App 3								
	0	0	0	3	7	13	27	61	91	111	356
Replicate 11											
0-7.5 cm	274	484	743	648	608	462	326	322	182	153	62.9
	292	430	953								
7.5-15 cm	<LOQ	<LOQ	28.7	24.6	35.1	12.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	29.2								
15-30 cm	<LOQ	<LOQ	10.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	10.1								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
Replicate 12											
0-7.5 cm	253	488	635	630	560	527	266	224	278	140	65.4
	223	466	751								
7.5-15 cm	<LOQ	10.1	24.2	17.5	<LOQ	12.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	17.5								
15-30 cm	<LOQ	<LOQ	<LOQ	15.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								

Average apparent soil residues of dimethomorph declined from a high of 809 µg kg^{-1} immediately after the third application to 64.2 µg kg^{-1} at 356 days after the third treatment (356 DAT3). The "B" layer (7.5 -15 cm) had average apparent residues from immediately after the third treatment (0.1 DAT3) until 13 DAT3. The "C" layer (15-30 cm) had average apparent residues from immediately after the third treatment (0.1 DAT3) until 3 DAT3. No average apparent residues of dimethomorph (10 µg kg^{-1} or greater) were found in the "D" layer (30-45 cm) layer during the study.

Dissipation times

The DT_{50} and DT_{90} values for dimethomorph in this soil were 25 and 275 days, respectively (correlation coefficient= 0 97).

Mean recovery from both replicate plots was equivalent to 232 and 197 g a.i. ha^{-1}, which is 103% and 88% of the nominal amount, respectively.

Average apparent soil residues of dimethomorph in soil from the control plot were always less than 3.81 µg kg^{-1}.

Soil samples were not analyzed for transformation products of dimethomorph and a route of dissipation could not be determined.

III. CONCLUSION

The DT_{50} and DT_{90} values for dimethomorph in this soil were 25 and 275 days.

Soil samples were not analyzed for transformation products of dimethomorph and a route of dissipation could not be determined.

Report: CA 7.1.2.2.1/4
Leonard R.C., 1998b
CL 336379 (Dimethomorph) - Rate of dissipation of CL 336379 residues in soil after treatment with Acrobat MZ (90/600) WP fungicide applied to bare ground
DK-620-034
Guidelines: Agriculture Canada Trade Memorandum T-1-255
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The purpose of this study was to determine soil rate of dissipation of dimethomorph after treatment with ACROBAT MZ (90/600) WP fungicide to soil at the proposed label rate for potatoes.

This field portion of this study was conducted in North Rose, New York in Wayne County at a test site with a loamy sand soil texture. ACROBAT MZ (90/600) WP was broadcast applied to each treated plot at the rate of between 223-235 g a.i. ha^{-1}. Samples of soil were collected from 0.1 days after the first treatment (0.1 DAT1) until 365 days after the third treatment (365 DAT3). The soil samples were analyzed for apparent dimethomorph residues according to Cyanamid method M 2656, which has a validated sensitivity (Limit of Quantitation, LOQ) of 10.0 µg kg^{-1}.

Average apparent soil residues of dimethomorph declined from a high of 523 µg kg^{-1} immediately after the third application (0 IDAT) to 34.6 at 365 days after the third treatment (365DAT3). No average apparent residues of dimethomorph (10 µg kg^{-1} or greater) were found in the 30-45 cm ("D" layer) and only two average apparent residues were found in the 15-30 cm ("C" layer) during the study.

The half-life of dimethomorph residues in this soil during the 1996 "growing season" (from July 1996 application until November 1996) was calculated to be 59 days. The DT_{50} and DT_{90} dimethomorph residues in this soil for all time intervals (0.1 DAT3 - 365 DAT3) was determined to be 8 and 209 days, respectively. As indicated by the respective correlation coefficients (r^2 =0.64; r^2 =0.87), the square root of the 1st order model is a more accurate depiction of the presented data.

Apparent soil residues of dimethomorph in untreated soil were always less than 1.14 µg kg^{-1}. The limit of detection was approximately 1.0 µg kg^{-1}.

I. MATERIAL AND METHODS

A. MATERIALS

Test Substance Characterization

Test item (formulation): Acrobat MZ (90/600) WP Fungicide
Active substance (a.s.): 9% Dimethomorph (BAS 550 F), 60% Mancozeb, 31% inert ingredients
Type of formulation: WP (wettable powder)
Assay of lot number: AC 9531-85R
CAS number (CL 336379) 110488-70-5

Test Sites

Two untreated control and two treated plots each measured 6.1 m x 21.3 m. A buffer of 10.7 m was used to separate each of the treated and untreated plots from each other. The test site had a slope of approximately 0-1%. The depth of the water table was >9.1 meters.

Twenty 45 cm soil cores were taken from the test plot prior to the application of the test substance for the characterization of the test system. Similar depths for each core were composited and labeled A (0-7 5 cm), B (7 5-15cem), C (15-30 em), and D (30-45 cm). After analysis by an independent laboratory (AGVISE Laboratories, Northwood, ND/Branson, MN), soil texture was determined to be a loamy sand (sand=85%, silt=7%, clay=8%) in the top 7 5 cm of soil (series Oakville), with organic matter 3.3% and soil pH of 6.9.

Table 7.1.2.2.1-19: Soil characteristics of the trial site

	Depth [cm]			
	0 – 7.5	7.5 - 15	15 - 30	30 - 45
Textural classification	Loamy sand	Loamy sand	Sand	Sand
% sand	85	87	91	93
% silt	7	5	3	3
% clay	8	8	6	4
pH	6.9	6.7	6.2	6.1
organic matter (%)	3.3	2.4	1.1	0.6
CEC (meq/100 g)	9.2	7.9	5.6	4.2
bulk density - disturbed (g/cm³)	1.26	1.26	1.33	1.38
soil moisture at 1/3 bar (%)	13.6	15.3	10.4	10.9

CEC = cation exchange capacity

In 1993, the test plots were an orchard planted with apples and PENNCOZEB 75DF, RUBIGAN 1E, ASANA XL, THIODAN 3E, GUTHION 3F, OMITE 30W, CAPTAN 50W, PENNCAP M, VYDATE 2L, TOPSIN M 70W, OMITE 6E, and SEVIN 80S were applied as maintenance pesticides.

In 1994 and 1995, the test plots were left fallow with no pesticides applied. On June 29, 1996, a cultimulcher was used at the test site to level and pack the soil to a depth of 5-7.5 cm.

No crops were planted at the test site during this bare ground trial. Fertilizer was not applied to the test site during this trial.

B. STUDY DESIGN

Experimental conditions

For the analysis of the dissipation behaviour of dimethomorph in soil, two untreated control plots and two treated plots were examined.

The product, formulated as a wettable powder, was broadcast applied to bare soil in a threefold application (5 days apart) at the rate of between 2.5-2.6 kg ha^{-1} formulated product (223-235 g a.i. ha^{-1}) dissolved in 206-245 L ha^{-1} of spray solution. Applications took place using a CO_2 backpack sprayer with 3 m (hand held boom. The boom was set up with six flat-fan nozzles spaced in 50.8 cm increments. In addition, the dose was verified by means of sampling Petri dishes filled with 50 g of top soil collected from the treated plot. The petri dishes, without their lids, were buried at ground level in the treated plots (six/plot, randomly placed) prior to application.

The application rate for the third application was verified by analyzing the petri dish samples. The determined application rate was equivalent to 235 g a.i. ha^{-1} for both treated plots, which is 104% of the nominal amount.

Using the previously calibrated equipment, dimethomorph was mixed and applied three times as follows

Table 7.1.2.2.1-20: Application parameters for dimethomorph to test plots

Date	Plot number	550 F (g)	Spray Solution (mL)	Boom Height (cm)	Actual (L ha^{-1})	Rate (kg form product ha^{-1})	Rate (kg a.i. ha^{-1})
07/01/96	11	91.1	8518	38	236	2.5	227
07/01/96	12	91.1	8518	38	231	2.5	223
07/06/96	11	112.1	9220	38	206	2.5	225
07/06/96	12	112.1	9220	38	212	2.6	232
07/11/96	11	91.1	8518	38	242	2.6	233
07/11196	12	91.1	8518	38	245	2.6	235

GRAMOXONE Extra 2.5S was applied to the test plots for weed control four times in 1996 at the rate of 0.56 kg ai ha^{-1}.

Climatic Conditions

GRAMOXONE Extra 2.5S was applied to the test plots for weed control four times in 1996 at the rate of 0.56 kg ai ha^{-1}. No irrigation was supplemented.

With one exception, daily weather and rainfall data were collected on site by the ACDS Research weather station located <0.40 kms east of the test site From October 14-21, 1996, weather and rainfall data were collected off site approximately 3.2 km west of the test site.

Table 7.1.2.2.1-21: Summary of climatic conditions at field trial sites used to investigate the dissipation of dimethomorph

Climatic conditions	Air Temp [°C]		∑ Precipitation [mm]	∑ Irrigation [mm]
Month	Avg. min	Avg. max		
Aug 96	14	27	122	0
Sep 96	11	21	307	0
Oct 96	5	16	144	0
Nov 96	-1	5	147	0
Dec 96	-1	4	117	0
Jan 97	-5	0	60	0
Feb 97	-3	3	60	0
Mar 97	-3	4	175	0
Apr 97	1	11	42	0
May 97	6	15	50	0
Jun 97	14	25	62	0
Aug 97	16	26	41	0

Soil Sampling

Twenty soil cores were collected from outside each plot on July 1, 1996, prior to application, and from the control plot at various intervals throughout the course of the study. Immediately after each treatment, 0.1DAT1, 0.1DAT2, and 0.1DAT3, twenty soil cores and six petri dishes (containing topsoil) were taken from each of the two treated plots. Samples were further collected in the same manner from the treated plots on the following dates 3. 7, 14, 28, 60, 90, 119 and 365 days after the third application.

Collection of cores from the treated plots was performed by excavating cores 5.7 cm in diameter and 15 cm deep. Then a 3.8 cm wide by 30 cm long core (15-45 cm depth) was pulled from the center of the first core. A hand held Concord hydraulic probe with acetate liners was used by the field investigator to collect all soil samples. The top of each tube was capped with a red cap, and the acetate liner with the sample inside was removed from the soil probe.

Details about the sampling are presented in Table 7.1.2.2.1-22.

Table 7.1.2.2.1-22: Details of sampling

Method of sampling		**Random**
Sampling intervals	Application 1	0 days
	Application 2	0 days
	Application 3	0, 3, 7, 13, 27, 61, 91, 111 and 356 days
Method of collection		Cores were taken in two stages, 0-15 cm and 15-45 cm
Number of cores collected per plot		40 (day 0 samples, all applications) or 20 (all other intervals)
Number of segments per core (after sectioning)		4 (0-7.5, 7.5-15, 15-30 and 30-45 cm)
Length of soil segments		7.5 or 15 cm
Core diameter		5.7 cm for the 0-15 cm cores and 3.8 cm for the 15-45 cm cores

Sample Handling and Freezer Stability

The soil samples were collected and transferred into a freezer within four hours of the start of sampling. The freezer's temperatures ranged from -10 °C to -24 °C. During preparation for analysis, the soil was allowed to thaw only as much as necessary for homogenization. Freezer storage stability studies on dimethomorph residues in soil are in progress according to American Cyanamid protocol DM97PT03. The soil samples were frozen for no more than 14 months from the time of sampling to analysis.

Analytical procedure

Samples were analyzed following American Cyanamid method M2656 [*CA 4.1.2/1*]. The final determination of dimethomorph is made by gas chromatography with a nitrogen-phosphorus detector. The limit of quantification (LOQ) of the method is 10 µg kg^{-1} (ppb) and the limit of detection (LOD) is ca. 1 µg kg^{-1} (ppb).

The validity of the analytical method was proven within the present study by analysis of fortified field samples (10-5000 µg kg^{-1}).

Storage stability experiments

The freezer storage stability study is presented below [see KCA 7.1.2.2.1/7, BASF DocID DK-326-026]. Data show that dimethomorph (CL 336379) residues are stable in soil for up to 22 months when stored under frozen conditions.

Calculation of dissipation times

A linear regression (1st order) by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues over the entire study (0.1 DAT3-365 DAT3). The equation for this linear regression is shown below.

LogY= -0.00273X + 2.41 (Correlation Coefficient – R^2 = 0.68)

An additional linear regression (1st order) by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues from only the 1996 "growing season" (0.1 DAT3 – 119 DAT3). The equation for this linear regression is shown below.

LogY= -0.0051427X + 2.51092 (Correlation Coefficient - R^2 = 0.64)

The half-life was calculated from the slope as follows.

Half-life= Log 0.50/slope = -0.301/-0.0051427= 59 days

The DT_{50} and DT_{90} values for dimethomorph in this soil for all sampling intervals (0.1DAT3- 356 DAT3) were determined using Timme and Frehse. The dimethomorph value of day 0.1 was used as the starting point (100% value) for the calculation of disappearance. Dimethomorph dissipated in the soil according to a square root 1st order kinetic model.

II. RESULTS AND DISCUSSION

Analytical Results

Apparent residues of dimethomorph were determined in soil in 7.5 cm increments to a depth of 15 cm, and in 15 cm increments from 15 to 45 cm. All averages were calculated using 5 µg kg^{-1} as a default value for <10 µg kg^{-1}. After averaging the two replicates of each increment at each sampling interval, the sum of all positive averages was calculated for the analyte at each sampling interval. Results are presented in Table 7.1.2.2.1-23.

Apparent soil residues of dimethomorph declined from 527 µg kg^{-1} after the third application to 34.6 µg kg^{-1} at 365 days after the third treatment. No average apparent residues of dimethomorph (10 µg kg^{-1} or greater) were found in the 30-45 cm ("D" layer) and two average apparent residues were found in the 30-45 cm ("C" layer) during the study.

Table 7.1.2.2.1-23: Residues of dimethomorph under field conditions in soil

Sampling Intervals (days)	Concentration (µg kg^{-1})										
	App 1	App 2	App 3								
	0	0	0	3	7	14	28	60	90	119	365
Replicate 11											
0-7.5 cm	91.6	303	553	423	219	406	102	151	101	96.7	45.2
	186	377	501								
7.5-15 cm	13.4	15.3	23.1	11.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	23.9	35.4								
15-30 cm	<LOQ	<LOQ	13	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	10.0	<LOQ								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								
Replicate 12											
0-7.5 cm	152	385	436	361	175	241	100	182	60.7	98.5	24.0
	156	375	376								
7.5-15 cm	11.6	19.8	28.5	30.3	22.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	23.9	48.2								
15-30 cm	<LOQ	<LOQ	17.3	14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	12.2								
30-45 cm	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ	<LOQ	<LOQ								

The DT_{50} and DT_{90} values for dimethomorph in this soil were 8 and 209 days, respectively (correlation coefficient of 0.87).

As indicated by their respective correlation coefficients (r^2 = 0 64, r^2 = 0 87), the square root of the 1st order model is a more accurate depiction of the presented data.

Apparent soil residues of dimethomorph found in petri dishes placed in the field prior to the last application were equivalent to showing an application rate of 235 g a i ha^{-1} (104% of theoretical) for plot 11 and 243 g a i ha^{-1} (108% of theoretical) for plot 12. This indicates that the test substance was applied to the soil according to label concentration.

Apparent soil residues of dimethomorph in untreated soil were always less than 1.14 µg kg^{-1}. The limit of detection was approximately 1 µg kg^{-1}.

III. CONCLUSION

The DT_{50} and DT_{90} values for dimethomorph in this soil were 8 and 209 days, respectively.

Soil samples were not analyzed for transformation products of dimethomorph and a route of dissipation could not be determined.

Report: CA 7.1.2.2.1/5
Lennon G.A., 1999a
CL 336379 (Dimethomorph) - Soil rate of dissipation study: AC 336379 residues in soil after treatment with Acrobat MZ fungicide applied to bare ground plot and a cropped processing tomato plot in 6 sequential applications (CA; 1996)
DK-620-040
Guidelines: EPA 164-1
GLP: no

Executive Summary

The objective of this study was to determine the rate of dissipation and potential for leaching of dimethomorph in soil after treatment on a bare ground plot and a processing tomato plot with ACROBAT MZ (90/600) WP fungicide in 6 sequential applications at the maximum use rate for vegetable crops.

The field portion was conducted in Madera, California. This test site had a sandy loam soil texture. ACROBAT MZ fungicide was applied to a bare soil and a cropped tomato plot in six weekly applications. The rate of application was 0.280 kg ha^{-1} in approximately 23.5 liters of spray solution ha^{-1}, for a total of 1.680 kg ha^{-1}. Soil cores from identical horizons were combined for analysis of dimethomorph residues according to American Cyanamid Company method M 2656 which has a validated sensitivity (Limit of Quantitation; LOQ) of 10 µg kg^{-1}.

Dimethomorph residues in soil from the bare ground plot (plot 1) declined from an average of 491 µg kg^{-1} at 0.1 days after the sixth application to an average of 47 µg kg^{-1} at 543 days after the sixth treatment.

Dimethomorph residues in soil from the cropped tomato plot (plot 2) declined from an average of 524 µg kg^{-1} at 7 days after the sixth application to an average of 60 µg kg^{-1} at 543 days after the sixth treatment.

Apparent soil residues of dimethomorph in untreated soil were always less than 2.8 µg kg^{-1}.

The half-life of dimethomorph soil residues in the bare ground plot was calculated to be 161 days and in the cropped tomato plot 175 days.

I. MATERIAL AND METHODS

A. MATERIALS

Test Substance Characterization

The test substance, ACROBAT MZ, is formulated as a wettable powder containing 9% dimethomorph; 60% mancozeb and 31% inert ingredients. This is a commercial formulation proposed for use as a fungicide on potatoes and tomatoes. The assay made prior to shipment of lot number AC 953l-85R used in this study showed an average concentration of 8.7%. ACROBAT MZ has been demonstrated to be stable for 12 months at 25°C and for 3 months at 45°C.

Site Description

The test site contained two treated plots (a bare ground plot and a processing tomato plot) and a control plot. The control plot and each of the treated plots had approximate dimensions of 23 m x 46 m. Each plot was divided into 6 subplots for soil sampling. The untreated and treated plots were separated by a buffer of >60m. The test site has a slope <2.0%.

Soil Characterization

Eighteen 91 cm soil cores were taken from the test plots prior to the application of the test substance for the characterization of the test system. Similar depths for each core were composited in 15 cm segments. After analysis by an independent laboratory (Agvise, Northwood, North Dakota), the soil texture was determined to be a sandy loam.

Table 7.1.2.2.1-24: Soil characteristics of the trial site

	Depth [cm]					
	0 – 15	15 - 30	30 - 45	45 - 60	60 - 75	75 - 90
Textural classification	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
% sand	72	72	72	72	70	70
% silt	18	16	16	16	18	18
% clay	10	12	12	12	12	12
pH	6.9	6.4	6.5	7.6	8.1	8.1
organic matter (%)	0.9	0.5	0.4	0.3	0.2	0.1
CEC (meq/100 g)	10.8	13.1	12.2	13.1	20.6	22.4
bulk density - disturbed (g/cm³)	1.39	1.33	1.38	1.41	1.40	1.35
soil moisture at 1/3 bar (%)	10.2	10.6	11.0	11.1	11.7	12.3

CEC = cation exchange capacity

B. STUDY DESIGN

Test Substance Application

ACROBAT MZ (Lot number AC 9531-85R) was applied to the treated plots as a broadcast spray using the previously calibrated equipment with the following parameters:

Table 7.1.2.2.1-25: Application parameters for dimethomorph to test plots

Date	ACROBAT MZ (g)	Spray Solution (mL)	Boom Height (cm)	GPA	Rate (kg a.i. ha^{-1})**
08/15/96	882.7	79485	45	29.2	1.172
08/22/96	882.7	79485	45	30.3	1.221
08/29/96	882.7	79485	45	29.8	1.221
09/05/96	882.7	79485	45	30.2	1.221
09/12/96	882.7	79485	45	30.8	1.270
09/19/96	882.7	79485	45	30.5	1.221

* boom height converted from inches
** kg a.i. ha^{-1} converted from lbs/acre

Climatic Conditions

Weather and rainfall data were collected at various weather stations during the duration of the study at the University of California IPM CIMIS Station in Firebaugh, CA (local off-site temperature data); University of California IPM Touchtone Station in Madera, CA (local off-site rainfall data); and an onsite weather station at the EXCEL Madera Research Station in Madera, CA. Monthly averages are presented in Table 7.1.2.2.1-26.

Table 7.1.2.2.1-26: Summary of monthly local weather data

Location	Date	Rainfall total (mm)	Average of max. daily air temperature (°C)	Average of min. daily air temperature (°C)
Local off-site*	06/96	7	32	14
	07/96	0	36	17
	08/96	0	34	11
	09/96	0	30	12
	10/96	51	24	8
	11/96	68	18	6
	12/96	118	13	6
	01/97	119	13	6
	02/97	4	17	4
	03/97	0	22	6
	04/97	0	24	9
Madera research station (on site)	05/97	0	32	13
	06/97	0	32	14
	07/97	0	35	16
	08/97	0	34	16
	09/97	1	33	14
	10/97	0	26	7
	11/97	52	19	7
	12/97	23	12	2
	01/98	63	13	5
	02/98	74	15	5
	03/98	48	18	6
	04/98	21	21	7

* Local off-site weather Madera, CA (rainfall) and Firebaugh, CA (temps) converted from inches of rainfall and °F

Maintenance Pesticides

Table 7.1.2.2.1-27: Maintenance pesticides applied to all plots in the test site for crop maintenance

Pesticide	Date Applied	Rate (kg ha^{-1})*	Reason
SEVIN XLR	9/4/96	2.24	Worm control
GRAMOXONE EXTRA	9/4/96 (applied to plots 0 & 1 only)	1.05	Weed control
GRAMOXONE EXTRA	11/15/96, 3/25/97, 6/17/97, 7/29/97, 8/19/97, 10/16/97, 3/6/98	1.05	Weed control
ROUNDUP ULTRA	10/2/96, 4/25/97, 7/2/97	1.12	Weed control

* converted from lbs $acre^{-1}$

Irrigation

Supplemental irrigation from an on site well (pH 6.0) was supplied by sprinkler. Irrigation was performed as indicated in the table below.

Table 7.1.2.2.1-28: Irrigation volume to test plots

Date	Water (mm)*	Date	Water (mm)*	Date	Water (mm)*	Date	Water (mm)*
08/12/96	2.54	03/20/97	1.83	06/21/97	1.70	08/07/97	1.98
08/16/96	2.18	03/27/97	2.08	06/24/97	1.73	08/10/97	2.59
08/23/96	1.73	04/14/97	1.45	06/26/97	1.96	08/18/97	1.70
08/28/96	3.05	04/2397	0.66	06/27/97	0.58	08/21/97	2.08
09/02/96	1.35	04/28/97	0.84	06/30/97	1.70	08/31/97	1.42
09/09/96	1.30	05/09/97	1.68	07/09/97	1.55	09/10/97	2.18
09/16/96	1.40	05/14/97	1.45	07/10/97	1.78	09/16/97	1.78
09/20/96	1.85	05/20/97	1.07	07/15/97	1.50	09/22/97	2.06
09/23/96	1.40	05/29/97	1.27	07/17/97	3.58	09/25/97	2.41
09/30/96	1.42	06/03/97	1.60	07/18/97	1.88	09/28/97	1.07
10/07/96	1.52	06/10/97	1.73	07/21/97	1.17	10/07/97	2.26
10/15/96	2.36	06/16/97	1.40	07/22/97	1.22	10/28/97	1.88
03/13/97	1.50	06/18/97	1.68	07/31/97	2.21	12/29/97	1.80

* converted from inches of water

Crop Disposition

In September 1996, the tomato plants in treated plot 2 were cut at the ground level and were manually removed from the plot area. The plants were brought to a barren field located in a remote location away from the test plot and disced twice.

Soil Sampling

Eighteen soil cores were taken randomly from plots 1 and 2 at the test site in 1996, prior to application (pre-treatment samples), and from the control plot at various intervals throughout the course of the study. Immediately after the first, third, and sixth application, eighteen soil cores were collected from each of the treated plots. When the crop was present in the processing tomato plot, the soil cores were obtained from the area located directly under the tomato plants.

Soil cores were collected first from the control plot (when collected) and then from the treated plots. Collection of soil cores from the treated plots was performed by excavating cores which were 15 cm deep. Then a 75 cm contiguous soil core (15-90 cm) was pulled from the center of the first core. The treated plots were divided into 6 subplots of approximately equal size. Three cores (each separated from the next by 30 cm) were taken from each of the six subplots, for a total of 18 cores for each treated plot.

Samples were further collected in the same manner from treated plots 1 and 2 at 0.1 days after the first application, and 0, 0.1 days after the third application and 0, 0.1, 3, 7, 14, 29, 76, 90, 134, 151, 180, 238, 300, 358, 459 and 543 days after the sixth application.

A four wheeled hydraulic soil corer was used by the field investigator to collect an approximately 90 cm long excavation core in a 120 cm long probe for each sampling time point, except for the 134 DAT6 sampling event. These samples had to be collected manually using a slide hammer due to excessive rainfall which prevented the tractor mounted probe from entering the test plots.

The top of each tube was capped with a red cap, and the acetate liner with the sample inside was removed from the soil probe. The bottom of the soil core was capped with a black cap. The six cores that constituted a given replicate from each treated plot, the eighteen pre-treatment cores, and the eighteen cores from the control plot received a unique identifier consisting of the protocol number, and a four-digit number indicating treatment number, replicate number, and sequence number based on the sampling interval.

Each group of eighteen excavated soil core segments constituting a replicate corresponding to their lower cores were labeled with the same label (sample number) used for their underlying cores. Prior to each application, approximately 50 g of top soil collected from the control plot was placed into 6 separate petri dishes. The petri dishes, without their lids, were placed in the bare ground plot (one per subplot) and their position marked with flags. Immediately after each application, the petri dishes were removed from the field. The lids were placed on the petri dish and wrapped with a sufficient amount of tape to secure the top to the bottom.

Analytical procedure

Samples were analyzed following American Cyanamid method M2656 [*CA 4.1.2/1*]. The final determination of dimethomorph is made by gas chromatography with a nitrogen-phosphorus detector. The limit of quantification (LOQ) of the method is 10 µg kg^{-1} (ppb) and the limit of detection (LOD) is ca. 1 µg kg^{-1} (ppb).

The validity of the analytical method was proven within the present study by analysis of fortified field samples (10-5000 µg kg^{-1}).

Storage stability experiments

The freezer storage stability study is presented below [see KCA 7.1.2.2.1/7, BASF DocID DK-326-026]. Data show that dimethomorph (CL 336379) residues are stable in soil for up to 22 months when stored under frozen conditions.

Calculation of dissipation

A linear regression by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues over the test period. The equation for this linear regression is shown below.

Log Y = -0.00187X + 2.63 (r correlation coefficient = 0.98) (Plot 1-Bare Ground)

Log Y = -0.00172X + 2.70 (r correlation coefficient = 0.92) (Plot 2-Tomato Crop)

The half-life was calculated from the slope as follows:
Half-life =Log 0.50/slope = -0.301/-0.00187 = 161 days (Plot 1-Bare Ground).
Half-life =Log 0.50/slope = -0.301/-0.00172 = 175 days (Plot 2-Tomato Crop).

II. RESULTS AND DISCUSSION

Analytical results for treated soil samples are summarized in Table 7.1.2.2.1-29 to Table 7.1.2.2.1-31. All averages were calculated using 5 µg kg^{-1} as a default value for <10 µg kg^{-1}. After averaging the three replicates of each increment at each sampling interval, the sum of positive averages was calculated for the analyte at each sampling interval.

Table 7.1.2.2.1-29: Dimethomorph residues in soil (plot 1)

Sample Sequence Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**
Sampling Interval (d) *	**-35(1)**	**-21(0)**	**-21(1)**	**0(0)**	**0(1)**	**3**	**7**	**14**	**29**
Soil Depth	[µg kg^{-1}]								
0-15 cm (A layer)	102	152	228	375	491	180	404	383	339
15-30 cm (B layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
30-45 cm (C layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	102	152	228	375	491	480	404	383	339

Sample Sequence Number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Sampling Interval (d)	**76**	**90**	**134**	**151**	**180**	**238**	**300**	**358**	**459**	**543**
Soil Depth	[µg kg^{-1}]									
0-15 cm (A layer)	276	N/C	282	202	191	166	90	91	59	47
15-30 cm (B layer)	<10	N/C	<10	16	<10	<10	<10	<10	<10	<10
30-45 cm (C layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	N/C	<10	<10	14	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	N/C	<10	N/C	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	276	-	282	218	201	166	90	91	59	47

* numerical dates are in relation to the sixth and final application
N/C not collected in field

Table 7.1.2.2.1-30: Dimethomorph residues in soil (plot 2)

Sample Sequence Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**
Sampling Interval (d) *	**-35(1)**	**-21(0)**	**-21(1)**	**0(0)**	**0(1)**	**3**	**7**	**14**	**29**
Soil Depth	[µg kg^{-1}]								
0-15 cm (A layer)	98	173	219	330	432	461	513	391	317
15-30 cm (B layer)	<10	<10	<10	16	<10	<10	11	<10	<10
30-45 cm (C layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	98	173	219	346	432	461	524	391	317

Sample Sequence Number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Sampling Interval (d)	**76**	**90**	**134**	**151**	**180**	**238**	**300**	**358**	**459**	**543**
Soil Depth	[µg kg^{-1}]									
0-15 cm (A layer)	453	-	423	279	275	220	137	104	68	60
15-30 cm (B layer)	11	N/C	46	29	14	16	<10	<10	<10	<10
30-45 cm (C layer)	<10	N/C	<10	11	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	464	-	469	319	289	236	137	104	68	60

* numerical dates are in relation to the sixth and final application
N/C not collected in field

Dimethomorph residues in soil from the bare ground plot (plot 1) declined from an average of 491 µg kg^{-1} at 0.1 days after the sixth application (0.1 DAT6) to an average of 47 µg kg^{-1} at 543 days after the sixth treatment (543 DAT6). Average dimethomorph residues were found at every interval above the LOQ (10 µg kg^{-1}) in the A-layer (0-6") layer, once in the B-layer (6-12") at 151 DAT6 and once in the D-layer (18-24") at 180 DAT6. Some sampling intervals (in one replicate only) contained detectable residues of dimethomorph in the B-layer at 180 DAT6, in the C-layer at 151 DAT6, 180 DAT6 and in the D-layer at 76 DAT6.

Dimethomorph residues in soil from the cropped tomato plot (plot 2) declined from an average of 524 µg kg^{-1} at 7 days after the sixth application (7 DAT6) to an average of 60 µg kg^{-1} at 543 days after the sixth treatment (543 DAT6). Average dimethomorph residues were found at every interval above the LOQ (10 µg kg^{-1}) in the A-layer (0-6") layer, m the B-layer (6-12") at 0 DAT6, 7 DAT6, 76 DAT6, 134 DAT6, 151 DAT6, 180 DAT6, 238 DAT6, and once in the C-layer at 151 DAT6.

Some sampling intervals (in one replicate only) contained detectable residues of dimethomorph in the B-layer at 3 DAT6, and the E and F-layers at 180 DAT6. These occasional residues in the lower soil layers from both plots may have been due to contamination in the field or laboratory portions of the study.

Apparent soil residues of dimethomorph in untreated soil were always less than 2.8 µg kg^{-1}.

Table 7.1.2.2.1-31: Summary of dimethomorph residues in petri dish soil

Sample Number	Amount [µg cm^{-2}]	Recovery [%]	Sample Number	Amount [µg cm^{-2}]	Recovery [%]	Sample Number	Amount [µg cm^{-2}]	Recovery [%]
1031	2.05	73	1043	2.24	80	1055	2.12	76
1032	2.25	80	1044	1.33	48	1056	3.6	129
1033	3.08	110	1045	2.29	82	1057	2.25	80
1034	2.05	73	1046	1.50	54	1058	2 55	91
1035	2.14	76	1047	2.26	81	1059	2.65	95
1036	2 41	86	1048	2.29	82	1060	2.42	86
1037	2.99	107	1049	2 58	92	1061	2.16	77
1038	2 64	94	1050	2 20	79	1062	2.54	91
1039	3 32	118	1051	2.71	97	1063	2.64	94
1040	3.52	126	1052	3.09	110	1064	3.12	111
1041	3.59	128	1053	1 28	46	1065	3.44	123
1042	3.29	118	1054	3.32	119	1066	2.40	86
Mean % recovery / standard deviation:								**92 ± 22%**
Theoretical amount of dimethomorph in petri dish soil = 2.80 µg cm^{-2}								

Slight discrepancies may be noted in application calculations based on the numbers supplied These discrepancies are due to rounding differences in data reported versus raw data

Petri dish soil samples were analyzed using Method 2656 for dimethomorph residues to confirm the application rates and accountability for the total material applied. Average test substance recovery and standard deviation were 92 ± 22% of theoretical (Table 7.1.2.2.1-31).

Calculation of dissipation

A linear regression by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues over the test period. The equation for this linear regression is shown below.

Log Y = -0.00187X + 2.63 (r^2 correlation coefficient = 0.98) (Plot 1-Bare Ground)
Log Y = -0.00172X + 2.70 (r^2 correlation coefficient = 0.92) (Plot 2-Tomato Crop)

The half-life was calculated from the slope as follows:

Half-life =Log 0.50/slope = -0.301/-0.00187 = 161 days (Plot 1-Bare Ground)
Half-life =Log 0.50/slope = -0.301/-0.00172 = 175 days (Plot 2-Tomato Crop)

III. CONCLUSION

The half-life of dimethomorph soil residues in the bare ground plot (plot 1) was calculated to be 161 days and in the cropped tomato plot (plot 2) 175 days.

Report:	CA 7.1.2.2.1/6 Lennon G.A.,Kleiner A., 1999a CL 336379 (Dimethomorph): Soil rate of dissipation study: AC336379 residues in soil after treatment with Acrobat MZ (90/600) WP fungicide applied to a bare ground plot and a cropped processing tomato plot in 6 sequential applications (OH; 1996) DK-620-042
Guidelines:	EPA 164-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The objective of this study was to determine the rate of dissipation and potential for leaching of dimethomorph in soil after treatment on a bare ground plot and a processing tomato plot with ACROBAT MZ (90/600) WP fungicide in six sequential applications at the maximum use rate.

The field portion of this study was conducted in New Holland, Ohio. This test site had a silty clay loam soil texture (plot 1) and a clay loam soil texture (plot 2). ACROBAT MZ fungicide was applied to a bare soil and a cropped tomato plot in six weekly applications. The rate of each application was 0.280 kg ha^{-1} in approximately 95 liters of spray solution per acre, for a total of 1.680 kg ha^{-1}. The treated plots were divided into six replicate subplots for sampling purposes, and eighteen soil core samples were collected at various intervals up to 540 days after the sixth and final application. Cores were cut into 15 cm increments down to 90 cm. Soil cores from identical horizons were combined for analysis of dimethomorph residues according to American Cyanamid Company method M 2656 which has a validated sensitivity (Limit of Quantitation; LOQ) of 10 parts per billion (µg kg^{-1}).

Dimethomorph residues in soil from the bare ground plot (plot 1) declined from an average of 752 µg kg^{-1} at 3 days after the sixth application to an average of 43 µg kg^{-1} at 449 days after the sixth treatment. Average dimethomorph residues above the LOQ (10 µg kg^{-1}) were found at every interval in the A-layer (0-15 cm) layer and in the B-layer (15-30 cm) at 14 DAT, 31DAT, 66 DAT, and 245 DAT.

Dimethomorph residues in soil from the cropped tomato plot (plot 2) declined from an average of 473 µg kg^{-1} at 3 days after the sixth application to an average of 27 µg kg^{-1} at 449 days after the sixth treatment (449 DAT). Average dimethomorph residues above the LOQ (10 µg kg^{-1}) were found at every interval in the A-layer (0-15 cm) layer and in the B-layer (15-30 cm) at 9 DAT and 155 DAT.

Some sampling intervals (in one replicate only) contained detectable residues of dimethomorph in the B-layer at 0.1 DAT, 31DAT,184 DAT, and the C-layer at 9 DAT. The dimethomorph residues in single replicates in the lower layers from both plots may have been due to contamination in the field or laboratory portions of the study.

Apparent soil residues of dimethomorph in untreated soil were approximately 1 µg kg^{-1} or less.

Petri dish soil samples were analyzed using Method 2656 for dimethomorph residues to confirm the application rates and accountability for the total material applied. Average test substance recovery and standard deviation from the 36 petri dish samples were 85 ± 21 % of theoretical (Table 7.1.2.2.1-39).

(In this summary, any measurements portrayed in US customary units were converted to SI, i.e. lbs $acre^{-1}$ to kg ha^{-1}, ppb to µg kg^{-1}, gallons to liters, temperature °F to °C, and length/rainfall in inches to cm/mm respectively.)

I. MATERIAL AND METHODS

A. MATERIALS

Test Substance Characterization

The test substance, ACROBAT MZ, is formulated as a wettable powder containing 9% dimethomorph; 60% mancozeb and 31 % inert ingredients. This is a commercial formulation proposed for use as a fungicide on potatoes and tomatoes. The assay conducted prior to shipment of lot number AC 9531-85R used in this study showed an average concentration of 8.7%. ACROBAT MZ has been demonstrated to be stable for 12 months at 25°C and for 3 months at 45°C.

Site Description

The test site contained two treated plots (a bare ground plot and a processing tomato plot) and a control plot. The control plot had approximate dimensions of 20 m x 52 m while the dimensions of the treated plots were approximately 26 m x 41 m. Each treated plot was divided into six subplots for soil sampling. The untreated and treated plots were separated by a buffer of approximately 23 m. The test site has a slope of approximately 1.0%.

Soil Characterization

Eighteen 90 cm soil cores were taken from the test plots prior to the application of the test substance for the characterization of the test system. Similar depths for each core were composited in 15 cm segments labeled A (0-15 cm), B (15-30 cm), C (30-45 cm), D (45-60 cm), E (60-75 cm), and F (75-90 cm). After analysis by an independent laboratory (Agvise, Northwood, North Dakota), the soil texture was determined to be a silty clay loam for plot 1 and a clay loam for plot 2. The soil composition in the A-layer for plot 1 was sand=19%, silt=53%, clay=28% with 1.8% organic matter and a soil pH of 7.1 . The soil composition in the A-layer for plot 2 was sand=23%, silt=49%, clay=28% with 1.7% organic matter and a soil pH of 6.7.

Table 7.1.2.2.1-32: Soil characteristics of the trial site

	Depth [cm]					
	0 – 15	15 - 30	30 - 45	45 - 60	60 - 75	75 - 90
Textural classification	Silty clay loam	Clay loam	Clay	Clay	Clay	Clay loam
% sand	19	21	19	27	25	25
% silt	53	47	39	31	35	37
% clay	28	32	42	42	40	38
pH	7.1	7.6	7.7	7.7	7.8	8.1
organic matter (%)	1.8	0.9	0.5	0.5	0.7	0.6
CEC (meq/100 g)	11.5	15.0	20.0	24.4	22.1	24.0
bulk density - disturbed (g/cm³)	1.18	1.20	1.20	1.20	1.20	1.18
soil moisture at 1/3 bar (%)	26.9	25.7	27.3	27.4	26.5	23.7

CEC = cation exchange capacity

Site History

The cultural practices listed below were employed on the test site in the three years prior to the initiation of this study:

Table 7.1.2.2.1-33: Application parameters for dimethomorph to test plots

Year	Crop	Pesticide	Application rate [kg a.i. ha^{-1}]*
1993	Soybeans	GALAXY	1.120
		PINNACLE 25 DF	0.00085
		FUSION	0.16
1994	Wheat	BANVEL	0.125
1995	Corn	ROUND UP	1.120

* kg a.i. ha^{-1} converted from lbs a.i. acre^{-1}

In June 1996, 44.8 kg ha^{-1} of nitrogen fertilizer was applied to the test plots.

B. STUDY DESIGN

Test Substance Application

ACROBAT MZ (Lot number AC 9531-85R) was applied to the treated plots as a broadcast spray using the previously calibrated equipment with the following parameters.

Table 7.1.2.2.1-34: Application parameters for dimethomorph to test plots

Date	ACROBAT MZ (g)	Spray Solution (mL)	Boom Height (cm)	GPA	Rate (kg a.i. ha-1)**
08/02/96	735	55828.8	33-38	25.2	0.279
08/09/96	735	55261.0	33-38	24.8	0.278
08/15/96	735	55261.0	33-38	25.2	0.282
08/22/96	735	55639.5	33-38	25.0	0.278
08/29/96	735	55639.5	33-38	25.2	0.280
09/09/96	735	55639.5	33-38	25.1	0.279
Total dimethomorph applied:					**1.68**

* boom height converted from inches
** kg a.i. ha^{-1} converted from lbs/acre (may not add up to total due to rounding)

Climatic Conditions

Weather data were collected on-site at New Holland, Ohio, with a Neogen Envirocaster weather station and supplemented with NOAA weather data collected at the Washington Court House Station, located approximately 16 km from the test site. All rainfall data were collected on-site.

Rainfall Data

Table 7.1.2.2.1-35: Summary of monthly local weather data

Location	Date	Rainfall total [mm]	Monthly mean of max. daily air temperature [°C]	Monthly mean of min. daily air temperature [°C]
on-site (rainfall) Washington Court House Station (air temp)	06/96	114	26	14
	07/96	97	28	16
	08/96	27	28	14
	09/96	140	17	11
	10/96	43	17	5
	11/96	89	7	-2
	12/96	94	7	-2
	01/97	44	3	-7
	02/97	49	7	-2
	03/97	157	12	1
	04/97	30	17	3
	05/97	113	20	8
	06/97	107	25	16
	07/97	88	28	15
	08/97	168	26	15
	09/97	40	23	12
	10/97	57	19	6
	11/97	88	8	0
	12/97	50	4	-3
	01/98	90	6	-1
	02/98	58	8	0
	03/98	0	7	-2

* Converted from inches of rainfall and °F

Maintenance Pesticides

Table 7.1.2.2.1-36: Maintenance pesticides applied to all plots in the test site for crop maintenance

Pesticide	Date Applied	Rate [kg ha^{-1}]*	Reason
TREFLAN	06/21/96	0.840	Worm control
SENCOR	06/21/96	0.213	Weed control
GRAMOXONE EXTRA with a non-ionic surfactant at 0.5% v/v	08/28/97	1.232	Weed control
LEXONE DF	08/28/97	0.840	Weed control

* converted from lbs $acre^{-1}$

Irrigation

Supplemental irrigation was supplied by an overhead sprinkler (50 mm ha^{-1}) used to irrigate the test site on July 10, 1996. The source of the irrigation water was Paint Creek with a pH of 7.0.

Crop Disposition

In September 1996, the tomato plants in treated plot 2 were cut near the soil with hand clippers to avoid disturbing soil surface. Plants with fruit attached were carried from the plot area and burned on a brush pile. Dropped fruit were removed from the plot area by hand.

Soil Sampling

Eighteen soil cores were taken randomly from plots 1 and 2 at the test site in August 1996, prior to application (pre-treatment samples), and from the control plot at various intervals throughout the course of the study. Immediately after each application, six petri dishes were collected from the bare ground plot, and immediately after the first application eighteen soil cores were collected from each of the treated plots. When the crop was present in the processing tomato plot, the soil cores were obtained from the area located directly under the tomato plants.

Further samples were collected at 0 an 0.1 days following the third application as well as on day 0, 0.1, 3, 9, 14, 31, 66, 92, 155, 184, 245, 302, 360, 449, 540 following the sixth application. On day 120 following the sixth application, no samples could be collected due to frozen ground.

Prior to each application, approximately 50 g of top soil collected from the control plot was placed into six separate petri dishes. The petri dishes, without their lids, were placed in the bare ground plot (l/subplot) and their position marked with stakes.

Using a Concord 3-stage probe, the field investigator collected soil cores first from the control plot (when collected) and then from the treated plots. For the pre-treatment and control samples, 18 cores (90 cm long) were taken with a 120 cm long probe equipped with an acetate liner and having a diameter of approx. 4.4 cm. The treated plots were divided into six subplots of approximately equal size, and three cores (each separated from the next by greater than 30 cm) were taken from each of the six subplots. Single cores from each of the subplots were combined to give three replicate samples of six cores each, for a total of 18 cores for each treated plot. These 18 cores consisted of two core segments each that were taken in the following manner: The top 30 cm of soil was excavated with a 5.6 cm diameter excavation attachment equipped with an acetate liner. The underlying 75 cm core was then taken with the 120 cm long, 4.4 cm diameter probe equipped with an acetate liner. Cores were trimmed to either 90 cm (pre-treatment and controls) or 75 cm (treated) in the field. Excavated cores were 15 cm in length, and required no trimming.

The top of each tube was capped with a red cap, and the acetate liner with the sample inside was removed from the soil probe. The bottom of the soil core was capped with a black cap. The six cores that constituted a given replicate from each treated plot, the eighteen pre-treatment cores, and the eighteen cores from the control plot received a unique identifier consisting of the protocol number, and a four-digit number indicating treatment number, replicate number, and sequence number based on the sampling interval. Each group of eighteen excavated soil core segments constituting a replicate corresponding to their lower cores were labeled with the same label (sample number) used for their underlying cores. However, for the excavated samples, the letter "A" was added as a suffix to the sample number on the excavated soil core to facilitate tracking.

Sample Handling and Freezer Stability

Treated and untreated samples were transferred to a freezer within 4 hours of sampling. The samples were maintained there until shipment. Temperatures were monitored with continuous recorders, and transcribed to show daily minimum and maximum temperatures. The soil samples remained under frozen conditions for the entire time at the field facility. On one occasion, the temperature of a freezer rose to 1 °C for no more than one hour when samples were first put into it on 8/2/96. During handling at American Cyanamid Company, the soil was allowed to thaw only as much as necessary for homogenization.

Analytical procedure

Samples were analyzed following American Cyanamid method M2656 [*CA 4.1.2/1*]. The final determination of dimethomorph is made by gas chromatography with a nitrogen-phosphorus detector. The limit of quantification (LOQ) of the method is 10 µg kg^{-1} (ppb) and the limit of detection (LOD) is ca. 1 µg kg^{-1} (ppb).

The validity of the analytical method was proven within the present study by analysis of fortified field samples (10-5000 µg kg^{-1}).

Storage stability experiments

The freezer storage stability study is presented below [see KCA 7.1.2.2.1/7, BASF DocID DK-326-026]. Data show that dimethomorph (CL 336379) residues are stable in soil for up to 22 months when stored under frozen conditions.

II. RESULTS AND DISCUSSION

Analytical results for treated soil samples are summarized in Table 7.1.2.2.1-37 to Table 7.1.2.2.1-38. All averages were calculated using 5 µg kg^{-1} as a default value for < 10 µg kg^{-1}. After averaging the three replicates of each increment at each sampling interval, the sum of positive averages was calculated for the analyte at each sampling interval.

Table 7.1.2.2.1-37: Dimethomorph residues in soil (plot 1)

Sample Sequence Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**
Sampling Interval (d) *	**-38(1)**	**-25(0)**	**-25(1)**	**0(0)**	**0(1)**	**3**	**9**	**14**	**31**
Soil Depth	**[µg kg^{-1}]**								
0-15 cm (A layer)	109	154	330	452	687	752	410	574	453
15-30 cm (B layer)	<10	<10	<10	<10	<10	<10	<10	11	10
30-45 cm (C layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	N/C	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	109	154	330	452	687	752	410	585	463

Sample Sequence Number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Sampling Interval (d)	**66**	**92**	**120**	**155**	**184**	**245**	**302**	**360**	**449**	**540**
Soil Depth	**[µg kg^{-1}]**									
0-15 cm (A layer)	446	273	-	254	235	162	104	69	43	68
15-30 cm (B layer)	15	<10	N/C	<10	<10	<10	<10	<10	<10	<10
30-45 cm (C layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	461	273	0	254	235	174	104	69	43	68

* Numerical dates are in relation to the sixth and final application
N/C Not collected in field

Table 7.1.2.2.1-38: Dimethomorph residues in soil (plot 2)

Sample Sequence Number	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**
Sampling Interval (d) *	**-38(1)**	**-25(0)**	**-25(1)**	**0(0)**	**0(1)**	**3**	**9**	**14**	**31**
Soil Depth	**[µg kg^{-1}]**								
0-15 cm (A layer)	18	142	134	529	408	473	443	336	302
15-30 cm (B layer)	<10	<10	<10	12	<10	<10	13	<10	<10
30-45 cm (C layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	<10	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	18	142	134	541		473	456		

Sample Sequence Number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Sampling Interval (d)	**66**	**92**	**120**	**155**	**184**	**245**	**302**	**360**	**449**	**540**
Soil Depth	**[µg kg^{-1}]**									
0-15 cm (A layer)	310	333	-	194	126	164	54	38	27	37
15-30 cm (B layer)	<10	<10	N/C	12	<10	<10	<10	<10	<10	<10
30-45 cm (C layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
45-61 cm (D layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
61-76 cm (E layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
76-91 cm (F layer)	<10	<10	N/C	<10	<10	<10	<10	<10	<10	<10
Sum of positive Avgs (µg L^{-1})	310	333	0	194	126	164	54	38	27	37

* Numerical dates are in relation to the sixth and final application
N/C Not collected in field

Dimethomorph residues in soil from the bare ground plot (plot 1) declined from an average of 752 µg kg^{-1} at 3 days after the sixth application (3 DAT6) to an average of 43 µg kg^{-1} at 449 days after the sixth treatment (449 DAT6). Average dimethomorph residues above the LOQ (10 µg kg^{-1}) were found at every interval in the A-layer (0-15 cm) layer and in the B-layer (15-30 cm) at 14 DAT6, 31 DAT6, 66 DAT6, and 245 DAT6. Some sampling intervals (in one replicate only) contained detectable residues of dimethomorph in the B-layer at 9 DAT6, 92 DAT6, 184 DAT6 and in the C-layer at 31 DAT6 and 449 DAT6.

Dimethomorph residues in soil from the cropped tomato plot (plot 2) declined from an average of 473 µg kg^{-1} at 3 days after the sixth application (3 DAT6) to an average of 27 µg kg^{-1} at 449 days after the sixth treatment (449 DAT6). Average dimethomorph residues above the LOQ (10 µg kg^{-1}) were found at every interval in the A-layer (0-15 cm) layer andin the B-layer (15-30 cm) at 9 DAT6 and 155 DAT6.

Some sampling intervals (in one replicate only) contained detectable residues of dimethomorph in the B-layer at 0.1 DAT6, 31DAT6, 184 DAT6, and the C-layer at 9 DAT6. The dimethomorph residues in single replicates in the lower layers from both plots may have been due to contamination in the field or laboratory portions of the study.

Apparent soil residues of dimethomorph in untreated soil were approximately 1 µg kg^{-1} or less, with the exception of one control sample which gave apparent values of approx. 3 and 6 µg kg^{-1} on two separate assays. (Note: Three additional assays of this sample gave apparent values of less than 1 g kg^{-1}.)

Petri dish soil samples were analyzed using Method 2656 for dimethomorph residues to confirm the application rates and accountability for the total material applied for applications 1-6. Average test substance recovery and standard deviation from the 36 petri dish samples were 85 ± 21 % of theoretical (Table 7.1.2.2.1-39).

Table 7.1.2.2.1-39: Summary of dimethomorph residues in petri dish soil

Sample Number	Amount (µg cm^{-2})	Percent Recovery (%)	Sample Number	Amount (µg cm^{-2})	Percent Recovery (%)	Sample Number	Amount (µg cm^{-2})	Percent Recovery (%)
1031	2.19	78	1043	3.42	122	1055	2.33	83
1032	1.59	57	1044	2.50	89	1056	2.29	82
1033	1.87	67	1045	2.46	88	1057	2.59	93
1034	1.59	57	1046	2.87	103	1058	1.94	69
1035	1.41	50	1047	3.39	121	1059	2.98	106
1036	2.54	91	1048	2.67	95	1060	2.90	104
1037	2.15	77	1049	2.38	85	1061	2.92	104
1038	2.50	89	1050	2.06	74	1062	2.66	95
1039	1.87	67	1051	2.68	96	1063	1.89	68
1040	2.62	94	1052	1.80	64	1064	2.40	86
1041	2.14	76	1053	2.57	92	1065	1.02	36
1042	3.70	132	1054	3.24	116	1066	1.56	56
Mean % Recovery / std deviation:							**85 ± 21%**	
Theoretical amount of dimethomorph in petri dish soil = 2.80 µg cm^{-2}								

Calculation of dissipation

A linear regression by the method of the least-squares was conducted on the sampling days vs the logarithm (base 10) of the concentration of the total apparent residue to show the dissipation of the residues over the test period. The equation for this linear regression is shown below.

Log Y = -0.00216X +2.75 (r^2 correlation coefficient = 0.92) (Plot 1-Bare Ground)
Log Y = -0.00239X + 2.63 (r^2 correlation coefficient = 0.92) (Plot 2-Tomato Crop)

The half-life was calculated from the slope as follows:

Half-life =Log 0.50/slope = -0.301/-0.00216= 139 days (Plot 1-Bare Ground)
Half-life =Log 0.50/slope = -0.301/-0.00239= 126 days (Plot 2-Tomato Crop)

III. CONCLUSION

The half-life of dimethomorph soil residues in the bare ground plot (plot 1) was calculated to be 139 days and in the cropped tomato plot (plot 2) 126 days.

Summary of supplementary field study information

In all studies, half-lives were estimated from the slope by linear regression of the least squares where the half-lives ranged from 53 to 209 days. No kinetic evaluations were presented in the latter two studies, while the calculated DT_{50} values of the first three studies (non-normalized and based on Timme and Frehse) ranged from 8 to 35 days and fall well into the range of 27.1 to 76.2 days used for endpoint derivation of the isomer mixture.

No additional information on E and Z isomers can be derived from the supplemental studies.

Storage stability

The following freezer storage stability study [see KCA 7.1.2.2.1/7 DK-326-026] is valid for all five supplementary field studies summarized above, however, the study was not completed at time of finalization of the field reports (so that in some of the supplementary field studies a reference is made to a storage stability study "in progress").

Report:	CA 7.1.2.2.1/7 Bixler T.A.,Babbitt B., 1999a CL 336379 (Dimethomorph): Freezer storage stability of CL 336379 residues in soil DK-326-026
Guidelines:	EPA 860.1380
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The objective of the study is to determine the stability of dimethomorph (CL 336379) residues in soil during freezer storage. The storage conditions chosen are equivalent to the storage conditions used for residue samples awaiting analysis.

The analytical work for this study was started on June 05, 1997, when soil samples were fortified and placed in the freezer. Thirty 20 g sub-samples of control soil (AC 6794-91) were weighed into pre-labeled glass jars. Half of the samples were fortified at 100 ppb by adding 2.0 mL of standard containing 1.0 µg/mL of dimethomorph. All the jars were sealed and placed into the freezer on June 05, 1997. The analysis intervals were 0, 6, 12, 18 and 22 months. The 0-month samples were extracted June 05, 1997.

The results from the analyses of dimethomorph in soil are summarized below. Data show that dimethomorph residues are stable in soil for up to twenty-two months when samples are stored in conditions equivalent to the storage conditions used for residue samples awaiting analysis.

I. MATERIAL AND METHODS

A. MATERIALS

American Cyanamid Company supplied the standard reference material. The standard reference material was labeled upon receipt at Maxim Technologies, Inc. as follows:

Table 7.1.2.2-40 Test material information

Compound	Lot Number	Maxim #	Purity	Expiration Date	Date Received
CL 336379	AC9978-68A	20634033	97.6%	23-AUG-97	l7-MAR-97

The reference standard dimethomorph was stored in a refrigerator at 5°C ± 4°C. Characterization and stability testing for the reference standard was performed by American Cyanamid Company and maintains the documentation. During the course of the study, sub-samples of the same lot number of this standard were received at Maxim. The purity was re-certified and expiration dates updated accordingly. All data can be found in the Facility Raw Data Package. Stock, fortification and calibration solutions were prepared as directed in the protocol and analytical method. These solutions were kept refrigerated at a temperature of 5°C ± 4°C when not in use.

B. STUDY DESIGN

Soil samples were analyzed by American Cyanamid Company Method M 2656 (final version signed May 07, 1997). The validated sensitivity (Limit of Quantitation; LOQ) for Method M 2656 is 10 ppb. The LOQ is the minimum concentration of analyte that has been proven tobe quantified from sample matrix.

There were two minor modifications to the method. These modifications were approved by the Study Director and had no impact on the outcome of the results:

a) 35 mL of 30% acetonitrile in methylene chloride was passed through the silica gel cartridge.
b) 7. 25 mL of 25 % methylene chloride in ethyl acetate was passed through the alumina-neutral cartridges at 1-2 drops/second.

II. RESULTS AND DISCUSSION

Table 7.1.2.2-41 summarizes the detailed analytical data of dimethomorph residues in soil (information on the dilution factor was added to the table). Data show that dimethomorph residues are stable in soil for up to 22 months when stored under frozen conditions.

Table 7.1.2.2-41: Summary of freezer stability data of dimethomorph residues in soil (0, 6, 12, 18, 22-month intervals)

Sample Number	Storage interval (month)	Dilution factor	dimethomorph residues found [ppb]	Percent recovered	Corrected recovery in stored sample*
DM97PT03-31 [a]	0	1	<1.27	-	-
DM97PT03-32 [b]	0	5	90.9	91	-
DM97PT03-33 [c]	0	5	86.6	87	96
DM97PT03-34 [d]	0	5	86.2	86	95
DM97PT03-21 [a]	6	1	<0.865	-	-
DM97PT03-22 [b]	6	5	97.5	98	-
DM97PT03-43 [c]	6	5	91.9	92	94
DM97PT03-44 [d]	6	5	89.3	89	91
DM97PT03-09 [a]	12	1	<0.881	-	-
DM97PT03-10 [b]	12	5	106	106	-
DM97PT03-37 [c]	12	5	96.3	96	91
DM97PT03-38 [d]	12	5	99.9	100	94
DM97PT03-13 [a]	18	1	<1.20	-	-
DM97PT03-14 [b]	18	5	97.5	97	-
DM97PT03-39 [c]	18	5	97.1	97	100
DM97PT03-40 [d]	18	5	93.0	93	96
DM97PT03-17 [a]	22	1	< 1.09	-	-
DM97PT03-18 [b]	22	5	102	102	-
DM97PT03-41 [c]	22	5	96.3	96	94
DM97PT03-42 [d]	22	5	98.2	98	96

a - Stored control sample
b - Stored control sample for concurrent recovery
c - Stored fortified sample 1
d - Stored fortified sample 2
* The corrected recovery was calculated by dividing the percent recovered by the respective concurrent recovery and multiplying by 100

III. CONCLUSION

Data show that dimethomorph residues are stable in soil for up to 22 months when stored under frozen conditions

CA 7.1.2.2.2 Soil accumulation studies

Since no DT_{90} greater than one year were found for dimethomorph, soil accumulation was not addressed.

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

The three already peer-reviewed adsorption/desorption studies with dimethomorph are considered still valid. The following table summarizes the peer-reviewed data:

Table 7.1.3.1.1-1: Adsorption of dimethomorph on different soils

Soil	Soil type	$K_{f,oc}$ [mL g^{-1}]	$K_{f,om}$ [mL g^{-1}]	1/n [-]	Reference
Soil I	Sandy loamy silt	515	299	0.834	BASF DocID 1991/7000102
Soil II	Sandy loam	377	219	0.814	BASF DocID 1991/7000102
Soil III	Sand	388	225	0.857	BASF DocID 1991/7000102
Soil IV	Silty sand	316	183	0.872	BASF DocID 1991/7000102
BBA 2.1	Sand	566	328	0.887	BASF DocID 1988/7000035
BBA 2.2	Humous sand	402	233	0.921	BASF DocID 1988/7000035
BBA 2.3	Sandy loam	290	168	0.814	BASF DocID 1988/7000035
Soil 190	Sand	456.5*	265	0.877	BASF DocID 1997/7000232
Soil 191	Silty clay Loam	557*	323	0.870	BASF DocID 1997/7000232
Soil 192	Sand	513*	298	0.881	BASF DocID 1997/7000232
Soil 193	Sandy loam	345*	200	0.820	BASF DocID 1997/7000232
Arithmetic mean		**-**	**-**	**0.86**	
Geometric mean		**419.4**	**243.3**	**-**	**-**

* mean value from the results for the E- and Z-isomer of dimethomorph

To complete information given in the previous Annex I renewal, further information on statistical values (R^2) and material balance are presented in the following, hereby adding information on fit and recovery data.

BASF DocID 1991/7000102

Table 7.1.3.1.1-2: Average soil distribution coefficients [1991/700102]

Soil	Kd_{ads}	Mean Kd_{ads}	K_{oc}	Mean K_{oc}	l/n	Mean 1/n	R^2
Soil I	4.96	4.94	517	515	0.823	0.834	0.9999
	4.91		512		0.844		0.9957
Soil II	8.57	8.51	379	377	0.810	0.814	0.9999
	8.45		374		0.818		0.9995
Soil III	2.67	2.72	382	388	0.861	0.857	1.0000
	2.76		394		0.853		0.9991
Soil IV	3.02	3.03	315	316	0.885	0.872	0.9994
	3.04		317		0.859		0.9995

The mass balance in the study based on data from the equilibrium condition test with 45 hours equilibration time range from 94.7 to 108.7% (mean value of 101.5%). Please also refer to Table 7.1.3.1.1-3.

Table 7.1.3.1.1-3: Mass balance in adsorption, desorption and soil samples [1991/7000102]

Soil	Original amount [dpm]	Amount recovered after adsorption [dpm]	Amount recovered in soil [dpm]	Sum of adsorption and soil [dpm]	Recovery [µg]
Soil I	270028	130348	161583	291931	108.1
		129276	126307	255583	94.7
Soil II		103304	190164	293468	108.7
		94429	179473	273902	101.4
Soil III		171543	94005	265548	98.3
		173197	90431	263628	97.6
Soil IV		161241	108290	269531	99.8
		157090	121232	278322	103.1

BASF DocID 1988/7000035

Table 7.1.3.1.1-4: Average soil distribution coefficients [1988/7000035]

Soil	Kd_{ads}	K_{oc}	l/n	R^2
BBA 2.1	4.47	566	0.887	0.9992
BBA 2.2	11.67	402	0.921	0.9989
BBA 2.3	2.09	290	0.814	0.9989

The mass balance in the study calculated for the three soils ranged from 91.2% to 99%, please also refer to Table 7.1.3.1.1-5.

Table 7.1.3.1.1-5: Mass balance in adsorption, desorption and soil samples [1988/7000035]

Soil	Original amount [µg]	Amount recovered after adsorption [µg]	Amount recovered after first desorption [µg]	Amount recovered after second desorption [µg]	Amount recovered in soil [µg]	Sum of adsorption, desorpion I and II and soil [µg]	Recovery [µg]
BBA 2.1	542	258.8	130.4	54.3	74.2	517.7	95.5
BBA 2.2	542	139.8	109.7	52.4	192.3	494.2	91.2
BBA 2.3	542	347.5	117.7	39.4	31.8	536.4	99.0

BASF DocID 1997/7000232

Table 7.1.3.1.1-6: Average soil distribution coefficients [1997/7000232]

Soil	Kd_{ads}	K_{oc}	Mean K_{oc}	Mean 1/n	R^2	Kd_{des}
Soil 190 E isomer	4.30	478	456.5	0.877	0.9997	4.91
Soil 190 Z isomer	3.92	435			0.9999	5.45
Soil 191 E isomer	10.3	574	557	0.870	1.0000	11.8
Soil 191 Z isomer	9.72	540			0.9997	10.9
Soil 192 E isomer	21.1	555	513	0.881	0.9998	29.9
Soil 192 Z isomer	17.9	471			0.9996	27.7
Soil 193 E isomer	4.84	346	345	0.820	0.9998	6.67
Soil 193 Z isomer	4.81	344			0.9989	6.28

The mass balance in the study calculated for the three soils ranged from 97.9% to 106.2%, please also refer to Table 7.1.3.1.1-7.

Table 7.1.3.1.1-7: Mass balance in adsorption, desorption and soil samples [1997/7000232]

Soil	Adsorbed test substance [%]	Adsorbed test substance desorbed [%]	Adsorbed test substance not desorbed [%]	Dosed test substance not desorbed [%]	Recovery [µg]
Soil 190 E isomer	51.7	59.6	50.4	26.5	104.4
Soil 190 Z isomer	46.3	54.3	45.7	20.8	98.1
Soil 191 E isomer	72.4	30.2	69.8	51.2	104.7
Soil 191 Z isomer	71.0	32.9	67.1	46.9	99.7
Soil 192 E isomer	85.2	15.4	84.6	73.0	106.2
Soil 192 Z isomer	82.0	16.4	83.6	67.2	98.6
Soil 193 E isomer	56.9	36.5	63.5	36.9	105.1
Soil 193 Z isomer	54.6	40.0	60.0	32.4	97.9

Supplementary sorption data

The results of the following study verify that the $K_{f,oc}$ values in Table 7.1.3.1.1-1 with a range of 290 to 566 mL g^{-1} (geometric mean of 419.4 mL g^{-1}, n=11) are conservative.

Report: CA 7.1.3.1.1/1
An D., 1998 a
Dimethomorph (AC 336379): Adsorption/desorption on soils
DK-620-038
Guidelines: EPA 163-1
GLP: no

Executive Summary

The objective of this study was to determine the adsorption/desorption behavior of dimethomorph on four soils.

The soil adsorption/desorption study was conducted at 20 ± 1°C using ^{14}C-labeled dimetomorph (chlorophenyl label). The test compound was applied at four measured concentrations. All of the phases of the study were conducted in triplicate at a 1:6 (w:w) soil:water (0.01 M $CaCl_2$) ratio using four soils collected from Missouri and New Jersey (sandy clay loam, sandy clay and two sandy loam). The kinetics test included 0, 2, 5, 21, 24, and 30 hour sample intervals. The results of the kinetics test showed that equilibration of adsorption was achieved within 24 hours (<5% change) for all four soils. Based on the results of the kinetics experiment, a 24-hour equilibration period was chosen to obtain the Freundlich isotherms for each soil in both the adsorption and desorption phases. Soils were extracted twice with 30 mL of acetonitrile. Following the extraction process, the soils were air-dried and the radioactivity was determined by combustion radioanalysis.

The ^{14}C-mass balance was determined on each test sample to be between 92.5% and 101.9%.

The HPLC analysis of aqueous samples from both the adsorption and desorption phases, and of the acetonitrile extracts, at the measured concentration of 0.344 ppm showed that dimethomorph was stable throughout the experiment for all four soils.

An average K_{oc} value of 1255 mL g^{-1} was obtained (787 to 1588 ml g^{-1}). The study demonstrates that dimethomorph has low mobility in the four soils studied.

I. MATERIAL AND METHODS

A. MATERIALS

Test Substance

The study was conducted with analytical grade, ^{14}C-radiolabeled dimethomorph provided by American Cyanamid Company. Before dosing, the total radioactivity per volume of the test article in the dosing solution was measured by liquid scintillation counting. The radiochemical purity of the test article was re-determined using TLC and HPLC prior to dosing.

Table 7.1.3.1.1-8: Test Substance

Chemical Name (IUPAC):	(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine
Source (Lot No.):	AC10011-58
Position of Radiolabel:	Uniformly labeled on chlorophenyl ring
Specific Activity:	44 µCi mg^{-1}
Radiochemical Purity:	96.4% by TLC and 95.5% by HPLC
Chemical Purity:	98.8 UV area % @254 nm
E/Z ratio:	42.7:57.3 @254 nm; 49.3:50.7 radiochemical detection
Storage Conditions:	-20°C
Expiration Date:	April 30, 1998

Reference Substance

Unlabeled dimethomorph (Lot No.: AC 9978-68A) with a chemical purity of 97.6% was used as a qualitative reference standard in HPLC analysis. The compound was provided by American Cyanamid Company.

Soils

The following four soils obtained from New Jersey and Missouri were used: Princeton sandy loam, New Jersey sandy loam, Missouri sandy clay loam, and Missouri sandy clay. The physico-chemical properties of the soils were characterized by Agvise Laboratories, and are presented in Table 7.1.3.1.1-9. All soils were sieved through a 2 mm screen before use.

Table 7.1.3.1.1-9: Soil information

Source	Textural Class	pH	CEC*	% Sand	% Silt	% Clay	% OM	% Moisture**
Missouri	Sandy Clay Loam	6.5	11.4	73	11	16	1.7	64.0
Princeton	Sandy Loam	6.8	9.1	62	25	13	1.5	14.1
Missouri	Sandy Clay	7.9	24.0	55	13	32	2.2	73.2
New Jersey	Sandy Loam	5.8	7.5	83	5	12	2.6	46.2

* Cation Exchange Capacity
** Moisture content was measured at 1/3 Bar for Princeton sandy loam, and measured at 1 cm water (MWHC) for the rest of the soils

B. STUDY DESIGN

Kinetics Test

The kinetics test was performed to determine the time required to reach adsorption equilibrium. Five grams of each of the four soils were placed in 50 mL glass centrifuge tubes (Pyrex/Corex) and dosed with 30 mL of dimetomorph at a measured concentration of 0.344 ppm in 0.01 M calcium chloride aqueous solution. The samples mixed thoroughly by shaking continuously on a reciprocal shaker at 20 ± 1°C. After two hours of shaking, the samples were centrifuged for 15 min at 20°C and approximately 2500 g in a Beckman GS-6R centrifuge. Triplicate aliquots of 0.1 mL supernatants were removed by pipette and radioassayed by liquid scintillation counting (LSC). Then the samples were returned to the shaker until the next sampling point. Sampling of the adsorption solution, by centrifugation and radioassay of the supernatants, was also done at 5, 21, 24, and 30 hours. The radioactivity at zero-time was the value of dosing solution.

Definitive Test

Adsorption: Solutions of [dime] were prepared at measured concentrations of 0.344, 0.173, 0.0353, and 0.0175 ppm in 0.01 M calcium chloride aqueous solution. The experiment was performed in triplicate for each of the four soils studied. Five grams of the soil and 30 mL of the solutions described above were placed in 50 mL glass centrifuge tubes. A control sample without soil was set up at each concentration to monitor any sorption of test compound to the walls of the tubes. The samples were mixed thoroughly by shaking continuously on a reciprocal shaker at 20 ± 1°C for about 24 hours to allow equilibrium to be reached. The samples were centrifuged for 15 min at 20°C and approximately 2500 g in a Beckman GS-6R centrifuge. Aliquots of aqueous solutions were taken by pipette and radioassayed by LSC. The remaining supernatants were then removed. The supernatants from the samples of the 0.344 ppm dosing rate were saved for HPLC analysis to check the stability of dimetomorph during the adsorption phase.

Desorption: After the adsorption phase, 30 mL of fresh 0.01 M calcium chloride solution was added to the soil remaining in each tube. Samples were shaken for approximately 24 hours at 20 ± 1°C, and centrifuged as previously described. Triplicate aliquots of the supernatants were taken and radioassayed by LSC. The supernatants from the samples of the 0.344 ppm dosing rate were saved for HPLC analysis to check the stability of dimetomorph during the desorption phase.

Soil Extraction: After removal of the desorption solution, soils were extracted twice with 30 mL of acetonitrile by shaking for approximately 1 hour on a reciprocal shaker at 20 ± 1°C. Samples were then centrifuged as previously described, and triplicate aliquots of the supernatants were radioassayed.

Soil Combustion: After solvent extraction, the soil samples were air-dried and ground to homogeneity by mortar and pestle. Five aliquots of approximately 0.5 (0.4997-0.5003) grams from each sample were weighed, combusted and radioassayed.

II. RESULTS AND DISCUSSION

The concentrations of stock solution were measured by radioassay of triplicate aliquots of the dosing solutions by liquid scintillation counting. The measured concentrations were determined to be 0.344, 0.173, 0.0353, and 0.0175 µg/mL. The kinetics test to determine the equilibration time showed that adsorption reached equilibrium within 24 hours of incubation at 20 ± 1°C. Therefore, an incubation period of approximately 24 hours was selected for the definitive test of adsorption and desorption phases. The control samples showed no adsorption of test compound to the walls of the test tubes.

In the definitive test, the recoveries of radioactivity were in the range of 98.4-101.7%, 96.0-99.3%, 92.5-101.9%, and 97.1-99.3% for the MO-sandy clay loam, Princeton-sandy loam, MO-sandy clay, and NJ-sandy loam, respectively. The stability of dimetomorph during the course of the definitive test was evaluated by reverse phase HPLC analysis of adsorption and desorption solutions and soil extracts from the 0.344 ppm treatment. All of the HPLC analyses revealed peaks of radioactivity representing the E- and Z-isomers of dimetomorph. Some minor peaks, with total radioactivity accounting for less than 5% of the analyzed radioactivity in any of the samples, were considered to be impurities from the test substance. The retention times of the radioactive peaks of the analyzed samples were consistent with those of authentic dimetomorph.

Adsorption of compounds by soils can be described empirically by the Freundlich equation. The adsorption coefficient, K_f, is a measure of the adsorption strength, and the constant, l/n, is a measure of the linearity of adsorption. The Freundlich adsorption coefficients were calculated using linear regression analysis. The adsorption coefficient normalized for soil organic carbon content, K_{oc}, was calculated from K_d.

The average soil distribution coefficients for adsorption, Kd_{ads} the adsorption coefficients normalized for organic carbon, K_{oc}, the Freundlich adsorption coefficients, Kr and 1 /n, and the correlation coefficients, R^2, as well as the desorption coefficients, Kd_{des} were determined to be as follows:

Table 7.1.3.1.1-10: Average soil distribution coefficients

Soil	Kd_{ads} [ml g^{-1}]	$K_{f,oc}$ [ml g^{-1}]	K_f [ml g^{-1}]	l/n [-]	R^2 [-]	Kd_{des} [ml g^{-1}]
MO-Sandy Clay Loam	15.7	1588	11.2	0.913	0.999	23.0
Princeton-Sandy Loam	10.1	1158	6.47	0.878	0.998	13.2
MO-Sandy Clay	19.0	1485	11.0	0.868	0.998	21.9
NJ-Sandy Loam	11.9	787	7.82	0.888	0.998	15.9

The adsorption coefficient, Kd_{ads} on the four soils correlated fairly well with the organic matter content of the soil, except that of the New Jersey sandy loam soil which was of high sand content and low pH value. Adsorption K_{oc} values for dimethomorph on the four soils were in the range of 787 to 1588 ml g^{-1} with an average value of 1255 ml g^{-1}, indicating that dimethomorph can be classified to have low mobility in soil.

III. CONCLUSION

The study is statistically sound with good recovery values. K_{oc} values of dimethomorph in the four soils were in the range of 787 to 1588 ml g^{-1} with an arithmetic mean of 1255 ml g^{-1}. These indicate that dimethomorph will have a low mobility in soil.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

No relevant breakdown products of dimethomorph were identified in the metabolism studies.

CA 7.1.3.2 Aged sorption

No experimental data are available. They are not considered necessary for leaching assessment of dimethomorph. PEC_{gw} calculations were performed with the regular Freundlich sorption coefficients as presented in M-CA 7.1.3.1.1.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No new experimental data are available. The column leaching study already peer-reviewed (formulation of dimethomorph and mancozeb) during the previous Annex I inclusion process is considered still valid. No residues of dimethomorph were detected in the leachates, except for one replicate of one sand soil, where a peak was detected at a level below the limit of determination, corresponding to 0.67% of the applied dose.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No new experimental data are available. The two aged soil column leaching studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

After ageing of 60-90 days the soils contained approximately 45.0-67% AR dimethomorph. The columns were leached with 200 mm (chlorophenyl label) and 510 mm (morpholine label) of water in two days. Following the leaching process, only 0.7-0.8% and 3.3-3.4% of column AR was found in the leachate. This leached radioactivity was composed of several compounds (up to seven compounds in the study with the chlorophenyl labeled dimethomorph), none of which accounted for greater than 0.5% of the dose. None of the products in the leachate chromatographed with dimethomorph or with a series of known or suspected degradation products.

CA 7.1.4.2 Lysimeter studies

No new experimental data are available. No lysimeter information was available during the previous Annex I application and the information is considered to be not required.

CA 7.1.4.3 Field leaching studies

No field leaching study was performed. The leaching assessment in the current renewal dossier is based on $PEC_{groundwater}$ calculations performed with updated endpoints according to the newest guidelines and guidance documents.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

Except for the new data requirement "aquatic mineralization" (accord. to OECD 309), no new experimental data were produced for the aquatic environment. The kinetic evaluation of the already peer-reviewed studies from the previous Annex I listing was however updated according to the newest guidelines and guidance documents.

An overview on the degradation rates obtained for the parent can be found at the end of this chapter. According to the PBT assessment, averaged DT_{50} values in aquatic systems are all under the cut-off values of 40 days (marine, fresh and estuarine water) and 120 days (sediments).

CA 7.2.1.1 Hydrolytic degradation

No new experimental data on hydrolysis of dimethomorph were produced. The old hydrolysis study is considered still valid. dimethomorph proved to be stable at all tested pH values in sterile buffers (pH 4 - pH 9).

CA 7.2.1.2 Direct photochemical degradation

No new experimental data on direct photolysis of dimethomorph was produced. The already peer-reviewed studies are considered still valid (aqueous photolysis and phototransformation in water).

Dimethomorph showed a degradation under aqueous photolytic conditions estimated from 28 days in a first study to 107 days in a second study. Dimethomorph was slowly degraded by UV irradiation to a number of minor photolysis products, none of which exceeded 10%. Highest peaks were found at 5.1 to 6.3 minutes with a maximum of 6.6% at the end of one study (DAT21, morpholine label), and fraction M2 at 2 to 3 minutes with a maximum of 5.8 to 6.5% from DAT4 to DAT15 in another study.

The average quantum yield of the direct photolysis of dimethomorph in buffered medium at pH 7 and 20ºC was $6.71 * 10^{-6}$, indicating that photolysis in water would be slow under environmental conditions.

The isomer ratio ended at approximately 30:70 to 20:80 (E:Z) in two of the studies.

CA 7.2.1.3 Indirect photochemical degradation

No new experiments on indirect photochemical degradation of dimethomorph were performed. No further information on the route of degradation in the aquatic environment can be given.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

The already peer-reviewed study is considered still valid. Dimethomorph was found to be not readily degradable according to OECD guideline 301 B and D, with indication to inherent, primary biodegradability.

Aerobic mineralisation in surface water

The following study on mineralization in surface water is a new requirement and not yet peer-reviewed.

Report:	CA 7.2.2.1/1 Yeomans P., 2015a 14C-Dimethomorph (BAS 550 F): Aerobic mineralisation in surface water 2014/1184744
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The purpose of this study was to determine the mineralization and degradation rates of the fungicidal active substance dimethomorph (BAS 550 F) in an aquatic system under dark conditions. The study was performed according to OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). The pelagic test system was chosen for this study.

The test was performed at two different dimethomorph (BAS 550 F) concentrations (10 µg L^{-1} and 100 µg L^{-1}) using two differently ^{14}C-labelled test items (morpholine and chlorophenyl labels), respectively. Sterile samples were tested for each label at the higher concentration. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples for the experiment were taken at 0, 3, 7, 14, 22, 36 and 59 days after treatment (DAT).

The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and radio-HPLC. Volatiles were trapped in 2 M sodium hydroxide and also analyzed by LSC. Parent substance identification was done by co-chromatography with the corresponding reference items of the E and Z isomer on HPLC.

From the obtained results it can be concluded that dimethomorph (BAS 550 F) is not significantly degraded in the natural water environment provided in this test. After 59 days, at least 86.2 and 91.6% of total applied radioactivity (TAR) was recovered as the unchanged active substance for the morpholine and chlorophenyl label, respectively. Several minor metabolites were observed during the study in small amounts of up to 4.0% TAR, However, one outlier was observed in one single sample (22 DAT, chlorophenyl label, low concentration) where a component with 7.6% TAR was detected. During the test, no systematic change to the isomer ratio was observed for either the morpholine or chlorophenyl label.

Radioactivity in the volatile traps did not exceed 2.1 or 0.7% TAR for the morpholine and chlorophenyl label respectively, indicating a low rate of mineralization.

Overall, the compound was considered to be stable in the test systems. Degradation kinetics were not reported as no significant degradation was observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS-Code:	BAS 550 F (dimethomorph)
Reg. No.:	247723
Chemical name:	(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
Molecular formula:	$C_{21}H_{22}ClNO_4$
Molar mass:	387.86 g mol^{-1} (unlabeled)

Label 1 (morpholine label)

Label:	morpholine-2,3-C14
Batch No.:	858-0201
Specific activity of a.s.:	5.57 MBq mg^{-1}
Radiochemical purity:	99.0%
Chemical purity:	97.6%

Label 2 (chlorophenyl label)

Label:	p-chlorophenyl-U-C14
Batch No.:	1068-1001
Specific activity of a.s.:	8 MBq mg^{-1}
Radiochemical purity:	98.7%
Chemical purity:	96.9%

2. Test system

Water and small amounts of sediment were collected from The Lake at Studley Royal (Ripon, United Kingdom). The physico-chemical properties of the system are summarized in Table 7.2.2.1-1.

Prior to use the sediment and water were stored together in the dark at 4 ± 2°C with free access to air. Water was filtered through a 0.1 mm sieve and sediment was passed through a 2 mm sieve prior to use and characterization. The concentration of sediment in the water was adjusted to an approximate concentration of 0.01 g sediment L^{-1}. The test system can still be considered as pelagic.

Table 7.2.2.1-1: Characterization of the water/sediment system

Designation **Origin**		**Fountains Abbey** **The Lake, Studley Royal, Ripon, UK**	
Water			
Temperature [a]	[°C]	17.2	
pH water [a]	-	9.35	
Oxygen concentration [a]	[mg L^{-1}]	17.09	
Redox potential (Eh) [a]	[mV]	137	
Hardness	[mmol L^{-1}]	112	
Total organic carbon	[mg L^{-1}]	5.98	
Total N	[%]	0	
Total P	[mg L^{-1}]	0.15	
Sediment			
Textural class		PSD	USDA
Sand	[%]	57	60
Silt	[%]	31	28
Clay	[%]	12	12
Soil type	-	Sandy loam	Sandy loam
pH [a]		9.02	
pH (H_2O)	-	8.2	
pH ($CaCl_2$)	-	7.3	
Redox potential (Eh) [a]	[mV]	-144	
Organic carbon (OC)	[%]	1.4	

[a] measured directly at sampling site

B. STUDY DESIGN

1. Experimental conditions

A total of 94 flasks was prepared: 18 flasks for each radiolabel (morpholine and chlorophenyl) and each nominal concentration (10 and 100 µg L^{-1}), 9 flasks for the sterile incubation (both labels; 100 µg L^{-1}), 2 flasks as system control with radiolabeled sodium benzoate and 2 flasks with sodium benzoate plus treatment solvent.

The vessels were filled with about 100 mL test water, using sterile techniques where necessary. Appropriate amounts of the respective application solutions were pipetted to the water surface to achieve a nominal application rate of 10 µg L^{-1} or 100 µg L^{-1}, respectively.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system. Each test vessel was connected to a volatile trapping system of two gas washing bottles containing trapping solutions (2x NaOH) for the ^{14}C-volatiles to be expected. Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs. Vessels were kept in the dark and were agitated by continuous stirring on magnetic stirrers throughout the incubation period.

2. Sampling

Samples, including the sterile groups, were taken at 0, 3, 7, 14, 22, 36 and 59 days after treatment (DAT).

For sampling, the flasks were removed from the rigs and the temperature, O_2 content, pH, conductivity and redox potential of the water was measured in a representative treated sample.

Throughout the test, traps were collected for sampled vessels. Remaining test samples had their traps collected and replenished with fresh solutions at 3, 7, 14, 22, 30, 36, 42 and 50 DAT. Reference vessels had traps collected at the same intervals.

3. Description of analytical procedures

The water in the test vessels was transferred into glass jars and weighed. The test vessels were then washed (with sonication) with acetonitrile. Weighed aliquots of the water and acetonitrile were mixed with scintillant for LSC.

For higher concentration (100 µg L^{-1}) samples, analysis by high performance liquid chromatography (HPLC) was carried out without further workup. For lower concentration (10 µg L^{-1}) samples, sub-samples of the water were partitioned twice with dichloromethane. The dichloromethane was concentrated to dryness and the samples reconstituted in acetonitrile prior to chromatography. Procedural recoveries were checked by LSC and were generally found to be 90% or greater. Where the radioactivity in water after partition was ≥ 5% of the total applied radioactivity (TAR) or if procedural recoveries were poor, HPLC fraction collection of unextracted water was used.

Volatiles trapped in sodium hydroxide were analyzed by LSC.

4. Calculation of the degradation/dissipation rates

Since dimethomorph was relatively stable in the test system, degradation kinetics were not calculated.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance and distribution of radioactivity are shown in Table 7.2.2.1-2 and Table 7.2.2.1-3.

The applied mass of test item per test vessel containing 100 mL of water was 10.1 µg (high concentration) and 0.99 µg (low concentration) for the morpholine label and 9.9 µg (high concentration) and 0.98 µg (low concentration) for the chlorophenyl label.

The material balances and the distributions of radioactivity in the pelagic test systems ranged from 93.9 to 103.4% TAR in the viable test vessels (with the exception of two low concentration vessels at 87.7 and 89.9% TAR) and from 94.9 to 99.4% TAR for the sterilized vessels.

At the end of the study (59 DAT), the radioactivity in the water accounted for 92.4 to 96.5% TAR for the viable test vessels and for 95.3 to 96.0% TAR for the sterilized vessels. Radioactivity in the volatile traps did not exceed 2.1 or 0.7% TAR for the morpholine and chlorophenyl label respectively, indicating a low rate of mineralization. Adsorption to the test vessel surface was negligible with only 0.6-2.8% TAR found in the rinsing solution of single test vessels.

Table 7.2.2.1-2: Material balance and distribution of radioactivity after application of [morpholine-2,3-^{14}C] dimethomorph to lake water (non-sterile and sterile)

Days after treatment (DAT)	Percent of applied radioactivity [% TAR]			
	Water	Vessel wash [a]	Total in NaOH Traps [b]	Material balance
Low concentration (10 µg L^{-1}) [c]				
0	98.0	0.7	NA	98.7
3	95.2	0.9	0.6	96.6
7	96.9	1.4	0.6	98.9
14	97.1	1.1	0.8	99.0
22	95.5	1.3	1.0	97.7
36	89.3	1.9	1.5	92.7
59	95.6	1.5	2.1	99.1
High concentration (100 µg L^{-1}) [c]				
0	96.5	1.2	NA	97.7
3	93.8	0.9	0.4	95.1
7	95.2	1.0	0.5	96.6
14	93.7	1.0	0.5	95.2
22	95.2	1.1	1.0	97.3
36	97.1	2.2	0.7	99.9
59	92.4	1.7	1.8	95.9
Sterilized lake water (100 µg L^{-1})				
0	97.2	0.9	NA	98.1
3	94.0	0.9	ND	94.9
7	98.5	0.9	ND	99.4
14	96.3	0.9	0.3	97.5
22	95.2	0.9	0.3	96.4
36	94.6	1.2	0.2	95.9
59	96.0	1.5	0.4	97.9

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or < 0.1% TAR)
[a] This is an acetonitrile wash of the incubation vessel
[b] Due to the nature of the trapping solutions, this may be presumed to be carbon dioxide
[c] Mean of two replicates

Table 7.2.2.1-3: Material balance and distribution of radioactivity after application of [p-chlorophenyl-U-14C] dimethomorph to lake water (non-sterile and sterile)

Days after treatment (DAT)	Percent of applied radioactivity [% TAR]			
	Water	Vessel wash [a]	Total in NaOH Traps [b]	Material balance
Low concentration (10 µg L^{-1}) [c]				
0	96.6	0.8	NA	97.4
3	92.7	1.2	ND	93.8
7	96.1	1.3	0.2	97.6
14	95.6	1.3	0.2	97.1
22	97.1	1.2	0.3	98.6
36	98.3	1.5	0.4	100.2
59	96.5	1.3	0.7	98.5
High concentration (100 µg L^{-1}) [c]				
0	95.7	1.5	NA	97.2
3	94.2	0.9	ND	95.1
7	95.6	1.3	0.1	97.0
14	93.2	1.2	0.2	94.5
22	96.2	1.4	0.3	97.8
36	93.3	1.8	0.3	95.3
59	95.6	1.6	0.6	97.8
Sterilized lake water (100 µg L^{-1})				
0	95.9	1.1	NA	97.0
3	93.9	1.0	ND	94.9
7	96.0	0.9	ND	96.9
14	94.8	1.1	ND	95.9
22	97.5	1.1	ND	98.6
36	97.0	1.4	ND	98.4
59	95.3	1.6	ND	96.9

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or < 0.1% TAR)
[a] This is an acetonitrile wash of the incubation vessel
[b] Due to the nature of the trapping solutions, this may be presumed to be carbon dioxide
[c] Mean of two replicates

B. TRANSFORMATION OF PARENT COMPOUND

Characterization and identification of residues in water extracts

Water
The results of the radio-HPLC analysis are summarized in Table 7.2.2.1-4 and Table 7.2.2.1-5.

No significant degradation of dimethomorph was observed during the test. After 59 days, between 86.2 and 92.7% TAR could still be recovered as unchanged parent for the different concentrations and radiolabels for the viable samples and 91.6% TAR for the sterile samples of both labels. Lower amounts of dimethomorph (BAS 550 F) were only observed in two single samples which had mass balance less than 90% TAR (36 DAT, morpholine label, and 3 DAT, chlorophenyl label).

Additional peaks in HPLC analysis only appeared in small amounts (up to 4.0% TAR) except in one single sample (22 DAT, chlorophenyl label, low concentration) where a component with 7.6% TAR was observed. This fraction collected component was not found in other samples and may be due to contamination.

The low amount of volatiles and metabolites detected indicate that only limited microbial degradation took place.

Control samples with benzoic acid
The control vessels treated with [^{14}C]-sodium benzoate showed that the test system was microbially active both without and with the addition of acetonitrile. The total recoveries of trapped volatile radioactivity after 59 days were 85.0 and 83.6% TAR and the material balances were 87.5 and 85.4% TAR for the samples without and with acetonitrile, respectively.

Sterilized samples
The very limited degradation observed in the viable test vessels resulted in no significant difference in test item concentration between the sterilized incubations and the viable vessels.

Identification of metabolites
The two isomers of which dimethomorph (BAS 550 F) is composed (E and Z isomers) were identified by co-chromatography with authentic standards during HPLC. Additional peaks only appeared in small amounts, not exceeding 4.6% TAR on average and were therefore not further investigated. Results were confirmed by thin layer chromatography (TLC) with co-chromatography.

Table 7.2.2.1-4: Metabolite overview for the water phase after application of [morpholine-2,3-14C] dimethomorph to lake water

Days After Treatment (DAT)	Percent of applied radioactivity [% TAR]							
	BAS 550 F (E isomer)	BAS 550 F (Z isomer)	BAS 550 F (sum of isomers)	Sum of Unknowns	Largest Unknown	Aqueous phase [a]	Back-ground	Total
Low concentration (10 µg L^{-1}) [b]								
0	29.5	63.2	92.8	1.1	0.6	3.4	0.7	98.0
3	33.5	57.3	90.8	1.0	1.0	3.2	0.3	95.2
7	26.8	65.6	92.4	0.6	0.6	3.4	0.6	96.9
14	27.2	65.8	93.0	ND	NA	3.7	0.4	97.1
22	-[c]	-[c]	89.9	4.8	1.6	NA	0.7	95.5
36	-[c]	-[c]	81.3	7.4	3.3	NA	0.5	89.3
59	-[c]	-[c]	87.4	7.1	2.5	NA	1.0	95.6
High concentration (100 µg L^{-1}) [b]								
0	36.8	56.9	93.7	2.7	1.5	NA	0.1	96.5
3	36.2	56.7	92.9	0.5	0.5	NA	0.4	93.8
7	35.5	57.5	93.0	1.9	1.5	NA	0.2	95.2
14	34.2	58.4	92.6	ND	NA	NA	1.0	93.7
22	33.5	58.5	92.0	2.9	1.4	NA	0.2	95.2
36	36.9	56.2	93.1	3.7	1.7	NA	0.2	97.1
59	33.1	53.1	86.2	5.9	1.6	NA	0.3	92.4
Sterilized lake water (100 µg L^{-1})								
0	37.7	57.2	94.9	2.3	1.0	NA	0.0	97.2
3	35.0	54.7	89.7	3.1	1.7	NA	1.2	94.0
7	38.5	57.4	95.9	2.6	1.8	NA	0.0	98.5
14	34.1	57.7	91.8	2.6	1.4	NA	1.9	96.3
22	39.2	54.3	93.5	1.6	1.6	NA	0.1	95.2
36	36.3	55.4	91.7	2.8	1.4	NA	0.1	94.6
59	32.0	59.7	91.6	4.2	1.5	NA	0.2	96.0

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or less than the limit of detection [LOD] of 0.3% TAR)
[a] Activity remaining in the aqueous phase after dichloromethane partition of the water (relevant for low concentration only)
[b] Mean of two replicates
[c] Analyzed using HPLC fraction collection, individual isomers not resolved

Table 7.2.2.1-5: Metabolite overview for the water phase after application of [p-chlorophenyl-U-^{14}C] dimethomorph to lake water

Days After Treatment (DAT)	Percent of applied radioactivity [% TAR]							
	BAS 550 F (E isomer)	BAS 550 F (Z isomer)	BAS 550 F (sum of isomers)	Sum of Unknowns	Largest Unknown	Aqueous phase [a]	Back-ground	Total
Low concentration (10 µg L^{-1}) [b]								
0	35.9	58.7	94.6	0.6	0.4	1.0	0.4	96.6
3	30.4	59.7	90.1	1.2	1.2	1.1	0.3	92.6
7	14.2	79.8	93.9	1.0	0.7	1.4	0.4	96.1
14	26.8	66.8	93.6	ND	NA	1.5	0.5	95.6
22	-[c]	89.6	89.6	7.4	4.6	NA	0.1	97.1
36	31.6	61.8	93.3	2.9	1.5	1.8	0.3	98.3
59	33.0	58.6	91.7	2.1	1.4	2.4	0.3	96.5
High concentration (100 µg L^{-1}) [b]								
0	34.7	59.1	93.8	1.8	1.1	NA	0.1	95.7
3	34.8	57.8	92.6	1.4	0.7	NA	0.1	94.1
7	36.1	58.1	94.3	1.2	1.2	NA	0.1	95.6
14	32.5	58.1	90.6	1.3	1.3	NA	1.3	93.2
22	35.4	57.9	93.2	2.9	1.3	NA	0.1	96.2
36	36.2	54.3	90.5	2.3	1.0	NA	0.5	93.3
59	33.0	59.1	92.1	3.3	1.2	NA	0.2	95.6
Sterilized lake water (100 µg L^{-1})								
0	36.3	58.5	94.8	1.0	0.6	NA	ND	95.9
3	33.1	59.1	92.2	1.7	1.3	NA	ND	93.9
7	36.4	58.7	95.1	0.8	0.8	NA	0.1	96.0
14	33.7	58.6	92.3	0.8	0.8	NA	1.8	94.8
22	34.5	61.2	95.6	1.8	0.9	NA	0.1	97.5
36	36.1	58.6	94.7	2.1	0.7	NA	0.3	97.0
59	31.6	60.0	91.6	3.5	1.1	NA	0.1	95.3

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or less than the limit of detection [LOD] of 0.3% TAR)
[a] Activity remaining in the aqueous phase after dichloromethane partition of the water (relevant for low concentration only)
[b] Mean of two replicates
[c] Analyzed using HPLC fraction collection, individual isomers not resolved

III. CONCLUSION

From the obtained results it can be concluded that dimethomorph (BAS 550 F) is not significantly degraded in the natural water environment provided in this test. For the morpholine label, after 59 days, at least 86.2% TAR was recovered as the unchanged active substance. For the chlorophenyl label, at least 91.7% TAR was recovered as the unchanged active substance after 59 days. During the test, several minor metabolites were observed in small amounts of up to 4.0% TAR. However, an outlier was observed in one single sample (22 DAT, chlorophenyl label, low concentration) where a component with 7.6% TAR was detected. Furthermore, no systematic change to the isomer ratio was observed for either the morpholine or chlorophenyl label during the test.

Radioactivity in the volatile traps did not exceed 2.1 or 0.7% TAR for the morpholine and chlorophenyl labels respectively, indicating a low rate of mineralization.

Overall, the compound was considered to be stable in the test systems. Degradation kinetics were not reported as no significant degradation was observed.

CA 7.2.2.2 Aerobic mineralisation in surface water

No new water/sediment study was performed. The already peer-reviewed study is considered still valid.

Two water/sediment studies were available. In the first study only the chlorophenyl-moiety of the molecule was labelled and the isomers were not determined separately. Two different systems (sediment OC: 1.84-5.0%, sediment pH: 7.3-7.5, water pH: 8.0-8.2) were investigated in this study. Dimethomorph quickly moved to the sediment and was fixed as bound residues (max. 57% AR at 105d study end, and 74% AR at 29d). The complete degradation was low (mineralisation rate: 14-22% AR at 105d study end). In the sediment phase, small amounts of demethylated metabolites were observed in one system (7.8% AR after 1d) and an unknown polar fraction (max. 14-16% AR after 105d) consisted of several components.

The second study was performed with both ^{14}C-morpholine and ^{14}C-chlorophenyl dimethomorph in two different systems (sediment OC: 1.3-4.5%, sediment pH: 7.8-8.0, water pH: 7.6-8.5). The dissipation of dimethomorph from water phase to sediment was slower, reaching a maximum of 41% AR after 7 days in both systems, and then declined to 7.8-9.6% AR at study end (100d). The majority of the applied radioactivity became associated with non-extractable sediment residues (max. 47-82% AR). The mineralisation rate was low, reaching a maximum of 8.6% AR after 100 days. Up to 17 different metabolite fractions were found, none exceeding 2.3% of the applied radioactivity.

The half-lives of dimethomorph in water/sediment systems were re-calculated according to the newest guidelines and guidance documents and the re-evaluation is presented in KCA 7.2.2.2/1 2014/1183303/

Report:	CA 7.2.2.2/1 Maleri M., 2015 c Kinetic evaluation of degradation of BAS 550 F - Dimethomorph in water/sediment systems: Determination of modeling endpoints according to FOCUS 2014/1183303
Guidelines:	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 2.0 (December 2014)
GLP:	no

Executive Summary

The degradation behavior of BAS 550 F – dimethomorph has been investigated in four natural water/sediment systems (Bickenbach system, Unter Widdersheim, Kellmetschweiher und Berhäuser Altrhein).

In systems with samples from Bickenbach and Unter Widdersheim, plots were treated with ^{14}C-labeled dimethomorph at a nominal field application rate of 385 g as ha^{-1} incubated in the dark at 20 ± 2°C for up to 105 days. In systems with samples from Kellmetschweiher (System A) and Berghäuser Altrhein (System B), plots were treated with chlorophenyl-^{14}C and morpholine-^{14}C-labeled dimethomorph at a nominal field application rate of 700 g as ha^{-1} incubated in the dark at 20 ± 2°C for up to 100 days

The aim of the present study was to evaluate the degradation kinetics of dimethomorph in the four water/sediment systems following the recommendations of the FOCUS workgroup on degradation kinetics to derive modeling endpoints.

Kinetic evaluation at Level P-I (one-compartment approach) was performed for dimethomorph for degradation in the total system as well as for dissipation from the water and sediment phase of the test systems. At Level P-II (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

A summary of modeling and trigger endpoints for dimethomorph in the total system, the water phase and the sediment phase, derived from kinetic analysis at Level P-I, is given in the table below.

The kinetic evaluation at Level P-II revealed no reliable fit for any of the evaluated water/sediment systems. Consequently, no modeling endpoints were calculated.

I. MATERIAL AND METHODS

A. STUDY DESIGN

Test system Bickenbach / Unter Widdersheim

In a first study, the distribution and degradation of ^{14}C labeled dimethomorph was studied in two natural systems of water and sediment under laboratory conditions. The water-sediment systems were taken from two locations called "Bickenbach" and "Unter Widdersheim", both located in the district Hesse, Germany.

For the test system Bickenbach / Unter Widdersheim, the test substance was applied to the water at a target rate of 128 mg a.s. per kg water which corresponded to a maximum recommended application rate of 385 g as ha^{-1}. Afterwards, the test systems were incubated in the dark at 20°C for up to 105 days.

Duplicate samples were taken at 0, 0.25, 1, 2, 7, 14, 29, 61 and 105 days after treatment (DAT). The water and sediment phases were separated, extracted, and the solvent extracts were analyzed by HPLC.

The mean material balance ranged from 93.6 to 105.5% of the total applied radioactivity (TAR) in Bickenbach system and from 93.3 to 105.9% TAR in Unter Widdersheim.

In both systems, the residues of the parent compound in the water phase decreased to <5% TAR at the end of the study. In the sediment phase, dimethomorph was detected at maximum amounts of 53.9% TAR at 0.25 DAT in Bickenbach system and of 65.7% TAR at 0 DAT in the Unter Widdersheim and dissipated to <5% TAR in both systems.

No major metabolites, i.e. metabolites occurring at amounts >5% TAR in two consecutive samples, were formed in the water or sediment phase of the two test systems.

Test system Kellmetschweiher/Berhäuser Altrhein

In a second study, the degradation of chlorophenyl-^{14}C and morpholine-^{14}C-labeled dimethomorph was studied in two different natural systems of water and sediment under laboratory conditions. The water sediment systems were taken from a pond (Kellmetschweiher, system A) and a pond-like side arm of a river (Berghäuser Altrhein, system B), both located near Ludwigshafen in Rhineland-Palatinate, Germany.

For the test system Kellmetschweiher/Berhäuser Altrhein, about 70 µg of ^{14}C-BAS 550 F was applied to the test vessels containing 185 g sediment and 295 mL water. The test substance was applied to the water at a rate of 15 µg a.s. per test vessel which corresponded to a maximum recommended application rate of 700 g as ha^{-1} when related to a 30 cm deep water body. Afterwards, the test systems were incubated in the dark at 20°C for up to 100 days.

Duplicate samples were taken at 0, 0.25, 1, 2, 7, 14, 30, 62 and 100 days after treatment (DAT). The water and sediment phases were separated, extracted, and the solvent extracts were analyzed by HPLC.

The mean material balance ranged from 98.1 to 99.1% of the total applied radioactivity (TAR) in Kellmetschweiher and from 98.9 to 99.1% TAR in Berghäuser Altrhein.

In both systems, the residues of the parent compound in the water phase decreased to <10% TAR at the end of the study. In the sediment, the parent reached a maximum of about 48% TAR after 14 days in system A (Kellmetschweiher) and about 42% TAR after 7 days in system B (Berghäuser Altrhein).

HPLC analysis of the water phase and the sediment extracts showed that the major part of the radioactivity always consisted of unchanged parent. The sum of all metabolites (up to 17) never exceeded 6.8% TAR in the water phase and 6.0% TAR in the sediment extracts. No single metabolite ever exceeded 2.1% TAR.

Kinetic modeling strategy

Kinetic evaluation was performed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [FOCUS Kinetics v2.0 (2006)]. According to FOCUS, degradation endpoints were derived for use as modeling inputs.

Kinetic evaluation at Level P-I (one-compartment approach) was performed for dimethomorph degradation in the total system as well as dissipation from the water and sediment phase of the test systems.

At Level P-II (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

Kinetic models included in the evaluation

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics.

Level P-I:

- Single-First-Order (SFO),
- Gustafson and Holden (FOMC),
- Double first-order in parallel (DFOP).
- Hockey-stick (HS).

Level P-II:

- Two-compartment model (water and sediment), SFO kinetics.

At Level P-I, modeling endpoints were derived preferably from the SFO model. If the SFO model was not appropriate, conservative pseudo-SFO degradation rates were derived from the appropriate bi-phasic model.

The appropriateness of a distinct kinetic model to describe degradation was tested with the following checks recommended by FOCUS [FOCUS Kinetics v2.0 (2006)]:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed
- t-test to evaluate whether estimated degradation parameters differ from zero

The visual fit was categorized as follows:

- Poor fit = the fit does not follow the pattern of the measured residues, not acceptable to derive modeling endpoints;
- Acceptable fit = the fit mainly follows the pattern of the measured residues with small deviations, acceptable to derive modeling endpoints;
- Good fit = the fit follows the pattern of the measured residues well, residuals are randomly scattered around zero, acceptable to derive modeling endpoints.

Furthermore, a kinetic model was considered appropriate for deriving modeling endpoints in water/sediment systems if the χ^2-error value was low (ideally below 15%) and the t-test for the degradation parameters was passed at 10% error level.

II. RESULTS AND DISCUSSION

The datasets for each water/sediment system were analyzed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics.

Dimethomorph has two isomers (E and Z) which were evaluated separately and as sum of both isomers (isomer mix).

Table 7.2.2.2-1: Summary of modeling endpoints for dimethomorph, Level P-I (mixture)

Compartment	Test system	Kinetic model	χ^2 error	Modeling $DegT_{50}$ [d]
Total sytem	Bickenbach	SFO	12.3	3.6
	Unter Widdersbach	SFO	11.7	2.6
	Kellmetschweiher (System A)	SFO	2.0	58.4
	Berghäuser Altrhein (System B)	SFO	4.9	15.4
Compartment	**Test system**	**Kinetic model**	**χ^2 error**	**Modeling $DisT_{50}$ [d]**
Water	Bickenbach	SFO*	13.8	1.2*
	Unter Widdersbach	DFOP**	2.3	0.9**
	Kellmetschweiher (System A)	SFO	12.1	15.3
	Berghäuser Altrhein (System B)	SFO	15.4	4.8
Sediment	Bickenbach	SFO	12.3	3.6
	Unter Widdersbach	SFO	12.8	3.1
	Kellmetschweiher (System A)	SFO	4.0	88.6
	Berghäuser Altrhein (System B)	SFO	12.5	20.6

* Outlier removed.

** DT_{50} calculated from slow k rate.

Table 7.2.2.2-2: Summary of modeling endpoints for dimethomorph, Level P-I (E isomer)

Compartment	Test system	Kinetic model	χ^2 error	Modeling $DegT_{50}/DisT_{50}$ [d] [a]
Total sytem	Bickenbach	SFO	13.4	3.1
	Unter Widdersbach	SFO	13.7	1.2
	System A	SFO	3.6	31.0
	System B	SFO	2.9	9.2
Water	Bickenbach	SFO*	10.2	1.6*
	Unter Widdersbach	DFOP**	3.4	0.9**
	System A	DFOP**	4.0	16.1
	System B	SFO	15.3	3.5
Sediment	Bickenbach	SFO	13.3	2.3
	Unter Widdersbach	SFO	16.0	1.1
	System A	SFO	10.3	38.9
	System B	SFO	11.4	14.2

[a] $DegT_{50}$: total system; $DisT_{50}$: water or sediment phase.

* Outlier removed.

** DT_{50} calculated as DT_{90} / 3.32.

Table 7.2.2.2-3: Summary of modeling endpoints for dimethomorph, Level P-I (Z isomer)

Compartment	Test system	Kinetic model	χ^2 error	Modeling $DegT_{50}/DisT_{50}$ [d] [a]
Total sytem	Bickenbach	SFO	13.5	4.5
	Unter Widdersbach	SFO	13.7	4.0
	System A	SFO	3.7	93.2
	System B	SFO	6.6	21.8
Water	Bickenbach	SFO*	15.7	1.0*
	Unter Widdersbach	DFOP**	1.4	1.1**
	System A	SFO	11.6	21.0
	System B	DFOP**	4.2	8.5
Sediment	Bickenbach	SFO	14.4	5.4
	Unter Widdersbach	SFO	16.6	4.3
	System A	SFO	1.6	147.0
	System B	SFO	13.1	28.2

[a] $DegT_{50}$: total system; $DisT_{50}$: water or sediment phase.

* Outlier removed.

** DT_{50} calculated as DT_{90} / 3.32.

III. CONCLUSION

The dissipation and degradation kinetics of dimethomorph in two water/sediment systems were evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [FOCUS Kinetics v2.0 (2006)]. The visual assessment and goodness-of-fit statistics of the respective models indicate plausible fits. Therefore, the resulting endpoints can be considered reliable.

A summary of modeling endpoints for dimethomorph in the total system, the water phase and the sediment phase, derived from kinetic analysis at Level P-I for the isomer mixture, is given in Table 7.2.2.3-1. Results for E- and Z-isomer are summarized in Table 7.2.2.3-2 and Table 7.2.2.3-3.

The kinetic evaluation at Level P-II revealed no reliable fit for any of the evaluated water/sediment systems. Consequently, no endpoints were summarized for Level P-II values.

CA 7.2.2.3 Water/sediment studies

No water/sediment study was performed.

CA 7.2.2.4 Irradiated water/sediment study

No irradiated water/sediment study was performed.

CA 7.2.3 Degradation in the saturated zone

Moderate adsorption coefficients of dimethomorph were determined in the soil adsorption/desorption studies, with K_{oc} values of 290-566 mL/g). $PEC_{groundwater}$ calculations from previous and also current dossier as well as the lysimeter study clearly showed no risk of displacement of active substance into deeper soil layers or even groundwater. Investigations on the degradation in the saturated zone are therefore considered not necessary.

CA 7.3 Fate and behaviour in air

Dimethomorph is characterized by a low vapor pressure (9.7 x 10^{-7} Pa at 20°C) and a negligible volatilization from soil and plant surfaces. Furthermore, it is rapidly degraded by photochemical processes (approximately DT_{50} of 3.6 hours).

CA 7.3.1 Route and rate of degradation in air

No new experimental data are available.

CA 7.3.2 Transport via air

Dimethomorph has a very low volatilization potential and is degraded very fast by photochemical processes. Consequently, there is no risk of long-range transport of dimethomorph.

CA 7.3.3 Local and global effects

No effects are expected since transport via air is very unlikely (for details see above).

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil: Dimethomorph (parent only)

Ground Water: Dimethomorph (parent only)

Surface Water: Dimethomorph (parent only)

Sediment: Dimethomorph (parent only)

Air: Dimethomorph (parent only)

No volatile metabolite was detected.

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil: Dimethomorph (parent only)

Ground Water: Dimethomorph (parent only)

Surface Water: Dimethomorph (parent only)

Sediment: Dimethomorph (parent only)

Air: Dimethomorph (parent only)

CA 7.5 Monitoring data

According to the knowledge of the applicant, there are currently no published environmental monitoring data available for dimethomorph, which would provide knowledge on the environmental behaviour not covered by this dossier.

During literature search a few publications were found dealing with environmental monitoring of various pesticides in surface water, groundwater and/or sediment outside EU (e.g. China, South Africa). Additionally most studies lacked standard methods or quantitative information. Frequency of detections were rare and concentrations not indicated or very low.

The following information could be retrieved from the literature review are summarized below:

- Dimethomorph was not found to be persistent in soil in intensively used agricultural areas (conventional agriculture).
- DT_{50} values of 10.3 to 31.5, 9.5 to 9.7 days or 14.6 days in soil were stated in several studies. These values confirm results of standard studies in this dossier.
- Direct photolysis was found to be no important pathway in water.
- A foliar DT_{50} of 5.2 days was provided in a study (10 days used in standard assessment).
- Wetlands showed to be effective mitigation measures for dimethomorph removal.

No full summaries were supplied for the given information since they all strongly confirmed information available from standard methods provided during the previous Annex I review and the present Annex I renewal.

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Dimethomorph

Document M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

Telephone [redacted]
Telefax
e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
30/09/2016	On request from RMS, the full OECD summaries of the studies included in the old DAR are added to the dossier, and all changes are highlighted in yellow. CA 8.1.1.1/2 : The acute oral toxicity (LD50) of CME 151 to the bobwhite quail, DK-505-001 CA 8.1.1.1/3 : The acute oral toxicity (LD50) of CME 151 to the mallard duck, DK-505-003 CA 8.1.1.2/1 : The dietary toxicity (LC50) of CME 151 to the bobwhite quail, DK-505-002 CA 8.1.1.2/2 : The dietary toxicity (LC50) of CME 151 to the mallard duck DK-505-004 CA 8.1.1.3/1 : Reproduction study with Dimethomorph technical (CL 336379) in the Northern bobwhite (Colinus virginianus), DK-505-009 CA 8.1.1.3/2 : Reproduction study with Dimethomorph technical (CL 336379) in the mallard duck (Anas platyrhynchos), DK-505-007 CA 8.2.1/1 : 96-hour acute toxicity (LC50) with CME 151 in the rainbow trout, DK-511-002 CA 8.2.1/3 : 96-hour acute toxicity (LC50) with CME 151 in the carp, DK-511-001 CA 8.2.1/5 : The acute toxicity of CME 151 to bluegill sunfish (Lepomis macrochirus), DK-511-003 CA 8.2.1/8 : Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to rainbow trout, Oncorhynchus mykiss, under flow-through test conditions, DK-511-007 CA 8.2.1/9 : Acute toxicity of AC 336379 (Dimethomorph) to the sheepshead minnow (Cyprinodon variegatus) under flow-through test conditions, DK511-005 CA 8.2.1/10 : Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to bluegill sunfish, Lepomis macrochirus under flow-through test conditions, DK-511-006 CA 8.2.2.1/1 : Toxicity of AC 336,379 (Dimethomorph) technical during the early life-stages of rainbow trout (Oncorhynchus mykiss), DK-512-002 CA 8.2.2.1/5 : Toxicity of Dimethomorph to rainbow trout (Oncorhynchus mykiss) in a prolonged flow-through test (21 days), DK-512-001 CA 8.2.4.1/1 : 48-hour acute toxicity of CME 151 to Daphnia magna (OECD immobilization test), DK-521-002 CA 8.2.4.1/3 : Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to Daphnia magna under static test conditions, DK-521-006 CA 8.2.4.2/1 : Acute toxicity of AC 336379 (Dimethomorph) to the mysid (Mysidopsis bahia)	MCA Section 8, Version 1, BASF DocID 2016/1000212

	under flow-through test conditions, DK-521-004 CA 8.2.4.2/2 : Effects of AC 336379 (Dimethomorph) on new shell growth in the Eastern oyster (Crassostrea virginica) under flow-through test conditions, DK-522-001 CA 8.2.5.1/1 : Influence of Dimethomorph on survival and reproduction of Daphnia magna in a semistatic test (22 days), DK-524-001 CA 8.2.5.1/4 : Chronic toxicity of AC 336,379 (Dimethomorph) technical during the complete life-cycle of Daphnia magna under flow-through test conditions, DK-523-001 CA 8.2.5.3/1 : Effects of AC 336,379 (Dimethomorph) on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system, DK-529-002 CA 8.2.6.1/1 : Acute toxicity of CME 151 to Scenedesmus subspicatus (OECD algae growth inhibition test conditions, DK-521-001 CA 8.2.6.1/2 : BAS 550 F - Determination of the inhibitory effect on the cell multiplication of unicellular green algae, DK-521-007 CA 8.3.1.1.1/2 : Acute toxicity of technical Dimethomorph (AC 336379) to the honey bee, Apis mellifera, DK-541-027 CA 8.3.1.1.2/2 : Acute toxicity of technical Dimethomorph (AC 336379) to the honey bee, Apis mellifera, DK-541-027 CA 8.4.1/1 : A chronic toxicity and reproduction test exposing the earthworm Eisenia fetida, to Dimethomorph technical (CL 336,379) in OECD artificial soil following the ISO-draft (ISO/DIS 11268-2), DK-534-001 CA 8.7/1 & 8.7/2 : Acute toxicity (LC50) study of CME 151 to earthworms, DK-531-001 & DK-123-135	
31/05/2017	After the draft assessment report from RMS, the dossier was updated and all changes are highlighted in blue. CA 8.1.5: Submission of study and inclusion of study summary on request of RMS CA 8.2 – Table 8.1-1: Endpoint value from study 2010/1177241 adapted, adaptions to footnote No. 3 CA 8.2.1/4: Endpoint recalculated by RMS added CA 8.2.1/7: Summary updated as requested by RMS CA 8.2.2.1/4 and CA 8.2.2.1/2: Summary updated as requested by RMS CA 8.2.3/1 and CA 8.2.3/2: Summary updated as requested by RMS CA 8.2.8/3: Summary updated as requested by RMS CA 8.2.8/4: Summary updated as requested by RMS	MCA Section 8, Version 3, BASF DocID 2016/1235732

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

On request from RMS, the full OECD summaries of the studies included in the old DAR are added to the dossier, and all changes are highlighted in yellow.

Updated after the draft assessment report from RMS. All changes are highlighted in blue.
Concerning the update from May 2017 on request from RMS, all changes concerning the request are highlighted in turquoise.

General remarks

The order of the study summaries is different compared to the information given in the application submitted for renewal of approval. Reason is that the order of the studies had to be altered to present. In case references are summarized which were not listed in the Application, or in case references listed in the application are not contained in this chapter, an additional comment will be made at the respective section.

An overview of the changes of order in chapter 8 compared to the Application is given in the table below for the reviewer's convenience:

Table 8.1-1: Overview of changes of documents submitted compared to originally listed documents in the Application

Data point in Application	Data point in current dossier	DocID	Changes to Application	Reason for change
n.a.	8.1/3	2015/1171916	data generation	Needed for refinement of mammalian risk assessment
n.a.	8.1/4	2015/1217511	data generation	Needed for refinement of mammalian risk assessment
n.a.	8.1.2.2/2	2015/1253632	data generation	Needed for refinement of mammalian risk assessment
8.1.5/1	n.a.	2014/1189839	not submitted although announced in Application	Document was proposed but will be written in the dossier itself
8.2.1/2	8.2.1/2	2010/1177242	change of docID	Data was already calculated and did not need to be redone
8.2.2.1/2	8.2.2.1/2	2014/1224007	data generation; change of docID	Recalculation of endpoint to EC10
8.2.2.1/6	8.2.2.1/6	2014/1224007	data generation; change of docID	Recalculation of endpoint to EC10
8.2.5.1/1	8.2.5.1/1	2015/1238096	data generation	Recalculation of endpoint to EC10
8.2.5.1/2	8.2.5.1/2	2015/1238096	data generation	Recalculation of endpoint to EC10
8.2.5.2/1	8.2.5.2/1	2015/1238096	data generation	Recalculation of endpoint to EC10
8.2.6.1/2	n.a.	2014/1224009	not submitted although announced in Application	Document was proposed but will be written in the dossier itself
8.3.1/1	n.a.	2014/1000183	not submitted although announced in Application	Study not needed as risk assessment is acceptable without data
8.3.1/2	n.a.	2015/10003896	not submitted although announced in Application	Study not needed as risk assessment is acceptable without data

n.a. Not applicable; study was not included in the original application or will not be submitted in the final dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

Introduction

Dimethomorph (BAS 550 F), a fungicide for use in for example strawberries, lettuce and vineyards, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive 2007/25/EC of 23 April 2007. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011.

All relevant information on the first Annex I review and the endpoints used in ecotoxicological risk assessments can be found in the review report for dimethomorph (SANCO/10040/06 - rev.3).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossier for the representative formulations.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Summaries of relevant and reliable public literature data on dimethomorph are provided in this section as appropriate. Further information on the literature assessment and respective justifications can be found in M-CA 9.

Details on the EU agreed studies which have been already evaluated within the previous Annex I inclusion of dimethomorph are provided in the EU Review documents of dimethomorph (*i.e.* Draft Assessment Report (DAR), Volume 1, Annex B.9., 2004; EFSA Scientific Report (2006) 82, 1 - 69).

For better transparency and traceability of the active substance history, an overview of all studies is summarized in Table 8.1-1:.

Table 8.1-1: Summary of EU-reviewed and agreed, as well as additional toxicity studies relevant for AIR3 for the active substance dimethomorph (BAS 550 F) for assessing the risk to birds and mammals [1)]

Test system	Test species	Reference BASF DocID	EU-agreed
BIRDS			
Acute oral toxicity	*Colinus virginianus*	DK-505-001	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	DK-505-003	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
	Serinus canaria	2014/1190617	No, new study
Short-term dietary toxicity	*Colinus virginianus*	DK-505-002	EFSA Scientific Report (2006) & DAR (2004) (no longer part of core data package according to EFSA/2009/1438)
	Anas platyrhynchos	DK-505-004	EFSA Scientific Report (2006) & DAR (2004) (no longer part of core data package according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Colinus virginianus*	DK-505-009	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	DK-505-007	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
MAMMALS			
Acute oral toxicity	Rat	DK-411-004	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
	Rat	2010/1091144 (amended by 2010/1091145)	No, new study
	Rat	2010/1210734	No, new study
2-Generation reproductive toxicity	Rat	DK-430-001	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	Rat	DK-432-002	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)
	Rabbit	DK-432-004	EFSA Scientific Report (2006) & DAR (2004) (still valid for AIR 3 according to EFSA/2009/1438)

1 EU agreed means assessed during the previous EU evaluation process, but not necessarily listed in the list of endpoint as in the Conclusion on the peer review of dimethomorph, *EFSA Scientific Report* (2006) 82, 1-69.

New studies conducted for use in the risk assessments for birds and mammals

In addition to studies on effects on birds or terrestrial vertebrates, new studies on the residue behaviour of dimethomorph on plants relevant for the higher tier risk assessment of bird and terrestrial vertebrates are summarized under this chapter from M-CA 8.1/1 to 8.1/4. The studies have not been peer-reviewed on EU level, which is indicated prior to the respective summary. For completeness some older studies are submitted additionally, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU).

On request of the RMS, the notifier provided more information abut the study below (Martin, 2015a) in an additional document [see KCA 8.1/5 2017/1099857].

Report:	CA 8.1/1 Martin T., 2015 a Study on the residue behavior of BAS 550 F (Dimethomorph) on wheat (young plants) after the application of BAS 550 02 F under field conditions in Germany, France (North), Netherlands, Italy and Spain, 2014 2014/1186694
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

The objective of the study was to determine the dissipation of residues of dimethomorph (BAS 550 F) on young wheat plants after one application of the product BAS 550 02 F at 10 L/ha (corresponding to 1.5 kg as/ha). Samplings were carried out directly after and at subsequent time intervals after application up to 14 days. Residue levels of dimethomorph in the wheat specimens taken 0 DALA (1 HALA) ranged from 100 – 190 mg/kg. They continuously decreased until the last sampling (14 DALA) to a range of 0.12 – 15 mg/kg.

MATERIAL AND METHODS

MATERIALS

Test Material:	BAS 550 02 F
Description:	BAS 550 02 F (dimethomorph)
Lot/Batch #:	FRE-001030; BAS 550 F: 150 g/L (BAS 550 02 F, DC)
CAS#:	dimethomorph (BAS 550 F): 110488-70-5
Crop parts(s) commodity:	wheat (young plants without roots; from BBCH 12 to 23)
Sample size:	24.0-119.3 g

STUDY DESIGN

Study site

During the 2014 growing season a total of eight trials were conducted in different locations of representative wheat growing areas in Germany, France (North), the Netherlands, Spain and Italy.

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.

The test item BAS 550 02 F (150 g/L of dimethomorph, DC) was foliar applied on plot 2 at a nominal application rate of 10 L product (1.5 kg dimethomorph /ha) per ha with a spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 12-13).

Sampling information

For this study treated specimens were collected as wheat whole plants without roots 1 hour after the last application (HALA) as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. Untreated specimens were collected also as wheat whole plants without roots 0, 5 and 14 days after the last application.

Untreated specimens were obtained prior to treated specimens when coincided at sampling timings.

All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analysed for dimethomorph (BAS 550 F) according to BASF method No. L0076/01 (535/1). The method has a limit of quantitation of 0.01 mg/kg.

The results of procedural recovery experiments averaged at about 86.5% for BAS 550 F at fortification levels between 0.01 and 1.00 mg/kg.

RESULTS AND DISCUSSION

The dimethomorph residues in the wheat specimens taken 0 DALA (1 HALA) ranged from 100 – 190 mg/kg. They decreased continuously to 130 – 170 mg/kg in the specimens taken 1 DALA and further to 54 – 150 mg/kg at 2 DALA. In the specimens taken 3 DALA 35 – 110 mg/kg were determined. The residue level in the specimens taken 4 DALA was 5.8 – 65 mg/kg, and in those taken 5 DALA 3.7 – 52 mg/kg were found. In the specimens taken 7 DALA 0.94 – 23 mg/kg were determined. The residue level in the specimens taken 10 DALA was 0.34 – 18 mg/kg and in those taken 12 DALA 0.21 – 23 mg/kg were found. At the last sampling (14 DALA) the residue level decreased to 0.12 – 15 mg/kg.

No residues of dimethomorph above the limit of quantitation were found in any of the analysed untreated specimens.

Table 8.1-2: Residues of dimethomorph in wheat (whole plant without roots)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	dimethomorph residues [mg/kg]
Trial no. L140363 Study site: Brandenburg, Germany	1 HALA	1	26.04.2014	12	100
	1 DALA	2	27.04.2014	12	140
	2 DALA	3	28.04.2014	12-13	70
	3 DALA	4	29.04.2014	13-21	55
	4 DALA	5	30.04.2014	13-21	35
	5 DALA	6	01.05.2014	13-21	19
	7 DALA	7	03.05.2014	21-23	10
	10 DALA	8	06.05.2014	21-23	12
	12 DALA	9	08.05.2014	21-23	1.9
	14 DALA	10	10.05.2014	21-23	0.59
Trial no. L140364 Study site: Kleve, Germany	1 HALA	1	10.04.2014	13	130
	1 DALA	2	11.04.2014	13	130
	2 DALA	3	12.04.2014	14	120
	3 DALA	4	13.04.2014	14	110
	4 DALA	5	14.04.2014	15	22
	5 DALA	6	15.04.2014	16	12
	7 DALA	7	17.04.2014	16	13
	10 DALA	8	20.04.2014	21	5.9
	12 DALA	9	22.04.2014	21	2.5
	14 DALA	10	24.04.2014	22	2.1
Trial no. L140365 Study site: Indre et Loire, France	1 HALA	1	23.04.2014	13	130
	1 DALA	2	24.04.2014	13	150
	2 DALA	3	25.04.2014	13	89
	3 DALA	4	26.04.2014	13	86
	4 DALA	5	27.04.2014	13	20
	5 DALA	6	28.04.2014	13	25
	7 DALA	7	30.04.2014	13	8.7
	10 DALA	8	03.05.2014	21	1.2
	12 DALA	9	05.05.2014	21	2.0
	14 DALA	10	07.05.2014	21	1.8
Trial no. L140366 Study site: Horst aan de Maas, Netherlands	1 HALA	1	10.04.2014	13	180
	1 DALA	2	11.04.2014	13	170
	2 DALA	3	12.04.2014	14	150
	3 DALA	4	13.04.2014	14	73
	4 DALA	5	14.04.2014	15	28
	5 DALA	6	15.04.2014	16	13
	7 DALA	7	17.04.2014	16	15
	10 DALA	8	20.04.2014	21	18
	12 DALA	9	22.04.2014	21	2.8
	14 DALA	10	24.04.2014	22	3.3

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	dimethomorph residues [mg/kg]
Trial no. L140367 Study site: Seville, Spain [1)]	1 HALA	1	04.11.2014	12	100
	1 DALA	2	05.11.2014	12	130
	2 DALA	3	06.11.2014	12	54
	3 DALA	4	07.11.2014	12	35
	4 DALA	5	08.11.2014	12-13	5.8
	5 DALA	6	09.11.2014	13	3.7
	7 DALA	7	11.11.2014	13	0.94
	10 DALA	8	14.11.2014	14	0.34
	12 DALA	9	16.11.2014	14	0.38
	14 DALA	10	18.11.2014	14	0.36
Trial no. L140368 Study site: Seville, Spain [1)]	1 HALA	1	04.11.2014	12	190
	1 DALA	2	05.11.2014	12	130
	2 DALA	3	06.11.2014	12	100
	3 DALA	4	07.11.2014	12	49
	4 DALA	5	08.11.2014	12-13	46
	5 DALA	6	09.11.2014	12-13	5.7
	7 DALA	7	11.11.2014	13	1.6
	10 DALA	8	14.11.2014	13-14	5.3
	12 DALA	9	16.11.2014	13-14	0.21
	14 DALA	10	18.11.2014	14	1.8
Trial no. L140369 Study site: Bologna, Italy	1 HALA	1	18.11.2014	12-13	140
	1 DALA	2	19.11.2014	12-13	150
	2 DALA	3	20.11.2014	12-13	86
	3 DALA	4	21.11.2014	12-13	66
	4 DALA	5	22.11.2014	12-13	13
	5 DALA	6	23.11.2014	13	5.3
	7 DALA	7	25.11.2014	13	2.1
	10 DALA	8	28.11.2014	13	0.34
	12 DALA	9	30.11.2014	13	0.22
	14 DALA	10	02.12.2014	13-14	0.12
Trial no. L140370 Study site: Foggia, Italy	1 HALA	1	28.11.2014	13	120
	1 DALA	2	29.11.2014	13	130
	2 DALA	3	30.11.2014	13	100
	3 DALA	4	01.12.2014	13-14	72
	4 DALA	5	02.12.2014	13-14	65
	5 DALA	6	03.12.2014	13-14	52
	7 DALA	7	05.12.2014	13-14	23
	10 DALA	8	08.12.2014	14	14
	12 DALA	9	10.12.2014	14	23
	14 DALA	10	12.12.2014	14	15

HALA: hours after last application; DALA: days after last application,
1 separate locations

CONCLUSION

The dimethomorph residues in the wheat specimens taken directly after application (1 HALA) ranged from 100 – 190 mg/kg. They continuously decreased until the last sampling (14 DALA) to a range of 0.12 – 15 mg/kg.

On request of the RMS, the notifier provided more information abut the study below (Galvez and Moreno, 2015a) in an additional document [see KCA 8.1/5 2017/1099857].

Report:	CA 8.1/2 Galvez O.,Moreno S., 2015 a Study on the residue behaviour of Dimethomorph (BAS 550 F) on peas (young plants) after treatment with BAS 550 02 F under field conditions in North and South Europe, season 2014 2014/1186693
Guidelines:	2004/10/EC, International guidelines for distribution and pesticides application AEPLA FAO 1985, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B, OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the magnitude of residues of dimethomorph (BAS 550 F) on young pea plants after one application of the product BAS 550 02 F at 10 L/ha (corresponding to 1.5 kg as/ha). Samplings were carried out directly after and at subsequent time intervals after application up to 14 days. Residue levels of dimethomorph in the pea specimens taken 0 DALA (1 HALA) ranged from 40-136 mg/kg. During the last sampling (14 DALA) residue levels of dimethomorph had decreased to a range of 0.55 – 28 mg/kg.

MATERIAL AND METHODS

MATERIALS

Test Material:	BAS 550 02 F
Description:	BAS 550 02 F (dimethomorph)
Lot/Batch #:	FRE-001030; BAS 550 F: 150 g/L (BAS 550 02 F, DC)
CAS#:	dimethomorph (BAS 550 F): 110488-70-5
Crop parts(s) commodity:	Peas (young plants without roots; from BBCH 12 to 35)
Sample size:	50.0-260.9 g (specimen weight)

STUDY DESIGN

Study site

During the 2014 growing season eight trials (L140371, L140372, L140373, L140374, L140375, L140376, L140377 and L140378) were conducted in representative growing areas for peas in Germany, France North, The Netherlands, Spain and Italy.

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot (plot 2). The applications were conducted using commercial or experimental equipment, which simulated commercial applications. No product containing the test item was used on the test plots during the season.

The test item BAS 550 02 F (150 g/L of dimethomorph, DC) was foliar applied on plot 2 at a nominal application rate of 10 L product (1.5 kg dimethomorph) per ha with a spray volume of 200 L/ha at two to three leaves unfolded growth stage (BBCH 12-13).

Sampling information

For this study treated specimens were collected as pea whole plants without roots 1 hour after the last application (HALA) as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. Control specimens were collected also as pea whole plants without roots before last application and at 5 and 14 days after the last application.

Control specimens were collected prior to the treated specimens to avoid any possible contamination.

Each specimen was collected in duplicate ('ship' and 'retain'), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis without replication.

All specimens were transferred to freezing storage at ≤ -18ºC on the day of sampling and shipped frozen from the test sites to the Specimen Management Laboratory in BASF SE Agricultural Center Limburgerhof. The homogenized specimens were transported to LGC in Cambridgeshire under deep-frozen conditions (-18° C or below) for analysis. The BASF SE kept a retain specimen.

Description of analytical methods

All specimens were analysed for dimethomorph (BAS 550 F) according to BASF method No. L0076/01. The method has a limit of quantitation of 0.01 mg/kg for BAS 550 F.

The results of procedural recovery experiments averaged at 83.0% for BAS 550 F at fortification levels between 0.01 and 1.00 mg/kg (n = 5).

RESULTS AND DISCUSSION

The dimethomorph residues in the pea specimens taken for trials L140371 – L140476: 0 DALA (1 HALA) ranged from 40 – 136 mg/kg. They decreased continuously to 22 – 120 mg/kg in the specimens taken 1 DALA and further to 14 – 110 mg/kg at 2 DALA. In the specimens taken 3 DALA 11 – 77 mg/kg were determined. The residue level in the specimens taken 4 DALA was 6.1 – 53 mg/kg, and in those taken 5 DALA 6.9 – 70 mg/kg were found. In the specimens taken 7 DALA 2.3 – 43 mg/kg were determined. The residue level in the specimens taken 10 DALA was 1.7 – 38 mg/kg and in those taken 12 DALA 0.75 – 45 mg/kg were found. At the last sampling (14 DALA) the residue level decreased to 0.55 – 28 mg/kg.

Two trials (No. L140377 and L140378) are not included in the summary table below as the analytics shows unexplainable high variability in the results including increased values up to DALA 5, which cannot be explained by the field raw data or analytical measurements, and are not coherent with the other trials. For more details please refer directly to the report.

Residues of BAS 550 F were below the LOQ (0.01 mg/kg) in all control specimens except for sample L1403770003 where it was 0.081 mg/kg.

Table 8.1-3: Residues of dimethomorph in pea (whole plant without roots)

Trial details	Sampling timing [1]	Sampling no.	Date	Crop growth stage (BBCH)	dimethomorph residues [mg/kg]
Trial no. L140371 Study site: Brandenburg, Germany	1 HALA	1	06.06.2014	12-13	70
	1 DALA	2	07.06.2014	13-21	58
	2 DALA	3	08.06.2014	13-21	40
	3 DALA	4	09.06.2014	13-21	39
	4 DALA	5	10.06.2014	13-21	6.1
	5 DALA	6	11.06.2014	14-21	7.4
	7 DALA	7	13.06.2014	14-21	2.6
	10 DALA	8	16.06.2014	17-21	2.1
	12 DALA	9	18.06.2014	18-21	0.75
	14 DALA	10	20.06.2014	18-21	0.55
Trial no. L140372 Study site: Brandenburg, Germany	1 HALA	1	03.09.2014	12	62
	1 DALA	2	04.09.2014	12	79
	2 DALA	3	05.09.2014	12-13	53
	3 DALA	4	06.09.2014	13-21	31
	4 DALA	5	07.09.2014	14-21	15
	5 DALA	6	08.09.2014	14-21	10
	7 DALA	7	10.09.2014	14-21	6.4
	10 DALA	8	13.09.2014	15-21	3.7
	12 DALA	9	15.09.2014	16-21	1.8
	14 DALA	10	17.09.2014	16-21	1.8
Trial no. L140373 Study site: Ambillou, France	1 HALA	1	11.04.2014	12	79
	1 DALA	2	12.04.2014	12	64
	2 DALA	3	13.04.2014	12	61
	3 DALA	4	14.04.2014	12	<0.01
	4 DALA	5	15.04.2014	13	53
	5 DALA	6	16.04.2014	13	49
	7 DALA	7	18.04.2014	13	38
	10 DALA	8	21.04.2014	14	32
	12 DALA	9	23.04.2014	14	11
	14 DALA	10	25.04.2014	14	9.8

Trial details	Sampling timing [1]	Sampling no.	Date	Crop growth stage (BBCH)	dimethomorph residues [mg/kg]
Trial no. L140374 Study site: Limburg, Netherlands	1 HALA	1	05.05.2014	13	94
	1 DALA	2	06.05.2014	13	52
	2 DALA	3	07.05.2014	14	14
	3 DALA	4	08.05.2014	14	11
	4 DALA	5	09.05.2014	15	6.9
	5 DALA	6	10.05.2014	15	6.9
	7 DALA	7	12.05.2014	16	3.1
	10 DALA	8	15.05.2014	17	3.0
	12 DALA	9	17.05.2014	18	2.4
	14 DALA	10	19.05.2014	19	2.0
Trial no. L140375 Study site: Seville Spain	1 HALA	1	08.12.2014	12	40
	1 DALA	2	09.12.2014	12	22
	2 DALA	3	10.12.2014	12	23
	3 DALA	4	11.12.2014	12	28
	4 DALA	5	12.12.2014	12	16
	5 DALA	6	13.12.2014	12-13	13
	7 DALA	7	15.12.2014	13	2.3
	10 DALA	8	18.12.2014	13	1.7
	12 DALA	9	20.12.2014	13	1.3
	14 DALA	10	22.12.2014	15/35	0.85
Trial no. L140376 Study site: Seville Spain	1 HALA	1	16.12.2014	12	136
	1 DALA	2	17.12.2014	12	120
	2 DALA	3	18.12.2014	12	110
	3 DALA	4	19.12.2014	12-13	77
	4 DALA	5	20.12.2014	12-13	42
	5 DALA	6	21.12.2014	13	70
	7 DALA	7	23.12.2014	14/31	43
	10 DALA	8	26.12.2014	14/31	38
	12 DALA	9	28.12.2014	14/31	45
	14 DALA	10	30.12.2014	14/32	28

1): HALA hours after last application; DALA days after last application

CONCLUSION

The dimethomorph residues in the pea specimens taken directly after the last application (1 HALA) ranged from 40-136 mg/kg. They continuously decreased until the last sampling (14 DALA) to a range of 0.55 – 28 mg/kg.

On request of the RMS, the notifier provided more information abut the study below (Shbaita, 2015a) in an additional document [see KCA 8.1/5 2017/1099857]. Please note that residue data refer to fresh weight.

Report: CA 8.1/3
Shbaita H., 2015 a
Calculation of DT50 dissipation times of BAS 550 F - Dimethomorph in wheat from field trails conducted in the Northern and Southern Zones of Europe
2015/1171916
Guidelines: none
GLP: no

The residue decline of BAS 550 F – Dimethomorph in wheat plants has been studied in multiple field trials at different sites in Northern and Southern Europe during the growing season 2014.

This modelling report provides kinetic analysis and estimation of the dissipation times (DT_{50} values) for dimethomorph (BAS 550 F).

MATERIAL AND METHODS

Since no specific recommendation is available how to carry out the kinetic evaluation for the described experiment, guidance of the FOCUS workgroup on degradation kinetics was used in order to derive degradation parameters for modelling purposes. This means the selected DT_{50} values are suitable input parameters for models that require single first order (SFO) DT_{50} values or conservative substitutes.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS (2006) guidance. The recommended kinetic models, i.e. the single first order kinetics (SFO) was applied. For visual inspection, the recommended graphical representations of observed and modelled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the $\chi 2$ minimum error level is provided.

The visual assessments of the residual plots show that the residuals are randomly scattered around the zero line and that the fits are acceptable for all datasets. The estimated dissipation rate constants are significantly different from zero as indicated by low t-test values. The DT_{50} values that are suitable for modelling purposes according to FOCUS and the respective statistical indices are presented in Table 8.1-4

Some high χ^2 error values were calculated. They were mainly caused by a few outlier values which can be seen in the graphs of the fits in the appendix. However no refinement of the fits by eliminating single outliers was varied out to improve the χ^2 error statistics. The visual inspection showed that the fits described the decline of the residues in all cases reasonably well. The residuals are equally distributed and do not show a pattern, therefore it can be concluded that the fits are acceptable and the estimated half-live according single first order kinetics can be considered as an adequate characterization of dimethomorph on plants.

RESULTS

An overview of the calculated DT50 values for dimethomorph in wheat plants is given in Table 8.1-4: .

Table 8.1-4: Calculated DT50 values for dimethomorph in wheat plants and statistical indices

Plant	Trial	Zone	Kinetic model	DT_{50} [d]	P (t-test)	χ^2 error
Wheat	L140363	North	SFO	2.49	0.001	31.13
Wheat	L140364	North	SFO	2.60	0.001	33.45
Wheat	L140365	North	SFO	2.33	<0.001	28.66
Wheat	L140366	North	SFO	2.13	<0.001	26.50
Wheat	L140367	South	SFO	1.74	0.002	44.61
Wheat	L140368	South	SFO	1.67	<0.001	13.76
Wheat	L140369	South	SFO	1.88	<0.001	31.31
Wheat	L140370	South	SFO	3.69	<0.001	12.80

CONCLUSION

Dissipation of dimethomorph residues on wheat plants is well described by single first order kinetics. The DT_{50} values obtained from field residue studies are suitable for modelling purposes according to FOCUS kinetics.

On request of the RMS, the notifier provided more information abut the study below (Shbaita, 2015b) in an additional document [see KCA 8.1/5 2017/1099857]. Please note that residue data refer to fresh weight.

Report:	CA 8.1/4 Shbaita H., 2015 b Calculation of DT50 dissipation times of BAS 550 F - Dimethomorph in peas from field trials conducted in the Northern and Southern Zones of Europe 2015/1217511
Guidelines:	none
GLP:	no

The residue decline of BAS 550 F – Dimethomorph in pea plants has been studied in multiple field trials at different sites in Northern and Southern Europe during the growing season 2014.

This modelling report provides kinetic analysis and estimation of the dissipation times (DT_{50} values) for dimethomorph (BAS 550 F).

MATERIAL AND METHODS

Since no specific recommendation is available how to carry out the kinetic evaluation for the described experiment, guidance of the FOCUS workgroup on degradation kinetics was used in order to derive degradation parameters for modelling purposes. This means the selected DT_{50} values are suitable input parameters for models that require single first order (SFO) DT_{50} values or conservative substitutes.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS (2006) guidance. The recommended kinetic models, i.e. the single first order kinetics (SFO) was applied. For visual inspection, the recommended graphical representations of observed and modelled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the $\chi 2$ minimum error level is provided.

The visual assessments of the residual plots show that the residuals are randomly scattered around the zero line and that the fits are acceptable for all datasets except for the trial number L140377 and L140378. For these trials, no kinetic modell was able to provide statistically significant DT_{50} values. For all other trials, the estimated dissipation rate constants are significantly different from zero as indicated by low t-test values. The DT_{50} values that are suitable for modelling purposes according to FOCUS and the respective statistical indices are presented in the following table.

RESULTS

An overview of the calculated DT50 values for dimethomorph in pea plants is given in Table 8.1-5: .

Table 8.1-5: Calculated DT_{50} values for dimethomorph in pea plants and statistical indices

Plant	Trial	Zone	Kinetic model	DT_{50} [d]	P (t-test)	χ^2 error
Pea	L140371	North	SFO	1.98	<0.001	23.04
Pea	L140372	North	SFO	2.39	<0.001	29.6
Pea	L140373	North	SFO	6.14	<0.001	9.42
Pea	L140374	North	SFO	0.96	<0.001	16.03
Pea	L140375	South	SFO	3.00	<0.001	13.45
Pea	L140376	South	SFO	5.00	<0.001	15.73
Pea	L140377	South	--*	--	--	--
Pea	L140378	South	--*	--	--	--

*No kinetic model was able to provide statistically significance

CONCLUSION

Dissipation of dimethomorph residues on pea plants is well described by single first order kinetics. Only for the field trials L140377 and L140378 no kinetic model was found with statistically significant DT_{50} values. All other DT_{50} values are suitable for modelling purposes according to FOCUS kinetics and will be used in the refined exposure calculations.

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

Report: CA 8.1.1.1/1
[redacted] 2014 a
BAS 550 F (Dimethomorph) - Acute toxicity in the canary (Serinus canaria) after single oral administration (LD50)
2014/1190617
Guidelines: EPA 850.2100, EPA 850.2000
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

An avian acute oral toxicity test with the substance dimethomorph was conducted. The objective of this study was to evaluate the acute toxicity of dimethomorph administered to the canary (*Serinus canaria*) as a single oral dose and to determine an LD_{50} and a no-observed-effect level (NOEL) for sublethal effects.

The test substance was administered via a single oral dose of 0 (control) and 2000 mg as/kg b.w. to groups of 30 weeks old canary. Ten birds (5 males and 5 females) were used in each test substance group. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights and feed consumption were recorded regularly.

There were no treatment related mortalities in the treatment group. There was 10% mortality at the control group. However, according to the test guideline 10% mortality is acceptable for the control group. When compared to the control group, there were no apparent treatment related effects on body weight and food consumption at the treatment group.

The acute oral LD_{50} value for canary (*Serinus canaria*) exposed to dimethomorph as a single oral dose was determined to be greater than 2000 mg a.s./kg. The no-observed effect level (NOEL) was greater than 2000 mg as/kg b.w.

I. MATERIAL AND METHODS

Test item:	Dimethomorph (BAS 550 F), Reg. No. 247723, batch no. COD-001646, purity 99.7% (± 1.0%)
Test species:	Canary (*Serinus canaria*) age of birds approximately 30 weeks at dosing; supplier: Zoowelt – [redacted]
Test design:	Birds were administered single doses of 0 (control) and 2000 mg dimethomorph /kg b.w.. The test substance was orally inserted in 2 gelatine capsules into the crop of each bird. The animals of the control group received 2 empty gelatine capsules. 5 males and 5 females per dose group were used. Following dosing, multiple observations were performed on day 0 of the test, with particular attention being paid for signs of regurgitation. From test initiation until termination, all birds were observed at least daily. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights were measured individually on the day prior to dosing to allow time for capsule preparation (day -1) and on days 7 and 14 of the test. Average estimated feed consumption was then measured weakly.
Endpoints:	Mortality, clinical signs of toxicity, food consumption and body weight data were conducted on all birds, gross-pathological examinations were conducted. LD_{50} and NOEL.
Test concentrations:	0 (control) and 2000 mg as/kg b.w.
Test conditions:	Birds were fasted for approximately 2 – 3 hours prior to dosing; temperature: 15 - 27 °C (limits); relative humidity: 45 – 70 % (limits); photoperiod: 10 hours light/14 hours dark, light intensity: approximately 114 lux.
Analytics:	No Analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.
Statistics:	No statistical calculation of the LD_{50} was performed since no mortality was observed in the tested dose. In order to assess effects on body weight and feed consumption student's t-test was performed. The NOEL was calculated using Fisher's exact test.

II. RESULTS AND DISCUSSION

Biological results:
There were no incidences of regurgitation noted among the control birds or among the birds at the 2000 mg a.s./kg dosage level.
There were no treatment related mortalities in the 2000 mg a.s./kg b.w. treatment. There was one dead female in the control group after 12 days. However, according to the test guideline 10 % mortality is acceptable for the control group.
When compared to the control group, there were no apparent treatment related effects on body weight and food consumption in the treatment group.

Diarrhoea on the day of dosing is a consequence of the fasting period and is observed in the dose group as well as in the control group and is not considered to be a toxic effect. In one female animal of the tested dose group slight apathy was observed 1 hour after dosing.

No substance-related findings were observed during the gross post-mortem examination. In two females of the tested dose group mites were detected. Since the female animals were apparently in good health during the last 13 days of the observation period, the finding had no influence on the results.

The acute toxicity data are summarized in Table 8.1.1.1-1: .

Table 8.1.1.1-1: Acute toxicity of dimethomorph (BAS 550 F) to the canary (*Serinus canaria*)

	Dose rate [mg a.s./kg bw]	
	0 (control)	2000
Number of birds per dose group	10	10
Number of dead birds	1	0
Dead birds percentage [%]	10	0
Endpoint	Dose [mg a.s./kg b.w.]	
Highest dose causing no substance-related mortality	2000	
LD_{50} (14 d)	≥ 2000	
NOEL	≥ 2000	

b.w. = body weight

III. CONCLUSION

The acute oral LD50 value for canary exposed to dimethomorph as a single oral dose was determined to be greater than 2000 mg a.s./kg, the dosage level where no mortalities occurred in this study. Since no clinical signs could be observed in the treatment group, the no-observed effect level (NOEL) was greater than 2000 mg a.s./kg.

Report:	CA 8.1.1.1/2 [redacted] 1986 a The acute oral toxicity (LD50) of CME 151 to the bobwhite quail DK-505-001
Guidelines:	EPA 71-1
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

EXECUTIVE SUMMARY

An avian toxicity test with the active substance dimethomorph was conducted. The study was designed to determine the acute oral toxicity (LD_{50}) of dimethomorph to the bobwhite quail. The test item was administered via a single-dose of 500, 1000 or 2000 mg a.s./kg b.w. to approx.. 2-month old northern bobwhite quails. Ten birds (5 males and 5 females) were used in each group. The test substance was dissolved in corn oil and administered to the birds by oral gavage. The control group was dosed only with corn oil.
Feed was removed for at least 15 hours prior to dosing.

All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. All groups received food and water *ad libitum* throughout the test. The test was terminated after 14 days.

All groups were observed for mortality, clinical signs, impact on food consumption, and body weight for 14 consecutive days post dosing. Gross-pathological examinations were conducted on all birds that died during the study and all birds in the highest dose group.

No clinical signs of toxicity were observed following dosing. One bird in the 500 mg/kg dose group was found dead on day 2, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LD_{50} was determined to be > 2000 mg/kg of b.w., the highest dose tested. Since there was no dose-related mortalities, the NOEL for mortality was 2000 mg/kg of b.w. Group mean body weight changes and food consumption were variable and there was no evidence of any treatment-related effect.

Post-mortem examination of the one bird found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest dose group that were examined macroscopically at test termination. The relevant endpoints are summarized in the table below.

The LD_{50} in this study was > 2000 mg/kg of body weight. The NOEL was 2000 mg/kg of body weight.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME151); Batch Number DW 11/86; purity: 96.6%.

B. STUDY DESIGN

Test Species:	Northern Bobwhite (*Colinus virginianus*) Approx. 2 month at dosing, Origin: [redacted]
Test design:	Birds were administered single doses of 500, 1000 and 2000 mg a.s./kg b.w. of the test substance dimethomorph. The test substance was dissolved in corn oil and administered to the birds by oral gavage. The control group was dosed only with corn oil. A total of 10 birds (5 males and 5 females) were used in each treatment and control group. Birds were housed in steel battery cages, with a total of 5 birds per cage. Males and females were housed separately.
	Birds were observed daily for mortality and clinical signs of toxicity for 14 days after dosing. Individual birds were weighed at test initiation and test termination to evaluate changes in body weight. Food consumption was evaluated during the test. All birds in the highest dose groups and any birds that died during the test were examined by gross necropsy.
Endpoints:	Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations (conducted for all birds that died during the study and all birds in the highest dose group). Calculation of LD_{50} and NOEL.
Test concentrations:	0 (corn oil control), 500, 1000, and 2000 mg/kg of b.w.
Test conditions:	Birds fasted for about 15 hours to 19 hours before administration of the test substance; temperature: 19 °C (minimum) and 24 °C (maximum), relative humidity: 85%. Photoperiod: 17 hours light, 7 hours dark.
Analytics:	No analytical determinations of the test substance in the diet were necessary since the test substance was directly in the gavage.
Statistics:	No statistical analysis was conducted.

II. RESULTS AND DISCUSSION

No clinical signs of toxicity were observed following dosing. One bird in the 500 mg/kg dose group was found dead on day 2, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LD_{50} was determined to be > 2000 mg/kg of b.w., the highest dose tested. Since there was no dose-related mortalities, the NOEL for mortality was 2000 mg/kg of b.w. Group mean body weight changes and food consumption were variable and there was no evidence of any treatment-related effect.

Post-mortem examination of the one bird found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest dose group that were examined macroscopically at test termination. The relevant endpoints are summarized in the table below.

Table 8.1.1.1-2: Acute toxicity of dimethomorph to the northern bobwhite (*Colinus virginianus*)

	Dose rate [mg a.s./kg b.w.]			
	0 (control)	500	1000	2000
Number of birds per dose group	10	10	10	10
Number of dead birds	0	1	0	0
Dead birds percentage [%]	0	10	0	0

Endpoints	Dose [mg a.s./kg b.w.]
Highest dose causing no substance-related mortality	2000
LD_{50} (14 d)	> 2000
NOEL[1)]	2000

Body weight = b.w.
1) NOEL is the highest tested concentration without significant substance related effects

III. CONCLUSION

The LD_{50} in this study was > 2000 mg/kg of body weight. The NOEL was 2000 mg/kg of body weight.

Report:	CA 8.1.1.1/3 [redacted], 1986 b The acute oral toxicity (LD50) of CME 151 to the mallard duck DK-505-003
Guidelines:	EPA 71-1
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

EXECUTIVE SUMMARY

An avian toxicity test with the active substance dimethomorph was conducted. The study was designed to determine the acute oral toxicity (LD_{50}) of dimethomorph to the bobwhite quail. The test item was administered via a single-dose of 500, 1000 or 2000 mg a.s./kg b.w. to approx.. 2-month old northern bobwhite quails. Ten birds (5 males and 5 females) were used in each group. The test substance was dissolved in corn oil and administered to the birds by oral gavage. The control group was dosed only with corn oil. Feed was removed for at least 15 hours prior to dosing.

All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. All groups received food and water *ad libitum* throughout the test. The test was terminated after 14 days.

All groups were observed for mortality, clinical signs, impact on food consumption, and body weight for 14 consecutive days post dosing. Gross-pathological examinations were conducted on all birds in the highest dose group.

No clinical signs of toxicity were observed following dosing. No mortalities occurred during the test. Therefore, the LD_{50} was determined to be > 2000 mg/kg of b.w., the highest dose tested. The NOEL for mortality was 2000 mg/kg of b.w.

Group mean body weight changes were considered to be within normal limits. Food consumption results were variable and were influenced by spillage. There was no evidence of any treatment-related effect. The NOEL for effects on body weight and feed consumption was 2000 mg/kg of b.w.

No abnormalities were detected in any of the birds in the highest dose group that were examined macroscopically at test termination.

The LD_{50} in this study was > 2000 mg/kg of body weight. The NOEL was 2000 mg/kg of body weight.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME151); Batch Number DW 11/86; purity: 96.6%.

B. STUDY DESIGN

Test Species:	Mallard duck (*Anas platyrhynchos*); Origin: [redacted] Birds were over 16 weeks old at dosing.
Test design:	Birds were administered single doses of 500, 1000 and 2000 mg a.s./kg b.w. of the test substance dimethomorph. The test substance was dissolved in corn oil and administered to the birds by oral gavage. The control group was dosed only with corn oil. A total of 10 birds (5 males and 5 females) were used in each treatment and control group. Birds were housed in steel battery cages, with a total of 5 birds per cage. Males and females were housed separately. Birds were observed daily for mortality and clinical signs of toxicity for 14 days after dosing. Individual birds were weighed at test initiation and test termination to evaluate changes in body weight. Food consumption was evaluated during the test. All birds in the highest dose groups and any birds that died during the test were examined by gross necropsy.
Endpoints:	Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations (all birds in the highest dose groups and any birds that died during the test were examined by gross necropsy). Calculation of LD_{50} and NOEL.
Test concentrations:	0 (corn oil control), 500, 1000, and 2000 mg/kg of b.w.
Test conditions:	Birds fasted for about 15 hours to 19 hours before administration of the test substance; temperature: 20 °C (minimum) and 22 °C (maximum), relative humidity: 73%. Photoperiod: 17 hours light, 7 hours dark.
Analytics:	No analytical determinations of the test substance in the diet were necessary since the test substance was directly in the gavage.
Statistics:	No statistical analysis was conducted.

II. RESULTS AND DISCUSSION

Biological results:
No clinical signs of toxicity were observed following dosing. No mortalities occurred during the test. Therefore, the LD_{50} was determined to be > 2000 mg/kg of b.w., the highest dose tested. The NOEL for mortality was 2000 mg/kg of b.w.

Group mean body weight changes were considered to be within normal limits. Food consumption results were variable and were influenced by spillage. There was no evidence of any treatment-related effect. The NOEL for effects on body weight and feed consumption was 2000 mg/kg of b.w.

No abnormalities were detected in any of the birds in the highest dose group that were examined macroscopically at test termination. Therefore, the NOEL for macroscopic abnormalities was 2000 mg/kg of b.w. The relevant endpoints are summarized in the table below. The relevant endpoints are summarized in the table below.

Table 8.1.1.1-3: Acute toxicity of dimethomorph to the northern bobwhite (*Colinus virginianus*)

Endpoints	Dose [mg a.s./kg b.w.]
Highest dose causing no substance-related mortality	2000
LD_{50} (14 d)	> 2000
NOEL[1)]	2000

Body weight = b.w.
1) NOEL is the highest tested concentration without significant substance related effects

III. CONCLUSION

The LD_{50} in this study was > 2000 mg/kg of body weight. The NOEL was 2000 mg/kg of body weight.

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available.

Report:	CA 8.1.1.2/1 [redacted] 1987 a The dietary toxicity (LC50) of CME 151 to the bobwhite quail DK-505-002
Guidelines:	EPA 71-2
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

EXECUTIVE SUMMARY

An avian dietary toxicity test was conducted. The study was designed to determine the dietary LC_{50} of dimethomorph to the bobwhite quail that was administered to juvenile bobwhite quail for 5 days.

The test item was administered at concentrations of 162.5, 325, 650, 1300, 2600 and 5200 mg a.s./kg diet. Ten 11-days old Bobwhite quails were used in each treatment group. All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight during the substance feeding period of 5 days and for a post exposure. All groups received feed and water *ad libitum* throughout the test.

No clinical signs of toxicity were observed during the test. One bird in the 650 mg a.s./kg treatment group was found dead on day 5, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LC_{50} was determined to be > 5200 mg a.s./kg diet, the highest dietary concentration tested. There was no evidence of any treatment-related effect on body weight. Food consumption was variable and there was no evidence of any treatment-related effect. Post-mortem examination of the one bird found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest treatment group that were examined macroscopically at test termination.

In a dietary toxicity test with bobwhite quail (*Colinus virginianus*), the LC_{50} of dimethomorph was found to be > 5200 mg a.s./kg diet. The NOEC was 5200 mg a.s./kg b.w./day.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME151); Batch Number DW 11/86; purity: 96.6%.

B. STUDY DESIGN

Test species:	Northern Bobwhite (*Colinus virginianus*); Origin: [redacted] The birds were 11 days old when placed on treatment.
Test design:	The test substance was administered via the diet for 5 days at concentrations of 162.5, 325, 650, 1300, 2600 and 5200 mg a.s./kg diet to 11-days old northern bobwhite quails. The test substance was mixed into bird diet without the use of a carrier vehicle. The treated diets were fed to the birds *ad libitum*. Groups of 10 juvenile birds were fed diet containing different concentrations of technical grade dimethomorph for 5 days, followed by a 3-day post-exposure observation period. Birds were housed in wooden pens, with a total of 10 birds per pen. Birds were observed daily for mortality and clinical signs of toxicity. Individual birds were weighed at test initiation and test termination to evaluate changes in body weight. Food consumption was evaluated during the test. All birds in the highest dose groups and any birds that died during the test were examined by gross necropsy.
Endpoints:	Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations (conducted for all birds that died during the study and all birds in the highest dose group). Determination of LC_{50} and NOEC.
Test concentrations:	0 (untreated control), 162.5, 325, 650, 1300, 2600, and 5200 mg/kg diet.
Test conditions:	Chicks were administered treated feed for 5 consecutive days followed by a post-exposure period of six days basal diet ad libitum without test substance; temperature: 20 °C – 25 °C; relative humidity: 73%.
Analytics:	Concentrations of the test substance in the test diets were confirmed by HPLC analysis.
Statistics:	No statistical analysis was conducted.

II. RESULTS AND DISCUSSION

Analytical measurements:
The mean measured concentrations of dimethomorph in the treated diets ranged from 91.7 to 105.4 % of the targeted nominal concentrations. Results of the homogeneity and stability evaluation indicated that the test substance was homogeneously mixed into the test feed, and that the test substance was stable in the test feed for at least 7 days.

Biological results:
No clinical signs of toxicity were observed during the test. One bird in the 650 mg a.s./kg treatment group was found dead on day 5, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LC_{50} was determined to be > 5200 mg a.s./kg diet, the highest dietary concentration tested. There was no evidence of any treatment-related effect on body weight. Food consumption was variable and there was no evidence of any treatment-related effect. Post-mortem examination of the one bird found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest treatment group that were examined macroscopically at test termination. The relevant results are summarized in the table below

Table 8.1.1.2-1: Avian dietary toxicity of dimethomorph to the northern bobwhite (*Colinus virginianus*)

	Dose rate [mg a.s./kg b.w.]						
	0 (control)	162.5	325	650	1300	2600	5200
Number of birds per dose group	10	10	10	10	10	10	10
Number of dead birds	0	0	0	1	0	0	0
Dead birds percentage [%]	0	0	0	10	0	0	0

	Endpoints [mg a.s./kg diet]
LC_{50}	> 5200
NOEC	5200

a.s. = active substance
b.w. = body weight

III. CONCLUSION

In a dietary toxicity test with bobwhite quail (*Colinus virginianus*), the LC_{50} of dimethomorph was found to be > 5200 mg a.s./kg diet. The NOEC was 5200 mg a.s./kg b.w./day.

Report:	CA 8.1.1.2/2 [redacted] 1987 b The dietary toxicity (LC50) of CME 151 to the mallard duck DK-505-004
Guidelines:	EPA 71-2
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

EXECUTIVE SUMMARY

An avian dietary toxicity test was conducted. The study was designed to determine the dietary LC_{50} of dimethomorph to the mallard duck that was administered to juvenile bobwhite quail for 5 days.

The test item was administered at concentrations of 162.5, 325, 650, 1300, 2600 and 5200 mg a.s./kg diet. Ten 9-days old mallard ducks were used in each treatment group. All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight during the substance feeding period of 5 days and for a post exposure. All groups received feed and water *ad libitum* throughout the test.

No clinical signs of toxicity were observed during the test. One bird in the 162.5 mg a.s./kg treatment group was found dead on day 4, without any previous symptoms of toxicity, and one bird in the 325 mg a.s./kg treatment group was found dead on day 7, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LC_{50} was determined to be > 5200 mg a.s./kg diet, the highest dietary concentration tested.
There was evidence of a treatment-related effect on body weight gain during the treatment period at 650, 1300, 2600, and 5200 mg a.s./kg diet. These groups showed reduced body weight gains over this period in comparison to the controls. Body weight gains during the post-treatment period were variable and there was less clear evidence of any treatment-related effect. Therefore, the NOEC for effects on body weight was 325 mg a.s./kg b.w/day.
There was evidence of a treatment-related depression of food consumption at 2600 mg a.s./kg diet and 5200 mg a.s./kg diet during the treatment phase. There was no evidence of any treatment-related effect on food consumption during the post-treatment observation period. Therefore, the NOEC for effects on feed consumption was 1300 mg a.s./kg b.w./day.
Post-mortem examination of the two birds found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest treatment group that were examined macroscopically at test termination.

In a dietary toxicity test with mallard duck (*Anas platyrhynchos*), the LC_{50} of dimethomorph was found to be > 5200 mg a.s./kg diet. The NOEC was 325 mg a.s./kg b.w./day.

II. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical grade dimethomorph (also known as CME151); Batch Number DW 11/86; purity: 96.6%.

B. STUDY DESIGN

Test species: Mallard duck (*Anas platyrhynchos*); Origin: [redacted] The birds were 9 days old when placed on treatment.

Test design: The test substance was administered via the diet for 5 days at concentrations of 162.5, 325, 650, 1300, 2600 and 5200 mg a.s./kg diet to 9-days old mallard ducks. The treated diets were fed to the birds *ad libitum*. The test substance was mixed into bird diet without the use of a carrier vehicle.

Groups of 10 juvenile birds were fed diet containing different concentrations of technical grade dimethomorph for 5 days, followed by a 3-day post-exposure observation period. Birds were housed in wooden pens, with a total of 10 birds per pen. Birds were observed daily for mortality and clinical signs of toxicity. Individual birds were weighed at test initiation and test termination to evaluate changes in body weight. Food consumption was evaluated during the test. All birds in the highest dose groups and any birds that died during the test were examined by gross necropsy.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations (conducted for all birds that died during the study and all birds in the highest dose group). Calculation of LC_{50} was not possible, but calculations of NOEC.

Test concentrations: 0 (untreated control), 162.5, 325, 650, 1300, 2600, and 5200 mg/kg diet.

Test conditions: Chicks were administered treated feed for 5 consecutive days followed by a post-exposure period of six days basal diet ad libitum without test substance; temperature: 28 °C – 30 °C; relative humidity: 67%.

Analytics: Concentrations of the test substance in the test diets were confirmed by HPLC analysis.

Statistics: No statistical analysis was conducted.

II. RESULTS AND DISCUSSION

Analytical measurements:
The mean measured concentrations of dimethomorph in the treated diets ranged from 91.6 to 105% of the targeted nominal concentrations. Results of the homogeneity and stability evaluation indicated that the test substance was homogeneously mixed into the test feed, and that the test substance was stable in the test feed for at least 7 days.

Biological results:
No clinical signs of toxicity were observed during the test. One bird in the 162.5 mg a.s./kg treatment group was found dead on day 4, without any previous symptoms of toxicity, and one bird in the 325 mg a.s./kg treatment group was found dead on day 7, without any previous symptoms of toxicity. No other mortalities occurred during the test. Therefore, the LC_{50} was determined to be > 5200 mg a.s./kg diet, the highest dietary concentration tested.
There was evidence of a treatment-related effect on body weight gain during the treatment period at 650, 1300, 2600, and 5200 mg a.s./kg diet. These groups showed reduced body weight gains over this period in comparison to the controls. Body weight gains during the post-treatment period were variable and there was less clear evidence of any treatment-related effect. Therefore, the NOEC for effects on body weight was 325 mg a.s./kg b.w/day.
There was evidence of a treatment-related depression of food consumption at 2600 mg a.s./kg diet and 5200 mg a.s./kg diet during the treatment phase. There was no evidence of any treatment-related effect on food consumption during the post-treatment observation period. Therefore, the NOEC for effects on feed consumption was 1300 mg a.s./kg b.w./day.
Post-mortem examination of the two birds found dead during the study indicated no abnormalities. In addition, no abnormalities were detected in any of the birds in the highest treatment group that were examined macroscopically at test termination. The relevant results are summarized in the table below

Table 8.1.1.2-2: Avian dietary toxicity of dimethomorph to the mallard duck (*Anas platyrhynchos*)

Parameter	Group [mg a.s./kg diet]						
	Control	162.5	325	650	1300	2600	5200
Number of birds per dose group	0.0	10	10	10	0.0	0.0	0.0
Number of dead birds	0	1	1	0	0	0	
Dead birds percentage [%]	0	10	10	0	0	0	0
	Endpoints [mg a.s./kg diet]						
LC_{50}	> 5200						
NOEC (b.w.)	325						

a.s. = active substance
b.w. = body weight

III. CONCLUSION

In a dietary toxicity test with mallard duck (*Anas platyrhynchos*), the LC_{50} of dimethomorph was found to be > 5200 mg a.s./kg diet. The NOEC was 325 mg a.s./kg b.w./day.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available.

Report:	CA 8.1.1.3/1 [redacted] 1997 a Reproduction study with Dimethomorph technical (CL 336379) in the Northern bobwhite (Colinus virginianus) DK-505-009
Guidelines:	EPA 71-4(a), OECD 206
GLP:	yes (certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

This 1-generation study was designed to determine possible effects of administration of dimethomorph technical via food on the reproduction in the bobwhite quail (*Colinus virginianus*). Effects on health, body weight development and feed consumption of adult birds as well as effects on different reproductive parameters were determined. The birds were offered the test item treated food over 23 weeks at concentrations of 0, 50, 200 and 800 mg a.s./kg diet. There were 17 replicates of two birds (1 male and 1 female) in each treatment group. Animals of all test groups received feed and water *ad libitum* throughout the test. The laid eggs were collected, incubated and the offspring was raised for 2 weeks.

Analysis of the control samples did not show any indication of the presence of the test substance.

There were no treatment-related adult mortalities at any of the concentrations tested and no overt signs of toxicity were observed during the course of the study. Incidental mortalities occurred in the controls and in all treatment groups. Due to the nature of the injuries (e.g., injuries due to pair aggression), these mortalities were considered not to be treatment-related. In addition, there were no apparent treatment-related effects upon reproductive performance (egg production), the eggshell thickness, or chick survival and body weight at any test concentration.

The NOEC for dimethomorph in the avian reproduction study with northern bobwhite was 800 mg a.s./kg diet, the highest dietary concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CL 336379); Lot Number AC-9978-131; purity: 98%.

B. STUDY DESIGN

Test species:	Northern bobwhite quails (*Colinus virginianus*), phenotypically indistinguishable from wild type; adults, age: 20 weeks of age at experimental start (before beginning of first egg-laying period); supplier: [redacted]
Test design:	Northern bobwhite quails approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 17 pens were allocated to the control and each treatment group. All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The study period was divided into phases: 1. Acclimation to laboratory conditions; 2. Start of treated feed; 3. Pre-egg laying (with photostimulation); 4. Adult termination (final incubation, hatching and 14-day offspring rearing period). The adult birds were maintained on a 7-hour light: 17-hour dark photoperiod during acclimation and during the first 8 weeks on treated feed. At the end of week 8, the light portion of the photoperiod was increased gradually over a 6-day period to 17 hours light: 7 hours dark. The photoperiod remained at this interval until termination of the adult portion of the study. The total time on the treated diet was 23 weeks and 3 days. Eggs were collected daily from the onset of egg production and set weekly for incubation. In addition, every other week throughout the egg laying period, eggs laid on one day were collected from every pen (when available) for egg shell thickness measurements. During incubation, the eggs were candled to check for embryo development. All hatchlings were observed after hatching for 14 days to assess hatchling survival.
Endpoints:	Adult birds: mortalities, abnormal behavior and signs of toxicity, gross necropsy, adult body weight and adult feed consumption.
	Reproductive parameters: Eggs laid/hen/day, eggs cracked of eggs laid, fertile eggs of eggs set, viable embryos of eggs set, hatchlings of 3 week-Embryos, body weight hatchlings, 14-day old survivors of hatchlings, hatchlings of eggs set, hatchlings of fertile eggs, hatchlings/pen/day, 14-day old survivors/pen/day, 14-day old survivors of eggs set, hatchling body weight and egg shell thickness.

Test concentrations:	0 (control), 50, 200, and 800 mg/kg of diet.
Test conditions:	Adult bobwhite study room: Temperature ranged from 16.7 to 26.7 °C; relative humidity: 33 to 82%; photoperiod: 7 hours light (week 1 – 8), lengthened photoperiod to 17 hours light (week 9 to the end of study), average of 10.4 foot candles. Egg collection and storage: Collected daily, stored in cold room: average temperature: 8.7 °C. Eggs set for incubation: temperature ranged from 36.8 to 37.7 °C, relative humidity 52 to 68%; the eggs were transferred to the hatcher on day 21: temperature: 37.3 °C, relative humidity 63 to 67 %. Hatchlings: Brooding compartment at average temperature of 35°C from hatching until the birds were 14 days of age; average ambient room temperature of 37.3 °C; photoperiod: 17 hours light per day.
Analytics:	Concentrations of dimethomorph in the test diets were analytically verified during the test using a validated HPLC method.
Statistics:	Data sets were tested for normality using Chi-square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data were Arcsine transformed. The various parameters were analyzed by ANOVA and Dunnett's test or ANOVA and Tukey's post-hoc test. If the data were not normal or were heterogeneous, they were analyzed with a Kruskal-Wallis ANOVA followed by Dunn's multiple pair wise comparison test.

II. RESULTS AND DISCUSSION

Analytical results: Samples of the test diets presented to the adult birds during the test averaged 49.9, 195, and 856 mg a.s./kg diet for the three treatment groups. No dimethomorph was detected in the control diet.

Biological results: There were no treatment-related adult mortalities at any of the concentrations tested and no overt signs of toxicity were observed during the course of the study. Incidental mortalities occurred in the controls and in all treatment groups. Due to the nature of the injuries (e.g., injuries due to pair aggression), these mortalities were considered not to be treatment-related. In addition, there were no apparent treatment-related effects upon reproductive performance (egg production), the eggshell thickness, or chick survival and body weight at any test concentration.

The effect of the various treatments on the reproductive parameters evaluated during the test are summarized in the tables below.

Table 8.1.1.3-1: Effects of dimethomorph on the parental generation of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
No. of replicates (1 male and 1 female per replicate/pen)	17	17	17	17
No. of substance-related mortalities of adult birds	3	2	1	2
Adult b.w. [g] at the end of study (male/female)	262/307	249/308	255/304	260/292
Gain of adult b.w. [g] at the end of study (male/female)[1)]	46/94	27/86	37/86	41/74

Table 8.1.1.3-2: Effects of dimethomorph on the reproduction of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
Number of surviving replicates	14	15	16	15
Total eggs laid	832	890	931	811
Eggs laid/hen	59.4	59.3	58.2	54.1
Eggs laid/hen/day	0.84	0.84	0.82	0.76
Eggs cracked	20	20	21	19
Mean egg shell thickness (mm)	0.197 (± 0.021)	0.196 (± 0.033)	0.186 (± 0.032)	0.186 (± 0.030)
Eggs set	755	810	840	736
Viable Embryos	691	706	766	669
Mean b.w. (g) of hatchlings per group	7.2 ± 0.7	7.1 ± 0.58	6.9 ± 0.65	6.9 ± 0.68
Mean bodyweight (g) of 14-day old survivors	28.6 ± 6.07	28.8 ± 6.81	27.9 ± 6.74	31.1 ± 6.71
Hatchlings	660	674	744	645
14-day old survivors	486	505	514	460
14-day old survivors/hen	34.7	33.7	32.1	30.7

Table 8.1.1.3-3: Effects of dimethomorph on the reproduction of the northern bobwhite quail (*Colinus virginianus*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
% viable embryos/eggs set	92	87	91	80
% hatchlings/eggs set	87	83	89	88
% 14-day old survivors/eggs set	64	62	61	63
% 14-day survivors of hatchlings	74	75	69	71
% cracked eggs of eggs laid	2	2	2	2

None of the reproductive parameters in any of the three treatment groups were statistically different from the controls.

III. CONCLUSION

The NOEC for dimethomorph in the avian reproduction study with northern bobwhite was 800 mg a.s./kg diet, the highest dietary concentration tested.

Report:	CA 8.1.1.3/2 [redacted]., 1997 b Reproduction study with Dimethomorph technical (CL 336379) in the mallard duck (Anas platyrhynchos) DK-505-007
Guidelines:	EPA 71-4(b), OECD 206
GLP:	yes (certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

This 1-generation study was designed to determine possible effects of administration of dimethomorph technical via food on the reproduction in the bobwhite quail (*Colinus virginianus*). Effects on health, body weight development and feed consumption of adult birds as well as effects on different reproductive parameters were determined. The birds were offered the test item treated food over 23 weeks at concentrations of 0, 50, 200 and 800 mg a.s./kg diet. There were 16 replicates of two birds (1 male and 1 female) in each treatment group. Animals of all test groups received feed and water *ad libitum* throughout the test. The laid eggs were collected, incubated and the offspring was raised for 2 weeks.

Analysis of the control samples did not show any indication of the presence of the test substance.

There were no adult mortalities in the controls or any treatment group. In addition, there were no apparent treatment-related effects upon reproductive performance (egg production), the eggshell thickness, or chick survival and body weight at any test concentration.

The NOEC for dimethomorph in the avian reproduction study with mallard duck was 800 mg a.s./kg diet, the highest dietary concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CL 336379); Lot Number AC-9978-131; purity: 98%.

B. STUDY DESIGN

Test species:	Mallard duck (*Anas platyrhynchos*), phenotypically indistinguishable from wild type; adults, age: 18 weeks, one day at experimental start (before beginning of first egg-laying period); supplier: [redacted]
Test design:	Mallard ducks approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 16 pens were allocated to the control and each treatment group. All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. . The study period was divided into phases: 1. Acclimation to laboratory conditions; 2. Start of treated feed; 3. Pre-egg laying (with photostimulation); 4. Adult termination (final incubation, hatching and 14-day offspring rearing period). The adult birds were maintained on a 7-hour light: 17-hour dark photoperiod during acclimation and during the first 8 weeks on treated feed. At the end of week 8, the light portion of the photoperiod was increased gradually over a 6-day period to 17 hours light: 7 hours dark. The photoperiod remained at this interval until termination of the adult portion of the study. The total time on the treated diet was 23 weeks and 3 days. Eggs were collected daily from the onset of egg production and set weekly for incubation. In addition, every other week throughout the egg laying period, eggs laid on one day were collected from every pen (when available) for egg shell thickness measurements. During incubation, the eggs were candled to check for embryo development. All hatchlings were observed after hatching for 14 days to assess hatchling survival.
Endpoints:	Adult birds: mortalities, abnormal behavior and signs of toxicity, gross necropsy, adult body weight and adult feed consumption.
	Reproductive parameters: Eggs laid/hen/day, eggs cracked of eggs laid, fertile eggs of eggs set, viable embryos of eggs set, hatchlings of 3 week-Embryos, body weight hatchlings, 14-day old survivors of hatchlings, hatchlings of eggs set, hatchlings of fertile eggs, hatchlings/pen/day, 14-day old survivors/pen/day, 14-day old survivors of eggs set, hatchling body weight and egg shell thickness.

Test concentrations: 0 (control), 50, 200, and 800 mg/kg of diet.

Test conditions: Adult bobwhite study room: Temperature ranged from 16.7 to 26.7 °C; relative humidity: 40 to 98%; photoperiod: 7 hours light (week 1 – 8), lengthened photoperiod to 17 hours light (week 9 to the end of study), average of 14.3 foot candles.

Egg collection and storage: Collected daily, stored in cold room: average temperature: 12.2 °C. Eggs set for incubation: temperature: 37.3 to 37.7 °C, relative humidity 51 to 65%; the eggs were transferred to the hatcher on day 21: temperature range from 36.6 to 37.5 °C, relative humidity 63 to 67 %.

Hatchlings: Brooding compartment from hatching until the birds were 14 days of age; average ambient room temperature of 31 °C; photoperiod: 17 hours light per day.

Analytics: Concentrations of dimethomorph in the test diets were analytically verified during the test using a validated HPLC method.

Statistics: Data sets were tested for normality using Chi-square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data were Arcsine transformed. The various parameters were analyzed by ANOVA and Dunnett's test or ANOVA and Tukey's post-hoc test. If the data were not normal or were heterogeneous, they were analyzed with a Kruskal-Wallis ANOVA followed by Dunn's multiple pair wise comparison test.

II. RESULTS AND DISCUSSION

Analytical results: Samples of the test diets presented to the adult birds during the test averaged 56.5, 234, and 985 mg a.s./kg diet for the three treatment groups. No dimethomorph was detected in the control diet.

Biological results: There were no adult mortalities in the controls or any treatment group. In addition, there were no apparent treatment-related effects upon reproductive performance (egg production), the eggshell thickness, or chick survival and body weight at any test concentration.

The effect of the various treatments on the reproductive parameters evaluated during the test are summarized in the tables below.

Table 8.1.1.3-4: Effects of dimethomorph on the parental generation of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
No. of replicates (1 male and 1 female per replicate/pen)	16	16	16	16
No. of substance-related mortalities of adult birds	0	0	0	0
Adult b.w. [g] at the end of study (male/female)	1175/1086	1169/1102	1184/1124	1200/1114
Gain of adult b.w. [g] at the end of study (male/female)[1)]	74/158	81/173	121/205	113/198

Table 8.1.1.3-5: Effects of dimethomorph on the reproduction of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
Number of surviving replicates	16	16	16	16
Total eggs laid	716	660	691	822
Eggs laid/hen	44.8	41.3	46.1	51.4
Eggs laid/hen/day	0.63	0.58	0.65	0.72
Eggs cracked	50	40	37	58
Mean egg shell thickness (mm)	0.357 (± 0.023)	0.359 (± 0.026)	0.351 (± 0.019)	0.343 (± 0.024)
Eggs set	608	564	596	697
Viable Embryos	438	377	416	498
Mean b.w. (g) of hatchlings per group	36.4 ± 3.0	36.4 ± 3.3	36.9 ± 3.5	35.7 ± 3.0
Mean bodyweight (g) of 14-day old survivors	209.7 ± 51.0	210.1 ± 57.4	214.3 ± 53.7	200.2 ± 56.4
Hatchlings	287	229	310	335
14-day old survivors	263	197	289	308
14-day old survivors/hen	16.4	12.3	19.3	19.3

Table 8.1.1.3-6: Effects of dimethomorph on the reproduction of the northern bobwhite quail (*Colinus virginianus*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	50	200	800
% viable embryos/eggs set	72	67	70	71
% hatchlings/eggs set	47	41	52	48
% 14-day old survivors/eggs set	43 (37)	35 (30)	48 (42)	44 (37)
% 14-day survivors of hatchlings	92	86	93	92
% cracked eggs of eggs laid	7	6	5	7

None of the reproductive parameters in any of the three treatment groups were statistically different from the controls.

III. CONCLUSION

The NOEC for dimethomorph in the avian reproduction study with mallard duck was 800 mg a.s./kg diet, the highest dietary concentration tested.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

Report: CA 8.1.2.1/1
[redacted] 2010 a
BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats
2010/1091144
Guidelines: OECD 423, EPA 870.1100, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

A summary is provided in CA 5.2.1

Report: CA 8.1.2.1/2
[redacted] 2010 b
Amendment No. 1 to the report - BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats
2010/1091145
Guidelines: OECD 423, EPA 870.1100, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

A summary is provided in CA 5.2.1

Report: CA 8.1.2.1/3
Class T., 2010 a
Concentration and homogeneity analysis of BAS 550 F in 0.5% CMC in water
2010/1140958
Guidelines: none
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

A summary is provided in CA 5.2.1

Report: CA 8.1.2.1/4
[redacted] 2010 a
BAS 550 F (Dimethomorph) - Acute oral toxicity study in rats
2010/1210734
Guidelines: OECD 423 (2001), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.1100, JMAFF No 12 Nosan No 8147
GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

A summary is provided in CA 5.2.1

Report: CA 8.1.2.1/5
Keller C.,Kamp H., 2011 a
BAS 550 F (Dimethomorph) - Homogeneity and concentration control analysis in 0.5% Carboxymethylcellulose in deionized water
2011/1071936
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A summary is provided in CA 5.2.1

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Report: CA 8.1.2.2/1
Anonymous, 2015 a
Dimethomorph: Long-term endpoint for the mammalian risk assessment
2014/1189840
Guidelines: none
GLP: no

Executive Summary

The current long-term endpoint of 20 mg/kg bw/d for dimethomorph was agreed in the 2006 EFSA conclusion. This value is equivalent to the NOAEL of 300 ppm from the two-generation reproductive toxicity study, and was based on a slight (but statistically significant) reduction in mean bodyweight in parental females at the highest dietary concentration of 1000 ppm (67 mg/kg bw/d) in this study. Derivation of the long-term endpoint is re-assessed, based on the existing reproductive and developmental toxicity dataset, a new extended one generation reproductive toxicity study (EOGRTS) and other supporting data.

Both the older two-generation study (performed at dietary concentrations of up to 1000 ppm) and the new EOGRTS (up to 1600 ppm) show a clear absence of effects of dimethomorph on fertility or reproductive capacity. In the absence of reproductive effects, other potentially population-relevant endpoints from the reproductive toxicity dataset are considered.

The marginal (<10%) effect on parental female mean bodyweight seen at 1000 ppm in the 2-generation study is without reproductive consequence and is not considered to be population-relevant. Bodyweight effects of smaller magnitude were seen in the EOGRTS at 1600 ppm. Bodyweight effects are also commonly reported in repeated dose dietary toxicity studies with dimethomorph (at dietary concentrations of 1000-5000 ppm), are frequently transient and are often associated with reduced food consumption. The clear absence of bodyweight effects in dimethomorph studies using repeated gavage administration at dose levels considerably higher (up to 750 mg/kg bw/d) than those causing bodyweight effects in studies using dietary administration, strongly indicates an adverse effect of dimethomorph on dietary palatability. The slight bodyweight effects seen in dietary studies with dimethomorph are therefore considered unlikely to be of toxicological significance, and are not of relevance to derivation of the long-term endpoint. An overall NOAEL for population-relevant parental bodyweight effects of 1600 ppm is therefore determined for dimethomorph.

A potentially population-relevant reduction (-13%) in mean pup weight at birth was seen in the EOGRTS at the highest dietary concentration of 1600 ppm. Findings of reduced pup weight in this study are consistent with developmental toxicity studies in the rat, in which reduced foetal weight is reported at a dose level of 300 mg/kg bw/d but not at 150 or 160 mg/kg bw/d. No effect on pup weight was seen in the EOGRTS at 800 ppm or at 1000 ppm in the 2-generation study. An overall NOAEL for effects on pup weight at birth of 1000 ppm can therefore be determined.

Developmental toxicity studies with dimethomorph in the rat also identify the population-relevant finding of increased resorption at dose levels of ≥150 mg/kg bw/d. Similar effects are not seen in the two-generation or EOGRTS studies, indicating that findings in the developmental studies are a consequence of gavage dosing and are not of direct relevance to the situation in the field. These effects are therefore discounted for the purposes of deriving the long-term endpoint.

Effects in offspring (reduced anogenital index, effects on organ/tissue weights of the male reproductive tract, delayed sexual maturation) potentially linked to an anti-androgenic activity of dimethomorph are seen in the EOGRTS, but are not of great magnitude. Effects were seen at dietary concentrations of 800 and 1600 ppm but, based on the clear absence of reproductive toxicity in the 2-generation study at 1000 ppm, are considered unlikely to impact on fertility or reproductive capacity. A NOAEL of 1000 ppm can therefore be determined for these anti-androgenic effects.
An overview of parameters measured in the 2-generation study and EOGRTS relevant to the derivation of the long-term endpoint for dimethomorph are shown in the table below.

Comparison of reproductive toxicity data

Dose level (ppm)	**2-generation study**	**100**		**300**		**1000**	
	EOGRTS		**300**		**800**		**1600**
Male bodyweight (pre-mate)		-	-	-	-	-	↓3%
Female bodyweight (pre-mate)		-		-		↓9%*	↓4%
Male food consumption (pre-mate)		-		-	-	-	-
Female food consumption (pre-mate)		-		-		↓*	-
Female bodyweight (gestation)		-	-	-	-	-	↓6%*
Female food consumption (gestation)		-	-	-	-	-	-
Female bodyweight (lactation)		-	-	-	-	-	↓4%*
Female food consumption (lactation)		-	-	-	-	-	↓6%*
Litter size		-	-	-	-	-	-
Pup survival		-	-	-	-	-	-
Pup weight (birth)		-	-	-	-	-	↓13%*
Pup weight (lactation)		-		-		-	↓9%*

**significantly different to controls*

Based on consideration of the relevant dataset for dimethomorph, an alternative population-relevant endpoint of 67 mg/kg bw/d (1000 ppm from the 2-generation study) is proposed. This value is the overall NOAEL for population-relevant effects from the reproductive toxicity studies (as shown in the table above), and is based on a LOAEL for reduced pup weight at birth at 1600 ppm from the EOGRTS.

Report: CA 8.1.2.2/2
[redacted] 2015 a
BAS 550 F - Oral teratogenicity study in rat - Endpoint post implantation loss
2015/1253632
Guidelines: none
GLP: no

Executive Summary

Benchmark-dose modeling is conducted using EPA's BMDS software [1].

A dose with an extra risk of 5% and 10% (BMD) and its 95% lower confidence limit (BMDL) was calculated.

Data

Data on the number of implantations and the number of fetuses lost were obtained from the raw data presented on pages 69-72 of the rat developmental study report (BASF DocID DK-432-002) to calculate % post-implantation loss.

Model

The Nested Logistic model [1] was used,

Prob.(response) = alpha + [1 - alpha] / [1+exp(-beta - rho*ln(Dose))] , if dose >0
Prob.(response) = alpha , if dose =0

with the parameters
rho – Power Parameter restrict to rho >= 1.
alpha – value at dose 0
beta
and
phi1-phi4 - the intra-female correlation coefficient varying among doses,
must be >0 or < 1

These parameters were estimated via the Maximum-Likelihood method using the beta-binomial distribution.

A dose with an extra risk of 5% and its 95% lower confidence limit were calculated.

An extra risk is defined as [p(d) – p(0)] / [1 - p(0)]

Results and Conclusion

The model was conducted in accordance with guidance by EFSA[1] and the US EPA[2], who recommend benchmark dose modelling as a scientifically valid methodology for making quantitative assessments of dose response relationships. The model output indicates that an increase in effect size by 5% (i.e. BMDL05, see EFSA[1] for more details) equals a mean post-implantation loss of 9.5% at a dose level of 75 mg/kg bw/d

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

No new study available.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to regulations (EU) 283/2013 and 284/2013, the risk to amphibians should be addressed. The aquatic guidance document states: "Even if the revised data requirements (Commission Regulation (EU) 283/2013) do not request toxicity tests for amphibian species, amphibians should be included in the aquatic and terrestrial RA of PPPs. Assessment of the risk to amphibians should be based on any existing relevant information. Available relevant data, including data from the open literature, for the substance under consideration should be presented and taken into account in the RA…". In the case of dimethomorph, respectively BAS 550 01 F and 550 02 F, there are no studies in the literature on the toxicity of this substance on amphibians.

With regard to the aquatic risk assessment, several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Aldrich, 2009; Fryday and Thompson, 2012). The most comprehensive one was published recently (Weltje et al., 2013). A common conclusion of these data evaluations is that other aquatic endpoints (generally available for pesticides) cover the potential toxicity to amphibians in water.

The available studies do not indicate any particular concern to amphibians and there is no information from the literature on adverse effects of dimethomorph or BAS 550 01 F or BAS 550 02 F. Accordingly, we consider that the risk assessment performed for fish as the most sensitive group of aquatic organisms covers the risk to amphibians.

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilothermic (*i.e.* do not maintain a constant body temperature) and as a result feeding activity will peak on warm days and will be zero during hibernation or on cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; its function is in general protection and as a barrier, and it is not an organ used for respiration or water/mineral exchange with the environment. Accordingly, reptiles are considered less vulnerable to dermal exposure compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed. For dimethomorph there are no studies in the literature addressing potential toxicity or adverse effects for reptiles.

CA 8.1.5 Endocrine disrupting properties

Mammals

For mammals, the endocrine disruption potential of dimethomorph is discussed in detail in chapter CA 5.8.3.

Birds

Currently there are no formal criteria available in the EU of what constitutes an endocrine disruptor under Regulation 1107/2009. For birds, there is also no internationally validated regulatory testing guideline available. Effects on avian reproduction are covered by the avian reproduction study, which is part of the standard data package for an active ingredient.
For dimethomorph, two avian reproduction studies are available, one in bobwhite quail and one in mallard duck (see CA 8.1.1). As guideline regulatory studies, the focus of these studies is on the general and reproductive toxicity of dimethomorph to birds. Therefore, and similar for the overwhelming majority of active substances, no specific assessments were included with regard to endocrine effects, since standard guideline studies are not designed to specifically investigate those parameters. Still, the bird reproduction study does cover reproductive endpoints that are under endocrine control. With their long-term exposure (≥ 21 weeks) and detailed assessment of fitness and reproductive parameters, and gross necropsy assessment, the studies provide adequate information on the overall effect pattern of the active substance dimethomorph in birds. Hence, the studies are considered suitable to allow for a full evaluation of the reproductive toxicity including any endocrine potential of dimethomorph that might impact the reproductive performance in birds.
This is further supported by recent conclusions of the US Environmental Protection Agency (EPA) within its Endocrine Disruptor Screening Program (EDSP)[1]. In June 2015, US EPA released its review[2] of the Tier 1 screening assay results for the list 1 chemicals (in total 52 chemicals) in the EDSP. Many plant protection products were amongst the chemicals screened in the EDSP, including some substances which are discussed in regards to indication for potential interaction with the androgen pathway. EPA clearly states that for these substances, the data obtained from the "avian reproduction studies (OCSPP 850.2300 [which is the final published version of the previous guideline OPPTS 850.2300]) are considered sufficient for evaluating potential reproductive effects to birds" and "additional testing is not recommended". Specifically note that for all EDSP-screened plant protection products the standard guideline avian

[1] The Endocrine Disruptor Screening Program (EDSP) of US EPA is a program to screen chemicals for their potential to affect the estrogen, androgen and thyroid hormone systems using a two-tiered screening and testing process. The results of the screening are evaluated in a weight of evidence (WoE) approach by EPA to determine whether a chemical has the potential to interact with the endocrine system and whether more thorough testing is required. The WoE conclusion on the tier 1 screening assays for list 1 chemicals was published in June 2015.

[2] United States Environmental Protection Agency Washington, D.C. 20460, Memorandum, June 29, 2015, EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals.

reproduction toxicity studies were the only available data on chronic bird toxicity, and hence were considered sufficient for the evaluation of potential reproductive effect to birds.

Based on the US EPA assessment for the EDSP, it is considered valid to conclude for the case of dimethomorph that the available data from the avian reproduction toxicity studies in bobwhite quail and mallard duck are suitable for the evaluation of potential reproductive effect to birds, and to derive an endpoint that is suitable to cover the endocrine potential of dimethomorph in the avian reproductive risk assessment.

Dimethomorph is discussed in the mammalian toxicology section in regard to indications for potential interaction with the androgen pathway. However, results concerning endocrine modulation from mammalian studies cannot be extrapolated directly to birds as literature suggests lower sensitivity of birds to anti-androgenic substances than mammals as birds. This reported decreased sensitivity is due to mammals being fundamentally different in their genetic base for gender, and in the hormonal regulation of sexual development and differentiation. During the development of birds, the critical parameter is the presence of estrogen, while sexual differentiation in mammals is primarily determined by testosterone levels. Consequently, birds are deemed to be less sensitive to androgen-active substances than mammals. This is detailed as follows in the OECD 2007 review paper[3] on the avian two-generation toxicity test:

> [...]. "*Sex determination and control of differentiation is linked to the heterogametic sex. In mammals, this is the male (XY); in birds, it is the female (ZW). The so-called default sex, which is the phenotype to which the embryo will develop in the absence of sex-specific hormones, is the homogametic sex. Mammals will develop as female (XX) and birds as male (ZZ) if no sex differentiation hormones are present. Therefore, birds require that estradiol be synthesized to cause differentiation of the gonad into an ovary. Lack of estrogen, regardless of androgen levels, will result in development of phenotypic males. In mammals, the reverse is true: embryos will develop into phenotypic females unless sufficient levels of androgens are present to induce gonadal differentiation into testicular tissue.*" [...]
>
> Based on the above, the authors of the OECD 2007 review paper concluded:
>
> [...]. "*Birds appear to be affected by androgenic or anti-androgenic substances to a much lesser extent than they are affected by estrogens and anti-estrogens." [...]*

The available avian reproduction studies with dimethomorph confirm the low toxicity of dimethomorph to avian species: No effects were observed up to and including the highest tested concentrations of 800 ppm both in the bobwhite quail and in the mallard duck. Furthermore, the standard data requirement for registration in the EU for reproductive testing is with one bird species; however, for dimethomorph avian reproduction studies are available for two species, hence reducing the risk linked to potential variations in species sensitivity. Under the conservative assumptions in both the study design (constant long-term exposure to high concentrations), the low toxicity observed in the avian reproduction studies, and the long-term bird risk assessment (which demonstrated that even under the very conservative assumptions of tier 1 the long-term TER values are far above the trigger value of 5), sufficient data is available to conclude that the risk to birds from the proposed uses of dimethomorph is acceptable.

[3] OECD. 2007. OECD series on testing and assessment number 74: Detailed review paper for avian two-generation toxicity test, OECD, ENV/JM/MONO(2007)21.

In summary, based on: i) the availability of two avian reproduction studies for dimethomorph, ii) the conclusions of the US EPA indicating avian reproductive studies for evaluating potential reproductive effects on birds are sufficient for identifying endocrine related activity for plant protection products in the EDSP, iii) literature suggesting lower sensitivity of birds to anti-androgenic substances than mammals in alignment with the low toxicity profile observed for dimethomorph in the avian reproduction studies, and iv) the conservative nature of the bird reproductive risk assessment, it can be reasoned for dimethomorph that the standard endpoints from the avian reproduction studies are sufficiently protective to address the potential endocrine activity and are suitable for their use in the avian reproductive risk assessment for dimethomorph.

Please note that the following study summary was included on request of the RMS. This publically available monitoring study does not provide any endpoints for the risk assessment of dimethomorph, and thus, the study is not considered relevant by the Notifier. In addition, the results of this study do not change the opinion of the notifier and the reasoning above that the standard endpoints from the avian reproduction studies for dimethomorph are sufficiently protective to address the potential endocrine activity.

Report:	CA 8.1.5/1 Bro E. et al., 2015 a Quantification of potential exposure of gray partridge (Perdix perdix) to pesticide active substances in farmlands 2017/1096025
Guidelines:	none
GLP:	no

Executive Summary

The authors propose an ecologically-relevant methodology to estimate potential exposure to active substances (a.s.) of a farmland bird, the gray partridge (*Perdix perdix)*. It is based on bird habitat use of fields at the time of pesticide applications. The potential exposure to 179 a.s. of 140 clutches during pre-laying, laying, and incubation phases, and of 75 coveys is identified and quantified. The data come from a large scale field study conducted in France combining radio telemetry and a farmer survey. The proportion of clutches potentially exposed to a given chemical was ≥5% for 32 a.s. 71% of clutches were potentially exposed to ≥ 1 a.s. and 67% to ≥2 a.s.. Mixtures involved 2 to 22 a.s. Active substances were fungicides (53%), herbicides (25%), and insecticides (16%) used on a variety of crops in April–June, when ground-nesting birds are breeding. The European Food Safety Authority conclusions report a long-term first-tier toxicity-to-exposure ratio (TER_{lt}) <5 for 11 out of 19 documented a.s., and higher-tier TER_{lt} <5 for 5 out of 10 a.s.. According to the authors, this suggests a potential risk for bird reproduction in farmlands. Globally 13% of coveys were potentially exposed to 18 a.s. during the first month (1–4 coveys per a.s.). The authors discuss the use of their field data in future research and risk assessment.

I. MATERIAL AND METHODS

A. MATERIALS

No test material used

B. STUDY DESIGN

The study was carried out in 12 sites located in north-central France. The total area reached ca. 14,500 ha. Main crops were winter wheat and winter barley, and, to a lesser extent, rapeseed, sugar beets and pastures. A radiotracking survey of gray partridges was conducted in spring and summer 2010–2011. Captures were made from late February to late March. 467 females were radio-tagged. The status (alive, dead or "missing") and the locations of the birds were determined twice a day. 281 clutches were detected and it was determined if they were infertile or contained a dead embryo. When an embryo was observed, its stage of development was determined using reference standards. The authors determined the incubation, laying, and pre-laying phases for each clutch by back-dating the beginning of incubation and egg-laying from hatching/failure date. A survey of farmers was conducted to record their operations at the field level. 142 farmers provided the following data: trade formulation of the pesticides used, tank mixtures, dates of application, and doses used. Data were collected for ca. 1000 fields and a total area of ca. 6500 ha. The authors recorded a total of 179 a.s. used between 1st of March and 31st of August 2010 and 2011. It was considered that a female (and then her eggs or her chicks) was potentially exposed to a pesticide if the area where it was tracked overlapped with treated fields. The exposure of clutches for the incubation, laying, and pre-laying phases, and of chicks during brooding was assessed. The potential exposure of chicks to a.s. was estimated for their four first weeks of life separately, as well as for their whole first month of life. The proportion of females (i.e., clutches and coveys) that were potentially exposed to this a.s. was calculated. Data on reproductive toxicity for each a.s. such as the no observed (adverse)-effect level (NO(A)EL) reported in the European Food Safety Authority (EFSA) conclusions were collected. Bird first-tier and higher-tier TER_{lt} were recorded.

II. RESULTS AND DISCUSSION

Partridge clutches (n = 140 clutches, N1600 eggs) were exposed to 108 a.s. during pre-laying, laying, and incubation phases over a total of 179 a.s. used between 1st March and 31st August 2010 and 2011. 71.4% of the clutches were exposed to at least one a.s.. The proportion of clutches potentially exposed to a given a.s. was ≥5% for 32 a.s.. These "top" a.s. were mainly fungicides (n = 17, 53.1%). The main period of use of top a.s. was from early April to early/ mid-June. Spring to mid-summer is also the time of laying, incubation, hatching, and brooding of the gray partridge and other ground nesting birds, indicating spatio-temporal correspondence between bird breeding and pesticide use. 67.1% of clutches were potentially exposed to mixtures, involving 2 up to 22 a.s.. 13.3% of coveys (n = 75) were potentially exposed to a.s.. Chicks were globally exposed to 18 a.s. during their first month of life. a.s. were mainly fungicides (66.7%) and insecticides (27.8%). Eleven out of 19 documented top a.s. are associated to a lowest first-tier TER_{lt} <5. These a.s. are mostly fungicides (8 a.s. out of 11). However, some a.s. used as herbicides, insecticides or plant growth regulators are also associated with a lowest first-tier TER_{lt} <5. Higher-tier TER_{lt} calculated for some passerine species (or unspecified species) tend to confirm a risk for some bird species for 5 out of 10 a.s.

III. CONCLUSION

A high proportion of clutches is potentially exposed to a diversity of a.s. and mixtures, mostly during egg formation. Chicks seem less potentially exposed to a.s.

CA 8.2 Effects on aquatic organisms

All changes concerning the update (May 2017) after the draft assessment report on dimethomorph are highlighted in blue. Deleted parts are struck through.

Since Annex I inclusion of dimethomorph (BAS 550 F), new toxicity studies on the active substance have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. Summaries of these new studies are provided below. For completeness this includes some older studies, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU) and also EU agreed studies that have been re-evaluated in the meantime (*e.g.* due to recalculations of endpoints or a new evaluation according to current guidelines).
Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of dimethomorph are provided in the EU Review documents of dimethomorph (Draft Assessment Report (DAR), Vol. 3, Annex B.9, December 2004; Final Addendum to the DAR, April 2006; EFSA Scientific Report (2006), 82, 1 - 69).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1: .

No relevant metabolites of dimethomorph have been detected in relevant amounts in aquatic test systems (please refer to CA 7.2 of this dossier). Hence, no ecotoxicological tests with metabolites on aquatic organisms are required and no such studies have been conducted.

Full references to cited literature used within the following chapters are given at the end MCA 8.2.

Table 8.2-1: List of studies and endpoints for aquatic organisms exposed to the active substance dimethomorph (BAS 550 F)

Organism	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked water study)	Reference (BASF Name / DocID)	EU agreed
Fish				
Oncorhynchus mykiss	96 h LC_{50}	3.4 (nominal)	DK-511-002 / 1986/7000035	Yes, but new recalculation of endpoint based on current standards
		6.1 (mean measured)	2010/1177242 (recalculations)	
Oncorhynchus mykiss	96 h LC_{50}	6.79	*DK-511-007 / 2001/7000326*	Yes
Cyprinodon variegates #	96 h LC_{50}	11.3	*DK-511-005 / 1997/7000203*	Yes
Cyprinus carpio	96 h LC_{50}	14.0	DK-511-001 / 1986/7000034	Yes, but new recalculation of endpoint based on current standards
		16.6 ~~18.1~~	2010/1177241 (recalculations)	
Lepomis macrochirus	96 h LC_{50}	> 25.0	DK-511-003 / 1988/7000033	No (study submitted in previous Annex I process but not valid according to old DAR)
		> 13.7	2010/1177243 (recalculations)	
Lepomis macrochirus	96 h LC_{50}	> 9.53	*DK-511-006 / 2001/7000325*	Yes
Pimephales promelas 1)	96 h LC_{50}	> 8.40	2014/7002827	No (new study; conducted for registrations outside of Europe)
Oncorhynchus mykiss	21 d NOEC	0.480	DK-512-001 / 1993/7000129	Yes, but new recalculation of endpoint based on current requirements (*i.e.* EC_{10})
	21 d EC_{10}	**> 1.53**	2014/1224005 (recalculations)	
Oncorhynchus mykiss (ELS study)	60 d NOEC	0.056	DK-512-002 / 1997/7000205	Yes, but new recalculation of endpoint based on current requirements (*i.e.* EC_{10})
	60 d EC_{10}	**0.116**	2014/1224007 (recalculations)	
Cyprinodon variegatus # (ELS study) 1)	40 d NOEC	0.136	2010/7012691 + 2014/1224007	No (new study; conducted for registrations outside of Europe)
	40 d EC_{10}	**0.150**		

Organism	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked water study)	Reference (BASF Name / DocID)	EU agreed
Pimephales promelas (ELS study) [1)]	34 d NOEC	0.107	2002/1011268 + 2014/1224007	No (new study; conducted for registrations outside of Europe)
	34 d EC_{10}	**> 0.92**		
Pimephales promelas [1)] (Fish Short Term Reproduction Assay)	21 d NOEC	≥ 0.48	2014/1187237	No (new study conducted in order to address endocrine activity)
Bioconcentration				
Lepomis macrochirus (bioconcentration study; 28 d uptake phase and 14 d depuration phase) [1)]	BCF_{SS} (whole fish, normalized to lipid content)	29 - 40	DK-519-001 / 1999/7000059	No (not submitted in previous Annex I process; conducted for registrations outside of Europe; included for completeness)
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**> 10.6**	*DK-521-006 / 2001/7000327*	Yes
Daphnia magna	48 h EC_{50}	48.9	DK-521-002 / 1986/7000037	No (study submitted in previous Annex I process but not valid according to old DAR)
		20.1	2010/1177164 (recalculations)	
Americamysis bahia (former name: *Mysidopsis bahia)* [#]	48 h LC_{50} [2)]	> 9.75 *	*DK-521-004 / 1997/7000206*	Yes
Crassostrea virginica [#]	96 h EC_{50}	4.42	*DK-522-001 / 1997/7000207*	Yes
Daphnia magna	22 d NOEC	0.100	DK-524-001 / 1993/7000130	Yes, but new recalculation of endpoint based on current requirements (*i.e.* EC_{10})
	22 d EC_{10}	0.152	2015/1238096 (recalculation)	
Daphnia magna	21 d NOEC	**0.220** [3)]	DK-523-001 / 1997/7000208	Yes, but new recalculation of endpoint based on current requirements (*i.e.* EC_{10})
	21 d EC_{10}	**0.421**	2015/1238096 (recalculation)	
Americamysis bahia [#, 1)]	28 d NOEC	0.241	2010/7008279	No (new study; conducted for registrations outside of Europe)
	28 d EC_{10}	0.238	2015/1238096 (recalculation)	

Organism	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked water study)	Reference (BASF Name / DocID)	EU agreed
Sediment dwelling aquatic invertebrates				
Chironomus riparius (spiked water study)	24 d NOEC	4.11 (22.7 mg/kg dry sediment) [4)]	DK-529-002 / 1997/7000209	Yes, but new recalculation of endpoint based on current requirements (*i.e.* EC_{10})
	24 d EC_{10}	3.02	2015/1238096 (recalculation)	
Algae [5)]				
Pseudokirchneriella subcapitata	72 h E_rC_{50} 72 h E_bC_{50}	**82.2** 41.4	*DK-521-007 / 2001/7001037*	Yes
Scenedesmus subspicatus	96 h E_bC_{50}	29.2	DK-521-001 / 1986/7000036	Yes (but not valid according to recent guidelines)

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), only the relevant endpoint(s) is used in the (tier 1) risk assessment presented in chapter 10.2 of the MCP dossier part for Annex I renewal.
Italic figures: The references in italic were submitted in the old dossier and peer reviewed. They are not summarized in this supplemental dossier.
Abbreviations: ELS = early life stage; BCF= bioconcentration factor

[#] Estuarine/Marine species.

***** The EU agreed upon endpoint and the endpoint found in the study summaries differs. Therefore, for the risk assessment only the EU agreed upon endpoint was considered.

[1)] Study has not been submitted during Annex I inclusion process of dimethomorph. A study summary is provided below.

[2)] The 48-h LC_{50} obtained in the 96 h study is used as relevant endpoint in the risk assessment according to EU Regulation 283/2013 on the data requirements for active substances and the EFSA Aquatic GD (2013).

[3)] At the time of the Annex I inclusion two chronic studies on *Daphnia magna* were conducted with dimethomorph under semi-static (22 d, DK-524-001) and flow-through (21 d, DK-523-001) conditions. Test concentrations in the study performed under semi-static conditions ranged from 0.1 - 9.6 mg a.s./L and the observed NOEC and LOEC were 0.1 and 0.31 mg a.s./L, respectively corresponding to an EC_{10} = 0.152 mg a.s./L. The range of concentrations tested under flow-through conditions (DK-523-001) was properly chosen and more accurate (5 concentrations in the range of 0.11 - 1.8 mg a.s./L). Hence, the obtained NOEC of 0.22 mg a.s./L (and a corresponding EC_{10} = 0.421 ~~0.452~~ mg a.s./L) is both more precise and relevant for the risk assessment and will be considered for TER calculations.

[4)] Based on sediment concentration in spiked water study at day 10 measured in the treatment at the NOEC concentration.

[5)] In accordance to the EFSA Aquatic Guidance Document (EFSA 2013) and OECD guidelines 201 (OECD, 2011), only the EC_{50} values for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers.

CA 8.2.1 Acute toxicity to fish

An acute toxicity study with rainbow trout (*Oncorhynchus mykiss*) conducted with dimethomorph was already evaluated during the previous Annex I inclusion process. The endpoint of this study is based on nominal concentrations and was used as relevant endpoint for former risk assessments. However measured concentrations of dimethomorph in the test solutions ranged from 133% to 270% of the targeted nominal concentrations. Therefor the study deviate from the current guideline OECD 203. Due to the variable analytical results, the results from the study have been re-evaluated based on measured concentrations and current standards. This document was not included in the application. The recalculated endpoint based on mean measured concentrations are used in the risk assessment for dimethomorph. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph. As requested by the RMS those summaries are also added below for sake of completeness.

Report:	CA 8.2.1/1 [redacted] 1986 a 96-hour acute toxicity (LC50) with CME 151 in the rainbow trout DK-511-002
Guidelines:	OECD 203, EEC 84/449 C 1, OECD Principles of Good Laboratory Practice Bundesanzeiger No. 42a (March 2 1983) FRG
GLP:	no

Executive Summary (originally submitted study)

In a static acute (96 h) toxicity laboratory study, rainbow trout were exposed to nominal concentrations of 0 (control and solvent control), 1.0, 2.0, 5.0, 7.0 and 10.0 mg a.s./L in groups of 10 animals in each treatment and control group. Fish were observed for survival at least daily during the test.

The biological results are based on nominal concentrations. After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 1.0 mg a.s./L, whereas 10%, 80%, 90% and 100% mortality occurred in the test item concentrations 2.0, 5.0, 7.0 and 10.0 mg a.s./L, respectively.

In a static acute toxicity study with *O. mykiss* the LC_{50} (96 h) value of dimethomorph was 3.4 mg a.s./L based on nominal concentrations and 6.2 mg a.s./L based on measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME 151); Batch Number DW 11/86; purity: 94.8%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); Origin: [redacted]
Test design:	Static system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 10 fish per treatment and aquarium; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (750 mg/L Tween 80); 1, 2, 5, 7, and 10 mg a.s./L
Test Conditions:	Temperature: 14.3 - 15.0 °C; pH:7.3 - 8.0; dissolved oxygen: 7.6 - 10.3 mg/L
Analytics:	Analytical verification of test item concentrations was conducted using HPLC method with UV detection
Statistics:	Descriptive statistics, The LC_{50} values were estimated using the Logit Model.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations at 2, 48 and 96 h. The measured concentrations of dimethomorph in the test solutions ranged from 133 to 270% of the targeted nominal concentrations. The variable analytical results were likely due to concentrations approaching the maximum solubility limit for the compound. The biological result are based on measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 1.0 mg a.s./L, whereas 10%, 80%, 90% and 100% mortality occurred in the test item concentrations 2.0, 5.0, 7.0 and 10.0 mg a.s./L, respectively. The result are summarized in table Table 8.2.1-1: .

Table 8.2.1-1: Acute toxicity (96 h) of dimethomorph to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1**	**2**	**5**	**7**	**10**
Concentration [mg a.s./L] (measured)	**--**	**--**	**2.4**	**4.0**	**8.0**	**10.3**	**14.1**
Mortality after 96 h [%]	0	0	0	10	80	90	100
Symptoms after 96 h	ns	ns	1D, 1He, 2Rr,	3F, 2D, 1He, 2Rs, 1Ha, 2Rr	2D, 2Ho, 1Ha, 3Rr	3D, 3Ho, 1Rb*, 2Rs, 2Ha, 3Rr	1F, 3D, 3Ho, 3Rb, 2Ha, 3Rr, 1C
Endpoints [mg a.s./L] (nominal)							
LC_{50} (96 h)	3.4						
Endpoints [mg a.s./L] (measured)							
LC_{50} (96 h)	6.2						
NOEC	2.4						

Symptoms of sub-lethal effects: ns = no symptoms, F = flatulence if the abdomen, D = discoloration, He = hypersensitivity, Ho = hyposensitivity, Rb = remaining near bottom, Rs = remaining near surface, Ha = hypoactivity, Rr = increased respiratory rate, C = convulsion; severity score: 1 = slight, 2 = moderate, 3 = severe, * = initially only

III. CONCLUSION

In a static acute toxicity study with *O. mykiss* the LC_{50} (96 h) value of dimethomorph was 3.4 mg a.s./L based on nominal concentrations and 6.2 mg a.s./L based on measured concentrations.

Report: CA 8.2.1/2
Habekost M., 2010 a
BAS 550 F - Re-calculation of toxicity values for study DK-511-002 (96-hour acute toxicity study (LC50) with CME 151 in the rainbow trout)
2010/1177242
Guidelines: none
GLP: no

Executive Summary (recalculations)

The LC_{50} endpoint derived in the study with *Oncorhynchus mykiss* (BASF DocID DK-511-002) was determined based on nominal concentrations only. A statistical recalculation of the LC_{50} value was conducted based on mean measured concentrations (2.4, 3.9, 7.9, 10.2 and 14.0 mg a.s./L). The statistical determination was done by probit analysis using linear minimum likelihood regression.

Mortality: LC_{50} = 6.1 mg a.s./L (95% confidence limits: 4.7 - 7.4 mg a.s./L), based on mean measured concentrations

An acute toxicity study with *Cyprinus carpio* conducted with dimethomorph was already evaluated during the previous Annex I inclusion process. The endpoint of this study is based on nominal concentrations. However measured concentrations of dimethomorph in the test solutions ranged from 115.6% to 129.2% of the targeted nominal concentrations at test initiation and from 110% to 142.5% at test termination. Therefor the study deviate from the current guideline OECD 203. Due to the variable analytical results, the results from the study have been re-evaluated based on measured concentrations and current standards. This document was not included in the application. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

This study performed with the active substance dimethomorph was not listed in the "Application" document submitted for the dimethomorph AIR 3 renewal process.

Report:	CA 8.2.1/3 [redacted] 1986 b 96-hour acute toxicity (LC50) with CME 151 in the carp DK-511-001
Guidelines:	OECD 203, EEC 84/449 C 1, OECD Principles of Good Laboratory Practice Bundesanzeiger No. 42a (March 2 1983) FRG
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Executive Summary (originally submitted study)

In a static acute (96 h) toxicity laboratory study, common carps were exposed to nominal concentrations of 0 (control and solvent control), 12.0, 14.0, 16.0 and 20.0 mg a.s./L in groups of 10 animals in each treatment and control group. Fish were observed for survival at least daily during the test.

The biological results are based on nominal concentrations. After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 12.0 mg a.s./L, whereas 70%, 70% and 100% mortality occurred in the test item concentrations 14.0, 16.0 and 20.0 mg a.s./L, respectively.

In a static acute toxicity study with *Cyprinus carpio* the LC_{50} (96 h) value of dimethomorph was 14 mg a.s./L based on nominal concentrations. The NOEC was determined to be 12 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME 151); Batch Number DW 11/86; purity: 94.8%.

B. STUDY DESIGN

Test species:	Common carp (*Cyprinus carpio*); Origin: [redacted].
Test design:	Static system (96 h); 4 test item concentrations plus a dilution water control and a solvent control, 10 fish per treatment and replicate; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (750 mg/L Tween 80), 12, 14, 16, and 20 mg a.s./L
Test Conditions:	Temperature: 21.4 - 22.8 °C; pH: 7.2 - 8.1; dissolved oxygen: 6.8 - 9.3 mg/L; photoperiod: 12 hours light:12 hours dark
Analytics:	Exposure concentrations of dimethomorph were analytically confirmed during the definitive test using an HPLC method.
Statistics:	Descriptive statistics, The LC_{50} values were estimated using the Logit Model.

II. RESULTS AND DISCUSSION

Analytical measurements: The measured concentrations of dimethomorph in the test solutions ranged from 115.6 and 129.2% of the targeted nominal concentrations at the start of the test and 110% to 142.5% at test termination. The variable analytical results were likely due to concentrations approaching the maximum solubility limit for the compound. Due to the variable analytical results, the results from the test were based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 12.0 mg a.s./L, whereas 70%, 70% and 100% mortality occurred in the test item concentrations 14.0, 16.0 and 20.0 mg a.s./L, respectively. The result are summarized in Table 8.2.1-2: .

Table 8.2.1-2: Acute toxicity (96 h) of dimethomorph to Common Carp (*Cyprinus carpio*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**12**	**14**	**16**	**20**
Mortality after 96 h [%]	0	0	0	70	70	100
Symptoms of fish after 96 h*	n	n	2F, 1He, 1Fa, 1Hea, 1Ha, 1Ri, 2Rr	1He, 1Fa, 2Ri, 2Rr, 3E, 2L	2Ho, 1Fa, 2Ri, 2Rr, 3E, 1L	1He, 1Ho, 1Hea, 2Ri, 3E, 2Sm, 1L
Endpoints [mg a.s./L] (nominal)						
LC_{50} (96 h)	14					
NOEC	12					

Symptoms of sub-lethal effects: ns = no symptoms, F = flatulence if the abdomen, He = hypersensitivity, Ho = hyposensitivity, Fa = fins adjacent, Hea = hyperactivity, Ha = hypoactivity, Ri = respiratory rate irregular, Rr = increased respiratory rate, E = loss of equilibrium, Sm = spasm with rotatory movement, L = lateral recumbency; severity score: 1 = slight, 2 = moderate, 3 = severe, * = initially only.

III. CONCLUSION

In a static acute toxicity study with *Cyprinus carpio* the LC_{50} (96 h) value of dimethomorph was 14 mg a.s./L based on nominal concentrations. The NOEC was determined to be 12 mg a.s./L (nominal).

Report: CA 8.2.1/4
Habekost M., 2010 b
BAS 550 F - Re-calculation of toxicity values for study DK-511-001 (96-hour acute toxicity study (LC50) with CME 151 in the carp)
2010/1177241
Guidelines: none
GLP: no

Executive Summary (recalculations)

The LC_{50} endpoint derived in the study with *Cyprinus carpio* (BASF DocID DK-511-001) was determined based on nominal concentrations only. A statistical recalculation of the LC_{50} value was conducted based on mean measured concentrations (16.3, 18.6, 20.4 and 22.6 mg a.s./L). The statistical determination was done by probit analysis using linear minimum likelihood regression.

Mortality: LC_{50} = ~~18.1~~ 16.6 mg a.s./L * ~~(95% confidence limits: 15.6 - 19.4 mg a.s./L)~~, based on mean measured concentrations

An acute toxicity study with *Lepomis macrochirus* conducted with dimethomorph was already evaluated during the previous Annex I inclusion process. The endpoint of this study is based on nominal concentrations. However measured concentrations of dimethomorph in the test solutions ranged from 46% to 74% of the targeted nominal concentrations. Therefor the study deviate from the current guideline OECD 203. Due to the variable analytical results, the results from the study have been re-evaluated based on measured concentrations and current standards. This document was not included in the application. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

This study performed with the active substance dimethomorph was not listed in the "Application" document submitted for the dimethomorph AIR 3 renewal process.

* Recalculation was performed by the RMS using nonlinear regression. This rendered an EC_{50} of 16.6 mg a.s./L.

Report:	CA 8.2.1/5 [redacted], 1988 a The acute toxicity of CME 151 to bluegill sunfish (Lepomis macrochirus) DK-511-003
Guidelines:	OECD 203, EPA 72-1
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Executive Summary (originally submitted study)

In a semi-static acute (96 h) toxicity laboratory study, bluegill sunfish were exposed to nominal concentrations of 0 (control and solvent control), 2.5, 4.5, 8.0, 14.0 and 25.0 mg a.s./L in groups of 20 animals in each treatment and control group. Fish were observed for survival at least daily during the test.

The biological results are based on nominal concentrations. After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of up to and including 4.5 mg a.s./L, whereas 5%, 5% and 25% mortality occurred in the test item concentrations 8.0, 14.0 and 25.0 mg a.s./L, respectively.

In a semi-static acute toxicity study with *Lepomis macrochirus* the LC_{50} (96 h) value of dimethomorph was >25 mg a.s./L based on nominal concentrations. The NOEC was determined to be 4.5 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME 151); Batch Number DW 11/86; purity: 96.6%.

B. STUDY DESIGN

Test species:	Bluegill Sunfish (*Lepomis macrochirus*); Origin: [redacted]
Test design:	Static system (96 h); 5 test item concentrations plus a dilution water control and a solvent control in 2 replicates, 10 fish per treatment and replicate; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (50µl/L mg/L acetone containing 1% Tween 80), 2.5, 4.5, 8.0, 14, and 25 mg a.s./L.
Test Conditions:	Temperature: 20.0 to 21.0 °C; pH: 7.3 to 7.7; Dissolved oxygen: 7.9 to 9.0 mg/L photoperiod: 16 hours light:8 hours dark
Analytics:	Exposure concentrations of dimethomorph were analytically confirmed during the definitive test using an HPLC method.
Statistics:	No statistical analysis was conducted

II. RESULTS AND DISCUSSION

Analytical measurements: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of up to and including 4.5 mg a.s./L, whereas 5%, 5% and 25% mortality occurred in the test item concentrations 8.0, 14.0 and 25.0 mg a.s./L, respectively.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of up to and including 4.5 mg a.s./L, whereas 5%, 5% and 25% mortality occurred in the test item concentrations 8.0, 14.0 and 25.0 mg a.s./L, respectively. The results are summarized in Table 8.2.1-3: .

Table 8.2.1-3: Acute toxicity (96 h) of dimethomorph to Bluegill Sunfish (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**2.5**	**4.5**	**8.0**	**14**	**25**
Mortality after 96 h [%]	0	0	0	0	10	10	50
Number of affected fish with symptoms after 96 h*	ns	ns	ns	8L	11L	19Ls	15Ls, 5P
Endpoints [mg a.s./L] (nominal)							
LC_{50} (96 h)	> 25						
NOEC	4.5						

Symptoms of sub-lethal effects: ns = no symptoms, L = lethargic, M = moribund, Ls = severe lethargy, P = increased pigmentation.

III. CONCLUSION

In a semi-static acute toxicity study with Lepomis macrochirus the LC_{50} (96 h) value of dimethomorph was >25 mg a.s./L based on nominal concentrations. The NOEC was determined to be 4.5 mg a.s./L (nominal).

Report: CA 8.2.1/6
Habekost M., 2010 c
BAS 550 F - Re-calculation of toxicity values for study DK-511-003 (The acute toxicity of CME 151 to bluegill sunfish (Lepomis macrochirus))
2010/1177243
Guidelines: none
GLP: no

Executive Summary (recalculations)

The LC_{50} endpoint derived in the study with *Lepomis macrochirus* (BASF DocID DK-511-003) was determined based on nominal concentrations only. A statistical recalculation of the LC_{50} value was conducted based on mean measured concentrations (1.9, 3.3, 5.3, 8.1 and 13.7 mg a.s./L).

Mortality: LC_{50} > 13.7 mg a.s./L based on mean measured concentrations

The following acute toxicity study with the fathead minnow with the active substance dimethomorph has not been evaluated previously on EU level.

Report: CA 8.2.1/7
[redacted] 2014 a
BAS 550 F: A 96-hour flow-through acute toxicity test with the fathead minnow (Pimephales promelas)
2014/7002827
Guidelines: EPA 850.1075, OECD 203
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile fathead minnow were exposed to a dilution water control, a solvent control and to dimethomorph at nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L (corresponding to mean measured concentrations of 0.60, 1.3, 2.5, 4.9 and 8.4 mg a.s./L) in groups of 10 animals in glass aquaria containing approximately 15 L water. Fish were observed for survival and symptoms of toxicity 6.5, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality and sub-lethal effects were observed in the controls and at all test item concentrations.

In a flow-through acute toxicity study with fathead minnow the LC_{50} (96 h) of dimethomorph was > 8.4 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be ≥ 8.4 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F, Reg. no.: 247723), batch no. COD-001646, purity: 99.7% ± 1.0%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), age: juveniles; mean body length of control fish: 2.8 cm (2.6 cm - 2.9 cm); mean wet weight of control fish: 0.13 g (0.086 g - 0.17 g); supplied by [redacted]

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading: 0.0091 g fish/L/day); assessment of mortality and sub-lethal effects at 6.5, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water); solvent control (0.1 mL/L dimethylformamide); 0.63, 1.3, 2.5, 5.0 and 10 mg dimethomorph/L (nominal), corresponding to mean measured concentrations of 0.60, 1.3, 2.5, 4.9 and 8.4 mg a.s./L.

Test conditions: 25 L Teflon lined stainless steel aquaria; test volume: approximately 15 L; dilution water: filtered well water; flow rate: approximately 10 volume additions of test water in each test chamber per day; hardness: 136 mg $CaCO_3$/L; temperature: 21.8 - 22.0°C; pH 8.0 - 8.2; oxygen content: 8.2 mg/L - 9.2 mg/L; conductivity: 341 µS/cm; photoperiod 16 h light : 8 h dark; light intensity at test initiation: approx. 318 lux; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a gas chromatography with mass spectrometric detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of dimethomorph concentrations was conducted in each test item concentration at the beginning of the test, after 48 h and at the end of the test. The analyzed contents of dimethomorph ranged from 79.8% to 107% of nominal concentrations at test initiation, from 81.3% to 101% after 48 h and from 90.4% to 99.6% of nominal concentrations at test termination. Recovery of the analytical method was 103%. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality and sub-lethal effects were observed in the controls and at all test item concentrations. The results are summarized in Table 8.2.1-4:

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Table 8.2.1-4: Acute toxicity (96 h) of dimethomorph to fathead minnow (*Pimephales promelas*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.60**	**1.3**	**2.5**	**4.9**	**8.4**
Mortality [%] (96 h)	0	0	0	0	0	0	0
Symptoms (after 96 h)	none	none	none	none	none	none	none
Endpoints [mg dimethomorph/L] (mean measured)							
LC_{50} (96 h)	> 8.4						
NOEC (96 h)	≥ 8.4						

III. CONCLUSION

In a flow-through acute toxicity study with fathead minnow the LC_{50} (96 h) of dimethomorph was > 8.4 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be ≥ 8.4 mg a.s./L (mean measured).

Report:	CA 8.2.1/8 [redacted] 2001 a Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to rainbow trout, Oncorhynchus mykiss, under flow-through test conditions DK-511-007
Guidelines:	OECD 203, EPA 72-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

In a flow-through acute (96 h) toxicity laboratory study, rainbow trout were exposed to nominal concentrations of 0 (control and solvent control), 2.6, 4.4, 7.2, 12, and 20 mg a.s./L in groups of 20 animals in each treatment and control group. Fish were observed for survival at least daily during the test.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 1.75 mg a.s./L, whereas 20%, 25%, 45% and 90% mortality occurred in the test item concentrations 3.13, 4.71, 7.84 and 12.0 mg a.s./L, respectively

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with rainbow trout was determined to be 6.79 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 1.75 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as BAS 550 F); Lot Number AC 9978-131; purity: 98%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); Origin: [redacted]
Test design:	Flow-through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control in 2 replicates, 10 fish per treatment and replicate; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50} and NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (0.1 mL/L dimethlyformamide), 2.6, 4.4, 7.2, 12, and 20 mg a.s./L
Test Conditions:	Temperature: 15.3 - 16.3 °C, pH: 7.5 - 7.6, dissolved oxygen: 9.7 - 10.2 mg/L, photoperiod: 16 hours light : 8 hours dark
Analytics:	Analytical verification of test item concentrations was conducted using HPLC method (Method MV 1341)
Statistics:	Descriptive statistics, The LC_{50} values were estimated using the Probit Model.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.10 (control and vehicle blank), 1.75, 3.13, 4.71, 7.84, and 12.0 mg a.s./L. These measured concentrations ranged from 80 to 95% of the nominal concentrations, after the nominal concentrations were corrected for the 76% recovery from the 20 mg a.s./L primary mixing chamber solution. The low recovery from the mixing chamber solution was due to the water solubility limit for the test substance being exceeded. The results of the study are based on the mean measured concentration of the test solutions.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 2.6 mg a.s./L (nominal), whereas 20%, 25%, 45% and 90% mortality occurred in the test item concentrations 4.4, 7.2, 12 and 20.0 mg a.s./L, respectively. The result are summarized in Table 8.2.1-5: .

Table 8.2.1-5: Acute toxicity (96 h) of dimethomorph to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**2.6**	**4.4**	**7.2**	**12**	**20**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.75**	**3.13**	**4.71**	**7.84**	**12.0**
Mortality after 96 h [%]	0	0	0	20	25	45	90
Number of affected fish after 96 h*	n	n	0	1	13	8	2
Endpoints [mg a.s./L] (mean measured)							
LC_{50} (96 h)	6.79						
$NOEC_{survival}$	1.75						

*Affected fish exhibited one or more of the following sub-lethal effects lethargy, loss of equilibirium, change in coloration, distended abdomen; N = no sub-lethal effect

III. CONCLUSION

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with rainbow trout was determined to be 6.79 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 1.75 mg a.s./L.

Report:	CA 8.2.1/9 [redacted] 1997 a Acute toxicity of AC 336379 (Dimethomorph) to the sheepshead minnow (Cyprinodon variegatus) under flow-through test conditions DK-511-005
Guidelines:	EPA 72-3(a)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

In a flow-through acute (96 h) toxicity laboratory study, sheepshead minnow were exposed to nominal concentrations of 0 (control and solvent control), 2.6, 4.4, 7.2, 12, and 20 mg a.s./L in groups of 20 animals in each treatment and control groups. Fish were observed for survival at least daily during the test.

The biological results are based on mean measured concentrations. Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 2.0 mg a.s./L (mean measured), whereas 10%, 5%, 15% and 85% mortality occurred in the test item concentrations .3.29, 5.27, 8.82 and 14.6 mg a.s./L, respectively.

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with sheepshead minnow was 11.3 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 2.00 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as AC 336379); Lot Number AC 9978-131; purity: 98.0%.

B. STUDY DESIGN

Test species:	Sheepshead minnow (*Cyprinodon variegatus*); Origin: [redacted]
Test design:	Flow-through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control in 2 replicates, 10 fish per treatment and replicate; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (0.4 mL/L dimethlyformamide), 2.6, 4.4, 7.2, 12, and 20 a.s./L
Test Conditions:	Temperature: 21.5 - 22.0 °C; pH: 7.6 - 8.0; dissolved oxygen: 5.7 - 8.0 mg/L; salinity: 17 parts per thousand; photoperiod: 16 hours light:8 hours dark
Analytics:	Analytical verification of test item concentrations was conducted using HPLC method (Method RU 151/35/90)
Statistics:	Descriptive statistics, The LC_{50} values calculated using the Probit Model or binomial/nonlinear interpolation methods.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.50 (control and vehicle blank), 2.00, 3.29, 5.27, 8.82, and 14.6 mg a.s./L. These measured concentrations ranged from 73 to 77% of the nominal concentrations. The results of the study are based on the mean measured concentration of the test solutions.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations of 2.0 mg a.s./L (mean measured), whereas 10%, 5%, 15% and 85% mortality occurred in the test item concentrations .3.29, 5.27, 8.82 and 14.6 mg a.s./L, respectively. The result are summarized in Table 8.2.1-6: .

Table 8.2.1-6: Acute toxicity (96 h) of dimethomorph to Sheepshead Minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**2.6**	**4.4**	**7.2**	**12**	**20**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**2.00**	**3.29**	**5.27**	**8.82**	**14.6**
Mortality after 96 h [%]	0	0	0	10	5	15	85
Number of affected fish after 96 h*	n	n	n	4	6	7	3
Endpoints [mg a.s./L] (mean measured)							
LC_{50} (96 h)	11.3						

*Affected fish exhibited lethargy and/or loss of equilibirium

III. CONCLUSION

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with sheepshead minnow was 11.3 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 2.00 mg a.s./L.

Report:	CA 8.2.1/10 [redacted]2001 b Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to bluegill sunfish, Lepomis macrochirus under flow-through test conditions DK-511-006
Guidelines:	OECD 203, EPA 72-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

In a flow-through acute (96 h) toxicity laboratory study, bluegill sunfish were exposed to nominal concentrations of 0 (control and solvent control), 2.6, 4.4, 7.2, 12, and 20 mg a.s./L in groups of 20 animals in each treatment and control groups. Fish were observed for survival at least daily during the test.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations to and including 6.75 mg a.s./L (mean measured), whereas 5% mortality occurred in the test item concentrations of 9.53 mg a.s./L.

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with bluegill sunfish was > 9.53 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 9.53 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as BAS 550 F); Lot Number AC 9978-131; purity: 98%.

B. STUDY DESIGN

Test species:	Bluegill sunfish (*Lepomis macrochirus*); Origin: [redacted]
Test design:	Flow-through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control in 2 replicates, 10 fish per treatment and replicate; the number of live and dead fish in each treatment group was recorded at least daily during the test.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water); solvent control (0.1 mL/L dimethlyformamide), 2.6, 4.4, 7.2, 12, and 20 a.s./L
Test Conditions:	Temperature: 22.1 - 23.0 °C; pH: 7.4 - 7.8; dissolved oxygen: 8.6 - 9.0 mg/L; photoperiod: 16 hours light:8 hours dark
Analytics:	Analytical verification of test item concentrations was conducted using HPLC method (Method MV 1341)
Statistics:	No statistical analysis was conducted

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.10 (control and vehicle blank), 1.62, 2.78, 4.19, 6.75, and 9.53mg a.s./L. These measured concentrations ranged from 87 to 121% of the nominal concentrations, after the nominal concentrations were corrected for the 53% recovery from the 20 mg a.s./L primary mixing chamber solution. The low recovery from the mixing chamber solution was due to the water solubility limit for the test substance being exceeded. The results of the study are based on the mean measured concentrations of the test solutions.

Biological results: After 96 hours of exposure, no mortality was observed in the control, in the solvent control and at test item concentrations to and including 6.75 mg a.s./L (mean measured), whereas 5% mortality occurred in the test item concentrations of 9.53 mg a.s./L. The result are summarized in Table 8.2.1-7: .

Table 8.2.1-7: Acute toxicity (96 h) of dimethomorph to Bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**2.6**	**4.4**	**7.2**	**12**	**20**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.62**	**2.78**	**4.19**	**6.75**	**9.53**
Mortality after 96 h [%]	0	0	0	0	0	0	5
Number of affected fish after 96 h*	n	n	n	n	n	n	n
Endpoints [mg a.s./L] (mean measured)							
LC_{50} (96 h)	> 9.53						
$NOEC_{survival}$	9.53						

*Affected fish immobilisation

III. CONCLUSION

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with bluegill sunfish was > 9.53 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 9.53 mg a.s./L.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following early life-stage test on rainbow trout (*Oncorhynchus mykiss*) was performed with the active substance dimethomorph. The study was already evaluated during the previous Annex I inclusion process. However, this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards. The recalculated endpoint will be used in the risk assessment for dimethomorph. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

Report:	CA 8.2.2.1/1 [redacted] 1997 a Toxicity of AC 336,379 (Dimethomorph) technical during the early life-stages of rainbow trout (Oncorhynchus mykiss) DK-512-002
Guidelines:	OECD 210, EPA 72-4, EEC 96/12, EEC 91/414 Annex II 8.2.2.2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

The chronic toxicity of dimethomorph (BAS 550 F) to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-day early life-stage test under flow-through conditions. The test was initiated with the addition of 30 rainbow trout eggs that were two hours of age to each incubation cup. There was one incubation cup per aquarium, with four aquaria per treatment and control group. The following concentrations were tested: 0.0 (control), 0.033, 0.065, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (corresponding to mean measured concentrations of 0.0341, 0.0562, 0.120, 0.240, 0.449 and 0.897 mg a.s./L). Hatchability and post-hatch survival rate were assessed daily throughout the study. At test termination the blotted wet weight and standard length of each remaining fish was determined.

The results are based on mean measured concentrations. The survival was not impaired in any of the concentration groups compared to the control group. The hatching success was similar in all test groups including the control and thus, was not affected by the test substance. There was a statistically significant reduction in standard length and wet weight between the control and the test substance concentrations ≥ 0.240 and ≥ 0.120 mg a.s./L, respectively.

In an early life stage study with *O. mykiss* the NOEC (96 d) of dimethomorph was determined to be 0.0562 mg a.s./L based on wet weight and mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as AC 336379); Lot Number AC 9978-131; purity: 97.6%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); Origin: [redacted]
Test design:	Flow through system (96 d); 6 test item concentrations plus a dilution water control, The test was initiated with the addition of 30 rainbow trout eggs that were two hours of age to each incubation cup. The embryo incubation cups were suspended in individual glass aquaria. There was one incubation cup per aquarium, with four aquaria per treatment and control group at the beginning of the test. This resulted in a total of 120 embryos in each treatment and control group at test initiation. The embryos were thinned to 15 per replicate (60 per treatment) on day 25. Daily assessment mortality of hatch, survival of fry, length an weight at test termination.
Endpoints:	NOEC value based on hatch rate, post-hatch survival and growth
Test concentrations:	Control (dilution water), 0.033, 0.065, 0.13, 0.25, 0.50, and 1.0 mg a.s./L (nominal)
Test conditions:	Temperature: 13.0 -14.6 ºC, pH: 7.94 - 8.41, dissolved oxygen: 9.5 - 9.7 mg/L, photoperiod: for embryos in the dark until hatch, after hatch illuminated at 50% light intensity for 8 hours per day, 100% and 16 hours light:8 hours dark after day 7 post; light intensity: 61 ± 3.9 foot-candles.
Analytics:	Analytical verification of test item concentrations was conducted at least once per week using an HPLC-method (Method RU 151/35/90).
Statistics:	Descriptive statistics Hatching and survival data were analyzed using contingency table methods. Differences between control and treatment groups were evaluated using a frequency analysis based on Chi-square statistic estimated from the two-way contingency table. For smaller sample sizes, one-tailed Fisher's exact test was used. Length and weight data were evaluated using analysis of variance methods (ANOVA) and Dunnett's Test.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph during the 96-day test were < 0.0052 (control), 0.0341, 0.0562, 0.120, 0.240, 0.449, and 0.897 mg/L. The measured concentrations ranged from 89.7 to 103% of the targeted nominal concentrations.

Biological results: The results are based on mean measured concentrations. The survival was not impaired in any of the concentration groups compared to the control group. The hatching success was similar in all test groups including the control and thus, was not affected by the test substance. There was a statistically significant reduction in standard length and wet weight between the control and the test substance concentrations ≥ 0.240 and ≥ 0.120 mg a.s./L, respectively. The results are summarized in Table 8.2.2.1-1: .

Table 8.2.2.1-1: Chronic toxicity of dimethomorph to rainbow trout (*Oncorhynchus mykiss*) in a fish early life stage test (96 d)

Concentration (nominal) [mg a.s./L]	**Control**	**0.033**	**0.065**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration (mean measured) [mg a.s./L]	**--**	**0.0341**	**0.0562**	**0.120**	**0.240**	**0.449**	**0.897**
Embryo survival until hatch [%]	100	98	100	100	100	98	100
Survival of larvae after hatch [%]	92	93	90	95	97	92	90
Mean length [mm]	46.1	45.5	45.6	45.2	**44.5**	**44.2**	**43.4**
Mean weight [g]	1.495	1.371	1.399	**1.367**	**1.284**	**1.234**	**1.110**
Endpoints [mg BAS 550 F/L] (mean measured)							
$NOEC_{wet\ weight}$	0.0562						

Values printed in **bold** show statistically significant differences compared to the control. Statistically significant differences compared to the control based on ANOVA ($p \leq 0.05$)

III. CONCLUSION

In an early life stage study with *O. mykiss* the NOEC (96 d) of dimethomorph was determined to be 0.0562 mg a.s./L based on wet weight and mean measured concentrations.

Report: CA 8.2.2.1/2
Brausch J.M., 2015 a
Calculation of EC10 values and geomean for chronic fish studies with BAS 550 F (Dimethomorph)
2014/1224007
Guidelines: none
GLP: no

Executive Summary (recalculations)

The endpoint derived in the study with *Oncorhynchus mykiss* (BASF DocID DK-512-002) was the No Observable Effects Concentration (NOEC); however, in the newest EFSA Guidance Document (EFSA Journal 2013) on a tiered risk assessment for plant protection products for aquatic organisms, the EC_{10} value is clearly preferred. Therefore, a new statistical evaluation was conducted in order to obtain the EC_{10} values for the most sensitive endpoints body weight and length using Probit analysis.

The recalculated values (as mean measured concentrations) for the study are as follows:

Length: $EC_{10} > 0.897$ mg a.s./L
Body weight: $EC_{10} = 0.116$ mg a.s./L (0.069 – 0.197 mg a.s./L)

The following flow-through chronic toxicity study on the fathead minnow with the active substance dimethomorph was conducted for registration outside of Europe and has not been evaluated previously on EU level. The study is submitted for completeness. During the study deviation from the current guideline OECD 210 occurred in consequence of technical problems with the dilution system. Furthermore this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards.

Report:	CA 8.2.2.1/3 [redacted] 2002 a BAS 550 F - Early life-stage toxicity test on the Fathead minnow (Pimephales promelas) 2002/1011268
Guidelines:	OECD 210, EPA 72-4 (a), EPA 540/9-86-138
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 8.2.2.1/2 Brausch J.M., 2015 a Calculation of EC10 values and geomean for chronic fish studies with BAS 550 F (Dimethomorph) 2014/1224007
Guidelines:	none
GLP:	no

Executive Summary

The chronic toxicity of dimethomorph to fathead minnow (*Pimephales promelas*) was evaluated in a 34-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to nominal test item concentrations of 0.01, 0.033, 0.1, 0.33 and 1 mg dimethomorph/L (corresponding to mean measured concentrations of 0.0082, 0.0310, 0.107, 0.347 and 0.92 mg a.s./L). Hatchability, post-hatch survival rate, time to hatch and swim-up, and growth parameters of fathead minnow embryos were assessed throughout the study.

The results are based on nominal and additionally on mean measured concentrations. The embryo survival rate until hatch was statistically significantly decreased in comparison to the control group in the concentration group of 0.33 mg a.s./L according to Fisher´s exact test. The Wilcoxon-test revealed statistically significant decreases for the concentration groups of 0.01, 0.33 and 1 mg a.s./L. However, the slight decrease in the 0.01 mg a.s./L group (statistically significant only with one of the 2 statistical methods (Wilcoxon-test)) was not considered to be an test item related effect. The hatch rate in all test item concentrations was above the minimum value of 66% which acceptable for the control group according to the test guideline. Generally, a tendency towards a decreased survival until hatch was observed in the 2 highest test item concentration groups. Survival from hatch until the end of swim-up (day 6) was not statistically significantly decreased at up to and including the highest test item concentration group in comparison to the control group. For fish survival from end of swim-up (day 6) to the end of exposure (day 34), there was no statistically significant effect in comparison to the control group in any of the concentration groups. For survival during days 0 - 34 the survival rates of the concentration groups 0.01, 0.033 and 0.1 mg a.s./L were generally higher than in the control group. In the two highest tested concentrations (0.33 and 1.0 mg a.s./L) the survival over the whole exposure period was slightly lower than in the control group. The deviation was statistically significant only in the 0.33 mg a.s./L concentration group. The biological relevance is questionable due to the lack of a clear concentration-effect-relationship.

Hatch in the replicates of the control group and of all concentration groups started at day 3 of exposure and was completed one day later. Swim-up occurred simultaneously in the control and the concentration group on days 5 and 6 of exposure. No substance-related effects were observed on the time to hatch and swim-up in any of the concentration groups.

No signs of test item-related toxicity or abnormalities were observed in the control and in any of the test item treatment groups.

In comparison to the control group body weight and length were statistically significantly increased in the surviving animals of the 0.33 mg a.s./L group. This might be explained by the lower number of in the replicates of this group due to the increased mortality rate until hatch. An effect of the test substance is not plausible, since body weight and length in the highest test concentration group were comparable to the control.

In an early life stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (34 d) for dimethomorph was determined to be 0.1 mg a.s./L based on nominal concentrations and 0.107 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F, Reg. no.: 247 723), batch no. AC 9978-131, purity: 98.3%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), eggs less than 6 hours old, source: [redacted]

Test design: Flow through system (34 d); 5 test item concentrations plus a dilution water control, 4 replicates per treatment with 25 fertilized eggs in each. Eggs and larvae were exposed in cylindrical glass vessels (egg cups); on day 6 egg cups were removed from test aquaria. The test solution flowed continuously from the mixing tank into an "udder" which splitted the test water into 4 equal parts for the 4 replicate test aquaria. On day 34 fish were sacrificed and the body length and weight of surviving larvae were determined. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behavior.

Endpoints: NOEC values based on hatch rate, post-hatch survival, toxic signs, growth and time spans to hatch and swim-up; EC_{10} values based on length and wet weight (recalculation).

Test concentrations: Control (dilution water), 0.01, 0.033, 0.1, 0.33 and 1 mg dimethomorph/L (nominal), corresponding to mean measured concentrations of 0.0082, 0.0310, 0.107, 0.347 and 0.92 mg a.s./L.

Test conditions: Test vessels: stainless steel aquaria (29 x 21 x 22 cm), water volume: 9 L; egg cups: cylinders of transparent glass, diameter 6 cm, 10 cm high, with a stainless-steel grid (0.2 x 0.2 mm), submerged in each test vessel. Dilution water: non-chlorinated, filtered drinking water (diluted with deionized water); temperature 25 °C; pH 7.6 - 7.9; oxygen content 6.6 mg/L - 8.3 mg/L; total hardness: 90 - 98 mg $CaCO_3$/L; conductivity: 218 - 223 µS/cm; acid capacity: 1.94 - 2.06 mmol/L. Light intensity: 118 - 233 lux; photoperiod: 16 hours light : 8 hours dark; flow rates: 1.9 L/hour/test vessel. Feeding: freshly hatched *Artemia nauplii* and commercial fish diet (AZ 100, supplied by Tetra-Werke) from day 6 on. Slight aeration from day 21 on.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; Dunnett`s test for weight and length data, one-sided Fisher`s exact test for survival data, one-sided Wilcoxon-test for variability between replicates.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations at weekly intervals until day 34. Mean measured concentrations of dimethomorph ranged from 82% to 107% of nominal over the exposure period. The mean analytically determined concentration values were within a range of ± 20% of the nominal values. The following biological results are based on nominal and additionally on mean measured concentrations.

Biological results: The embryo survival rate until hatch was statistically significantly decreased in comparison to the control group in the concentration group of 0.33 mg a.s./L according to Fisher´s exact test ($p \leq 0.05$). The Wilcoxon-test revealed statistically significant decreases for the concentration groups of 0.01, 0.33 and 1 mg a.s./L ($p \leq 0.05$). However, the slight decrease in the 0.01 mg a.s./L group (statistically significant only with one of the 2 statistical methods(Wilcoxon-test)) was not considered to be an test item related effect. The hatch rate in all test item concentrations was above the minimum value of 66% which is acceptable for the control group according to the test guideline. Generally, a tendency towards a decreased survival until hatch was observed in the 2 highest test item concentration groups. Survival from hatch until the end of swim-up (day 6) was not statistically significantly decreased at up to and including the highest test item concentration group in comparison to the control group. For fish survival from end of swim-up (day 6) to the end of exposure (day 34), there was no statistically significant effect in comparison to the control group in any of the concentration groups.

For survival during days 0 - 34 the survival rates of the concentration groups 0.01, 0.033 and 0.1 mg a.s./L were generally higher than in the control group. In the two highest tested concentrations (0.33 and 1.0 mg a.s./L) the survival over the whole exposure period was slightly lower than in the control group. The deviation was statistically significant only in the 0.33 mg a.s./L concentration group (Fisher`s exact test; $p \leq 0.05$). The biological relevance is questionable due to the lack of a clear concentration-effect-relationship.
Hatch in the replicates of the control group and of all concentration groups started at day 3 of exposure and was completed one day later. Swim-up occurred simultaneously in the control and the concentration group on days 5 and 6 of exposure. No substance-related effects were observed on the time to hatch and swim-up in any of the concentration groups.

No signs of test item-related toxicity or abnormalities were observed in the control and in any of the test item treatment groups.

In comparison to the control group body weight and length were statistically significantly increased in the surviving animals of the 0.33 mg a.s./L group (Dunnett`s test; $p \leq 0.01$). This might be explained by the lower number of in the replicates of this group due to the increased mortality rate until hatch. An effect of the test substance is not plausible, since body weight and length in the highest test concentration group were comparable to the control. The results are summarized in Table 8.2.2.1-2: .

Table 8.2.2.1-2: Chronic toxicity of dimethomorph to fathead minnow (*Pimephales promelas*) in a fish early life stage test (34 d)

Concentration (nominal) [mg a.s./L]	**Control**	**0.01**	**0.033**	**0.1**	**0.33**	**1**
Concentration (mean measured) [mg a.s./L]	**Control**	**0.0082**	**0.0310**	**0.107**	**0.347**	**0.92**
Embryo survival until hatch [%]	85	80	87	81	**71** *	75
Survival of larvae from hatch until end of swim-up (day 6) [%]	96	100	100	100	100	100
Survival of young fish (day 6 - 34) [%]	94	99	98	95	99	99
Survival from day 0 to test termination (34 d) [%]	77	79	85	77	**70** *	74
Start of hatch [day] End of hatch [day]	3 5	3 5	3 5	3 5	3 5	3 5
Start of swim-up End of swim-up	5 6	5 6	5 6	5 6	5 6	5 6
Symptoms	none	none	none	none	none	none
Mean weight (34 d) [mg]	126	135	131	130	**147** **	120
% of control	100	107	104	103	117	96
Mean length (34 d) [cm]	2.40	2.50	2.50	2.46	**2.55** **	2.37
% of control	100	104	104	103	106	99

	Endpoints [mg dimethomorph/L]
$NOEC_{overall}$ (34 d) (nominal)	0.1
$NOEC_{overall}$ (34 d) (mean measured)	0.107
EC_{10} based on mean length (mean measured, recalculated)	> 0.92
EC_{10} based on mean wet weight (mean measured, recalculated)	> 0.92

Values printed in **bold** show statistically significant differences compared to the control, however biological relevance is questionable due to the lack of a clear concentration-effect-relationship.

* Statistically significant differences compared to the control (one-sided Fisher`s exact test and one-sided Wilcoxon-test, both $p \leq 0.05$).

** Statistically significant differences compared to the control (Dunnett`s test, $p \leq 0.01$).

III. CONCLUSION

In an early life stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (34 d) for dimethomorph was determined to be 0.1 mg a.s./L based on nominal concentrations and 0.107 mg a.s./L based on mean measured concentrations. The recalculated EC_{10} values based on mean length and mean wet weight are > 0.92 mg a.s./L (mean measured), respectively.

The following early life stage toxicity study on the sheepshead minnow with the active substance dimethomorph was conducted for registration outside of Europe and has not been evaluated previously on EU level. The study is submitted for completeness. Furthermore this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards.

Report:	CA 8.2.2.1/4 [redacted] 2010 a BAS 550 F: Early life-stage toxicity test with the sheepshead minnow, Cyprinodon variegatus, under flow-through conditions 2010/7012691
Guidelines:	EPA 850.1400
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 8.2.2.1/2 Brausch J.M., 2015 a Calculation of EC10 values and geomean for chronic fish studies with BAS 550 F (Dimethomorph) 2014/1224007
Guidelines:	none
GLP:	no

Executive Summary

The chronic toxicity of dimethomorph to sheepshead minnow (*Cyprinodon variegatus*) embryos and fry was investigated in a 40-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to dimethomorph at nominal concentrations of 0.065, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L (corresponding to mean measured concentrations of 0.0630, 0.136, 0.266, 0.536, 1.02 and 2.01 mg a.s./L). Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The biological results are based on mean measured concentrations. Egg hatch began on day 6 for the control, 0.0630 and 0.536 mg a.s./L treatments and on day 7 for the remaining treatment levels. Day 0 post-hatch (*i.e.,* ≥ 95% hatch in the control treatment) was determined to be study day 8. All test item treatments reached 95% hatch by study day 15. Hatch was completed in all treatment replicates between study days 8 and 16. Overall hatching success in the control was 84%. Hatching success in the test item treatments ranged from 84% to 91%. There was no statistically significant reduction in hatching success or time to start of hatch for any of the test item treatments as compared to the control. There was a statistically significant effect on the time to 95% hatch and time to completion of hatch in the 0.266, 0.536, 1.02 and 2.01 mg a.s./L test item treatments, as compared to the control.
Post-hatch survival was 98% in the control and between 89% and 99% in all test item treatments. No statistically significant effects on the post-hatch survival were observed for any of the test item treatments as compared to the control.

The mean standard fish lengths in the test item treatments ranged from 12.8 mm to 15.0 mm compared to 15.4 mm in the control treatment. The mean blotted wet weight was 0.110 g in the control and ranged from 0.065 g to 0.106 g in the test item treatments. There was a statistically significant reduction in length and blotted wet weight in the 0.266, 0.536, 1.02 and 2.01 mg a.s/L test item treatments as compared to the control.
No morphological abnormalities were observed during the exposure. Some fry (*i.e.,* < 2% of treatment population) were observed to be laying on the bottom of the chamber, and the fry exhibiting this behavior were present prior to study day 16 in the 0.266 and 2.01 mg a.s./L test item treatments. A single fish in the 0.536 mg a.s./L test item treatment displayed loss of equilibrium on study day 18. One fish surfacing was noted on study days 38 and 39 in three of the four replicates of the 2.01 mg a.s./L test item treatment. There were no other behavioral abnormalities observed during the exposure.

In an early life stage study with sheepshead minnow (*Cyprinodon variegatus*) the overall NOEC (40 d) for dimethomorph was determined to be 0.136 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F, Reg. no.: 247723), batch no. AC 9978-131, purity: 97.5%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*); unfertilized eggs and milt were collected from mature sheepshead minnow reared in-house.

Test design: Flow-through system (40 d); 6 test item concentrations plus a dilution water control; 4 replicate test chambers per treatment with 25 fertilized eggs in each; a proportional diluter system was used for the preparation of test solutions and intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. On study day 16 (*i.e.,* day 8 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Survival of the fry was monitored daily following hatch by visually inspecting each test chamber and any behavioral or physical changes were recorded, including abnormalities. On day 40 (32 days post hatch) surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatchability, survival, toxic signs and growth; EC_{10} values based on mean length and mean wet weight (recalculation).

Test concentrations:	Control (dilution water), 0.065, 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimethomorph/L (nominal), corresponding to mean measured concentrations of < MQL, 0.0630, 0.136, 0.266, 0.536, 1.02 and 2.01 mg a.s./L.
Test conditions:	Test vessels: glass aquaria (15 x 22 x 24 cm) with a test volume of approx. 5 L; glass incubation cups (used during embryo stage) with 9 cm diameter and Nitex® screen replacing the bottom; one incubation cup per test chamber; dilution water: commercial sea salt mix added to filtered and sterilized laboratory freshwater; water temperature 24.5 °C - 25.9 °C; pH 7.9 - 8.2; dissolved oxygen: 5.7 mg/L - 7.1 mg/L; salinity: 19 - 20‰; light intensity: ranged from 548 to 730 lux (day 26); photoperiod: 16 h light : 8 h dark (30-minute simulated dawn and dusk transition period); flow rate: 5.9 volume additions (*i.e.* 29 L) per test chamber over a 24 h period; feeding: fish larvae were fed *ad libitum* brine shrimp (*Artemia*) nauplii from day 6 onwards (one day following start of hatch), standard commercial fish food was added to the daily food beginning on day 23; fish were fed *ad libitum* at least three times daily during the week and at least twice a day on weekends.
Analytics:	Analytical verification of dimethomorph concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; outlier procedure: a studentized residuals outlier test on the individual standard length and blotted wet weight data was conducted prior to performing statistical analysis on these parameters. The test was conducted to identify the individual standard length and blotted wet weight measurements having standard deviations of >|2.0| from the remaining measurements for that treatment. For those measurements identified to be an outlier, both the standard length and blotted wet weight measurements were omitted (i.e., if the standard length measurement was an outlier and/or the blotted wet weight measurement was an outlier – the individual fish measurements for both parameters were removed from the data set); Fisher's exact test and/or ANOVA followed by Dunnett's test (p = 0.05); Probit analysis for recalculation of EC_{10} values based on mean length and mean wet weight.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of dimethomorph concentrations were conducted in samples of each treatment two days prior to test initiation, on study days 7, 14, 21, 28 and 35 and at test termination after 40 d of exposure. Measured concentrations of dimethomorph in the test item treatments prior to test initiation of the definitive test ranged from 94% to 112% of the nominal concentrations. Measured concentrations of dimethomorph in the test item treatments on day 0 of the exposure ranged from 81% to 113% of the nominal concentrations. On days 7 through 40, measured values were between 94% and 115% of nominal. The mean measured concentrations of dimethomorph in the test item treatments for the 40-day exposure ranged from 97% to 107% of the nominal concentrations. Overall, the concentrations of dimethomorph in all test item treatments were maintained within 20% of the mean measured concentrations throughout the exposure period. The following biological results are based on mean measured concentrations.

Biological results: Egg hatch began on day 6 for the control, 0.0630 and 0.536 mg a.s./L treatments and on day 7 for the remaining treatment levels. Day 0 post-hatch (i.e., ≥ 95% hatch in the control treatment) was determined to be study day 8. All test item treatments reached 95% hatch by study day 15. Hatch was completed in all treatment replicates between study days 8 and 16. Overall hatching success in the control was 84%. Hatching success in the test substance treatments ranged from 84% to 91%. There was no statistically significant (Dunnett`s test, p = 0.05) reduction in hatching success or time to start of hatch for any of the test item treatments as compared to the control. There was a statistically significant (Dunnett`s test, p = 0.05) effect on the time to 95% hatch and time to completion of hatch in the 0.266, 0.536, 1.02 and 2.01 mg a.s./L test item treatments, as compared to the control.
Post-hatch survival was 98% in the control and between 89% and 99% in all test item treatments. No statistically significant effects on the post-hatch survival were observed for any of the test item treatments as compared to the control (Dunnett`s test, $p = 0.05$).
The mean standard fish lengths in the test item treatments ranged from 12.8 mm to 15.0 mm compared to 15.4 mm in the control treatment (outliers omitted). The mean blotted wet weight was 0.110 g in the control and ranged from 0.065 g to 0.106 g in the test item treatments (outliers omitted). There was a statistically significant (Dunnett`s test, p = 0.05) reduction in length and blotted wet weight in the 0.266, 0.536, 1.02 and 2.01 mg a.s/L test item treatments as compared to the control.
No morphological abnormalities were observed during the exposure. Some fry (i.e., < 2% of treatment population) were observed to be laying on the bottom of the chamber, and the fry exhibiting this behavior were present prior to study day 16 in the 0.266 and 2.01 mg a.s./L test item treatments. A single fish in the 0.536 mg a.s./L test item treatment displayed loss of equilibrium on study day 18. One fish surfacing was noted on study days 38 and 39 in three of the four replicates of the 2.01 mg a.s./L test item treatment. There were no other behavioral abnormalities observed during the exposure. The results are summarized in Table 8.2.2.1-3:

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Table 8.2.2.1-3: Chronic toxicity of dimethomorph to sheepshead minnow (*Cyprinodon variegatus*) in an fish early life-stage test (40 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.065**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**Control**	**0.0630**	**0.136**	**0.266**	**0.536**	**1.02**	**2.01**
Hatching success [%]	84	88	85	86	90	84	91
Start of hatch [d]	7	7	7	7	7	7	8
Time to 95% hatch [d]	8	10	10	13*	12*	13*	14*
End of hatch [d]	9	11	11	14*	13*	14*	14*
32-day post-hatch survival [%]	98	98	94	93	91	99	89
Mean standard length on day 40 ± SD [mm] §	15.4 ± 1.1	14.7 ± 1.27	15.0 ± 1.19	14.4 ± 1.38*	14.2 ± 1.41*	13.7 ± 1.91*	12.8 ± 1.80*
% of control #	--	95	97	94	92	89	83
Mean blotted wet weight on day 40 ± SD [mm] §	0.110 ± 0.023	0.096 ± 0.025	0.106 ± 0.024	0.093 ± 0.027*	0.087± 0.027*	0.080 ± 0.032*	0.065 ± 0.031*
% of control #	--	87	96	85	79	73	59
Endpoint [mg dimethomorph/L] (mean measured)							
$NOEC_{overall}$ (40 d)	0.136						
Recalculated endpoints [mg dimethomorph/L] (mean measured)							
EC_{10} (mean length)	0.759						
EC_{10} (mean wet weight)	0.150						

SD - standard deviation
* Statistically significant differences compared to the control (Dunnett's test; p = 0.05).
§ Statistical outliers were omitted prior to determining replicate/treatment means and standard deviations.
# Calculated on the basis of the mean values.

III. CONCLUSION

In an early life stage study with sheepshead minnow (*Cyprinodon variegatus*) the overall NOEC (40 d) for dimethomorph was determined to be 0.136 mg a.s./L based on mean measured concentrations. The recalculated EC_{10} values based on mean length and mean wet weight are 0.759 and 0.150 mg a.s./L (mean measured), respectively.

A chronic toxicity study with rainbow trout (*Oncorhynchus mykiss*) conducted with dimethomorph was already evaluated during the previous Annex I inclusion process. However, this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

Report:	CA 8.2.2.1/5 [redacted] 1993 a Toxicity of Dimethomorph to rainbow trout (Oncorhynchus mykiss) in a prolonged flow-through test (21 days) DK-512-001
Guidelines:	OECD 204
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie und Bundesangelegenheiten, Wiesbaden)

[and see KCA 8.2.2.1/7 DK-123-082]

Executive Summary (originally submitted study)

In a prolonged (21 d) flow-through test juvenile rainbow trouts (*Oncorhynchus mykiss*) were exposed to nominal concentrations of 0 (control and solvent control), 0.015, 0.048, 0.15, 0.48 and 1.53 mg a.s./L in groups of 10 animals in each treatment and control group. Fish were observed for survival and signs of toxicity daily during the test.

The biological results are based on nominal concentrations. After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of 0.048, 0.15 and 0.48 mg a.s./L, whereas 10% mortality occurred in the solvent control and in the test item concentrations 0.015 and 1.53 mg a.s./L. There was no test substance related, statistically significant effect on survival or growth during the test. In the highest treatment group symptoms such as reduced food intake, irregular swimming behavior and body discoloration were common.

In a prolonged flow-through toxicity study with *O. mykiss* the NOEC (21 d) of dimethomorph was determined to be 0.48 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph; Batch Number DW 11/86; purity: 95.6%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); Origin: [redacted]
Test design:	Flow-through system (21 h); 5 test item concentrations plus a dilution water control and a solvent control, 10 fish per treatment, test water continually flowed into the mixing vessels and then into the test vessels, the number of live and dead fish and symptoms of intoxication were recorded at least daily during the test, growth at the end of the test.
Endpoints:	NOEC, mortality and symptoms of intoxication
Test concentrations:	Control (dilution water); solvent control (0.1 mL/L acetone), 0.015, 0.048, 0.15, 0.48, and 1.53 mg a.s./L
Test Conditions:	Temperature: 13.0 - 14.6 ºC, pH: 7.5 - 8.1, dissolved oxygen: 6.5 mg/L, photoperiod: 16 hours light:8 hours dark, light intensity: 50 – 300 lux
Analytics:	Analytical verification of test item concentrations was conducted using HPLC method (Method RU 151/35/90).
Statistics:	Descriptive statistics, Differences in weights and lengths were evaluated using William's Test.

II. RESULTS AND DISCUSSION

Analytical measurements: Measured concentrations of dimethomorph during the 21-day flow-through test ranged from 89.6 to 116.9% of the targeted nominal concentrations. Therefore, results of the test were based on nominal exposure levels.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of 0.048, 0.15 and 0.48 mg a.s./L, whereas 10% mortality occurred in the solvent control and in the test item concentrations 0.015 and 1.53 mg a.s./L. There was no test substance related, statistically significant effect on survival or growth during the test. In the highest treatment group symptoms such as reduced food intake, irregular swimming behavior and body discoloration were common. The result are summarized in Table 8.2.2.1-4: .

Table 8.2.2.1-4: Prolonged toxicity (21 h) of dimethomorph to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.015**	**0.048**	**0.15**	**0.48**	**1.53**
Mortality after 21 d [%]	0	0	10	0	0	0	10
Symptoms after 21 d [%] [1)]	0	0	10	0	0	0	70*
Mean length [cm]	6.4	6.2	6.0	6.7	6.6	6.2	5.8
Mean weight [g]	2.5	2.1	2.0	2.9	2.7	2.3	1.9
Endpoints [mg a.s./L] (nominal)							
$NOEC_{overall}$	0.48						

1) Symptoms of intoxication included stong ventilation, change in body color, fish swimming mainly on the surface of the water, reduced food intake, irratic swimming, and fish mainly on the bottom.
* Significantly different from controls

III. CONCLUSION

In a prolonged flow-through toxicity study with *O. mykiss* the NOEC (21 d) of dimethomorph was determined to be 0.48 mg a.s./L based on nominal concentrations.

Report: CA 8.2.2.1/6
Brausch J.M., 2014 a
Calculation of EC10 values for a 21-day chronic fish study with BAS 550 F (Dimethomorph)
2014/1224005
Guidelines: none
GLP: no

Executive Summary (recalculations)

The endpoint derived in the study with *Oncorhynchus mykiss* (BASF DocID DK-512-001) was the No Observable Effects Concentration (NOEC); however, in the newest EFSA Guidance Document (EFSA Journal 2013) on a tiered risk assessment for plant protection products for aquatic organisms, the EC_{10} value is clearly preferred. A statistical recalculation of the EC_{10} was not conducted as no endpoints had a greater than 10% difference from the controls. However, due to the lack of effect an EC_{10} could be estimated for the endpoints mortality, body weight and length.

The estimated values (as mean measured concentrations) for the study are as follows:

Mortality: $EC_{10} > 1.53$ mg a.s./L
Length: $EC_{10} > 1.53$ mg a.s./L
Body weight: $EC_{10} > 1.53$ mg a.s./L

CA 8.2.2.2 Fish full life cycle test

The chronic toxicity to fish is fully addressed by three early life stage studies, a fish short term reproduction assay and a chronic study (21 days) with rainbow trout. The last-named study was already evaluated during the previous Annex I inclusion process and summaries for the other studies are provided above. No additional fish full life cycle study is required and no (new) study has been conducted.

CA 8.2.2.3 Bioconcentration in fish

A bioconcentration study is not required for registration in the EU since the $logP_{ow}$ is smaller than 3, consequently the following study has not been evaluated previously on EU level. The bioconcentration study was performed with dimethomorph and bluegill sunfish for registrations outside of Europe. However, the study is submitted for completeness.

Report:	CA 8.2.2.3/1 [redacted] 1999 a Dimethomorph (AC 336379): Uptake, depuration, bioconcentration and metabolism of carbon-14 labeled AC 336379 in bluegill sunfish (Lepomis macrochirus) under flow-through conditions DK-519-001
Guidelines:	EPA 850.1730, OECD 305
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The objectives of this study were to determine the bioaccumulation potential and metabolic fate of dimethomorph in bluegill sunfish, to quantitate and identify the metabolites of dimethomorph in the edible and inedible portions of the fish, and to quantitate and identify the dimethomorph at nominal concentrations of 0.020 and 0.200 mg a.s./L (corresponding to mean measured test concentrations of 0.021 and 0.210 mg a.s./L).

In a flow-through bioconcentration study, bluegill sunfish were exposed to ^{14}C-dimethomorph at nominal concentrations of 0.020 mg/L and 0.200 mg/L (corresponding to mean measured concentrations of 0.021 mg/L and 0.210 mg/l). Steady state was achieved by day 14 of the uptake period. The computer generated dimethomorph BCF_k values for whole fish tissue, based on analysis of water, fillet, and viscera samples at selected intervals, were estimated to be 2.4 in the treatment group B (0.021 mg a.s./L) and 3.2 in the treatment group C (0.210 mg a.s./L).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F; equivalent to AC 336379; Reg. no.: 247723), batch no. AC9978-68A, purity: 97.6%.

^{14}C-Dimethomorph (^{14}C-BAS 550 F, equivalent to AC 336379; Reg. no.: 247 723), batch no. AC1166-5, radiochemical purity: 97.6%, specific activity: 3.86 µCi/mg, label: p-chlorophenyl-U-^{14}C.

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*), juveniles (less than one year old), mean total length of 60 (± 5.2) mm and a mean blotted wet weight of 4.044 g (± 1.150 g) at study initiation; source: [redacted]

Test design: Flow-through test (42-day) consisting of a 28-day exposure period followed by a 14-day depuration period conducted on two treatment groups plus a solvent control group, during depuration period the addition of DMF and of ^{14}C-test item was discontinued; no replicates, 120 fish in each treatment group aquaria; at test initiation and twice daily fish were observed for mortality and adverse behavior. Radioanalysis of fish tissue was performed periodically during the definitive test; fish tissues were extracted for analysis on days 7, 14, 21 and 28 of the uptake phase and on days 1 and 3 of the depuration phase; all fish were dissected into fillet/edible (body, muscle, skin, skeleton) and viscera/non-edible (fins, head, internal organs). Whole fish residues were calculated from the mean percent contribution of fillet and viscera to whole fish for each treatment group as determined from each sampling day of the definitive test.

Endpoints: Uptake, depuration, bioconcentration, and metabolism.

Test concentrations: Solvent control (0.1 mL DMF/L), 0.020 mg a.s./L (treatment group B) and 0.200 mg a.s./L (treatment group C) (nominal), corresponding to mean measured test concentrations of 0.021 and 0.210 mg a.s./L.

Test conditions: 90 L glass aquaria, 70 L test volume; dilution water: well water; flow-through-system: turnover rates ranged from 5.2 to 6.0 volume additions per day; temperature: 21 - 22 °C, pH: 7.8 - 8.3, dissolved oxygen: 6.3 mg/L - 8.5 mg/L, hardness: 260 mg/L - 280 mg $CaCO_3$/L; diet: calculated amount of Salmon Starter (approximately 2% of their weight) daily.

Analytics: The test item concentrations and ^{14}C-residues in water were analyzed using HPLC with UV detection. Radioactivity measurements and TRR (total radioactive residues) analyses were made using Liquid Scintillation Counting (LSC).

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Lipid content of fish

The lipid content of whole fish for day 28 of exposure was calculated from the mean percent contribution of fillet and viscera to whole fish for each treatment group. The lipid content of bluegill sunfish was 8.41 % and 8.07% for whole fish in treatment groups B and C, respectively. The mean percent lipid value was 7.07% for whole fish at day 0 and 7.31% for whole fish in treatment group A at day 28 of exposure.

Total Radioactive Residues (TRR) in Water and Fish and daily bioconcentration factors

The concentration of the test substance in the treated aquaria was maintained within ± 20% of the mean measured values during the uptake phase. The mean measured water TRR concentrations during the 28-day exposure were 0.021 ± 0.52 mg a.s./L and 0.210 ± 0.0 mg a.s./L for treatment groups B and C, respectively.

The mean percent contributions of fillet and viscera to whole fish residues were 55% and 45% for treatment group B, and 54% and 46% for treatment group C, respectively.

Based on the TRR in the whole fish during the uptake phase, steady state was achieved by day 14 of the uptake period.

Radioanalysis of fish tissue was performed periodically during the definitive test. Daily TRR in whole fish during the exposure period ranged from 100 µg/kg to 380 µg/kg and from 1200 µg/kg to 5600 µg/kg for treatment groups B and C, respectively. Daily TRR in fillet tissue during the exposure period ranged from 42 µg/kg to 97 µg/kg and from 550 µg/kg to 1000 µg/kg for treatment groups B and C, respectively. Daily TRR in the viscera tissue during the exposure period ranged from 170 µg/kg to 730 µg/kg and from 550 µg/kg to 1000 µg/kg for treatment groups B and C, respectively.

After 14-day depuration, the TRR in fillet, viscera and whole fish was below the nominal limits of detection (NLD; >99% depuration) for treatment Group B and 41 µg/kg in fillet and in viscera (96% depuration) (98% depuration) and 100 µg/kg in whole fish (98% depuration) for treatment Group C.

Table 8.2.2.3-1: Total radioactive residues as ^{14}C- dimethomorph in test water and fish tissue (group B, 0.020 mg/L nominal concentration) during 28 days exposure and 14 days depuration with bluegill sunfish (*Lepomis macrochirus*)

Total ^{14}C Concentration (µg/L or µg/kg) as ^{14}C- dimethomorph								
Study Day	Water		Fillet		Viscera		Whole Fish	
	TRR (µg/L)	dimethomorph (µg/L) E/Z	TRR (µg/kg)	TRR BCF *	TRR (µg/kg)	TRR BCF *	TRR (µg/kg)	TRR BCF *
Exposure								
0	21	8.33/ 10.71	---	---	---	---	---	---
0.17	20	---	42	2.1	170	8.5	100	5.0
0.5	20	---	55	2.8	330	17	180	9.0
1	21	---	63	3.0	450	21	240	11
2	21	---	68	3.2	550	26	280	13
3	21	---	67	3.2	340	16	190	9.0
7	21	9.57/ 10.37	76	3.6	560	27	290	14
14	21	9.03/10.43	97	4.6	730	35	380	18
21	20	8.11/ 10.07	83	4.2	520	26	280	14
28	20	8.66/ 10.29	89	4.5	690	35	360	18
Depuration								
1	<NLD	<NLD	26	---	370	---	180	---
3	<NLD	<NLD	<NLD	---	250	---	110	---
7	<NLD	---	<NLD	---	110	---	50	---
10	<NLD	---	<NLD	---	<NLD	---	<NLD	---
14	<NLD	---	<NLD	---	<NLD	---	<NLD	---

NLD = Nominal Limit of Detection; --- = Not Determined
E = E isomer; Z = Z Isomer
* Daily bioconcentration factors were obtained by dividing the fish tissue concentration by the daily water concentration for the respective sampling day.

Table 8.2.2.3-2: Total radioactive residues as ^{14}C-dimethomorph in test water and fish tissue, (group C, 0.200 mg/L nominal concentration) during 28 days exposure and 14 days depuration with bluegill sunfish (*Lepomis macrochirus*)

Total ^{14}C Concentration (µg/L or µg/kg) as ^{14}C- dimethomorph								
Study Day	Water		Fillet		Viscera		Whole fish	
	TRR (µg/L)	dimethomorph (µg/L) E/Z	TRR (µg/kg)	TRR BCF *	TRR (µg/kg)	TRR BCF *	TRR (µg/kg)	TRR BCF *
Exposure								
0	210	99.82/ 102.71	---	---	---	---	---	---
0.17	210	---	550	2.6	550	9.5	1200	5.7
0.5	210	---	630	3.0	630	20	2300	11
1	210	---	590	2.8	590	21	2300	11
2	210	---	840	4.0	840	41	4400	21
3	210	---	810	3.9	810	26	3000	14
7	210	95.69/ 103.79	940	4.5	940	33	3700	18
14	210	97.74/ 103.14	960	4.6	960	38	4200	20
21	210	98.58/ 105.61	930	4.4	930	33	3700	18
28	210	97.28/ 103.10	1000	4.8	1000	52	5600	27
Depuration								
1	0.29	<NLD	300	---	300	---	2700	---
3	<NLD	<NLD	140	---	140	---	1300	---
7	<NLD	---	73	---	73	---	170	---
10	<NLD	---	65	---	65	---	170	---
14	<NLD	---	41	---	41	---	100	---

NLD = Nominal Limit of Detection; --- = Not Determined
E = E isomer; Z = Z Isomer
* Daily bioconcentration factors were obtained by dividing the fish tissue concentration by the daily water concentration for the respective sampling day.

BCF_k (bioconcentration factor calculated from kinetic rate constants)

To determine the TRR BCF_k (bioconcentration factor calculated from kinetic rate constants) values for treatment groups B and C, the whole fish data was subjected to estimating the K_1, K_2, and steady-state values by hand-calculation. Hand-calculated TRR BCF_k values were 14 and 20 for treatment groups B and C, respectively.
Additionally, BIOFAC modelling computer program was used to analyze the calculated whole fish uptake/depuration data for treatment groups B and C. The BIOFAC calculated TRR BCF_k values of 16 and 22 were 114% and 110% of the hand-calculated TRR BCF_k values for treatment groups B and C, respectively. The BIOFAC results, based on TRR concentrations and parent (dimethomorph) concentrations are presented in Table 8.2.2.3-3 and Table 8.2.2.3-4, respectively.

Table 8.2.2.3-3: BIOFAC results based on the TRR concentrations

Treatment group (mean measured conc.)	B (0.021 mg a.s/L)	C (0.210 mg a.s./L)
K_1, Uptake rate constant [ppb fish / ppb water / Day]	12	15
K_2, Depuration rate constant [Day^{-1}]	0.73	0.70
Time for 50% depuration[days]	0.95	0.99
Time for 95% depuration [days]	4.1	4.3
TRR Bioconcentration Factor (BCF_k)	16	22
Time to 90% Steady-State [days]	3.1	3.3
Lipid Content Corrected BCF (BCF_{SS})	190	270

Table 8.2.2.3-4: BIOFAC results based on the parent (dimethomorph) concentrations

Treatment group (mean measured conc.)	B (0.021 mg a.s/L)	C (0.210 mg a.s./L)
K_1, Uptake rate constant [ppb fish / ppb water / Day]	3.6	6.8
K_2, Depuration rate constant [Day^{-1}]	1.5	2.1
Time for 50% depuration [days]	0.46	0.32
Time for 95% depuration [days]	2.0	1.4
TRR Bioconcentration Factor (BCF_k)	2.4	3.2
Time to 90% Steady-State [days]	1.5	1.1
Lipid Content Corrected BCF (BCF_{SS})	29	40

The BIOFAC data demonstrated a rapid depuration for dimethomorph derived-radioactivity in bluegill sunfish. In general, there was no difference observed in the kinetics for dimethomorph-derived radioactivity from the data of the two treatment groups.

Identification and Characterization of Metabolites

Selected water samples were analyzed by HPLC. The TRR concentration of the unaltered parent was steady from Day 0 through Day 28 (210 µg/L).

Selected fillet and viscera samples were analyzed by HPLC.

HPLC/^{14}C analysis revealed that the radiometabolite profiles of the residues extracted from the aquarium water, fillet and viscera were not qualitatively similar. Dimethomorph and metabolites CL 900986 (4-hydroxy-3-methoxyphenyl analog) and CL 900987 (3-hydroxy-4-methoxylphenyl analog) were the significant components of the fillet residue. CL 1010861 and CL 1010862, corresponding to the O-glucuronide conjugates of CL 900986 and CL 900987, were the major metabolites in the viscera. CL 1010863 and CL 1010864, corresponding to the sulfate conjugates of CL 900986 and CL 900987, were minor metabolites in the viscera. It was estimated that about 80% of these polar conjugates was due to the glucuronide conjugates and about 20% was due to the sulfate conjugates, based on the result of enzyme hydrolysis. The quantitation of the metabolites in whole fish was determined from the analysis of the fillet and viscera.
The range of the percent contribution and TRR levels for dimethomorph and the metabolites during the steady-state period (uptake day-14 to day-28) for the treatment groups B and C for whole fish are summarized in Table 8.2.2.3-5. Dimethomorph was the predominant component in the aquarium water, and the amount of metabolites that were excreted into the water by the fish was very little.

Table 8.2.2.3-5: Quantitative distribution of ^{14}C-dimethomorph -derived residues in whole fish

Component	[^{14}C] dimethomorph treatment day-14 to day-28 (steady-state) for whole fish			
	Group B, 0.021 mg a.s/L		Group C, 0.210 mg a.s./L	
	%	ppb	%	ppb
dimethomorph	12.8 to 15.1	43 to 51	14.2 to 16.4	572 to 799
CL 900986	1.8 to 4.0	7 to 13	2.5 to 5.9	103 to 255
CL 900987	5.8 to 7.5	21 to 25	7.6 to 10.6	310 to 443
CL 1010861 and CL 1010862 glucuronide conjugates [a] and CL 1010863 and CL 1010864 sulfate conjugates [b]	59.3 to 70.2	166 to 267	56.7 to 61.6	2098 to 3447
3 unidentified polar metabolites	1.8 to 6.1	7 to 23	4.3 to 4.4	164 to 240
5 unidentified non polar metabolites	2.5 to 6.4	11 to 18	4.3 to 5.9	220 to 242

[a] Estimated that about 80% was due to the glucuronide conjugates.
[b] Estimated that about 20% was due to the sulfate conjugates.

III. CONCLUSION

In a flow-through bioconcentration study, bluegill sunfish were exposed to ^{14}C-dimethomorph at nominal concentrations of 0.020 mg/L and 0.200 mg/L (corresponding to mean measured concentrations of 0.021 mg/L and 0.210 mg/l). Steady state was achieved by day 14 of the uptake period. The computer generated dimethomorph BCF_k values for whole fish tissue, based on analysis of water, fillet, and viscera samples at selected intervals, were estimated to be 2.4 in the treatment group B (0.021 mg a.s./L) and 3.2 in the treatment group C (0.210 mg a.s./L).

CA 8.2.3 Endocrine disrupting properties

The following short-term reproduction assay on the fathead minnow with the active substance dimethomorph has not been evaluated previously on EU level.

Report:	CA 8.2.3/1 [redacted] 2014 a BAS 550 F (Dimethomorph) - Fish short term reproduction assay on the fathead minnow (Pimephales promelas) 2014/1187237
Guidelines:	OECD 229, EPA 890.1350
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 8.2.3/2 Kusebauch B., 2015 a Concentration control analysis of BAS 550 F, Reg.No.247723 in mixing-water, GV/T Project No.83F0271/01E008 2014/1221163
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The potential endocrine activity of dimethomorph in fathead minnow (*Pimephales promelas*) was evaluated in a 21-day short-term reproduction assay under flow-through conditions. Fish were exposed to a dilution water control and to nominal test item concentrations of 0.047, 0.15 and 0.48 mg dimethomorph/L (equivalent to mean measured concentrations of 0.046, 0.143 and 0.488 mg dimethomorph/L). The evaluated endpoints were survival, fecundity, fertilization success, nuptial tubercle score, fish weight and length, blood plasma vitellogenin (VTG) concentration, histological examination of gonadal tissues as well as behavior and appearance, including secondary sexual characteristics.

The biological results are based on mean measured concentrations. Percent survival of males, females and the survival rates based on combined data sets in the control were 100%. No statistically significant effects on fish wet weight and total length compared to the control were observed at any test concentration. The vitellogenin concentration in the blood plasma of male fish in comparison to the control group was statistically significantly increased in the 0.15 mg a.s./L test group only by using the Wilcoxon test. However, the median vitellogenin values of the males were not dose-dependently altered. The percentage of fertilized eggs in the control and all test item concentrations was 99.8%. The mean number of eggs per female per day was not statistically significantly different in comparison to the control group. Tubercles were not observed in females; therefore, they were not scored. For males, no statistically significant differences in mean tubercle scores compared to the control were determined in any test concentration. Furthermore, no notable abnormalities were observed with regards to behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad. Dimethomorph did not demonstrate any (anti-)estrogenic or (anti-)androgenic potential when tested at the maximum tolerated dose in fathead minnow.

In a short-term reproduction assay with fathead minnow (*Pimephales promelas*), the overall NOEC (21 d) for dimethomorph was determined to be ≥ 0.488 mg a.s./L based on mean measured concentrations. Dimethomorph did not demonstrate any (anti-) estrogenic or (anti-)androgenic potential when tested at the maximum tolerated dose in fathead minnow.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F, Reg. no. 247 723), batch no. COD-001646, purity: 99.7%, ratio E-/Z-isomers 41:59.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), obtained from an in-house laboratory supply of reproductively mature animals (approx. 5 months old at test initiation, in spawning condition). Fish stock originally from "[redacted]

Test design: Flow-through system (21 d); 3 test item concentrations plus a dilution water control, 4 replicates per treatment with four female and two male fish in each, a proportional diluter system was used for continuous introduction of the solutions to the test chambers. Wet weight of fish was determined at test initiation. During the exposure period, survival, toxic signs (appearance and behavior), fecundity and fertilization success were assessed daily. At test termination, fish were sacrificed, measured for total length and wet weight and observed for secondary sexual characteristics. Blood samples were taken for plasma vitellogenin (VTG) analysis. Fish were preserved for subsequent tubercle scoring.

Endpoints: NOEC values based on survival (male, female and combined), toxic signs (behavior and appearance, including secondary sex characteristics), fecundity (number of eggs/female/day), fertilization success, nuptial tubercle score, vitellogenin (VTG) concentration (male and female), histological examination of gonadal tissues and weight and length (male and female).

Test concentrations: Control (dilution water), 0.047, 0.15 and 0.48 mg dimethomorph/L (nominal); equivalent to mean measured concentrations of 0.046, 0.143 and 0.488 mg dimethomorph/L.

Test conditions:	9 L stainless steel aquaria equipped with two stainless steel spawning tiles; dilution water: aerated non-chlorinated drinking water additionally purified through a charcoal filter, mixed with deionized water and sanitized by UV treatment; flow rate: ≥ 2.25 L/h (6 volume additions per 24 hours); biological loading: ≤ 0.2 g fish/L/day at the end of exposure; water temperature: 24.8 °C - 25.8 °C; pH: 7.8 - 8.1; dissolved oxygen content: 6.1 - 7.6 mg/L; total hardness: 105 - 111 mg $CaCO_3$/L; conductivity: 264 - 279 µS/cm; light intensity: approx. 144 - 251 lux; photoperiod: 16 h light : 8 h dark; feeding: fish were fed twice a day with commercial diet (Tetramin, Tetra-Werke, Germany) and with live brine shrimp nauplii (*Artemia salina*) and frozen brine shrimp ad libitum. Feeding was withdrawn one day before the end of exposure.
Analytics:	Analytical verification of dimethomorph concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics; Dunnett's Multiple Comparison Test and/ or Williams Test for weight and length data; Wilcoxon Test and Jonckheere-Terpstra Test for fecundity, fertilization success, VTG and tubercle score data.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of dimethomorph concentrations in the application solutions of each treatment were conducted at test initiation, day 7 and 14 and at test termination. Measured concentrations of dimethomorph ranged from 80.7% to 92.7% of nominal concentrations at test initiation and from 110.7% to 115.2% of nominal at test termination. The mean recovery rates over the whole study period were 98.9%, 95.0% and 101.6% of nominal in the test concentrations of 0.047, 0.15 and 0.48 mg a.s./L, respectively. As analytical data confirmed correct application of the test item, the following biological results are based on mean measured concentrations.

Biological results: Percent survival of males, females and the survival rates based on combined data sets in the control were 100%. No statistically significant effects on fish wet weight and total length compared to the control were observed at any test concentration (Dunnett's Multiple Comparison Test, $p \leq 0.05$). The vitellogenin concentration in the blood plasma of male fish in comparison to the control group was statistically significantly increased in the 0.15 mg a.s./L test group only by using the Wilcoxon test ($p < 0.05$). However, the median vitellogenin values of the males were not dose-dependently altered. The percentage of fertilized eggs in the control and all test item concentrations was 99.8%. The mean number of eggs per female per day was not statistically significantly different in comparison to the control group (Jonckheere-Terpstra Test, $p < 0.05$). Tubercles were not observed in females; therefore, they were not scored. For males, no statistically significant differences in mean tubercle scores compared to the control were determined in any test concentration. Furthermore, no notable abnormalities were observed with regards to behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad. Dimethomorph did not demonstrate any (anti-)estrogenic or (anti-)androgenic potential when tested at the maximum tolerated dose in fathead minnow. The results are summarized in Table 8.2.3-1.

Table 8.2.3-1: Chronic toxicity and endocrine activity of dimethomorph to fathead minnow (*Pimephales promelas*) in a short-term reproduction assay (21 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.047**	**0.15**	**0.48**
Concentration [mg a.s./L] (mean measured)	**Control**	**0.046**	**0.143**	**0.488**
Male survival [%]	100	100	100	100
Female survival [%]	100	100	100	100
Combined (male& female) survival [%]	100	100	100	100
Fertilization success [% fertilized eggs]	99.8 ± 0.00	99.8 ± 0.02	99.8 ± 0.04	99.8 ± 0.01
Fecundity [number of eggs/female/day]	18.2 ± 4.5	18.1 ± 5.9	19.5 ± 4.3	17.7 ± 2.4
Mean male total length on day 21 [cm]	5.1 ± 0.2	5.5 ± 0.2	5.5 ± 0.2	5.4 ± 0.3
Mean female total length on day 21 [cm]	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.0
Mean male wet weight on day 21 [g]	2.18 ± 0.20	2.46 ± 0.37	2.65 ± 0.09	2.42 ± 0.40
Mean female wet weight on day 21 [g]	1.02 ± 0.02	0.97 ± 0.10	0.98 ± 0.06	1.04 ± 0.05
Mean male GSI [%] #	--	--	--	--
Mean female GSI [%] #	--	--	--	--
Male VTG concentration [ng/mL]	869.9	10964	3394.6 *	920.8
Female VTG concentration [ng/mL]	6.98 x 10^6	7.19 x 10^6	6.61 x 10^6	7.91 x 10^6
Male median tubercle score	13.9	15.8	16.4	15.9
Symptoms +	none	none	none	none
	Endpoints [mg dimethomorph/L] (mean measured)			
$NOEC_{overall}$ (21 d)	≥ 0.488			

GSI = Gonadal Somatic Index (gonad weight/body weight x 100)

\# The preserved gonads were not evaluated, since the results did not indicate a high necessity of this evaluation.

* Statistically significantly different compared to the control (Wilcoxon Test, $p < 0.05$) and not considered to be a substance-related effect.

\+ Symptoms: fish were observed for behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad.

III. CONCLUSION

In a short-term reproduction assay with fathead minnow (*Pimephales promelas*), the overall NOEC (21 d) for dimethomorph was determined to be ≥ 0.488 mg a.s./L based on mean measured concentrations. Dimethomorph did not demonstrate any (anti-) estrogenic or (anti-)androgenic potential when tested at the maximum tolerated dose in fathead minnow.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

The following study performed with the active substance dimethomorph was not listed in the "Application" document submitted for the dimethomorph AIR 3 renewal process.

An acute toxicity study with *Daphnia magna* conducted with dimethomorph was already evaluated during the previous Annex I inclusion process. The endpoint of this study is based on nominal concentrations. However measured concentrations of dimethomorph in the test solutions ranged from 5.8% to 64.0% of the targeted nominal concentrations at test initiation and from 5.8% to 89.6% at test termination. Therefor the study deviate from the current guideline OECD 202. Due to the variable analytical results, the results from the study have been re-evaluated based on measured concentrations and current standards. For a detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

Report:	CA 8.2.4.1/1 Ellgehausen H., 1986 c 48-hour acute toxicity of CME 151 to Daphnia magna (OECD immobilization test) DK-521-002
Guidelines:	EPA 72-2, OECD 202 Part I (1984)
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Executive Summary (originally submitted study)

In a static acute (48 h) toxicity laboratory study, daphnids were exposed in 50 mL beakers containing 20 mL test medium to nominal concentrations of 0 (control and solvent control), 7.813, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 mg a.s./L in groups of 10 animals with two replicates for each treatment and control group. Immobilization data were determined after 24 and 48 h.

The biological results are based on nominal concentrations. After 48 hours of exposure, no immobilization was observed in the control and at test item concentrations of 7.813 mg a.s./L, whereas 15%, 25%, 20%, 70 and 95% immobilization occurred in the solvent control and in the test item concentrations 15.625, 31.25, 62.5 and 125 mg a.s./L, respectively. In the three highest test concentrations 100% immobilization occured.

In a static acute toxicity study with *Daphnia magna* the EC_{50} (48 h) value of dimethomorph was 48.87 mg a.s./L based on nominal concentrations. The NOEC was determined to be 7.813 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as CME 151); Batch Number DW 11/86; purity: 94.8%.

B. STUDY DESIGN

Test species:	*Daphnia magna;* Origin: In-house cultures of RCC Laboratories, Itingen, Switzerland
Test design:	Static system (48 hours), 8 test item concentrations plus control (dilution water) and a solvent control (0.01% Tween 80), 2 replicates with 10 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	NOEC and EC_{50} based on immobility of daphnids.
Test concentrations:	0 (control), 0 (solvent control), 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 mg a.s./L
Test conditions:	Temperature: 20 $\pm$ 2 °C; pH: 8.2 - 8.4; dissolved oxygen: 8.4 -8.7 mg/L; photoperiod: 100% dark;
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC method.
Statistics:	Descriptive statistics, EC_{50} values were estimated using the Logit Method

II. RESULTS AND DISCUSSION

Analytical measurements: The measured concentrations in the test solutions were significantly lower than the nominal concentrations. The measured concentrations were lower due to that fact that only clear solutions were analyzed and the water solubility of dimethomorph was exceeded in the higher test concentrations

Biological results: After 48 hours of exposure, no immobilization was observed in the control and at test item concentrations of 7.813 mg a.s./L, whereas 15%, 25%, 20%, 70 and 95% immobilization occurred in the solvent control and in the test item concentrations 15.625, 31.25, 62.5 and 125 mg a.s./L, respectively. In the three highest test concentrations 100% immobilization occured. For results see Table 8.2.4.1-1

Table 8.2.4.1-1: Effects of BAS 550 F on *Daphnia magna* mobility

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**7.81**	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**	**1000**
Immobility (24 h) [%]	0	0	0	0	0	10	55	90	100	100
Immobility (48 h) [%]	0	15	0	25	20	70	95	100	100	100
	Endpoints [mg BAS 550 F/L] nominal)									
EC_{50} (48 h)	48.87									
NOEC (48 h)	7.813									

III. CONCLUSION

In a static acute toxicity study with *Daphnia magna* the EC_{50} (48 h) value of dimethomorph was 48.87 mg a.s./L based on nominal concentrations. The NOEC was determined to be 7.813 mg a.s./L (nominal).

Report: CA 8.2.4.1/2
Habekost M., 2010 d
BAS 550 F - Re-calculation of toxicity values for study DK-521-002
2010/1177164
Guidelines: none
GLP: no

The EC_{50} endpoint derived in the study with *Daphnia magna* (BASF DocID DK-521-002) was determined based on nominal concentrations only. A statistical recalculation of the EC_{50} value was conducted based on mean measured and calculated mean measured concentrations (3.6, 11.8, 19.0, 27.3, 35.9, 42.7, 49.9 and 58.0 mg a.s./L). The statistical determination was done by probit analysis using linear minimum likelihood regression.

Immobilization: EC_{50} = 20.1 mg a.s./L (95% confidence limits: 14.4 – 25.1 mg a.s./L), based on mean measured concentrations

Report:	CA 8.2.4.1/3 Mitchell G.C. et al., 2001 c Acute toxicity of BAS 550 F (AC 336379, Dimethomorph) to Daphnia magna under static test conditions DK-521-006
Guidelines:	OECD 202, EPA 72-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 48-hour static acute toxicity laboratory study, the effect of BAS 550 F on water flea neonates was investigated. Daphnids were exposed to nominal concentrations of 0 (control and vehicle blank), 1.3, 2.5, 5.0, 10, and 20 mg a.s./L This approach resulted in mean measured concentrations of 1.26, 2.38, 4.66, 6.98, and 10.6 mg BAS 550 F/L. Additionally, a dilution water control and a solvent control was set up. Daphnids were exposed in 2 replicates per concentration, containing 10 daphnids each. The daphnids were observed for immobility 24 and 48 hours after start of exposure.

The following biological results are based on mean measured concentrations. After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 6.98 mg a.s./L, whereas 5% immobility was observed at the highest test item concentration of 10.6 mg a.s./L. For results see **Error! Reference source not found.**.

The 48-hour EC_{50} value for technical grade dimethomorph during a static acute toxicity test with *Daphnia magna* was > 10.6 mg a.s./L. The no-observed effect concentration (NOEC) was 10.6 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethomorph (technical grade, also known as BAS 550 F); Lot Number AC 9978-131; purity: 98.3%

B. STUDY DESIGN

Test species:	*Daphnia magna*; Origin: In-house cultures of T.R. Wilbury Laboratories that were originally obtained from Aquatic Biosystems, Inc., Fort Collins, CO, U.S.A.
Test design:	Static system (48 hours), 5 test item concentrations plus control (dilution water) and a solvent control (0.1 ml/L dimethylformamide), 2 replicates with 10 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	NOEC and EC_{50} based on immobility of daphnids.
Test concentrations:	0 (Control), 0 (solvent control), 1.3, 2.5, 5.0, 10 and 20 mg a.s./L,
Test conditions:	Temperature: 20.2 – 20.9 °C, pH: 7.4 - 7.6, dissolved oxygen: 8.2 - 8.8 mg/L, photoperiod: 16-hour light:8-hour dark
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC method (Method MV 1341).
Statistics:	No statistical analysis was conducted

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.10 (control and vehicle blank), 1.26, 2.38, 4.66, 6.98, and 10.6 mg a.s./L. These measured concentrations ranged from 53 to 97% of the nominal concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 6.98 mg a.s./L (mean measured), whereas 5% immobility was observed at the highest test item concentration of 10.6 mg a.s./L. For results see Table 8.2.4.1-2

Table 8.2.4.1-2: Effects of Dimethomorph on *Daphnia magna* mobility

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.5**	**5.0**	**10**	**20**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.26**	**2.38**	**4.66**	**6.98**	**10.6**
Immobility (24 h) [%]	0	0	0	0	0	0	5
Immobility (48 h) [%]	0	0	0	0	0	0	5
	Endpoints [mg a.s./L] (mean measured)						
EC_{50} (48 h)	> 10.6						
NOEC (48 h)	10.6						

III. CONCLUSION

The 48-hour EC_{50} value for technical grade dimethomorph during a static acute toxicity test with *Daphnia magna* was > 10.6 mg a.s./L. The no-observed effect concentration (NOEC) was 10.6 mg a.s./L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

EU agreed endpoints available.

Report:	CA 8.2.4.2/1 Mitchell G.C. et al., 1997 b Acute toxicity of AC 336379 (Dimethomorph) to the mysid (Mysidopsis bahia) under flow-through test conditions DK-521-004
Guidelines:	EPA 72-3(c)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, saltwater mysids (*Americamysis bahia*) were exposed to a dilution water control, a solvent control and to nominal concentrations of 1.3, 2.2, 3.5, 6.0, and 10 mg BAS 550 F/L (corresponding to mean measured concentrations of <0.500, <0.500, 1.21, 2.16, 3.45, 5.89, 9.75 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and at test item concentrations of 1.21 mg a.s./L, whereas 5%, 5% and 10% mortality were observed at 2.16, 6.45, 5.89 mg a.s./L. At the highest concentration 80% mortality and sub-lethal effects (*i.e.* lethargy and loss of equilibrium) were observed.

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with the mysid was 7.92 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 5.89 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethomorph (technical grade, also known as AC 336379); Lot Number AC 9978-131; purity: 98.0%.

B. STUDY DESIGN

Test species:	Saltwater mysid (*Mysidopsis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.
Test design:	Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate; daily assessment of mortality and symptoms of toxicity.
Endpoints:	LC_{50} (48 h), mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.2 mL dimethylformamide/L) and 1.3, 2.2, 3.5, 6.0, and 10 mg BAS 550 F/L (nominal), corresponding to mean measured concentrations of <0.500, <0.500, 1.21, 2.16, 3.45, 5.89, 9.75 mg a.s./L.
Test conditions:	Temperature: 21.2 - 22.6 °C, pH: 7.8 - 8.1; dissolved oxygen: 5.7 - 8.0 mg/L; salinity: 16 - 17 parts per thousand; photoperiod: 16 hours light:8 hours dark
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC method (Method RU 151/35/90).
Statistics:	Descriptive statistics; LC_{50} values were calculated by the binomial/nonlinear interpolation methods.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.50 (control and vehicle blank), 1.21, 2.16, 3.45, 5.89, and 9.75 mg a.s./L. These measured concentrations ranged from 93 to 99% of the nominal concentrations. The results of the study are based on the mean measured concentration of the test solutions.

Biological results: After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and at test item concentrations of 1.21 mg a.s./L, whereas 5%, 5% and 10% mortality were observed at 2.16, 6.45, 5.89 mg a.s./L. At the highest concentration 80% mortality and sub-lethal effects (*i.e.* lethargy and loss of equilibrium) were observed. The results are summarized Table 8.2.4.2-1

Table 8.2.4.2-1: Acute toxicity (48h) of Dimethomorph to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.2**	**3.5**	**6.0**	**10**
Concentration [mg a.s./L] (mean measured)	--	--	**1.21**	**2.16**	**6.45**	**5.89**	**9.75**
Mortality [%] (48 h)	0	0	0	5	5	10	80
Number of affected mysids after 48 h [#]	none	none	none	none	none	none	4
Endpoints [mg a.s./L] (mean measured)							
LC_{50} (48 h)	7.92						
$NOEC_{survival}$ (48)	5.89						

[#] Symptoms after 48 h: L = lethargy, E = loss of equilibrium

III. CONCLUSION

The 96-hour LC_{50} value for technical grade dimethomorph during a flow-through acute toxicity test with the mysid was 7.92 mg a.s./L. The no-observed effect concentration (NOEC) based on survival was 5.89 mg a.s./L.

Report:	CA 8.2.4.2/2 Mitchell G.C. et al., 1997 c Effect of AC 336379 (Dimethomorph) on new shell growth in the Eastern oyster (Crassostrea virginica) under flow-through test conditions DK-522-001
Guidelines:	EPA 72-3(b)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study the effect of BAS 550 F on shell growth of eastern oysters (*Crassostrea virginica*) was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to nominal concentrations of 1.3, 2.2, 3.5, 6.0, and 10 mg BAS 550 F/L (corresponding to mean measured concentrations of 1.33, 2.24, 3.63, 6.15 and 10.1 mg a.s./L) in groups of 10 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality of oysters occurred in the control and the solvent control and at all test item concentrations except 10.1 mg a.s./L. At 10.1 mg/L one dead oyster could be observed. Shell growth was statistically significantly inhibited at the three highest test item concentrations compared to the control (Kruskal-Wallis Test, $\alpha < 0.05$).

The 96-hour EC_{50} value for technical grade dimethomorph during a shell growth inhibition test with the eastern oyster was 4.42 mg a.s./L. The no-observed effect concentration (NOEC) based on inhibition of shell growth was 2.24 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph (also known as AC 336379); Lot Number AC 9978-131; purity: 98.0%.

B. STUDY DESIGN

Test species:	Eastern Oyster (*Crassostrea virginica*); Origin: P. Cummins Oyster Company, Chester, MD, U.S.A
Test design:	Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.
Endpoints:	EC_{50} and NOEC for mortality, shell growth inhibition and symptoms of toxicity.
Test concentrations:	Control (dilution water), solvent control (0.2 mL dimethylformamide/L), 1.3, 2.2, 3.5, 6.0, and 10 mg BAS 550 F/L (nominal), corresponding to mean measured concentrations of 1.33, 2.24, 3.63, 6.15 and 10.1 mg a.s./L.
Test conditions:	Temperature: 19.0 - 20.5 ºC; pH: 7.8 - 8.0; dissolved oxygen: 5.8 - 8.0 mg/L; salinity: 34 parts per thousand; photoperiod: 16 hours light:8 hours dark
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC method (Method RU 151/35/90).
Statistics:	Descriptive statistics; the EC_{50} was calculated using weighted least squares non-linear regression. The NOEC was calculated using TOXSTAT 3.3. Control and vehicle blank data were compared with a parametric "t" test ($\alpha = 0.05$). A Chi-square test for normal distribution and Bartlett's test to determine that the data were heterogeneous. New shell growth data in the treatments were compared to the pooled control using the Kruskal-Wallis test.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean measured concentrations of dimethomorph in the test solutions were < 0.50 (control and vehicle blank), 1.33, 2.24, 3.63, 6.15, and 10.1 mg a.s./L. These measured concentrations ranged from 101 to 104% of the nominal concentrations. The results of the study are based on the mean measured concentration of the test solutions.

Biological results: After 96 hours of exposure, no mortality of oysters occurred in the control and the solvent control and at all test item concentrations except 10.1 mg a.s./L. At 10.1 mg/L one dead oyster could be observed. Shell growth was statistically significantly inhibited at the three highest test item concentrations compared to the control (Kruskal-Wallis Test, $\alpha < 0.05$).The results are summarized Table 8.2.4.2-2

Table 8.2.4.2-2: Acute toxicity (96 h) of Dimethomorph to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.2**	**3.5**	**6.0**	**10**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.33**	**2.24**	**3.63**	**6.15**	**10.1**
Inhibition of shell growth after 96 h [mm]	2.9	2.7	2.8	2.5	1.6*	1.4*	0.2*
	Endpoints [mg a.s./L] (mean measured)						
EC_{50} (96 h)	4.42						
NOEC (96 h)	2.24						

* Statistically different from the pooled controls ($p \leq 0.05$).

III. CONCLUSION

The 96-hour EC_{50} value for technical grade dimethomorph during a shell growth inhibition test with the eastern oyster was 4.42 mg a.s./L. The no-observed effect concentration (NOEC) based on inhibition of shell growth was 2.24 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Two chronic toxicity studies with *Daphnia magna* conducted with dimethomorph were already evaluated during the previous Annex I inclusion process. However, these studies were re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards. For detailed summaries for the originally submitted studies, references are made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

Report:	CA 8.2.5.1/1 Memmert U.,Knoch E., 1993 b Influence of Dimethomorph on survival and reproduction of Daphnia magna in a semistatic test (22 days) DK-524-001
Guidelines:	OECD 202 Part II (1984), EEC XI/681/86
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie und Bundesangelegenheiten, Wiesbaden)

Report:	CA 8.2.5.1/2 Anonymous, 1993 a Determination of the concentrations of Dimethomorph (SAG 151) in test medium DK-123-221
Guidelines:	none
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie und Bundesangelegenheiten, Wiesbaden)

Executive Summary (originally submitted study)

In a 22-day semi-static toxicity test, effects of dimethomorph to water fleas (*Daphnia magna*) were examined. Daphnids were exposed to nominal concentrations of 0.0 (control, solvent control), 0.10, 0.31, 0.96, 3.1 and 9.6 mg a.s./L. All treatment groups and the control consisted of 10 replicates with one parent daphnid in each. Adult survival and offspring production were recorded three times per week over the exposure period of 22 days.

The biological results are based on nominal concentrations. After 22 days of exposure no parent mortality was observed in the solvent control and at the lowest concentration 0.10 mg a.s./L, whereas 10% mortality was observed in the control and at 0.31 mg a.s./L. 30%, 20% and 100% mortality were observed at the concentrations 0.96, 3.1 and 9.6 mg a.s./L, respectively. The number of offspring ranged between 0 and 99.9 in the test item treatments, compared to 84.2 in the control and 98.3 in the solvent control. Statistically significant effects on reproduction were observed at the four highest tested concentrations of 0.31 to 9.6 mg a.s./L.

In a 22-day semi-static toxicity study with *Daphnia magna* the NOEC of dimethomorph was determined to be 0.10 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical grade dimethomorph; Batch Number Batch Dw 11/86; purity: 95.6%.

B. STUDY DESIGN

Test species:	*Daphnia magna*; Origin: In-house cultures of RCC Umweltchemie GmbH & Co., Roßdorf, Germany
Test design:	Semi-static system (22 days), 5 test concentrations plus control and solvent control, 10 replicates per treatment; one animal per test vessel; assessment of parent immobility and reproduction 3 times per week
Endpoints:	NOEC, parent mortality, reproduction.
Test concentrations:	Control (dilution water), solvent control (32 mg/L Tween 80), 0.005, 0.10, 0.31, 0.96, 3.1, and 9.6 mg a.s./L (nominal);
Test conditions:	Temperature: 20.2 - 21.0 ºC; pH: 7.8 - 9.0; dissolved oxygen: ≥ 9.3 mg/L; photoperiod: 16 hours light:8 hours dark; light intensity: 200 - 600 lux; feeding: daily (except day 3 and 4) with algae (*Desmodesmus subspicatus*); no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC method (Method RU 151/35/90).
Statistics:	Descriptive statistics; Williams test for determination of the NOEC values ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical results: The measured concentrations ranged from 86.9 to 105.5% of the nominal concentrations. Therefore, all results were reported based on nominal test concentrations.

Biological results: After 22 days of exposure no parent mortality was observed in the solvent control and at the lowest concentration 0.10 mg a.s./L, whereas 10% mortality was observed in the control and at 0.31 mg a.s./L. 30%, 20% and 100% mortality were observed at the concentrations 0.96, 3.1 and 9.6 mg a.s./L, respectively. The number of offspring ranged between 0 and 99.9 in the test item treatments, compared to 84.2 in the control and 98.3 in the solvent control. Statistically significant effects on reproduction were observed at the four highest tested concentrations of 0.31 to 9.6 mg a.s./L. The results are summarized Table 8.2.5.1-1

Table 8.2.5.1-1: Effects of dimethomorph on *Daphnia magna* parent mortality, reproduction and growth after 22 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.1**	**0.31**	**0.96**	**3.1**	**9.6**
Parent mortality [%]	10	0	0	10	30	20	100*
Offspring / surviving adults	84.2	98.3	99.9	60.0*	53.3*	14.0*	0*
	Endpoints [mg a.s./L] (nominal)						
$NOEC_{survival}$ (22 d)	3.1						

* Statistically significant effects compared to the controls (ANOVA; $p < 0.05$).

III. CONCLUSION

In a 22-day semi-static toxicity study with *Daphnia magna* the NOEC of dimethomorph was determined to be 0.10 mg a.s./L based on nominal concentrations.

Report: CA 8.2.5.1/3
Brausch J.M., 2015 b
Calculation of EC10 values for chronic invertebrate studies with BAS 550 F (Dimethomorph)
2015/1238096
Guidelines: none
GLP: no

Executive Summary (recalculations)

The endpoint derived in the study with *Daphnia magna* (BASF DocID DK-524-001) was the No Observable Effects Concentration (NOEC); however, in the newest EFSA Guidance Document (EFSA Journal 2013) on a tiered risk assessment for plant protection products for aquatic organisms, the EC_{10} value is clearly preferred. The statistical recalculation of the EC_{10} was conducted using PROBIT analysis.

The recalculated value (as nominal concentration) for the study is as follows:

Offspring per female: EC_{10} = 0.152 mg a.s./L

Report:	CA 8.2.5.1/4 Murrell H.R. et al., 1997 a Chronic toxicity of AC 336,379 (Dimethomorph) technical during the complete life-cycle of Daphnia magna under flow-through test conditions DK-523-001
Guidelines:	EPA 72-4 (b), EEC 91/414 Annex II 8.2.5, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 8.2.5.1/5 Mitchell G.C., 1997 a Amendment - Chronic toxicity of AC 336,379 (Dimethomorph) technical during the complete life-cycle of Daphnia magna under flow-through test conditions DK-123-226
Guidelines:	EPA 72-4 (b), EEC 96/12, EEC 91/414 Annex II 8.2.5
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

In a 21-day flow-through toxicity test, effects of dimethomorph to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.0 (control), 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L. All treatment groups and the control consisted of 4 replicates with ten parent daphnid in each. Adult survival and offspring production were recorded daily over the exposure period of 21 days. The total length and dry weight of each remaining adult daphnid was determined at test termination.

The biological results are based on nominal concentrations. After 21 days of exposure no statistically significant parent mortality was observed in the test treatments compared to the control. The number of offspring per day and adult ranged between 8.48 and 11.87 in the test item treatments, compared to 11.78 in the control. Statistically significant effects on reproduction were observed at the two highest tested concentrations of 0.95 and 1.8 mg a.s./L, whereas statistically significant effects on body length were observed at the three highest tested concentrations of 0.46, 0.95 and 1.8 mg a.s./L.

In a 21-day flow-through toxicity study with *Daphnia magna* the NOEC of dimethomorph was determined to be 0.22 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethomorph (technical grade, also known as AC 336379); Lot Number AC 9978-131; purity: 97.6%.

B. STUDY DESIGN

Test species:	*Daphnia magna;* Origin: < 24 hours old at test initiation, in-house cultures of ABC Laboratories, Columbia, Missouri, U.S.A.
Test design:	Flow-through system (21 days), 5 test concentrations plus control, 4 replicates per treatment; 10 animal per test vessel; assessment of parent mortality and reproduction daily; determination of total length and dry weight of remaining adults at test termination.
Endpoints:	NOEC, parent mortality, reproduction.
Test concentrations:	Control (dilution water), 0.13, 0.25, 0.50, 1.0, and 2.0 mg a.s./L (nominal);
Test conditions:	Temperature: 19.6 - 20.0 °C; pH: 8.30 - 8.54; dissolved oxygen: 7.8 - 8.7 mg/L; photoperiod: 16 hours light:8 hours dark; light intensity: 524 - 583 lux; feeding: twice daily (except day 3 and 4) with algae (*Selenastrum capricornutum and Ankistrodesmus falcaltus*);
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC method (Method RU 151/35/90).
Statistics:	Descriptive statistics; Survival data: contingency table methods; Differences between control and treatments: Chi-square statistics two-way contingency); for smaller sample sizes: one-tailed Fisher`s exact test; Reproduction and growth data: Anova with following Dunnet`s Test.

II. RESULTS AND DISCUSSION

Analytical results: The measured concentrations ranged from 85 to 95% of the nominal concentrations. All results were reported based on mean measured test concentrations.

Biological results: After 21 days of exposure no statistically significant parent mortality was observed in the test treatments compared to the control. The number of offspring per day and adult ranged between 8.48 and 11.87 in the test item treatments, compared to 11.78 in the control. Statistically significant effects on reproduction were observed at the two highest tested concentrations of 0.95 and 1.8 mg a.s./L, whereas statistically significant effects on body length were observed at the three highest tested concentrations of 0.46, 0.95 and 1.8 mg a.s./L. The results are summarized Table 8.2.5.1-2

Table 8.2.5.1-2: Effects of dimethomorph on *Daphnia magna* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**--**	**0.11**	**0.22**	**0.46**	**0.95**	**1.8**
Survival of adult [%]	95	93	98	100	100	100
Offspring/adult/day	11.78	10.84	10.90	11.87	9.83*	8.48*
Mean length [mm]	4.56	4.52	4.50	4.49*	4.43*	4.32*
Mean weight [mg]	0.91	0.83	0.91	0.86	0.91	0.77
	Endpoints [mg a.s./L] (mean measured)					
$NOEC_{survival}$ (22 d)	0.22					

* Statistically significant effects compared to the controls (ANOVA with following Dunnett`s Test; $p < 0.05$).

III. CONCLUSION

In a 21-day flow-through toxicity study with *Daphnia magna* the NOEC of dimethomorph was determined to be 0.22 mg a.s./L based on mean measured concentrations.

Report: CA 8.2.5.1/3
Brausch J.M., 2015 b
Calculation of EC10 values for chronic invertebrate studies with BAS 550 F (Dimethomorph)
2015/1238096
Guidelines: none
GLP: no

Executive Summary (recalculations)

The endpoint derived in the study with *Daphnia magna* (BASF DocID DK-523-001) was the No Observable Effects Concentration (NOEC); however, in the newest EFSA Guidance Document (EFSA Journal 2013) on a tiered risk assessment for plant protection products for aquatic organisms, the EC_{10} value is clearly preferred. The statistical recalculation of the EC_{10} was conducted using PROBIT analysis.

The recalculated values (as nominal concentration) for the study are as follows:

Offspring per female: EC_{10} = 0.421 mg a.s./L (95% confidence interval: 0.039 - 0.748)
Length: EC_{10} > 2.0 mg a.s./L
Body Weight: EC_{10} = 1.343 mg a.s./L

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

The following chronic toxicity study on the marine saltwater mysid *Americamysis bahia* performed with the active substance dimethomorph is not required for registration in the EU and has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for the sake of completeness. However, this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards.

Report:	CA 8.2.5.2/1 Hicks S.L., 2010 b BAS 550 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions 2010/7008279
Guidelines:	EPA 850.1350, EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 8.2.5.2/2 Brausch J.M., 2015 b Calculation of EC10 values for chronic invertebrate studies with BAS 550 F (Dimethomorph) 2015/1238096
Guidelines:	none
GLP:	no

Executive Summary

The chronic toxicity of dimethomorph to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control and to dimethomorph at nominal concentrations of 0.065, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (corresponding to mean measured concentrations of 0.0582, 0.119, 0.241, 0.502 and 0.997 mg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Body length of the males and females was determined at test termination.

The biological results are based on mean measured concentrations. Survival of F_0 and F_1 saltwater mysids was not statistically significantly affected compared to the control at all test item concentrations after 28 and 10 days of exposure, respectively. The mean day of first-brood release by F0 mysids was 17.6 days in the control and no statistically significant differences were detected between control and all test item concentrations. The mean number of total young produced per female for the 0.502 and 0.997 mg a.s./L test item concentrations was significantly less than the control. At test termination the mean length of males exposed to 0.241 mg a.s./L and of females exposed to 0.119 and 0.241 mg a.s./L was significantly less than the mean length of the controls. The effects observed at these test item concentrations do not indicate a concentration dependent response.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for dimethomorph was determined to be 0.241 mg a.s./L based on mean measured concentrations. The recalculated EC_{10} value was 0.238 mg a.s./L based on offspring per female and nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F, Reg. no.: 247723), batch-no: AC9978-131, purity: 97.5%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture.

Test design: Flow-through system (28 d); 5 test item concentrations plus a control, 3 replicates for each test item concentration and the control; 30 mysids per glass aquaria (15 mysids per test chamber); mysids were maintained in test chambers until sexual maturity; at time of sexual maturity (day 13) male-female pairs were transferred into pairing chambers (7 pairs per replicate); remaining mysids from the pairing chambers that were not paired were pooled and placed in separate test chambers within glass aquaria; dead parental mysids and juveniles released during the test were removed; daily assessment of survival and symptoms of toxicity, reproduction (number of offspring produced by each female); determination of body length at test termination; post-larval F1 mysids were assigned to retention baskets within the same test chambers as the F0-mysid exposure; observed daily for mortality until they reached 10 days of age.

Endpoints: NOEC, EC_{10} (recalculation) based on survival, reproductive success and body length.

Test concentrations: Control (dilution water), 0.065, 0.13, 0.25, 0.50 and 1.0 mg dimethomorph/L (nominal), corresponding to mean measured concentrations of < MQL (minimum quantifiable limit), 0.0582, 0.119, 0.241, 0.502 and 0.997 mg a.s./L.

Test conditions:	20 L glass aquaria with two test chambers (one test chamber: 22 cm x 38 - 77 cm x 30 cm); two cylindrical retention baskets per test chamber, one containing mysids for the reproduction observations and the other for growth observations; basket consisted of a glass petri dish base (approximately 1.5 x 15 cm) with a nylon screen collar (Nitex mesh with 355 µm mesh size); dilution water: commercial sea salt mix added to laboratory freshwater, filtered and ultraviolet sterilized; flow rate: 0.67 L/per cycle/aquarium resulting in approximately 5 volume additions/aquarium/24 h; salinity: 19.1 - 20.8 ‰; temperature: 23.9 - 25.9 °C; pH 7.64 - 8.31; oxygen content: 6.62 mg/L - 7.34 mg/L; photoperiod 14 h light : 10 h dark; light intensity: 237 - 368 lux; mysids were fed with live brine shrimp nauplii (*Artemia* sp. < 48 h old) at least two times daily.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; one-way ANOVA and one-tailed Dunnett´s test to estimate the NOEC based on percentage of survival, growth as body length of each individual, and replicate mean number of total young per female ($p < 0.05$); recalculation of EC_{10} value using PROBIT analysis.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and after 7, 14, 21, and 28 days. Measured concentrations for dimethomorph were between 110% and 122% of nominal at test initiation. Measured concentrations after 7, 14, and 21 days ranged from 78% to 90%, from 87% to 95% and from 88% to 97% of nominal, respectively. At test termination, measured concentrations were between 85% and 99% of nominal. The following biological results are based on mean measured concentrations.

Biological results: Survival of F_0 and F_1 saltwater mysids was not statistically significantly affected compared to the control at all test item concentrations after 28 and 10 days of exposure, respectively (Dunnett´s test, $p < 0.05$). The mean day of first-brood release by F0 mysids was 17.6 days in the control and no statistically significant differences were detected between control and all test item concentrations (Dunnett´s test, $p < 0.05$). The mean number of total young produced per female for the 0.502 and 0.997 mg a.s./L test item concentrations was significantly less than the control (Dunnett´s test, $p < 0.05$). At test termination the mean length of males exposed to 0.241 mg a.s./L and of females exposed to 0.119 and 0.241 mg a.s./L was significantly less than the mean length of the controls (Dunnett´s test, $p < 0.05$). The effects observed at these test item concentrations do not indicate a concentration dependent response. The results are summarized in Table 8.2.5.2-1

Table 8.2.5.2-1: Chronic toxicity (28 d) of dimethomorph to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**0.065**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration [mg a.s./L] (mean measured)	**--**	**0.0582**	**0.119**	**0.241**	**0.502**	**0.997**
F_0: Survival on day 28 [%]	93	95	89	89	93	93
F_1: Survival on day 10 [%]	98	100	98	100	100	98
Reproductive success during 28-day exposure [Offspring per female ± SD]	27.2 ± 3.50	28.3 ± 3.38	26.0 ± 3.04	24.6 ± 0.733	21.3 ± 1.74 *	20.5 ± 2.31 *
F_0: Body length on day 28, males [mm ± SD]	6.25 ± 0.173	6.12 ± 0.0639	6.15 ± 0.0879	5.94 ± 0.119 *	6.43 ± 0.140	6.45 ± 0.0182
F_0: Body length on day 28, females [mm ± SD]	6.49 ± 0.115	6.38 ± 0.112	6.17 ± 0.201 *	6.22 ± 0.0678 *	6.46 ± 0.0799	6.63 ± 0.0713
F_0: Day of first brood ± SD	17.6 ± 0.247	17.9 ± 0.644	17.3 ± 0.378	17.7 ± 0.165	17.5 ± 0.218	17.7 ± 0.145

	Endpoint [mg dimethomorph/L] (mean measured)
$NOEC_{overall}$ (28 d)	0.241
	Recalculated endpoints [mg dimethomorph/L] (nominal)
EC_{10} (offspring per female)	0.238 (95% confidence interval: 0.034 - 0.400)
EC_{10} (female length, day 14)	> 0.997
EC_{10} (male length, day 14)	> 0.997
EC_{10} (female length, day 28)	> 0.997
EC_{10} (male length, day 28)	> 0.997

SD - standard deviation
* Statistically significant differences compared to the control (Dunnett´s test, $p < 0.05$).

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for dimethomorph was determined to be 0.241 mg a.s./L based on mean measured concentrations. The recalculated EC_{10} value was 0.238 mg a.s./L based on offspring per female and nominal concentrations.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study with *Chironomus riparius* was already evaluated during the previous Annex I inclusion process. However, this study was re-evaluated for the AIR 3 submission and the EC_{10} endpoint value was recalculated based on current standards. For a detailed summary for the originally submitted study, references is made to the EU dossier submitted during Annex I inclusion process for dimethomorph.

Report:	CA 8.2.5.3/1 England D.C. et al., 1997 a Effects of AC 336,379 (Dimethomorph) on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system DK-529-002
Guidelines:	BBA proposal: Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in water-sediment system 1995
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study)

In a 24-day static spiked water study with artificial sediment, non-biting midge larvae (*Chironomus riparius*) were exposed to dimethomorph. Nominal test concentrations were 0.0 (control), 1.13, 2.25, 4.50, 9.00 and 18.0 mg a.s./L (corresponding to initially mean measured concentrations: 1.08, 2.15, 4.11, 7.97 and 15.6 mg a.s./L). All test item concentrations and the water control had 8 replicates. 25 larvae were added to each test vessel.

The biological results are based on initially measured concentrations. First emerged midges were observed on DAI 13 (= day after insertion of larvae). No statistically significant differences were found for the emergence rates at any test item concentration when compared to the control. For the endpoints time to emergence and weight statistically significant differences compared to the control were observed at the two highest test item concentrations of 7.97 and 15.6 mg a.s./L (initially mean measured).

In a 24-day static spiked water test with *Chironomus riparius* and artificial sediment the NOEC of dimethomorph was determined to be 4.11 mg a.s./L based on larval weights and time to adult emergence (initially mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	^{14}C-radiolabeled dimethomorph (also known as AC 336379); Lot Number AC 10011-71; Specific Activity: 25.3 µCi/mg; purity: 99.3% radiopurity.

B. STUDY DESIGN

Test species:	Non-biting midge (*Chironomus riparius*), first instar larvae; source: in-house culture of ABC Laboratories, Columbia, Missouri, U.S.A..
Test design:	Static system (24 days); 5 test concentrations plus a water control, 14 replicates per treatment group, with 8 biological replicates containing test organisms and 6 replicates reserved for analytical sampling; 25 larvae were added to each test vessel; mortality was assessed on day 10 and 24, emergence at day 24, larval dry weight and time to emergence at day 10.
Endpoints:	NOEC, survival, growth, emergence rate and time to emergence.
Test concentrations:	Control (dilution water), 1.13, 2.25, 4.50, 9.00, and 18.0 mg a.s./L, corresponding to initial measured concentrations of 1.08, 2.15, 4.11, 7.97 and 15.6 mg a.s/L.
Test conditions:	2000 mL pyrex glass beakers artificial sediment; Temperature: 19.7 to 21.5 °C; pH: 7.5 - 8.3; dissolved oxygen: 4.2 - 8.1 mg/L; photoperiod: 16 hours light:8 hours; light intensity: 545 - 751 lux.
Analytics:	Analytical verification of ^{14}C-dimethomorph concentrations in the water column on day 0, 10 and 24 using a liquid scintillation counting.
Statistics:	Descriptive statistics, Survival, growth and emergence data were evaluated using analysis of variance methods (ANOVA) coupled with Dunnett's Test.

II. RESULTS AND DISCUSSION

On day 0, the mean measured water concentrations of ^{14}C-dimethomorph equivalents were: 1.08, 2.15, 4.11, 7.97, and 15.6 mg/L for treatment levels 1 through 5, representing 96, 96, 91, 89, and 87% of the initial nominal water concentrations. Water column concentrations decreased by 36 to 46% from day 0 to day 10, and were approximately one-third of the initial nominal concentrations at test termination on day 24. Interstitial water and sediment concentrations of ^{14}C-dimethomorph equivalents increased over the 24-hour exposure period. Interstitial water concentrations were 0.0280 to 0.268 mg/L over the five treatment concentrations, or 1.7 to 2.6% of the water concentrations on day 0. Interstitial water concentrations increased by 100 to 300% by day 10, and by an additional 20 to 50% from day to day-24. Sediment concentrations of ^{14}C-dimethomorph equivalents were 1.56 to 9.92 mg/kg on day-0, and increased by factors ranging from 300 to 600% by day 10, and by an additional 5 to 40% from day 10 to day-24.The following biological results are based on initial measured sediment concentrations.

Biological results: First emerged midges were observed on DAI 13 (= day after insertion of larvae). No statistically significant differences were found for the emergence rates at any test item concentration when compared to the control. For the endpoints time to emergence and weight statistically significant differences compared to the control were observed at the two highest test item concentrations of 7.97 and 15.6 mg a.s./L (initially mean measured). The results are summarized in Table 8.2.5.3-1

Table 8.2.5.3-1: Effects of ^{14}C-dimethomorph on survival, growth and emergence of *Chironomus riparius*

Concentration [mg a.s./L] (nominal)	**Control**	**1.13**	**2.25**	**4.50**	**9.00**	**18.0**
Concentration [mg a.s./L] (initial measured)	**--**	**1.08**	**2.15**	**4.11**	**7.97**	**15.6**
Survival (day 10) [%]	92	92	85	91	100	97
Weight (day 10) [mg]	1.5	1.4	1.3	1.3	1.1*	1.1*
Emergence (day 24) [%]	94	93	96	93	98	88
Time to emergence [days]	13.0	13.2	13.0	13.8	14.0*	14.3*
Endpoints [mg a.s./L] (initial measured)						
$NOEC_{weight, time\ to\ emergence}$ (10 d)	4.11					

* statistically different from control (ANOVA with following Dunnett`s test $p < 0.05$)

III. CONCLUSION

In a 24-day static spiked water test with *Chironomus riparius* and artificial sediment the NOEC of dimethomorph was determined to be 4.11 mg a.s./L based on larval weights and time to adult emergence (initially mean measured).

Report: CA 8.2.5.3/2
Brausch J.M., 2015 b
Calculation of EC10 values for chronic invertebrate studies with BAS 550 F (Dimethomorph)
2015/1238096
Guidelines: none
GLP: no

Executive Summary (recalculations)

The endpoint derived in the study with *Chironomus riparius* (BASF DocID DK-529-002) was the No Observable Effects Concentration (NOEC); however, in the newest EFSA Guidance Document (EFSA Journal 2013) on a tiered risk assessment for plant protection products for aquatic organisms, the EC_{10} value is clearly preferred. The statistical recalculation of the EC_{10} was conducted using PROBIT analysis.

The recalculated values (as nominal concentration) for the study are as follows:

Dry weight:	EC_{10} = 3.022 mg a.s./L
Female emergence:	EC_{10} > 15.6 mg a.s./L
Male emergence:	EC_{10} > 15.6 mg a.s./L
Female development time:	EC_{10} > 15.6 mg a.s./L
Male development time:	EC_{10} > 15.6 mg a.s./L

CA 8.2.5.4 Sediment dwelling organisms

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Due to the reasons given below, the algae study on *Scenedesmus subspicatus* (DocID DK-521-001 / 1986/7000036) is not considered to be valid according to current standard and is thus not considered for the aquatic risk assessment.

Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- at the time the study was conducted GLP was not compulsory; however the study was conducted according to the principle of Good Laboratory Practices
- measured concentrations were between 29% and 80% at test initiation and between 24% and 123% at test termination. The measured concentrations were lower due to that fact that only clear solutions were analyzed and the water solubility of dimethomorph was exceeded in the higher test concentrations. The biological results are based on nominal concentrations, but should be based on mean measured concentrations.
- in accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 96 h study are considered as relevant endpoint. Relevant information required according to OECD 201 are missing in the study report; i.e. no raw data available (number of cells in each replicate). Therefore it's not possible to recalculate the 72 h endpoints.

Nevertheless, for better transparency, the summary of this study was extracted from the originally submitted EU dossier of dimethomorph and is provided below.

Report:	CA 8.2.6.1/1 Ellgehausen H., 1986 d Acute toxicity of CME 151 to Scenedesmus subspicatus (OECD algae growth inhibition test) DK-521-001
Guidelines:	OECD 201
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Executive Summary (originally submitted study)

In a 96-hour static toxicity laboratory study, the effect of dimethomorph on the growth of the green alga *Scenedesmus subspicatus* was investigated. The following concentrations were applied: 0.0 (control, solvent control), 10, 20, 40, 80 and 160 mg a.s./L. Assessment of growth was conducted 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on nominal concentrations. Morphological effects on algae were not reported.

In a 96-hour algae test with *Scenedesmus subspicatus*, the E_bC_{50} of dimethomorph was determined to be 29.2 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethomorph (technical grade, also known as CME 151); Batch Number DW 11/86; purity: 94.8%.

B. STUDY DESIGN

Test species:	Fresh water green alga, *Scenedesmus subspicatus*,; obtained from Umweltbundesamt, Berlin, Germany
Test design:	Static system; test duration 96 hours; 5 test concentrations, each with 3 replicates per treatment and control; daily assessment of growth.
Endpoints:	EC_{50}, growth (area under the growth curve).
Test concentrations:	0 (control, algal medium), 0 (solvent control, 0.01% Tween 80) 20, 40, 80, and 160 mg a.s./L,
Test conditions:	test medium: algal nutrient solution; initial cell densities 1 x 10^4 cells/mL; temperature:20 ± 2 °C; pH: 7.2 - 8.2; dissolved oxygen: ≥ 9.3 mg/L; photoperiod: 24 hours light; light intensity – 8000 lux
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-methos.
Statistics:	Descriptive statistics; EC_{50} values were estimated using the Logit Method.

II. RESULTS AND DISCUSSION

Analytical measurements: The measured concentrations at the higher treatment levels were significantly lower than the nominal concentrations. At 20 mg/L, the measured concentrations ranged from 80 to 123% of the nominal concentrations. At 80 and 160 mg/L, the measured concentrations ranged from 23.5 to 45.1% of the nominal values. The measured concentrations were lower due to that fact that only clear solutions were analyzed and the water solubility of dimethomorph was exceeded in the higher test concentrations

Biological results: The biological results are based on nominal concentrations. Morphological effects on algae were not reported. The effects on algal growth are summarized in Table 8.2.6.1-1

Table 8.2.6.1-1: Effect of dimethomorph on the growth of the green alga *Scenedesmus subspicatus*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**10**	**20**	**40**	**80**	**160**
Area under the growth curve (AUC)	4778	5684	4658	4129	942	81.6	--
Inhibition in 96 h [%]	--	--	2.5	13.6	80.3	98.3	100
Endpoints [mg a.s./L] (nominal)							
EC_{50} (96 h)	29.2						

III. CONCLUSION

In a 96-hour algae test with *Scenedesmus subspicatus*, the E_bC_{50} of dimethomorph was determined to be 29.2 mg a.s./L based on nominal concentrations.

Report:	CA 8.2.6.1/2 Jatzek H.-J., 2001 a BAS 550 F - Determination of the inhibitory effect on the cell multiplication of unicellular green algae DK-521-007
Guidelines:	OECD 201, EPA 850.5400, EEC 92/69 A V C 3
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 72 hour static acute toxicity laboratory study, the effect of dimethomorph on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. Algae were exposed to nominal concentration of 6.25, 12.5, 25, 50 and 100 mg a.s./L. Additionally, a dilution water control and a solvent control (N,N-dimethylformamide (DMF)) were set up. Assessment of growth was conducted 24, 48 and 72 h after test initiation.

The biological results are based on nominal concentrations. The percent cell densities compared to the control in the concentrations of 6.25, 12.5, 25, 50 and 100 mg a.s./L were determined to be 1%, 20%, 30%, 70% and 94%, respectively.

The most sensitive endpoint in *Pseudokirchneriella subcapitata* to technical grade dimethomorph was effects on biomass. The EC_{50} for effects on biomass (i.e., E_bC_{50}) was 41.1 mg/L. The EC_{50} for effects on growth rate (i.e., E_rC_{50}) was 82.2 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethomorph (technical grade, also known as BAS 550 F); Batch Number AC 9978-131; purity: 98.3%.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG (Collection of agal cultures in Göttingen)
Test design:	Static system; test duration 72 hours; 5 test concentrations, each with 3 replicates per treatment plus a control with 6 replicates, each; assessment of growth at 0, 24 and 72 hours.
Endpoints:	EC_{50} with respect to growth rate and biomass.
Test concentrations:	Control, 6.25, 12.5, 25, 50, and 100 mg a.s./L (nominal)
Test conditions:	Temperature: 23 $\pm$ 2 °C; pH: 8.0 - 8.3; photoperiod: 24 hours light; light Intensity: 60 – 120 µE/(m^2 *S) 400 – 700 nm, initial cell densities 1 x 10^4 cells/mL;
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method.
Statistics:	Descriptive statistics; EC_{50} values were estimated using the Logit Method

II. RESULTS AND DISCUSSION

Analytical measurements: The analytical recoveries from the test substance treatment groups ranged from 75.8 to 82.5% of the nominal concentrations at 0 hours, to 77.6 to 94.0% of the nominal concentrations at 72 hours. The 100 and 50 mg/l test solutions were turbid. The results of the test are based on nominal test concentrations.

Biological results: The percent cell densities compared to the control in the concentrations of 6.25, 12.5, 25, 50 and 100 mg a.s./L were determined to be 1%, 20%, 30%, 70% and 94%, respectively. The effects on algal growth are summarized in Table 8.2.6.1-2

Table 8.2.6.1-2: Effect of Dimethomorph on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration (mg a.s./L) (nominal)	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Inhibition of cell density in 72 h [%]	--	1	20	30	70	94
Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	> 100					
E_bC_{50} (72 h)	> 100					

III. CONCLUSION

The most sensitive endpoint in *Pseudokirchneriella subcapitata* to technical grade dimethomorph was effects on biomass. The EC_{50} for effects on biomass (i.e. E_bC_{50}) was 41.1 mg/L. The EC_{50} for effects on growth rate (i.e. E_rC_{50}) was 82.2 mg/L.

CA 8.2.6.2 Effects on growth of an additional algal species

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.7 Effects on aquatic macrophytes

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.8 Further testing on aquatic organisms

From the literature search the following two peer-reviewed scientific studies on *Lemna minor* were considered relevant and reliable (with restrictions; RI 2). In these studies, *L. minor* were exposed to copper sulfate, dimethomorph, the formulation 'FORUM', which contains dimethomorph as active substance, or to combinations of copper sulfate and dimethomorph or 'FORUM'. In the following summary only the experimental data and results for dimethomorph and the dimethomorph containing formulation 'FORUM' are presented. Due to the fact that the following two studies present the same results, the following summary is valid for both.

Report:	CA 8.2.8/1 Megateli S. et al., 2009 a Toxicity of Copper/Dimethomorph combination for Lemna minor and depuration of the fungicides by aquatic plant 2009/1131842
Guidelines:	none
GLP:	no

Report:	CA 8.2.8/2 Megateli S. et al., 2013 a Simultaneous effects of two fungicides (Copper and Dimethomorph) on their phytoremediation using Lemna minor 2013/1419680
Guidelines:	none
GLP:	no

Executive Summary

The effect of dimethomorph, as single substance or in in the formulation 'FORUM', on the growth of the aquatic plant *Lemna gibba* was studied in a 168 h static toxicity test. The following nominal concentrations were applied: 0 (control), 0.040, 0.200, 0.400, 0.800 and 1.000 mg dimethomorph/L as pure ingredient or used as formulation FORUM. When *L. minor* were exposed to copper sulfate and dimethomorph in combination, the concentration of dimethomorph was set at 0.25, 0.50 and 1.00 mg a.s./L as pure ingredient or as formulation 'FORUM'. Assessment of plant growth (counting visible fronds) was conducted after 96 h and at the end of the test. Percent growth inhibition relative to the control was calculated for each test concentration based on the parameter frond number.

The biological results are based on nominal concentrations. Information about the growth or morphological changes of the duckweed population in the control vessels are not reported. After 96 h and 168 h the maximum inhibition reached was 45.45% with 0.500 mg dimethomorph/L (FORUM). In case dimethomorph was applied as pure ingredient inhibition was even lower and reached 19.04% and 33.33% in the highest test concentration after 96 h and 168 h, respectively.

In several 168 h aquatic-plant tests with *Lemna minor,* the growth inhibition based on frond numbers was determined to be maximum 33.33% due to exposition with dimethomorph as pure active substance at the highest tested concentration of 1.000 mg a.s./L (nominal) and 45.25% due to exposition with dimethomorph as the formulation "FORUM" at the concentration of 0.500 mg a.s./L (nominal). After 96 h the inhibition was maximum 19.04% and 45.45% in the treatments with 1.000 mg a.s./L (pure substance) and 0.500 mg a.s./L ("FORUM"), respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (E,Z): BAS 550 F, Reg. no.: 247 723, purity:97%, purchased from BASF Agro, France;
FORUM: 150 g dimethomorph/L, density: 1.080 g/cm³, purchased from BASF Agro, France.

B. STUDY DESIGN

Test species: Duckweed (*Lemna minor*), collected from ponds in the Ardennes, France; inocula 10 days old cultures; cultures maintained in-house.

Test design: Static system; test duration 168 h, 5 test item concentrations for dimethomorph as single substance and formulation, 3 test item concentrations for the combination of copper sulfate and dimethomorph; each with 2 replicates per treatment plus a control with 2 replicates; each toxicity test was repeated three times; total number of fronds at test initiation: 20 (50 ± 0.10 mg fresh weight) per replicate; assessment of growth after 96 h and 168 h.

Endpoints: Not reported.

Test concentrations: Control, 0.040, 0.200, 0.400, 0.800 and 1.000 mg dimethomorph/L (nominal) as pure ingredient or used as formulation FORUM;
Combined toxicity test: 0.25, 0.50 and 1.00 mg dimethomorph/L as pure ingredient or used as formulation FORUM.

Test conditions: 100 mL Erlenmeyer flasks, test volume: 50 mL sterile inorganic growth medium (Chollet, 1993); pH of nutrient solution 6.5 ± 0.1; water temperature: 22 ± 1 °C, photoperiod: 16 h light : 8 h dark; light intensity: 60 µmol PAR/m²/s.

Analytics: Analytical measurements were conducted using an HPLC-method.

Statistics: Descriptive statistics; Student´s t-test ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: After 96 h removal of dimethomorph (FORUM) was 40%, 31% and 15% in the test concentrations 0.250, 0.500 and 1.000 mg a.s./L, respectively, whereas in case of pure dimethomorph removal was lower reaching 30%, 19% and 10%. The following biological results are based on nominal concentrations.

Biological results: Information about the growth or morphological changes of the duckweed population in the control vessels are not reported. After 96 h and 168 h the maximum inhibition reached was 45% with 0.500 mg dimethomorph/L (FORUM). In case dimethomorph was applied as pure ingredient inhibition was even lower and reached 19% and 33% in the highest test concentration after 96 h and 168 h, respectively. Effects on growth are summarized in Table 8.2.8-1

Table 8.2.8-1: Effect of dimethomorph or 'FORUM' on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L](nominal)	**0.250**	**0.500**	**1.000**
Inhibition by dimethomorph after 96 h [%] (based on frond no.)	12.38 ± 2.29 *	15.23 ± 1.8 *	19.04 ± 2.77 *
Inhibition by FORUM after 96 h [%] (based on frond no.)	38.00 ± 10.6 *	45.45 ± 6.4 *	43.76 ± 5.33 *
Inhibition by dimethomorph after 168 h [%] (based on frond no.)	16.51 ± 1.40 *	30.80 ± 3.11 *	33.33 ± 3.77 *
Inhibition by FORUM after 168 h [%] (based on frond no.)	43.5 ± 2.8 *	45.25 ± 5.3 *	43.76 ± 11 *

* Statistically different to the control ($p < 0.05$).

III. CONCLUSION

In several 168 h aquatic-plant tests with *Lemna minor,* the growth inhibition based on frond numbers was determined to be maximum 33.33% due to exposition with dimethomorph as pure active substance at the highest tested concentration of 1.000 mg a.s./L (nominal) and 45.25% due to exposition with dimethomorph as the formulation "FORUM" at the concentration of 0.500 mg a.s./L (nominal). After 96 h the inhibition was maximum 19.04% and 45.45% in the treatments with 1.000 mg a.s./L (pure substance) and 0.500 mg a.s./L ("FORUM"), respectively.

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this peer-reviewed scientific study two green algae species were exposed to dimethomorph at various concentrations and effects were determined by measuring growth rate and algal chlorophyll fluorescence emission. According to the current OECD guideline 201 for alga testing (OECD, 2011) the relevant endpoint for toxicity tests with algae is inhibition of growth. Therefore, in the summary provided below, only the results based on the effect parameter growth rate are presented. The results derived from this study show no unique endpoint values. Furthermore the endpoints are based on a test duration of 96 h, whereas the current OECD guideline 201 for alga testing (OECD, 2011) a test duration of 72 h preferred. Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report: CA 8.2.8/3
Dosnon-Olette R. et al., 2010 a
Fungicide and herbicide removal in Scenedesmus cell suspensions
2010/1232972
Guidelines: none
GLP: no

Executive Summary

In a 96 h static acute toxicity laboratory study, the effect of dimethomorph on the growth of the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda* were investigated. Actual concentrations of 0.200, 0.400, 0.600 and 0.800 mg dimethomorph/L were applied. Additionally different control treatments were set up. Assessment of growth was conducted once at the beginning and every 24 h thereafter.

The biological results are based on actual test item concentrations. After 96 hours of exposure, growth rate of *S. obliquus* was statistically significantly reduced at all tested concentrations compared to the control, whereas statistically significantly differences in growth rate of *S. quadricauda* compared to the control occurred only at the highest tested concentration of 0.800 mg a.s./L. After 96 h of exposure at the highest concentration tested, the growth rate inhibition reached a maximum of 31%.

In a 96 h algae test with the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda*, the EC_{50} for dimethomorph was determined to be > 0.800 mg a.s./L for both algae species.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethomorph (BAS 550 F; Reg. no.: 247 723); tested as formulated product Forum (BASF Agro, Belgique) containing 150 g a.s./L (nominal).

B. STUDY DESIGN

Test species: Green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda* (SAG 276-3a and 276-4b; Göttingen, Germany).

Test design: Static system; test duration 4 d (96 hours); 4 test item concentrations plus control, all experiments were repeated three times and each sample was run in triplicate; three different controls were carried out in parallel: # 1 algae in a pesticide free medium, # 2 dead-frozen algae in a medium containing pesticides and # 3 medium containing pesticides but free of algae; daily assessment of growth by measurement of optical density.

Endpoint: Inhibition of growth rate after exposure over 4 days (96 h).

Test concentrations: Control; 0.200, 0.400, 0.600 and 0.800 mg a.s./L. Pesticide concentrations given in this study refer to the actual, and not to the nominal concentrations of dimethomorph.

Test conditions: 250 mL Erlenmeyer flasks containing 100 mL culture medium; exponentially growing cell cultures with an initial cell concentration of 1.4×10^6 cells/mL; data on physical/chemical test conditions (pH, conductivity, light intensity, temperature and hardness) not reported.

Analytics: Analytical measurements were conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, significant differences between controls and test item treatments were determined by One Way ANOVA tests ($p < 0.05$).

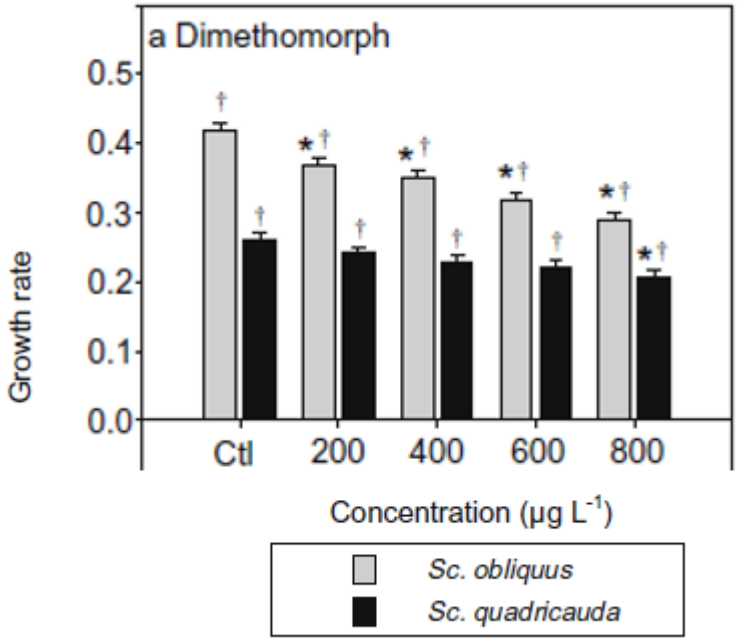
II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements were conducted daily in the controls. No pesticide was detected in control # 1. The following biological results are based on actual test item concentrations.

Biological results: After 4 days of exposure, growth rate of *Sc. obliquus* was statistically significantly reduced at all tested concentrations compared to the control, whereas statistically significantly differences in growth rate of *Sc. quadricauda* compared to the control occurred only at the highest tested concentration of 0.800 mg a.s./L (One Way ANOVA ($p < 0.05$). After 4 days of exposure at the highest concentration tested the growth rate inhibition reached a maximum of 31%.

The results are shown in Figure 8.2.8-1

Figure 8.2.8-1: Effect of dimethomorph on the growth rate of *S. obliquus* and *S. quadricauda* after 4-d exposure to dimethomorph



* Data are significantly different from control and † for data significantly different between the two algae ($P < 0.05$).

III. CONCLUSION

In a 96 h algae test with the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda,* the E_rC_{50} for dimethomorph was determined to be > 0.800 mg a.s./L for both algae species.

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this peer-reviewed scientific study two duckweed species were exposed to dimethomorph at various concentrations and effects were determined by measuring growth rate and in vivo chlorophyll fluorescence emissions. According to the current OECD guideline 221 for *Lemna sp*. testing (OECD, 2006) the relevant endpoint for toxicity tests with ~~algae~~ Lemna is inhibition of growth. Therefore, in the summary provided below, only the results based on the effect parameter growth rate are presented. The results derived from this study are not sufficient to calculate an E_rC_{50} value. Furthermore, dimethomorph is a fungicide and no TER calculations for aquatic plants are required. Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/4 Dosnon-Olette R. et al., 2010 b Influence of initial pesticide concentrations and plant population density on Dimethomorph toxicity and removal by two duckweed species 2010/1232973
Guidelines:	none
GLP:	no

Executive Summary

In a 96 h static toxicity laboratory study, the effect of dimethomorph, tested as the formulation Forum, on the growth of the duckweed *Lemna minor* and *Spirodela polyrhyza* ~~*S. polyrhiza*~~ was investigated. The following actual concentrations were applied: 0.025, 0.050, 0.075, 0.150, 0.300 and 0.600 mg a.s./L. Assessment of growth was conducted at test termination. The percentage growth inhibition, relative to the control, was calculated for each test concentration based on growth rates for the parameters frond number.

The biological results are based on actual concentrations. The increase of dimethomorph concentrations (0.025 to 0.600 mg/L) in the medium resulted in a decreased growth rate, with an inhibition up to 21% and 19% at 0.600 mg/L for *L. minor* and *S. polyrhiza* after 96 h of exposure, respectively. Growth rate was significantly affected from 0.300 mg/L for *L. minor* and from 0.150 mg/L for *S. polyrhiza*. However, no significant difference of sensitivity was observed between the two species.

In a 96 h aquatic plant test with *Lemna minor* and *S. polyrhiza* the E_rC_{50} of dimethomorph was determined to be > 0.600 mg a.s./L based on frond no. for both species.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Forum; content of a.s.: dimethomorph (BAS 550 F, Reg. no. 247 723): 165 g/L (nominal: 150 g/L).

B. STUDY DESIGN

Test species: *Lemna minor*; collected from ponds in the Champagne area (France); *Spirodela polyrhiza*, stock obtained from University of Jena, Germany; both cultures were maintained in-house and subcultured twice a week.

Test design: Static system (96 h); 8 treatment groups (6 test item concentrations, 2 controls) with 3 replicates; controls: #1 – plants in a pesticide free medium; #2 – medium containing pesticides but free of plants; furthermore, population density: 0.10 g of fresh weight per replicate (*L. minor*: 64 fronds; *S. polyrhiza*: 12 fronds) at test initiation; assessment of growth after 96 h.

Endpoints: Inhibition of growth rate after exposure over 4 days (96 h).

Test concentrations: Control, 0.025, 0.050, 0.075, 0.150, 0.300 and 0.600 mg a.s./L. Pesticide concentrations given in this study refer to the actual, and not to the nominal concentrations of dimethomorph.

Test conditions: 250 mL Erlenmeyer flasks, test volume: 100 mL, growth medium (Chollet, 1993), data on physical/chemical test conditions (pH, conductivity, light intensity, temperature and hardness) not reported.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; one-way ANOVA for comparison of growth rate of controls and treatment levels.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the end of the test. The analyzed contents of dimethomorph ranged from 64% - 81% and from 68% to 87% of actual concentration at test termination in the *L. minor* and *S. polyrhiza* trials, respectively. No pesticide was detected in control # 1. The following biological results are based on actual test item concentrations.

Biological results: The increase of dimethomorph concentrations (0.025 to 0.600 mg/L) in the medium resulted in a decreased growth rate, with an inhibition up to 21% and 19% at 0.600 mg/L for *L. minor* and *S. polyrhiza* after 96 h of exposure, respectively. Growth rate was significantly ($p<0.05$) affected from 0.300 mg/L for *L. minor* and from 0.150 mg/L for *S. polyrhiza*. However, no significant difference of sensitivity was observed between the two species. Effects on growth rate are summarized below (see Table 8.2.8-2)

Table 8.2.8-2: Effect of dimethomorph (tested as formulated product Forum) on the growth of duckweed *Lemna minor* and *S. polyrhiza*

Concentration [mg a.s./L] (actual)	Test organism	0.025	0.050	0.075	0.150	0.300	0.600
Inhibition after 96 h [%] (growth rate based on frond no.)	*L. minor*	3 ± 3	3 ± 3	6 ± 3	9 ± 3	12 ± 3 *	21 ± 3 *
	S. polyrhiza	3 ± 3	6 ± 3	8 ± 3	11 ± 3	14 ± 3 *	19 ± 3 *

* Significantly different from control ($p<0.05$).

III. CONCLUSION

In a 96 h aquatic plant test with *Lemna minor* and *S. polyrhiza* the E_rC_{50} of dimethomorph was determined to be > 0.600 mg a.s./L based on frond no. for both species.

References

EFSA (2013) EFSA Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.

European Commission (2013) Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

OECD (1992) OECD Guidelines for the Testing of Chemicals, Guideline 203, Fish, Acute Toxicity test. OECD Publishing. Adopted: 17 July 1992.

OECD (2004) OECD Guidelines for the Testing of Chemicals, Guideline 202, *Daphnia* sp., Acute Immobilisation Test. OECD Publishing. Adopted: 13 April 2004.

OECD (2011) OECD Guidelines for the Testing of Chemicals, Guideline 201, Freshwater Algae and Cyanobacteria, Growth Inhibition Test. OECD Publishing. Adopted: 23 March 2006, Annex 5 corrected: 28 July 2011. pp. 25.

CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of the active substance dimethomorph (BAS 550 F), new studies on bees have been performed with the active substance. As a result there are new endpoints, which are considered in the honey bee risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.3.1-1.
Originally, it was intended to use the technical grade of the active ingredient dimethomorph (BAS 550 F) for the studies, but preliminary tests showed that the solubility in the food solution was very low and could not be improved neither using an ultrasonic bath nor adding acetone. Thus, it was decided to use the representative formulation BAS 510 01 F.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance dimethomorph (BAS 550 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
dimethomorph	honeybee	48 h acute oral LD_{50}	> 32.4 µg a.s./bee	*DK-541-027*	yes
		48 h acute contact LD_{50}	> 102.0 µg a.s./bee		
		10 d chronic LD_{50}	n.d. *	2013/1178040	no, new study
		10 d chronic NOED	≥ 273.3 µg a.s./bee/day *		
		10 d chronic LC_{50}	n.d.*		
		10 d chronic NOEC	≥ 10 g a.s./kg food *		
	honeybee larvae	72 h NOED	37.4 µg a.s./larva *	2013/1178041	no, new study
		72 h NOEC	1.103 g a.s./kg food *		
	bumblebee	96 h acute oral LD_{50}	n.d. *	2015/1000605	no, new study
		96 h acute oral NOED	≥ 300 µg a.s./bee/day *		

* Studies were conducted with the solo formulation BAS 550 01 F (50% dimethomorph).
Italic figures: The references in italic were submitted in the old dossier and peer reviewed. ~~They are not summarized in this supplemental dossier.~~ Summaries are presented in CA 8.3.

In addition to the laboratory studies listed above, two potentially relevant studies from the public literature are available.

Report:	Stoner K A, Eitzer B D, 2013 Using a Hazard Quotient to evaluate pesticide residues detected in pollen trapped from honey bees (Apis mellifera) in Connecticut. PLoS One, 8(10), e77550 CODEN: POLNCL; ISSN: 1932-6203
Guidelines:	none
GLP:	no

Executive Summary

Pollen samples of honeybees returning to the hive were collected from apiaries in five locations in Connecticut, including urban, rural, and mixed agricultural sites, for periods from two to five years. Pollen was analyzed for pesticide residues using a standard extraction method widely used for pesticides (QuEChERS) and liquid chromatography/mass spectrometric analysis. Sixty pesticides or metabolites were detected. The dose lethal to 50% of adult worker honey bees (LD_{50}) is the only toxicity parameter available for a wide range of pesticides, and among the found pesticides there were contact LD_{50} values ranging from 0.006 to >1000 µg per bee. Contact and oral LD_{50} values were used to calculate Pollen Hazard Quotients (PHQ = concentration in ppb ÷ LD_{50} as µg/bee) when both were available. In this study, pesticide Pollen Hazard Quotients ranged from over 75,000 to 0.01. The pesticides with the greatest Pollen Hazard Quotients at the maximum concentrations found in our study were (in descending order): phosmet, imidacloprid, indoxacarb, chlorpyrifos, fipronil, thiamethoxam, azinphos-methyl, and fenthion, all with at least one Pollen Hazard Quotient (using contact or oral LD_{50}) over 500. At the maximum rate of pollen consumption by nurse bees, a Pollen Hazard Quotient of 500 would be approximately equivalent to consuming 0.5% of the LD_{50} per day. The maximum residues of dimethomorph in pollen were 69 ppb. Based on the pollen consumption of bees and an acute contact LD_{50} of > 10 µg/bee, the authors found that worker bees would ingest only 0.1% of the LD_{50} per day.

I. MATERIAL AND METHODS

Test species:	*Apis mellifera* (honey bee)
Test design:	Pollen was collected using Sundance™ I bottom-mounted pollen traps (Ross Rounds, Albany, NY). All pollen was collected from the trap twice weekly, with two samples put immediately into 50 ml centrifuge tubes, frozen upon return from the field, and held at -20° C until analysis. Pollen was collected from a single hive in the apiary unless the amount of pollen per sample decreased below sufficient levels for analysis, or the health of the hive declined, and then the trap was moved to a new hive in the same apiary. Pollen was analyzed for pesticide residues using a standard extraction method widely used for pesticides (QuEChERS) and liquid chromatography/mass spectrometric analysis.

II. RESULTS AND DISCUSSION

Sixty pesticides or metabolites were detected. Pesticide Pollen Hazard Quotients ranged from over 75,000 to 0.01. The pesticides with the greatest Pollen Hazard Quotients at the maximum concentrations found in our study were (in descending order): phosmet, imidacloprid, indoxacarb, chlorpyrifos, fipronil, thiamethoxam, azinphos-methyl, and fenthion, all with at least one Pollen Hazard Quotient (using contact or oral LD_{50}) over 500. At the maximum rate of pollen consumption by nurse bees, a Pollen Hazard Quotient of 500 would be approximately equivalent to consuming 0.5% of the LD_{50} per day. The maximum residues of dimethomorph in pollen were 69 ppb. Based on the pollen consumption of bees and an acute contact LD_{50} of > 10 µg/bee, the authors found that worker bees would ingest only 0.1% of the LD_{50} per day.

III. CONCLUSION

The maximum residues of dimethomorph in pollen were 69 ppb. Based on the pollen consumption of bees and an acute contact LD_{50} of > 10 µg/bee, the authors found that worker bees would ingest only 0.1% of the LD_{50} per day. The risk assessment made by the authors confirms no risk for bees through dimethomorph.

However, the article is not considered relevant by the Notifier for several reasons:
a) The multi-year studies on the residue analysis of bee-collected pollen were conducted in Connecticut (U.S.A.), where cropping systems, climate and whether conditions differ from the European conditions.
b) The criteria applied for site selection for hives appear arbitrarily, e.g. on roof of Experimental Station
c) However, the use of a LD_{50} for contact exposure rather than for oral exposure for residues in bee relevant matrices appears inappropriate. In addition, it is unclear where the LD_{50} (contact) > 10 µg a.s./bee is taken from.

Even though this study cannot be related to the EU regulatory requirements the risk assessment made by the authors confirms no risk for bees through dimethomorph. Conesquently, it can be concluded that the results do not raise further concerns about the use of dimethomorph and bee safety.

Report:	Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Buechler R, Berg S, Ritter W, Muehlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P, 2010
	The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie, 41(3), 332-352 CODEN: APDGB5; ISSN: 0044-8435
Guidelines:	none
GLP:	no

Executive Summary

Over a period of 4 years, 1200 bee colonies from ~120 apiaries were monitored with respect to factors / stressors that potentially have an impact on the overwintering success of bee colonies. These factors comprised mainly pathogenic factors, but also the potential impact of pesticides, including the fungicide dimethomorph.
While dimethomorph has been detected in a few samples, the authors did not specify which amount of dimethomoprh has been detected.
Overwintering success of honey bee colonies was influenced by (i) high varroa infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. Effects of plant protection products on the overwintering success of bee colonies did not occur.

I. MATERIAL AND METHODS

Test species:	*Apis mellifera* (honey bee)
Test design:	Population size of colonies was estimated before (October) and after (March/April) wintering, preferably in colonies with little or no brood, i.e. after and before massive brood rearing. The quotient of the population size before and after the wintering of the colonies were calculated as "overwintering quotient" and represented a measure of the weakening of the colonies over winter. During the spring survey the number of collapsed colonies was counted. A colony was considered dead if (i) no bees were present any more or (ii) the colony was too weak to have a chance to recover during spring (approximately less than three bee frames occupied by bees after winter). For the pathogen sampling and analysis of adult honey bees 150 specimen were collected in autumn, spring and summer and were analyzed fort he occurrence of *Varroa destructor, Nosema* spec. and for five relevant honey bee viruses (Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), sacbrood virus (SBV), deformed wing virus (DWV), and Israeli acute paralysis virus (IAPV). Samples of bee bread (appr. 10 × 10 cm) collected after the blooming period of oilseed rape (*Brassica napus*) were used for residue analysis. In the years 2005 and 2006, fifty apiaries were selected for residue analysis based on the microscopic pollen analysis of the honey. Only those which revealed a high input of rape nectar were considered suitable due to a potential exposure to pesticides. All samples were split in two parts, one for the pollen analysis and one for the residue analysis. For the chemical analyses a multi-method (LCMS/ MS, GC-MS) was adapted which allowed the detection and quantification of 258 active ingredients. 5 g beebread were extracted with acetonitrile. After removal of fat and remaining proteins by cooling to –20 ◦C overnight, solvent was cleaned-up using gel-permeation-chromatography (GPC). The extract was further cleaned by SPE cartridges containing C18, aminopropyl and graphitized carbon black. The final extract was analyzed by GC-MS and LC-MS/MS for 258 pesticides and pesticide metabolites. The limits of quantification were between 3 and 10 µg/kg, in a few cases 15 µg/kg.

II. RESULTS AND DISCUSSION

The German Beee Monitoring presents a four-year study involving more than 1200 bee colonies from about 120 apiaries which were monitored for the entire study period. Bee samples were collected twice a year to analyze various pathogenic factors including the ectoparasitic mite Varroa de-structor, fungi (Nosema spec., Ascosphaera apis), the bacterium Paenibacillus larvae, and several viruses. Data on environmental factors, beekeeping management practice, and pesticides were also collected. All data were statistically analyzed in respect to the overwintering mortality of the colonies.
It was demonstrated for several factors that they are significantly related to the observed winter losses of the monitored honey bee colonies: (i) high varroa infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. No effects could be observed for Nosema spec. or pesticides.

III. CONCLUSION

According to the results of the study the overwintering success of honey bee colonies was influenced by (i) high varroa infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. Effects of plant protection products on the overwintering success of bee colonies did not occur.

As the study did not provide endpoints for the risk assessment, cannot be related to the EU bee risk assessment and covers a generic monitoring approach, the study is not considered relevant by the Notifier.

Even though this study cannot be related to the EU bee risk assessment it was demonstrated that no effects could be observed for pesticides, which comprises dimethomorph, too. Consequently, it can be concluded that dimethomorph does not affect the overwintering success of bee colonies and that the results do not raise further concerns about the use of dimethomorph and bee safety.

CA 8.3.1.1 Acute toxicity to bees

The EU agreed acute oral and contact laboratory studies with BAS 550 F on honeybees (BASF DocID DK-541-027) were included in the appendix of studies. As requested by the RMS, study summaries are also added below for sake of completeness.

CA 8.3.1.1.1 Acute oral toxicity

Report:	CA 8.3.1.1.1/1 Verge E., 2014 a BAS 550 01 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions 2015/1000605
Guidelines:	EPPO PP 1/170 (4) (2010), OECD 213 (1998), OECD 214 (1998), Van der Steen (2001)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In an acute oral toxicity test, bumblebees (young adult worker bumblebees of *Bombus terrestris L.*) were exposed to BAS 550 01 F. The toxicity of the test item was determined at nominal doses of 100.0, 150.0, 200.0, 250.0 and 300.0 µg a.s./bumblebee (referring to the nominal a.s. content of BAS 550 01 F), resulting in an actual uptake of 96.3, 144.4, 186.8, 223.0 and 283.2 µg a.s./bumblebee (corresponding to 192.6, 288.8, 373.6, 446.0 and 566.4 µg BAS 550 01 F/bumblebee).

Additionally, bumblebees were treated with BAS 152 11 I as a reference item at the dose of 1.5 µg dimethoate/bumblebee (nominal) or with a 50% (w/v) aqueous sucrose solution as a control.

After 96 hours of oral exposure, 3.33% mortality was observed in the control group. At the end of the 96 hour exposure period no mortality (corrected mortality -3.4%) was recorded at the nominal dose of 300.0 µg a.s./bumblebee. The maximum mortality of 3.33% (corrected mortality 0.0%) was recorded at the nominal doses of 100.0 and 200.0 µg a.s./bumblebee. At the assessments after 4 and 48 hours one affected bee was observed at the nominal doses of 250.0 and 300.0 µg a.s./bumblebee, respectively. At the assessments 24, 72 and 96 hours after test start no abnormal behaviour were observed.

In an oral toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The NOED (No Observed Effect Dose) is assumed to be ≥ 283.2 µg a.s./bumblebee (equivalent to ≥ 566.4 µg BAS 550 01 F/bumblebee).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 550 01 F; batch no. FRE-001045; content of a.s.: dimethomorph (BAS 550 F, Reg. No. 247 723): 50.0% (nominal), 48.1% (analyzed).

B. STUDY DESIGN

Test species: *Bombus terrestris* (bumblebee), young adult worker bumblebees derived from a healthy and queen-right colony; source: Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands; collected from the outer combs of the bumblebee hive one day before test start.

Test design: In a 96 hour test, adults of worker bumblebees of *Bombus terrestris* were exposed orally to 5 doses of BAS 550 01 F (50% (w/v) aqueous sucrose solution). In total, 7 treatment groups were set up (5 dose rates of the test item, 1 untreated control group and 1 dose rate of the reference item) with 3 replicates per dose and 10 bumblebees per replicate. Assessment of bumblebee mortality and behavioral effects was done after 4, 24, 48, 72 and 96 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test concentrations: Control (50% (w/v) aqueous sucrose solution)

Test item:

Nominal a.s. dose rate (µg/bumblebee)	Consumed product (µg/bumblebee) *	Consumed a.s. (µg/bumblebee)
100.0	192.6	96.3
150.0	288.8	144.4
200.0	373.6	186.8
250.0	446.0	223.0
300.0	566.4	283.2

* Calculation based on the nominal content of a.s. in BAS 550 01 F (dimethomorph: 50.0%)

Test conditions: Temperature: 23.5 °C – 26.2 °C, relative humidity: 50.1% – 66.7%, photoperiod: 24 h darkness, food: 50% (w/v) aqueous sucrose solution, *ad libitum.*

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni-Holms Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 96 hours of oral exposure, 3.33% mortality was observed in the control group. At the end of the 96 hour exposure period no mortality (corrected mortality -3.4%) was recorded at the nominal dose of 300.0 µg a.s./bumblebee. The maximum mortality of 3.33% (corrected mortality 0.0%) was recorded at the nominal doses of 100.0 and 200.0 µg a.s./bumblebee. At the assessments after 4 and 48 hours one affected bee was observed at the nominal doses of 250.0 and 300.0 µg a.s./bumblebee, respectively. At the assessments 24, 72 and 96 hours after test start no sub-lethal effects or abnormal behaviors were observed.
In an oral toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The results are summarized in Table 8.3.1.1.1-1

Table 8.3.1.1.1-1: Toxicity of BAS 550 01 F to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Uptake of test item	Mortality [%]				Corrected mortality [%]			
[µg a.s./bumblebee]	[µg BAS 550 01 F/ bumblebee]	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	--	0.0	3.33	3.33	3.33	-	-	-	-
100.0	192.6	0.0	0.0	0.0	3.33	0.0	-3.4	-3.4	0.0
150.0	288.8	0.0	0.0	0.0	0.0	0.0	-3.4	-3.4	-3.4
200.0	373.6	0.0	0.0	0.0	3.33	0.0	-3.4	-3.4	0.0
250.0	446.0	0.0	0.0	0.0	0.0	0.0	-3.4	-3.4	-3.4
300.0	566.4	0.0	0.0	0.0	0.0	0.0	-3.4	-3.4	-3.4
	Endpoint (based on actual uptake)								
	[µg a.s./bumblebee]				[µg BAS 550 01 F/bumblebee]				
LD_{50} (96 h)	n.d.				n.d.				
NOED (96 h)	≥ 283.2				≥ 566.4				

The corrected mortality for the reference item in the oral toxicity test at the end of 96 hours was 96.6%.

III. CONCLUSION

In an oral toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The NOED (No Observed Effect Dose) is assumed to be ≥ 283.2 µg a.s./bumblebee (equivalent to ≥ 566.4 µg BAS 550 01 F/bumblebee).

Report:	CA 8.3.1.1.1/2 Strnad S.P.,Mulligan E., 2002 a Acute toxicity of technical Dimethomorph (AC 336379) to the honey bee, Apis mellifera DK-541-027
Guidelines:	EEC 91/414 Annex II 8.3.1.1, OECD 213, OECD 214, EPPO 170 (1992)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In an acute oral toxicity test, honeybees (young adult worker honeybees of *Apis mellifera ligustica*.) were exposed to BAS 550 F. The toxicity of the test item was determined at a nominal dose 32.4 µg consumed a.s./honeybee. Additionally, honeybees were treated with technical grade dimethoate as a reference item at 0.05, 0.1 and 0.2 µg dimethoate/honeybee (nominal), or with one of three controls: a 50% (w/w) aqueous sucrose solution, 100 and 800 µg sucrose solution containing 5% acetone. Assessment of honeybee mortality and behavioral effects was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, 0.0% mortality was observed in the test item treatment group compared to 2.0% in the sucrose control and 0% and 4.0% in the 100 and 800 µg sucrose solution control containing 5% acetone, respectively. At test termination (48-h), only one bee in the 100 µg sucrose solution control containing 5% acetone was affected (moribund or otherwise disoriented).

In an oral toxicity study with BAS 550 F on honeybees, the LD_{50} value is assumed to be > 32.4 µg BAS 550 F/honeybee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 550 F (dimethomorph, Reg. No. 247 723); Lot no. AC9978-131; 98% purity.

B. STUDY DESIGN

Test species: *Apis mellifera ligustica* L. (honeybee), young adult worker honeybees derived from a healthy and queen-right colony; source: M Bee's apiaries, Belle Mead, NJ, USA; collected from healthy and queen-right non-brood frames one day before test start.

Test design: In a 48 hour test, adults of worker honeybees of *Apis mellifera ligustica* L. were exposed orally to 1 dose of BAS 550 F (in 50% (w/w) aqueous sucrose solution). In total, 5 treatment groups were set up (1 dose rate of the test item, 3 untreated control groups and 3 dose rate of the reference item) with 5 replicates per dose and 10 honeybees per replicate. Assessment of honeybee mortality and behavioral effects was done after 4, 24 and 48 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: Technical grade dimethoate (98% purity). Tested at 0.05, 0.1 and 0.2 µg dimethoate/bee.

Test concentrations: Untreated diet control (50% (w/w) aqueous sucrose solution)
Acetone controls: 100 and 800 µg sucrose solution containing 5% acetone
Test item: 32.4 µg actual consumed dimethomorph/honeybee

Test conditions: Temperature: 23.7 °C, relative humidity: 71.00% – 79.78%, photoperiod: continuous illumination under ambient room conditions, food: 50% (w/w) aqueous sucrose solution, *ad libitum.*

Statistics: Descriptive statistics. Fisher Exact test for lethal and sublethal treatment effects.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, 0.0% mortality was observed in the test item treatment group compared to 2.0% in the sucrose control and 0% and 4.0% in the 100 and 800 µg sucrose solution control containing 5% acetone, respectively. At test termination (48-h), only one bee in the 100 µg sucrose solution control containing 5% acetone was affected (moribund or otherwise disoriented). The results are summarized below.

Table 8.3.1.1.1-2: Toxicity of BAS 550 F to *Apis mellifera ligustica* (honeybee) in an oral toxicity test

Treatment	Mortality [%]	
	24 h	48 h
50% sucrose control	0.0	2.0
100 µg sucrose solution / 5% acetone	0.0	0.0
800 µg sucrose solution / 5% acetone	4.0	4.0
32.4 µg BAS 550 F/honeybee	0.0	0.0
	Endpoint (based on actual uptake) [µg BAS 550 F/honeybee]	
LD_{50} (48 h)	> 32.4	

The mortality for the reference item in the oral toxicity test at the end of 48 hours was 62% at 0.20µg dimethoate/bee. The 24- and 48-hr oral LD_{50} values for dimethoate were 0.12 and 0.11 µg/bee, respectively.

III. CONCLUSION

In an oral toxicity study with BAS 550 F on honeybees, the LD_{50} value is assumed to be > 32.4 µg BAS 550 F/honeybee.

CA 8.3.1.1.2 Acute contact toxicity

Report:	CA 8.3.1.1.2/1 Verge E., 2014 a BAS 550 01 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions 2015/1000605
Guidelines:	EPPO PP 1/170 (4) (2010), OECD 213 (1998), OECD 214 (1998), Van der Steen (2001)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In an acute contact toxicity test with BAS 550 01 F, young adult worker bumblebees (*Bombus terrestris L.*) were exposed over a period of 96 hours to dose rates of 200.0, 300.0, 400.0, 500.0 and 600.0 µg BAS 550 01 F/bumblebee, which corresponds to 100.0, 150.0, 200.0, 250.0 and 300.0 µg a.s./bumblebee (based on nominal content of a.s.). Additionally, bumblebees were treated with BAS 152 11 I as a reference item at the dose of 12.0 µg dimethoate/bumblebee or with tap water as a control.

After 96 hours of contact exposure, no mortality occurred in the control group. After 96 hours of exposure no mortality was observed in the BAS 550 01 F treatment groups. No abnormal behaviors were observed during the entire 96 hour test period.

In a contact toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The NOED is assumed to be ≥ 300.0 µg a.s/bumblebee (equivalent to ≥ 600.0 µg BAS 550 01 F/bumblebee).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 550 01 F; batch no. FRE-001045; content of a.s.: dimethomorph (BAS 550 F, Reg. No. 247 723): 50.0% (nominal), 48.1% (analyzed).

B. STUDY DESIGN

Test species: *Bombus terrestris* (bumblebee), young adult worker bumblebees derived from a healthy and queen-right colony; source: Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands; collected from the outer combs of the bumblebee hive one day before test start.

Test design: In a 96 hour test, adults of worker bumblebees of *Bombus terrestris* were exposed to 5 doses of BAS 550 01 F dissolved in tap water. In total, 7 treatment groups were set up (5 dose rates of the test item, 1 untreated control group and 1 dose rate of the reference item) with 3 replicates per dose and 10 bumblebees per replicate. Assessment of bumblebee mortality and behavioral effects was done after 4, 24, 48, 72 and 96 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test concentrations: Water control (tap water)
Test item:

Nominal product dose rate (µg/bumblebee)	Nominal a.s. dose rate (µg/bumblebee)
200.0	100.0
300.0	150.0
400.0	200.0
500.0	250.0
600.0	300.0

* Calculation based on the nominal content of a.s. in BAS 550 01 F (dimethomorph: 50.0%)

Test conditions: Temperature: 23.7 °C – 26.1 °C, relative humidity: 53.1% – 64.6%, photoperiod: 24 h darkness, food: 50% (w/v) aqueous sucrose solution, *ad libitum.*

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 96 hours of contact exposure, no mortality was observed in the control. After 96 hours of exposure no mortality was observed in the BAS 550 01 F treatment groups. No sub-lethal effects or abnormal behaviors were observed during the entire 96 hour test period.
In a contact toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The results are summarized in Table 8.3.1.1.2-1

Table 8.3.1.1.2-1: Toxicity of BAS 550 01 F to *Bombus terrestris* (bumblebee) in a contact toxicity test

Treatment		Mean mortality [%]			
[µg BAS 550 01 F/bumblebee]	[µg a.s./bumblebee]	24 h	48 h	72 h	96 h
Control	Control	0.0	0.0	0.0	0.0
200.0	100.0	0.0	0.0	0.0	0.0
300.0	150.0	0.0	0.0	0.0	0.0
400.0	200.0	0.0	0.0	0.0	0.0
500.0	250.0	0.0	0.0	0.0	0.0
600.0	300.0	0.0	0.0	0.0	0.0
Endpoints					
		[µg a.s./bumblebee]		[µg BAS 550 01 F/bumblebee]	
LD_{50} (96 h)		n.d.		n.d.	
NOED (96 h)		≥ 300.0		≥ 600.0	

The mortality for the reference item in the contact toxicity test at the end of 96 hours was 83.3%.

III. CONCLUSION

In a contact toxicity study with BAS 550 01 F on bumblebees, no LD_{50}/LC_{50} value could be determined. The NOED is assumed to be ≥ 300.0 µg a.s/bumblebee (equivalent to ≥ 600.0 µg BAS 550 01 F/bumblebee).

Report:	CA 8.3.1.1.2/2 Strnad S.P.,Mulligan E., 2002 a Acute toxicity of technical Dimethomorph (AC 336379) to the honey bee, Apis mellifera DK-541-027
Guidelines:	EEC 91/414 Annex II 8.3.1.1, OECD 213, OECD 214, EPPO 170 (1992)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In an acute contact toxicity test, honeybees (young adult worker honeybees of *Apis mellifera ligustica*.) were exposed to BAS 550 F. The toxicity of the test item was determined at a nominal dose of 102 µg a.s./honeybee. Additionally, honeybees were treated with technical grade dimethoate as a reference item at 0.05, 0.1 and 0.2 µg dimethoate/honeybee (nominal), or with one of three controls: water alone, 1.0 µL acetone alone or 4.0 µL acetone alone. Assessment of honeybee mortality and behavioral effects was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, 2.0% mortality was observed in the test group compared to 2% in the water and 1.0 µL acetone controls, and 6.0% in the 4.0 µL acetone control. At test termination (48-h), no affected bees were observed in any of the treatments.

In an oral toxicity study with BAS 550 F on honeybees, the LD_{50} value is assumed to be > 102 µg BAS 550 F/honeybee.

I. MATERIAL AND METHODS

B. MATERIALS

Test item: BAS 550 F (dimethomorph, Reg. No. 247 723); batch no. AC9978-131; 98% purity.

B. STUDY DESIGN

Test species: *Apis mellifera ligustica* L. (honeybee), young adult worker honeybees derived from a healthy and queen-right colony; source: M Bee's apiaries, Belle Mead, NJ, USA; collected from healthy and queen-right non-brood frames one day before test start.

Test design: In a 48 hour test, adults of worker honeybees of *Apis mellifera ligustica* L were exposed one dose of BAS 550 F (in acetone) via direct application to the dorsal thorax. In total, 5 treatment groups were set up (1 dose rate of the test item, 3 untreated control groups and 3 dose rate of the reference item) with 5 replicates per dose and 10 honeybees per replicate. Assessment of honeybee mortality and behavioral effects was done after 4, 24 and 48 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: Technical grade dimethoate (98% purity). Tested at 0.05, 0.1 and 0.2 µg dimethoate/bee.

Test concentrations: Water control
Acetone controls:
-1.0 µL acetone,
-4.0 µL acetone,
Test item: 102 µg dimethomorph/honeybee

Test conditions: Temperature: 23.7 °C, relative humidity: 76.35% – 77.80%, photoperiod: continuous illumination under ambient room conditions.

Statistics: Descriptive statistics. Fisher Exact test for lethal and sublethal treatment effects.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, 2.0% mortality was observed in the test item treatment group compared to 2% in the water and 1.0 µL acetone controls, and 6.0% in the 4.0 µL acetone control. At test termination (48-h), no affected bees were observed in any of the treatments. The results are summarized below.

Table 8.3.1.1.2-2: Toxicity of BAS 550 F to *Apis mellifera ligustica* (honeybee) in a contact toxicity test

Treatment	Mortality [%]	
	24 h	48 h
water control	2.0	2.0
1.0 µL acetone control	0.0	2.0
4.0 µL acetone control	4.0	6.0
102 µg BAS 550 F/honeybee	0.0	2.0
	Endpoint [µg BAS 550 F/honeybee]	
LD_{50} (48 h)	> 102	

The reference item caused 100% mortality after 48 hours in the 0.20 µg dimethoate/honeybee concentration. The 24- and 48-hr contact LD_{50} values for dimethoate were 0.15 and 0.11 µg/bee, respectively.

III. CONCLUSION

In an oral toxicity study with BAS 550 F on honeybees, the LD_{50} value is assumed to be > 102 µg BAS 550 F/honeybee.

CA 8.3.1.2 Chronic toxicity to bees

Report: CA 8.3.1.2/1
Schmitzer S., 2013 a
Chronic oral toxicity test of BAS 550 01 F on the honeybee (Apis melllifera L.) in the laboratory
2013/1178040
Guidelines: OECD 213 (1998), CEB No. 230 (2003)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 10-day chronic oral toxicity test, one day old worker honeybees (*Apis mellifera carnica* P.) were exposed to a daily application of BAS 550 01 F diluted in the bee food (50% aqueous sucrose solution). The chronic toxicity of the test item was determined at nominal doses of 25, 50, 100, 200 and 400 µg a.s./bee/day (effective doses were 21.9, 43.5, 77.7, 163.7 and 273.3 µg a.s./bee/day), corresponding to concentrations of 625, 1250, 2500, 5000 and 10000 mg a.s./kg food, respectively. Additionally, honeybees were treated with Perfekthion EC (a.s.: dimethoate) as a reference item at a nominal dose of 0.031 µg a.s./bee/day. Untreated diet served as a control.

After 10 days of exposure, the untreated control showed a mean mortality of 6.7%. In the test item group, bees consuming doses of 21.9, 43.5, 77.7, 163.7 and 273.3 µg a.s./bee/day showed corrected mortalities of -7.2, -7.2, -3.6, -3.6 and 3.5%, respectively.
In the course of the study, only a few bees showed behavioral conspicuities. A dose of 163.7 µg a.s./bee/day led to uncoordinated movements of a single bee from day 4 to 10, except on day 9. At a dose of 273.3 µg a.s./bee/day seven bees displaying moving coordination problems or intensive cleaning were observed on day 10. No further test item related behavioral impairments occurred during the trial in any of the other test item dosed bees.

In a 10 day chronic toxicity feeding study with BAS 550 01 F, no LD_{50} /LC_{50} could be determined. The daily and overall NOEDs were ≥ 273.3 µg a.s./bee/day and ≥ 2733 µg a.s./bee, respectively, and the NOEC was ≥ 10000 mg a.s./kg food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 550 01 F (dimethomorph, Reg. No. 247 723), batch no. 01638729U0; 49.5% analyzed (50.0% nominal).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera* L.); female worker bees (1 day old); obtained from a healthy and queen-right colony; source: in-house culture.

Test design: 10-day chronic oral feeding test in the laboratory (dose response test). The honeybees were provided daily with one of 5 doses of the test item treated sugar solutions (50% aqueous sucrose solution) *ad libitum*. 6 treatment groups were set up: 5 doses of the test item and 1 untreated control group. Further, 1 dose of the reference item was tested. All doses with 3 replicates each consisting of 10 bees per replicate. Assessments of bee mortality and behavioral effects were done daily over the 10 day test period.

Endpoint: Mortality, behavior (including calculation of LC_{50}, $LD_{50\ (daily)}$, $LD_{50\ (overall)}$, NOEC, $NOED_{(daily)}$, $NOED_{(overall)}$).

Reference item: Perfekthion EC (BAS 152 11 I, dimethoate, 400.0 g/L nominal).

Test concentrations: Control: untreated diet (50% aqueous sucrose solution);
Test item: 625, 1250, 2500, 5000, 10000 mg a.s./kg food (corresponding to nominal doses of 25, 50, 100, 200 and 400 µg a.s./bee/day).
Reference item: 0.04 µg dimethoate/bee/day (nominal).

Test conditions: Temperature: 33.0°C – 36.0°C, relative humidity: 46% – 82%, photoperiod: 24 h darkness (except during assessments), food: 50% aqueous sucrose solution.

Statistics: Descriptive statistics; Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$,) for calculation of NOEC, $NOED_{daily}$ and $NOED_{overall}$.

II. RESULTS AND DISCUSSION

After 10 days of exposure, the untreated control showed a mean mortality of 6.7%. In the test item group, bees consuming doses of 21.9, 43.5, 77.7, 163.7 and 273.7 µg a.s./bee/day showed corrected mortalities of -7.2, -7.2, -3.6, -3.6 and 3.5%, respectively.

A dose of 163.7 µg a.s./bee/day led to uncoordinated movements of a single bee from day 4 to 10, except on day 9. At a dose of 273.7 µg a.s./bee/day, seven bees displaying moving coordination problems or intensive cleaning were observed on day 10. No further test item related behavioral impairments occurred during the trial in any of the other test item dosed bees.

The results are summarized in Table 8.3.1.2-1

Table 8.3.1.2-1: Endpoints and cumulative mortality of honeybees exposed to BAS 550 01 F in a 10 day chronic oral toxicity test

Treatment [BAS 550 01 F]			Mortality after 10 days	
Nominal dosage [µg a.s./bee/day]	**Consumed dosage [µg a.s./bee/day]**	**Concentration [mg a.s./kg food]**	**Absolute mortality [%]**	**Corrected mortality [%] [1)]**
Control	Control	Control	6.7	--
25	21.9	625	0.0	-7.2
50	43.5	1250	0.0	-7.2
100	77.7	2500	3.3	-3.6
200	163.7	5000	3.3	-3.6
400	273.7	10000	10.0	3.5

Endpoints		10 days
Test item doses	**LD_{50}**	n.d.
	NOED	≥ 273.3 µg a.s./bee/day
Test item concentrations	**LC_{50}**	n.d.
	NOEC	≥ 10000 mg a.s./kg food

[1)] Corrected mortality according to Abbott (1925).

The reference item dimethoate caused 100% mortality at day 10 at a concentration of 1.0 mg dimethoate/kg food, corresponding to a nominal dose of 0.031 µg dimethoate/bee/day.

III. CONCLUSION

In a 10 day chronic toxicity feeding study with BAS 550 01 F, no LD_{50} /LC_{50} could be determined. The daily and overall NOEDs were ≥ 273.3 µg a.s./bee/day and ≥ 2733 µg a.s./bee, respectively, and the NOEC was ≥ 10000 mg a.s./kg food.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report: CA 8.3.1.3/1
Kleebaum K., 2014 a
Acute toxicity of BAS 550 01 F to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro)
2013/1178041

Guidelines: OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an acute oral larval toxicity test, four day old honeybee larvae (*Apis mellifera*) were exposed to one application of BAS 550 01 F diluted in the larvae food.

The toxicity of the test item was determined with doses of 25.4, 37.4, 55.0, 80.9 and 118.9 µg a.s./larva (corresponding to 50.9, 74.8, 110.0, 161.8 and 237.9 µg BAS 550 01 F/larva). The concentrations of test item in the diet were 0.750, 1.103, 1.622, 2.386 and 3.509 g a.s./kg food. Additionally, honeybee larvae were treated with dimethoate as the reference item. Untreated diet served as a control.

After 72 hours of exposure, a mortality of 2.8% was observed in the control. In the test item group, mortalities ranged between 11.1% and 41.7% after 72 hours. Statistically significant effects occurred at the three highest test item treatment doses, 55.0, 80.9 and 118.9 µg a.s./larva.

After 72 hours of exposure, no deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in test item treatment group.

In an acute oral larval toxicity study with BAS 550 01 F on honeybee larvae, no LD_{50}/LC_{50} could be determined. The NOED (72 h) was determined to be 37.4 µg a.s./larva (equivalent to a NOEC (72 h) of 1.103 g a.s./kg food).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 550 01 F (dimethomorph, Reg. No. 247 723), batch no. 01638729U0; 49.5% analyzed (50.0% nominal).

B. STUDY DESIGN

Test species: *Apis mellifera carnica* P. (honeybee); synchronized first instar larvae (one day old); derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: One day old honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. After this, in a 72 hour acute test, the 4 day old larvae were exposed to a single application of BAS 550 01 F diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 6 treatment groups were set up: 5 doses of the test item, 1 untreated control group and 1 dose of the reference item were tested. All doses with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 and 48 hours later. Assessments of larval mortality were done after 24, 48 and 72 hours. Additionally, other observations such as small body size or large quantities of remaining food after 72 hours were noted. In an analytical phase of the study, the concentration of the active substance in the test item stock solution was determined.

Endpoints: Mortality, quantitative observations: body size, remaining food, body weight.

Reference item: Dimethoate technical (analyzed purity: 99.8%).

Test doses: Control (50% aqueous sugar solution with 50% royal jelly)
Test item treatments:

Nominal dose/concentration	
Doses [µg a.s./larva]	**Concentrations [g a.s./kg food]**
25.4	0.750
37.4	1.103
55.0	1.622
80.9	2.386
118.9	3.509

Reference item treatment: 1.1, 2.2, 4.4, and 8.8 µg dimethoate/larva.

Test conditions: Temperature: 34.0° C – 35.0° C; relative humidity: 93% - 96% with two short periods of lower humidity, photoperiod: 24 h darkness (except during assessments); food: 50% aqueous sugar solution with 50% royal jelly.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data and no effect levels (one-sided greater, $\alpha = 0.05$).Williams Multiple Sequential t-test procedure was used to compare the larvae's body weights.

II. RESULTS AND DISCUSSION

After 72 hours of exposure, a mortality of 2.8% was observed in the control. In the test item group, mortalities ranged between 11.1% and 41.7% after 72 hours. Statistically significant effects occurred at the three highest test item treatment doses, 55.0, 80.9 and 118.9 µg a.s./larva (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

After 72 hours of exposure, no deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in test item treatment group.

The results are summarized in Table 8.3.1.3-1

Table 8.3.1.3-1: Toxicity of BAS 550 01 F to *Apis mellifera* (honeybee) in an acute oral larval toxicity test after 72 hours

Treatment group	Dosage [µg a.s./ larva]	Concentration [g a.s./ kg food]	72 hour Mean mortality [%]	
			absolute	corrected [1)]
Control	--	--	2.8	--
Test item	25.4	0.750	11.1	8.6
	37.4	1.103	16.7	14.3
	55.0	1.622	38.9 *	37.1
	80.9	2.386	33.3 *	31.4
	118.9	3.509	41.7 *	40.0

Treatment	Endpoints	72 h
Test item doses	LD_{50} [µg a.s./larva] (95% CL / lower-upper)	n.d.
	NOED [µg a.s./larva]	37.4
Test item concentrations	LC_{50} [g a.s./kg food] (95% CL / lower-upper)	n.d.
	NOEC [g a.s./kg food]	1.103

1) According to Schneider-Orelli (1947).
* Statistically significant compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

Larva dosed with 8.8 µg dimethoate/larva revealed a corrected mortality of 60.0% after 72 hours.

III. CONCLUSION

In an acute oral larval toxicity study with BAS 550 01 F on honeybee larvae, no LD_{50} value (72 h) could be determined. The NOED (72 h) was determined to be 37.4 µg a.s./larva (equivalent to NOEC (72 h) 1.103 g a.s./kg food).

CA 8.3.1.4 Sub-lethal effects

No new studies are available.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available.

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance dimethomorph (BAS 550 F), no new studies on soil macro-organisms have been performed with the active substance. Therefore, for the active substance dimethomorph, EU agreed endpoints are used for the risk assessment on earthworms and other soil non-target macro-organisms. An overview on studies and endpoints is given in Table 8.4-1

Table 8.4-1: Toxicity to non-target soil meso- and macrofauna of dimethomorph

Substance	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)	EU agreed
dimethomorph	*Eisenia fetida*	$LC_{50\ CORR}$	> 500 *	*DK-531-001* Amendment: *DK-123-135*	yes
dimethomorph		$NOEC_{CORR}$	≥ 60 *	*DK-534-001*	yes

* Toxicity endpoint is adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), since the log P_{ow} of the substance is > 2.

Italic figures: The references in italic were submitted in the old dossier and peer reviewed. ~~They are not summarized in this supplemental dossier.~~ A summary for the acute earthworm study can be found in CA 8.7 and a summary for the chronic earthworm study can be found below in CA 8.4.1.

CA 8.4.1 Earthworms – sub-lethal effects

No new studies are available.

The EU agreed chronic laboratory study with BAS 550 F on earthworms (BASF DocID DK-534-001) was included in the appendix of studies. As requested by the RMS, this summary is also added below for the sake of completeness.

Report:	CA 8.4.1/1 Naudin S. et al., 1998 a A chronic toxicity and reproduction test exposing the earthworm Eisenia fetida, to Dimethomorph technical (CL 336,379) in OECD artificial soil following the ISO-draft (ISO/DIS 11268-2) DK-534-001
Guidelines:	ISO 11268-2 (1998), EEC 91/414 Annex II 8.4, EEC 96/12
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Executive Summary

The effects of BAS 550 F (CL 336379) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a chronic laboratory study over 56 days. Six application rates (3.75, 7.50, 15.0, 30.0, 60.0, and 120 mg dimethomorph/kg dry soil) were incorporated into artificial soil (10% peat) with four replicates per treatment (each containing 10 worms). An untreated control and a solvent control were included (each with 4 replicates, 10 worms each). Assessment of worm mortality, body weight and feeding activity were carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure, there was one recorded mortality in the 60 mg a.s./kg treatment (2.5%). In both controls and in the remaining treatments, mortality was 0%. Body weight changes compared to the solvent control were not statistically significantly different at any of the tested rates. Likewise, the reproduction was not statistically significantly affected at any concentration compared to the solvent control.

In a 56-day earthworm reproduction study with BAS 550 F (CL 336379), no adverse effects on survival, biomass development and reproduction could be determined up to the highest tested concentration of 120 mg dimethomorph/kg soil dry weight. The NOEC was therefore ≥ 120 mg dimethomorph/kg dry soil.

I. MATERIAL AND METHODS

A. Materials

Test item:	BAS 550 F (dimethomorph, Reg. No. 247 723 (CL 336379)); batch no. AC9978-131; 98% purity.

B. Methods

Test species:	Earthworm (*Eisenia fetida)*, adult worms (with clitellum and average fresh weight of 447 mg); source: in-house culture.
Test design:	56-day test in treated artificial soil according to OECD 207; different concentrations of the test item were incorporated into soil, 8 treatment groups (6 test item concentrations, control, solvent control); 4 replicates for the control, solvent control and each test item concentrations with 10 worms each. Assessment of adult worm mortality, biomass development was carried out after 28 days. Reproduction rate was determined after an additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, reproduction rate.
Reference item:	Benlate (50% benomyl). The effects of the reference item were investigated in a separate study.
Test rates:	Control, solvent control, 3.75, 7.50, 15.0, 30.0, 60.0, and 120 mg BAS 550 F/kg dry soil (nominal).
Test conditions:	Artificial soil according to OECD 207 (10% peat); pH 6.05 – 6.61 at test initiation, pH 6.68 – 6.79 at test termination; mean water content 67.6% of total water holding capacity (WHC) at test initiation and 76.5% of max. WHC at test termination; temperature: 20 ± 2°C; photoperiod: 16 h light: 8 h dark, light intensity: 473 - 685 lux.
Statistics:	Descriptive statistics, Dunnett's test for weight changes and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure, there was one recorded mortality in the 60 mg a.s./kg treatment (2.5%). In both controls and in the remaining treatments, mortality was 0%. Body weight changes compared to the solvent control were not statistically significantly different at any of the tested rates (Dunnett's t-test, $\alpha = 0.05$). Likewise, the reproduction was not statistically significantly affected at any concentration compared to the solvent control (Dunnett's t-test, $\alpha = 0.05$). The results are summarized in Table 8.4.1-1

Table 8.4.1-1: Effect of BAS 550 F (CL 336379) on earthworms (*Eisenia fetida)* in a 56-day reproduction study

BAS 550 F [mg/kg soil dry weight]	**Control**	**Solvent control**	**3.75**	**7.5**	**15.0**	**30.0**	**60.0**	**120**
Mortality (day 28) [%]	0	0	0	0	0	0.0	2.5	0
Weight change (day 28) [%]	81.8	65.0	62.1	65.6	63.9	69.0	68.4	75.3
No. of juveniles / adult earthworm alive (day 56)	2.9	10.1	13.3	8.1	11.6	10.9	9.3	9.2
Endpoints [mg BAS 550 F/kg soil dry weight]								
$NOEC_{mortality,\ growth,\ reproduction}$	> 120							

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 550 F (CL 336379), no adverse effects on survival, biomass development and reproduction could be determined up to the highest tested concentration of 120 mg dimethomorph/kg soil dry weight. The NOEC was therefore ≥ 120 mg dimethomorph/kg dry soil.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

No new studies are available.

CA 8.4.2.1 Species level testing

No new studies are available.

CA 8.5 Effects on nitrogen transformation

No new studies are available.

A study has been conducted during the previous Annex I inclusion of BAS 550 F with the representative formulation BAS 550 09 F (minor formulation change from BAS 550 02 F).

For further information please refer to M-CP 10.5 of BAS 550 02 F and the relevant EU documents.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Non-target plant GLP studies with the representative formulations are available. Further screening data are not required.

CA 8.6.2 Testing on non-target plants

No new studies are available.

Studies have been conducted with the representative formulations and are described in M-CP 10.6.2.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

Studies with the active substance are not required. Studies conducted with the representative formulations are described in M-CP 10.7 (including studies which are not required under 1107/2009).

The EU agreed acute laboratory study with BAS 550 F on earthworms (BASF DocID DK-531-001 + amendment BASF DocID DK-123-135) were included in the appendix of studies. As requested by the RMS, a study summary is also added below for the sake of completeness.

Report:	CA 8.7/1 Ellgehausen H., 1986 e Acute toxicity (LC50) study of CME 151 to earthworms DK-531-001
Guidelines:	OECD 207
GLP:	yes (certified by Eidgenoessisches Departement des Inneren, Bern, Schweiz)

Report:	CA 8.7/2 Ellgehausen H., 1987 a Acute toxicity (LC50) study of CME 151 to earthworms DK-123-135
Guidelines:	OECD 207
GLP:	no

Executive Summary

In an acute toxicity test, adult earthworm of the species *Eisenia fetida* were exposed BAS 550 F (CME 151). The test item was mixed into artificial soil (10% peat) at rates of 62.5, 125, 250, 500, and 1000 mg BAS 550 F/kg dry soil. For the control treatment, the soil was left untreated. Additionally, a Tween control containing 0.18% Tween 80 was included. All treatments were comprised of four replicates per treatment, each containing 10 worms. Worm mortality was assessed on days 7 and 14.

After 14 days of exposure, there was one mortality in the water control group, and no mortalities in the Tween 80 control group (0.18% Tween 80). In addition, there were no mortalities at test concentrations of 62.5, 125, and 250 mg dimethomorph/kg dry soil. There were a total of 3 mortalities (7.5%) at 500 mg a.s./kg, and 1 mortality (2.5%) at 1000 mg a.s./kg. No effects on the average body weight of worms when compared to the control was observed at the highest tested concentration, 1000 mg dimethomorph/kg dry soil. No behavioral abnormalities were observed at any tested concentration.

In a 14-d toxicity study on earthworms (*Eisenia fetida)* with BAS 550 F (CME 151), the LC_{50} was determined to be > 1000 mg dimethomorph/kg dry soil.

I. MATERIAL AND METHODS

A. Materials

Test item:	BAS 550 F (dimethomorph, Reg. No. 247 723 (CME 151)); batch no. DW 11/86; 96.6% purity (± 0.8%).

B. Methods

Test species:	Earthworm (*Eisenia fetida)*, adult worms (with clitellum and average fresh weight of 200 - 270 mg); source: Dr. P. Wilde, Farm Research, Bad Saeckingen, Germany.
Test design:	14-d exposure in treated artificial soil; different concentrations of the test item were mixed homogeneously into the soil which is filled in glass vessels before the earthworms are introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, Tween control); 4 replicates per test item concentrations with 10 worms each. Assessment of worm mortality was done after 7 and 14 d, measurement of weight change as sub-lethal parameter after 14 d.
Endpoints:	Mortality, weight change.
Test rates:	Control, Tween control (0.18% Tween 80), 62.5, 125, 250, 500, and 1000 mg BAS 550 F/kg dry soil.
Test conditions:	Artificial soil according to OECD 207 (10% peat); pH 6.5 at test initiation; water content 35.0% at test initiation; temperature: 21.5 °C; photoperiod: continuous illumination.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

After 14 days of exposure, there was one dead worm in the water control group, and no mortalities in the Tween 80 control group (0.18% Tween 80). In addition, there were no mortalities at test concentrations of 62.5, 125, and 250 mg BAS 550 F/kg dry soil. There were a total of 3 mortalities (7.5%) at 500 mg BAS 550 F/kg dry soil, and 1 mortality (2.5%) at 1000 mg BAS 550 F/kg dry soil. No effects on the average body weight of worms when compared to the control was observed at the highest tested concentration, 1000 mg dimethomorph/kg dry soil. No behavioral abnormalities were observed at any tested concentration. The results are summarized below.

Table 8.7-1: Effect of BAS 550 F (CME 151) on earthworms (*Eisenia fetida)* in a 14-day acute study

BAS 550 F [mg/kg soil dry weight]	**Control**	**Tween control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	1	0	0	0	0	3	1
Weight change (day 28) [%]	60.7	37.5	45.0	49.9	63.1	66.3	55.9
Endpoints [mg BAS 550 F/kg soil dry weight]							
LC_{50}	> 1000						

III. CONCLUSION

In a 14-d toxicity study on earthworms (*Eisenia fetida)* with BAS 550 F (CME 151), the LC_{50} was determined to be > 1000 mg BAS 550 F/kg dry soil.

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed study on the active substance are still valid and are summarized in Table 8.8-1: . No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 550 F (dimethomorph)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	≥ 1000	*DK-690-008*	yes

Italic figures: The references in italic were submitted in the old dossier and peer reviewed. They are not summarized in this supplemental dossier.

Concludingly, no influence on the waste water treatment procedures in sewage plants after use of dimethomorph according to agricultural practice is expected.

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available, which assess ecotoxicological effects of dimethomorph (BAS 550 F).

Dimethomorph

Document M-CA, Section 9

LITERATURE DATA

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
30/Sep/2016	MCA Section 9 The document DocID 2015/1280707 was missing in the original dossier and added in this version	MCA Section 9, Version 1 BASF DocID 2016/1000213
31/May/2017	Following the dRAR, in order to complete the search report submission of additional data DocID 2015/1282307	MCA Section 9, Version 3 BASF DocID 2016/1275368

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA

A literature search on dimethomorph and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on dimethomorph describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

According to the ESFA Guidance (*Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092))*, a separate literature review report was prepared for impurities to prevent tables reporting multiple search strategies or multiple data requirements to become too large".

In addition a literature search on some metabolites has been performed

The complete search report is provided in K-CA 9 (BASF DocID 2016/1000305, BASF DocID 2015/1280707 and BASF DocID 2015/1282307).

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records). The "ballast" was not further processed.

The "hits" were further evaluated by the scientific experts and categorized into "not relevant", "not reliable", and "used for dossier". This is documented in EXCEL files which are attached to the search report in K-CA 9 with the file names as listed below (alphabetical order):

Analytics:	Dimethomorph Literature Analytics
Ecotoxicology:	Dimethomorph Literature Ecotox aquatic
	Dimethomorph Literature Ecotox general
	Dimethomorph Literature Ecotox terrestric
	Dimethomorph Literature Ecotox wildlife
E-fate:	Dimethomorph Literature E-fate
Consumer Safety:	Dimethomorph Literature Metabolism and Residues in Animals
	Dimethomorph Literature Metabolism and Residues in Plants
Toxicology:	Dimethomorph Literature Toxicology and OPEX

All hits, considered reliable and contributing to the risk assessment were therefore further discussed in the dossier.

Dimethomorph

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for dimethomorph:

Table 10-1: Harmonised classification of dimethomorph according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Aquatic Chronic 2 Hazard statement code: H411	Pictogram signal word code: GHS09 Hazard statement code: H411	

No classification for human health according to criteria in directive 67/548/EEC or Regulation (EC) N° 1272/2008

New evaluation leads to a change of classification for aquatic chronic hazard, since the lowest chronic endpoint for dimethomorph is below 100 µg/L (see Table 10-3, 60d ELS fish NOEC). Therefore, BASF proposed the following classification and labelling for dimethomorph:

Regarding toxicology the evaluation conducted within this supplemental dossier submission has not changed the classification. In particular the new studies/data submitted do not affect the health effect classification and labelling. Thus, the harmonized classification as laid down in Regulation (EU) No. 1272/2008 (CLP) based on the evaluation ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009) is still applicable to dimethomorph.

Table 10-2: Proposed Classification and Labelling for Dimethomorph according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Aquatic Chronic 1 Hazard statement code: H410	Pictogram signal word code: GHS09 Warning Hazard statement code: H410	M-factor = 1

Table 10-3: Ecotoxicology/Environment data relevant for Classification of dimethomorph [1]

Study Type (duration)	Results	Reference (BASF Name / DocID)
Oncorhynchus mykiss (96 h)	96 h LC_{50} = 6.1 mg/L	DK-511-002 / 1986/7000035 -- / 2010/1177242
***Oncorhynchus mykiss* (96 d)**	**96 d NOEC = 0.056 mg/L**	**DK-512-002 / 1997/7000205**
	96 d EC_{10} = 0.116 mg/L	**2014/1224007**
***Crassostrea virginica* (96 h)**	**96 h EC_{50} = 4.42 mg/L [2]**	**DK-522-001 / 1997/7000207**
Daphnia magna (22 d)	22 d NOEC = 0.100 mg/L	DK-524-001 / 1993/7000130
	22 d EC_{10} = 0.152 mg/L	2015/1238096
Pseudokirchneriella subcapitata (72 h)	72 h E_rC_{50} = 82.2 mg/L	DK-521-007 / 2001/7001037
	72 h E_rC_{10} = 27.3 mg/L	
Biodegradation	Dimethomorph is not readily biodegradable	DK-690-001 /-- EFSA Scientific Report (2006) 82, 1-69

1 The lowest acute and chronic endpoint (basis for classification) is marked in **bold**.

2. According to Regulation EC No 1272/2008 no Acute aquatic hazard Category is triggered, as the lowest $L(E)C_{50}$ > 1 mg/L.