Dimethenamid-P

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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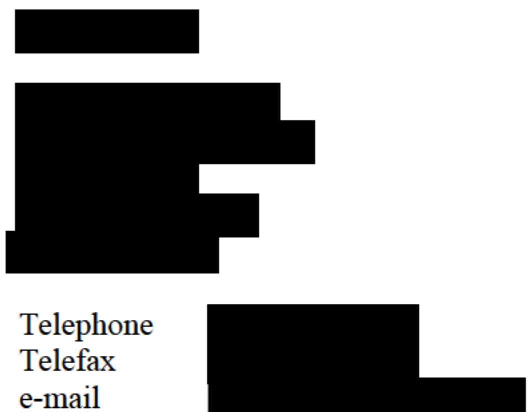

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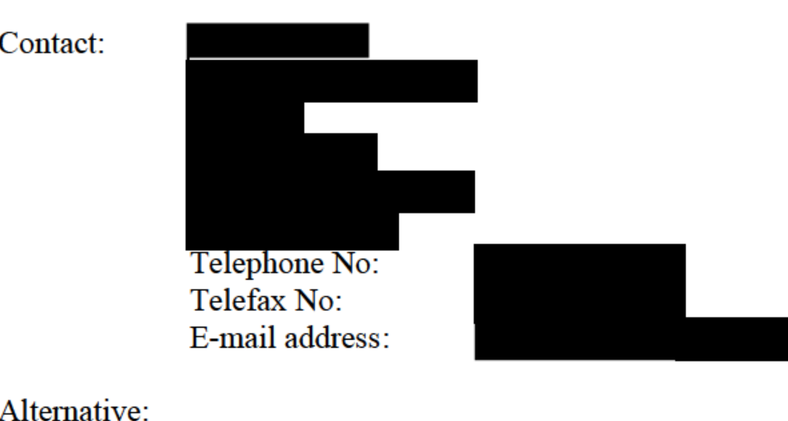
CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF Corporation
100 Park Avenue
Florham Park, New Jersey 07932
United States

Contact:



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E-mail address:

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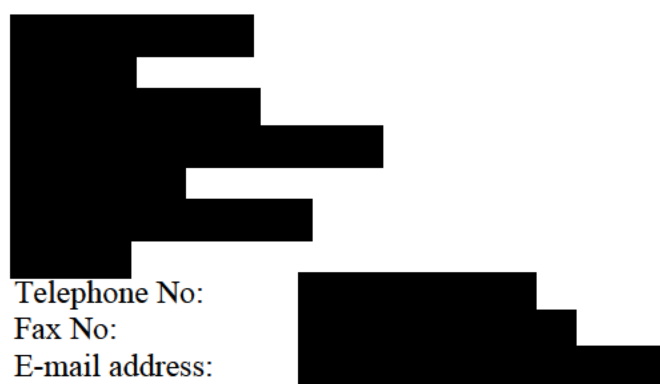
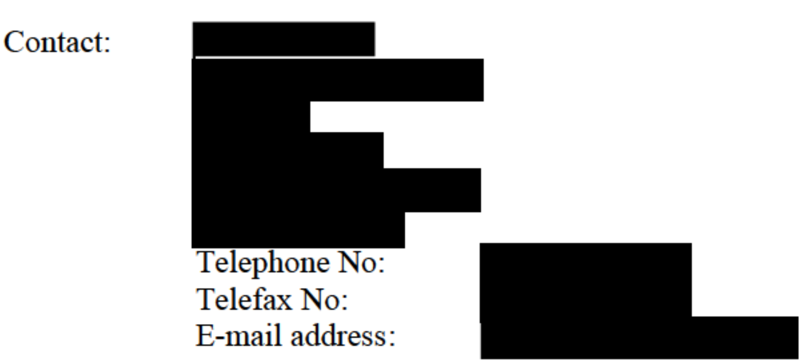


Telephone No:
Fax No:
E-mail address:

CA 1.2 Producer

Manufacturer of Dimethenamid-P (legal entity):

Contact:



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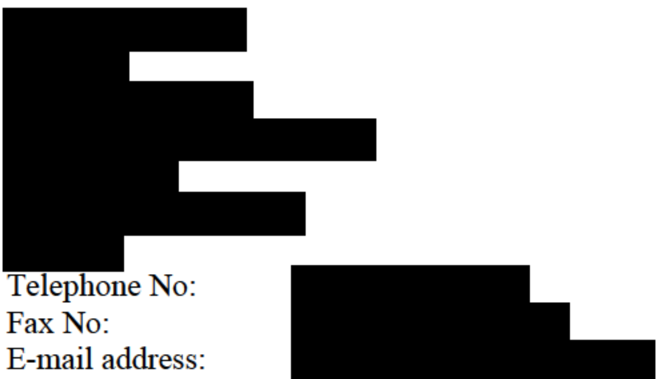

Telephone No:
Fax No:
E-mail address:

Location of the manufacturing site of Dimethenamid-P:

Confidential information - data provided separately (Doc J).

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Dimethenamid-P

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

Chemical name (IUPAC)	S-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide
Chemical name (CA)	Acetamide, 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-[(1R)-2-methoxy-1-methylethyl]-

CA 1.5 Producer's Development Code Numbers

BASF Code Number	BAS 656P H
BASF Registry Number	363851

CA 1.6 CAS, EC and CIPAC Numbers

CAS Number	163515-14-8
EC Number	n.a.
CIPAC Number	638

CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{12}H_{18}ClNO_2S$

Structural formula:

Molar mass: 275.8 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: 890 g/kg (preliminary values based on a pilot plant; Directive 2003/84/EC)
930 g/kg (new specification proposal according to commercial production)

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

CONFIDENTIAL information - data provided separately (Document J)

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

Dimethenamid-P

DOCUMENT M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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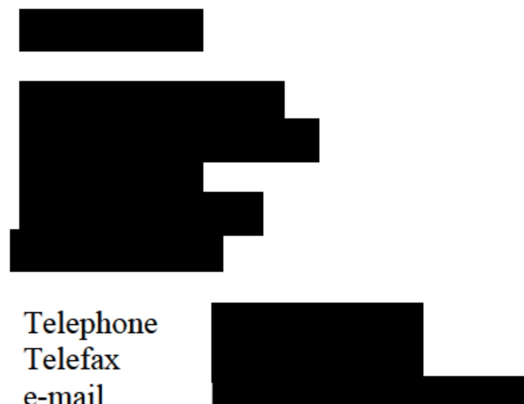

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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	OECD Guideline 102 OECD Guideline 103 (Siwoloboff, DSC)	RS-1289-010496: 94.0%	For the TGAI, no thermal effect was observed at normal pressure between -50°C and 400°C which could be related to solidification, melting or boiling respectively. Above 101 °C a weak broad endothermic effect shows up in the DSC curve accompanied by loss of 80% of the sample weight. The phenomenon is assigned to vaporization of the cracked products.	Y	[see 2004/1010746 Daum A. 2004 a]
CA 2.2 Vapour pressure, volatility			Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) Vapour pressure: $2.5 \cdot 10^{-3}$ Pa (25 °C) Henry's law constant: $4.8 \cdot 10^{-4}$ P m^3 mol^{-1} (25 °C)		Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003
CA 2.3 Appearance (Physical state, colour)	FP0038/005, FP0039/05, FP0040/006, FP0062/004	RS-1289-010496: 94.0%	colour: slightly brown (at diffused daylight) physical state: liquid odour: faint intrinsic odour condition: clear liquid	Y	[see 2004/1010746 Daum A. 2004 a]
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity			Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) UV/VIS absorption (max.): 7560 L mol^{-1} cm^{-1} (at 236nm, purity: 99.4 %)	Y	Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.5 Solubility in water			Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) pH 6.16 ± 0.28: 1449 ± 17 mg/l (no dissociation in water and therefore no pH dependency)		Review Report for Dimethenamid-P, SANCO/1402/2001 -Final, 3 July 2003
CA 2.6 Solubility in organic solvents		BEAU201204: 96.4%	In toluene, dichloromethane, methanol, acetone, ethyl acetate and acetonitrile dimethenamid-P has a solubility of > 1000 g of substance per L of solvent. Thus the test item can be considered completely miscible with these solvents. In n-hexane and in n-heptane (aliphatic hydrocarbons) dimethenamid-P has a solubility of about 310 to 330 g (0.32 ± 0.01 kg) of substance per L of solvent.	Y	[see 2006/1032688 Class T. 2006 b]
CA 2.7 Partition co-efficient n-octanol/water			Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) log P_{OW} = 1.89 ± 0.10 Effect of pH was not investigated since there is no dissociation in water.		Review Report for Dimethenamid-P, SANCO/1402/2001 -Final, 3 July 2003
CA 2.8 Dissociation in water • dissociation constant(s) (pKa values) • identity of dissociated species • dissociation constant(s) (pKa values) of the active			Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) No dissociation at pH 1 – 11		Review Report for Dimethenamid-P, SANCO/1402/2001 -Final, 3 July 2003

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
principle					
CA 2.9 Flammability and self-heating			Information already reported and peer-reviewed previously (Dimethenamid-P EU Monograph, 12 September 2000) An auto-ignition temperature of 396 °C was determined for the technical active substance Information already reported and peer-reviewed previously (see Review Report for Dimethenamid-P, SANCO/1402/2001-Final, 3 July 2003) Assessment of flammability not required. Technical Dimethenamid-P is a liquid and does not evolve highly flammable gases.		Dimethenamid-P EU Monograph, 12 September 2000 Review Report for Dimethenamid-P, SANCO/1402/2001 -Final, 3 July 2003
CA 2.10 Flash point			Information already reported and peer-reviewed previously (Dimethenamid-P EU Monograph, 12 September 2000) A flash point of 79 °C was determined for the technical active substance.		Dimethenamid-P EU Monograph, 12 September 2000
CA 2.11 Explosive properties	OECD Guideline 102, FP0091/002, Differential Scanning Calorimetry / Thermo Gravimetry	BEAU201204: 96.4%	Technical DMTA-P is thermally stable, as the DSC/TG-curve does not show any thermal effect at normal pressure between -5°C and 170°C which could be related to solidification, melting or boiling respectively. Above ca. 170°C two endothermic peaks appear accompanied by a two-step mass loss of more than 90% in total. These effects are assigned to decomposition of the test item and vaporization of the cracked products. Further tests for explosive properties were omitted, as no exothermic effects were observed. For the TGAI, no thermal effect was observed at normal pressure between -50°C and 400°C which could be related to solidification, melting or boiling respectively. Above 101 °C a weak broad endothermic effect shows up in the DSC curve accompanied by loss of 80% of the sample weight. The phenomenon is assigned to vaporization of the cracked products.	Y	[see 2006/1032689 Kroehl T. 2006 a] [see 2004/1010746 Daum A. 2004 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.12 Surface Tension			Information already reported and peer-reviewed previously (Dimethenamid-P EU Monograph, 12 September 2000) 52.0 ± 0.01 mN/m PAI, 0.1% 50.7 ± 0.00 mN/m PAI, 0.5%		Dimethenamid-P EU Monograph, 12 September 2000
CA 2.13 Oxidising properties			Information already reported and peer-reviewed previously (Dimethenamid-P EU Monograph, 12 September 2000) Study of reactions with oxidising and reducing agents indicated that dimethenamid exhibits mild reaction with potassium permanganate. Contact with strong oxidising agents should be avoided.		Dimethenamid-P EU Monograph, 12 September 2000
CA 2.14 Other studies			None		

Dimethenamid-P

DOCUMENT M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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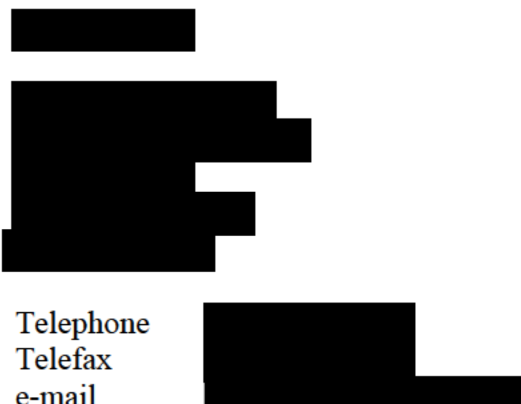

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Dimethenamid-P is evenly distributed by spray application at dose rates up to 864 g ai/ha using water as a spray carrier to control a wide range of annual broadleaf weeds and grasses in different crops. Spectrum is mainly a soil acting herbicide, which can be applied pre- and post-emergence of the crop. For optimum weed control weeds should not be more advanced than cotyledone stage or slightly beyond. Spectrum provides very efficient control of a broad range of mono- and dicotyledonous weed species. Regarding post-emergence application the foliar uptake is minor, but the prevention of new emerging weeds is a valuable property.

CA 3.2 Function

Dimethenamid-P is used as an herbicide.

CA 3.3 Effects on Harmful Organisms

Dimethenamid-P is providing soil residual and, to little extend, foliar activity with application either before or shortly after weed emergence, leading to the inhibition of cell division. In germinating monocotyledonous weed species Dimethenamid-P is predominantly absorbed via the emerging coleoptile. In dicotyledonous weed species Dimethenamid-P enters the plant primarily via root uptake (radicule) and via the germinating shoots (hypocotyls). After uptake Dimethenamid-P is hardly translocated within the plant. Typical symptoms of the aerial parts of broadleaf weed species that emerge include severe stunting, intense green coloration and a leathery appearance of the cotyledons. Emerged grasses are stunted and twisted.

CA 3.4 Field of Use Envisaged

Agriculture

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

Dimethenamid-P is used in a wide range of crops including (representative uses in bold):

Cereals crops

- **Maize**
- Sorghum
- Millet
- Moha
- Miscanthus

Oilseed crops

- **Sunflower**
- **Soybeans**
- **Winter Oilseed Rape**
- Oilseed Pumpkin

Beet roots

- **Sugarbeet**
- Red beets
- Cichory roots

Legume crops

- Dry pulses (dry harvest)
  - o beans: field beans,
  - o peas: chickpeas, field peas, chickling vetch
  - o lupins
- Legume vegetables (fresh harvest)
  - o beans (with & without pods):
    green bean (french beans, snap beans), scarlet runner bean, slicing bean
  - o peas (with & without pods):
    garden pea, green pea, sugar peas

Vegetables

- Brassica Vegetables
  - o Head cabbage
  - o Leafy cabbage
  - o Flowering cabbage
  - o Kohlrabi
- Bulb Vegetables
  - o Onion, welsh onions
  - o Shallot
  - o Garlic
  - o Chives, salad onions
- Root & Tuber Vegetables

  - Carrots
  - Potato
  - Horse radish
  - Turnips, swedes
  - Parsnip
  - Celeriac
- Stem Vegetables
  - Leek
  - Celery
  - Asparagus
  - Fennel
  - Rhubarb
- Fruiting Vegetables
  - Cucumber, gherkin
  - Pattison
  - Melon,
  - Pumpkin, zucchini
- Leafy Vegetables & Herbs
  - Lettuce
  - Cichory, witloof

Biannual / perennial crops
- Berries & Small Fruits
  - Currants
  - Raspberries
- Pome & Stonefruit
- Tree Nuts

Others
- Ornamentals
- Tree nursery
- Temporary fallow fields

Dimethenamid-P is used to control the most important broadleaf weeds such as:
- *Aethusa cynapium*,
- *Amaranthus* sp.,
- *Ambrosia artemisifolia*,
- *Anagallis arvensis*,
- *Atriplex patula*,
- *Capsella bursa-pastoris*,
- *Chenopodium album*,
- *Datura stramonium*,
- *Fumaria officinalis*,
- *Galeopsis tetrahit*,
- *Galinsoga parviflora*,
- *Galium aparine*,
- *Lamium* sp.,
- *Matricaria* sp.,

- *Melandrium noctiflorum*,
- *Polygonum lapathifolium*,
- *Polygonum persicaria*,
- *Portulaca oleracea*,
- *Solanum nigrum*,
- *Senecio vulgare*,
- *Sinapsis* sp.,
- *Sonchus arvensis*,
- *Stellaria media*,
- *Thlaspi arvense*,
- *Veronica* sp.,

and annual grasses such as:

- *Avena fatua*,
- *Digitaria* sp.,
- *Echinochloa* sp.,
- *Lolium* sp.,
- *Panicum* sp.,
- *Poa annua*,
- *Setaria* sp.,
- *Sorghum halepense*

CA 3.6 Mode of Action

Dimethenamid-P, classified by HRAC into group K3, belongs to the chemical class of chloroacetamides. The mode of action of the chloroacetamide herbicides is believed to function through the inhibition of the synthesis of very-long-chain fatty acids (VLCFAs) in the lipid biosynthesis pathway. Dimethenamid-P belongs to the chemical group of chloroacetamides. In contrast to other representatives of this chemical family Dimethenamid-P is not based on a benzene ring, but contains a sulphur based thiophene ring.

The activity is based on the influence on cell division and cell growth. A major effect in inhibiting the cell division is the alkylation of sulfhydryl groups in different enzymes. However, the molecular mechanism of Dimethenamid-P has not yet been clarified in detail. To date inhibitions of fatty acid and lipid biosynthesis are known. Results from recent studies indicate that in particular the elongation of C_{18} fatty acids to very long-chain fatty acids is blocked by chloroacetamides. The interaction with the fatty acid or lipid biosynthesis possibly explains the observed reductions in the cuticular waxlayer on seedlings after treatment with chloroacetamides. Furthermore, chloroacetamides affect protein synthesis and inhibit flavonoid (including anthocyane) and isoprenoid biosynthesis. From this metabolic pathway the phytohormone gibberelline derives from and its synthesis is affected by chloroacetamides at the level of hydroxylation. In the plant Dimethenamid-P is split into several metabolites, which in turn are detoxified by glutathion compounds.

The currently popular hypothesis which offers a possible explanation for the individual effects is based on the alkylating properties of chloroacetamides and the formation of conjugates with acetyl-coenzymeA as well as with other molecules of the SH-groups. As a result of these interventions, the plant cell division and cell elongation processes as well as tissue differentiation are inhibited. The root and shoot growth is suppressed. The result is shortened seedlings with reduced growth, subsequently giving rise to stunting, deformation and death of weed seedlings.

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

Following extract is taken out of the Resistance Risk Assessment explaining the situation for the solo product of Dimethenamid-P (BAS 656 12 H) reflecting the actual situation. Detailed information is present in the original document (DocID 2013/1334851).

Summary:

BAS 656 12 H contains the active substance Dimethenamid-P which inhibits the formation of very-long-chain-fatty-acids (VLCFA) in the lipid biosynthesis (HRAC-group K3). A total of 21 genes encoding VLCFA elongases are identified in Arabidopsis thaliana, which are showing a complex pattern of substrate specificity to VLCFA-inhibitors, may explaining why resistance to these herbicides are rare.
According to the International Survey of Herbicide Resistant Weeds, there are only seven cases reported worldwide on four grass weed species that have been shown to be resistant to other herbicides from the HRAC-group K3, with most of the cases restricted to the Australasia or North America regions with only one case in Europe on *Alopecurus*.

BAS 656 12 H and other Dimethenamid-P containing ready-mixtures with active substances of different mode of action can be used in a wide range of crops enabling the potential continuous use of Dimethenamid-P throughout the rotation.
However, even in such a situation, BAS 656 12 H poses no unacceptable risk of resistance evolution against Dimethenamid-P due to

- The low inherent risk rating of VLCFAs with their complex enzyme substrate specificity and multiple sites of action
- The very low incidences of weed resistance within HRAC-group K3
- No reported cases of weed resistance against Dimethenamid-P despite more than 20 years of use in Europe
- The most likely integration of Dimethenamid-P into tankmix- and sequence application with other herbicides of alternative mode of action, which are considered as a successful resistance management strategy

In conclusion, the use of BAS 656 12 H does not require specific resistance management strategies to be recommended.

BASF promotes a general awareness of herbicide resistance management in product and technical leaflets, training sessions to sales personnel, distributors and growers' associations and through its Agricentre websites. BASF actively participates in HRAC and WRAG meetings. In this way every attempt is made to formulate and promote general resistance management strategies and the rational use of its herbicides. In the case that BASF obtains information about a loss of field performance of BAS 656 12 H or Dimethenamid-P and that resistance is identified as the cause, BASF will inform HRAC / WRAG and the relevant authorities. This will result in modifications to the recommended resistance management strategy.

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

Exposure Controls/Personal Protection

Control parameters

Components with workplace control parameters
No occupational exposure limits known.

Exposure controls

Personal protective equipment
Respiratory protection:
Suitable respiratory protection for higher concentrations or long-term effect: Combination filter for gases/vapours of organic, inorganic, acid inorganic and alkaline compounds (e.g. EN 14387 Type ABEK).

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:
Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy. Vapours may form ignitable mixture with air.

Conditions for safe storage, including any incompatibilities

Segregate from foods and animal feeds.

Further information on storage conditions: Keep away from heat. Protect from direct sunlight.

Protect from temperatures above: 30 °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:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention.

Most important symptoms and effects, both acute and delayed

Symptoms: The most important known symptoms and effects are described in the classification and labelling. Further important symptoms and effects are so far not known.

Indication of any immediate medical attention and special treatment needed

Treatment: Treat according to symptoms (decontamination, vital functions), no known specific antidote.

Transport Information

Land transport

ADR

Hazard class:	9
Packing group:	III
ID number:	UN 3082
Hazard label:	9, EHSM
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains DIMETHENAMID-P)

RID

Hazard class:	9
Packing group:	III
ID number:	UN 3082
Hazard label:	9, EHSM
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains DIMETHENAMID-P)

Inland waterway transport

ADN

Hazard class:	9
Packing group:	III
ID number:	UN 3082
Hazard label:	9, EHSM
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains DIMETHENAMID-P)

Sea transport

IMDG

Hazard class:	9
Packing group:	III
ID number:	UN 3082
Hazard label:	9, EHSM
Marine pollutant:	YES
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains DIMETHENAMID-P)

Air transport

IATA/ICAO

Hazard class:	9
Packing group:	III
ID number:	UN 3082
Hazard label:	9, EHSM
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains DIMETHENAMID-P)

Fire-Fighting Measures

Extinguishing media
Suitable extinguishing media:
water spray, dry powder, foam, carbon dioxide.

Special hazards arising from the substance or mixture
carbon monoxide, hydrogen chloride, Carbon dioxide, nitrogen oxides, sulfur 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:
Keep containers cool by spraying with water if exposed to fire. Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. In case of fire and/or explosion do not breathe fumes. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations.

CA 3.9 Procedures for Destruction or Decontamination

Waste treatment methods

Must be disposed of or incinerated in accordance with local regulations. Combustion in a licensed incinerator is required. 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 should be emptied as far as possible and disposed of in the same manner as the substance/product. Users are requested to triple rinse empty primary packages as described in the ECPA "Guidelines for the rinsing of agrochemical containers", 1993. Pressure rinsing or integrated pressure rinsing of the packaging material achieves a similar or even better result. The rinsing water must be added to the spray liquid. To minimize waste of packages it is recommended that empty and rinsed containers are delivered to local container collection stations. If these do not exist, empty and rinsed containers must be rendered unusable and disposed according to local regulations.

CA 3.10 Emergency Measures in Case of an Accident

Personal precautions, protective equipment and emergency procedures
Use personal protective clothing. Avoid contact with the skin, eyes and clothing. Do not breathe vapour/spray.

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

Methods and material for containment and cleaning up
For small amounts: Pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder, kieselguhr).
For large amounts: Dike spillage. Pump off product.
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.

BASF
The Chemical Company

Dimethenamid-P

DOCUMENTM-CA, Section 4

ANALYTICAL METHODS

Compiled by:

Regulatory Manager
BASF Nederland B.V.,
P O Box 1019
6801 MC Arnhem,
The Netherlands

Telephone +31 26 3717286
Telefax +31 26 3717122
e-mail basf.com

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[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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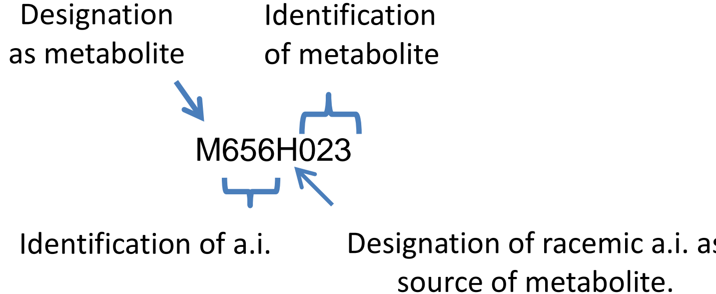
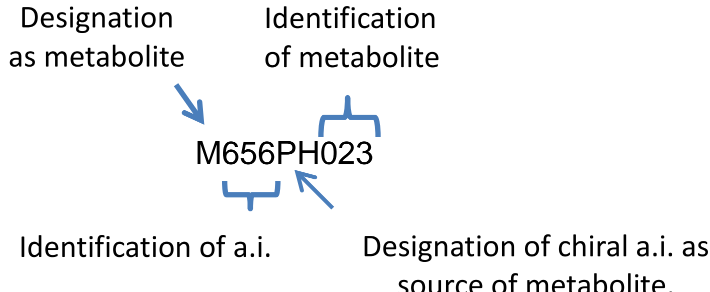
CA 4 ANALYTICAL METHODS

General explanation on metabolite nomenclature in relation to stereoisomery.

Dimethenamid-P is the S-enantiomer of the racemic dimethenamid. For the active substance a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic pathway synthesis. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example M23:



Metabolites where the source of identification and the synthesis route could clearly be attributed to the chiral compound dimethenamid-P were assigned as follows:



The metabolic pathways in soil, water, mammals, and plants are equivalent for the racemic dimethenamid and dimethenamid-P (S-enantiomer). The metabolites derived from either racemic or enantio-enriched source are considered toxicologically equivalent and were taken into account for the assessment within this dossier. The table below is a concordance tables for all metabolites presented in MCA, Section 4, Chapters 4.0-4.2.

Table 4-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Method[1] found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
BAS 656 H (275.8)	**360720**	**-**	**87674-68-8**	Active Substance	
BAS 656 PH	**363851**	**-**	**163515-14-8**	Active Substance Water L0109/02 L0109/01 519/0 L0167/01 L0179/02 R0038/01 R0037/01	
M656PH003	**5886782**	**M3**	**Not assigned**	Surface Water	
M656PH010	**5931836**	**M10**	**Not assigned**	Surface Water	
M656H023	**360715**	**M23**	**Not assigned**	L0109/02 L0109/01 Water 519/0 L0179/02	
M656PH023	**5886780**	**M23**	**Not assigned**	Surface Water	
M656H026	**360716**	**M26**	**Not assigned**	L0179/02 R0038/01 R0037/01	

Table 4-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Method[1] found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H027	Not assigned	M27	Not assigned	L0109/02 L0109/01 Water 519/0 L0179/02	
M656PH027	5912598	M27	Not assigned	Surface Water	
M656H030	Not assigned	M30	Not assigned	L0179/02 R0038/01 R0037/01	
M656PH031	5886777	M31	Not assigned	L0109/02 Water	
M656PH031	5886777	M31	Not assigned	Surface Water	
M656PH032	5886785	M32	Not assigned	Surface Water	
M656PH043	5917262	M43/M44	Not assigned	Surface Water	
M656PH045	5917261	M45/M46	Not assigned	Surface Water	

Table 4-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Method[1] found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656PH047	**5917260**	**M47/M48**	**Not assigned**	Surface Water	
M656PH054	**Not assigned**	**M54/M58**	**Not assigned**	Surface Water	

1 Metabolites are listed as they are in the description of the method. Please refer to the method to determine how isomers were determined

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 Nemitz A.,Genari G., 2013a Determination of active ingredient S-dimethenamid and its isomer R-dimethenamid in Dimethenamid-P technical grade active ingredient (TGAI) by means of HPLC 2013/1066432
Guidelines:	<none>
GLP:	no

Report:	CA 4.1.1/2 Sonnenschein L., 2013a Validation of the analytical method APL0665/01: Determination of active ingredient S-Dimethenamid and its isomer R-Ddimethenamid in Dimethenamid-P technical grade active ingredient (BAS 656 H) by means of high performance liquid chromatography (HPLC) 2013/1066433
Guidelines:	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act), EU Regulation 1107/2009 with Regulation 283/2013, EPA 830.1800, SANCO/3030/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Principle of the method

Technical Dimethenamid-P is dissolved in a mixture of heptane and 2-propanol and chromatographed on a normal phase HPLC system applying a chiral separation column. Quantitation of the active substance S-dimethenamid and its Enantiomer R-dimethenamid is performed by UV detection and external standard calibration using authentic reference items of known purity.

Identity

The identity of the peaks was confirmed by comparison of the retention times and the UV-DAD spectra of the reference items with the test substance.

Specificity

The specificity of the method was proven by comparison of the spectra and the retention times of the reference item, the test item and the blank solvent injection. No interference was observed in the specificity.

Linearity
The linear ranges for the determination of the enantiomers of dimethenamid-P (BAS 656 PH) were determined by injection of six solutions at different concentration levels (prepared from two stock solutions per enantiomer).
Concerning the S-enantiomer the separated peaks for the two atropisomers (S-dimethenamid I and S-dimethenamid II) were evaluated separately. In the final method the peak areas for the atropisomers are supposed to be summarized. Anyhow - when every single peak fulfills the requirements demanded by SANCO/3030/99 rev. 4 - the sums of the peak areas will fulfil these requirements as well. The equation parameters are given below. For the atropisomers with determined peak area ratios of 1:1 the sample weights of the authentic reference item were devided by two and assigned to each single atropisomer-peak. Each solution was injected twice.

Calibration equation parameters R-dimethenamid:

y-axis intercept (b) :	-3010.047
slope (m):	10886.50
correlarion factor:	0.999682
concentration range:	9.6 – 299.9 mg/L (4.8 – 150.0 g/kg)

Calibration equation parameters S-dimethenamid (atropisomer I):

y-axis intercept (b) :	-212765.9
slope (m):	10265.03
correlarion factor:	0.994822
concentration range:	781.1 – 1229.6 mg/L (390.6 – 614.8 g/kg)

Calibration equation parameters S-dimethenamid (atropisomer II):

y-axis intercept (b) :	-262617.3
slope (m):	12079.56
correlarion factor:	0.994868
concentration range:	781.1 – 1229.6 mg/L (390.6 – 614.8 g/kg)

Precision (Repeatability)
Repeatability was performed by five independent replicate sample determinations of BAS 656 PH (TGAI). The acceptability of the % RSD values (relative standard deviation) for precision was proved by the Horwitz equation, an exponential relationship between the inter laboratory relative standard deviation (RSDR) and concentration C (expressed as decimal fraction):

$$\%RSDR = 2^{(1-0.5\log C)}$$

which is modified for the estimation of repeatabilities (RSDr internal laboratory) to:

$$\%RSDr = \%\ RSDR \times 0.67$$

Horwitz results for the repeatability test with BAS 656 PH TGAI:

Item	nominal conc. [%] [4]	corresp. conc. \`C\`	%RSDR (Inter Lab. RSD)	%RSDr (Intra Lab. RSD)	%RSD analyzed	%RSD accepted
S-Dimethenamid I [1]	48,12	0,481	2,233	1,496	0,97	yes
S-Dimethenamid II [2]	48,12	0,481	2,233	1,496	1,00	yes
S-Dimethenamid (sum) [3]	96,23	0,962	2,012	1,348	0,98	yes
R-Dimethenamid	2,60	0,026	3,464	2,321	1,79	yes

[1] first atropisomer of Dimethenamid-P
[2] second atropisomer of Dimethenamid-P
[3] Sum of both atropisomers of Dimethenamid-P
[4] calculated from Dimethenamid purity of 98.8% and S-Dimethenamid : R-Dimethenamid ratio of

Applicability of CIPAC Methods
There is no CIPAC method available for the simultaneous determination of S-dimethenamid and R-dimethenamid

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

CONFIDENTIAL information - data provided separately (Document J)

CA 4.1.2 Methods for risk assessment

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

Soil

Report:	CA 4.1.2/1 Tilting N.,Sopena-Vazquez F., 2014a Validation of Analytical Method L0109/02: Determination of Dimethenamid-P (BAS 656 H) and its metabolites Reg No. 360715 (M23), Reg No. 360714 (M27) and Reg No. 360712 (M31) in soil and sediment by HPLC/MS-MS 2013/1110235
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods	Soil samples (5 g) were placed into a plastic centrifuge tube and extracted with 20 mL of a mixture of methanol and water (60/40, v/v) by mechanical shaking for 30 min at 225 rpm. Subsequently, the sample was centrifuged, the liquid phase was decanted and the extraction was repeated with another 20 mL of the methanol/water mixture. The extracts were combined and diluted to 50 mL with water. An additional dilution with 50 mL of water was performed (Final Volume = 100 mL) before LC-MS/MS measurement. The method was validated at two fortification levels (0.005 and 0.05 mg/kg) for two soils (LUFA 2.2 and LUFA 5M soils) and one sediment (Berghäuser Altrhein sediment).
Recovery findings	Method No L0109/02 was proved to be suitable for determining residues of dimethenamid-P (BAS 656 PH) and its metabolites Reg No 360715 (M23), Reg No 360714 (M27) and Reg No 360712 (M31) in soil and sediment using HPLC-MS/MS with a limit of quantitation (LOQ) of 0.005 mg/kg and a limit of detection (LOD) of 0.01 ng/mL. Validation experiments were conducted in two different soils for dimethenamid-P and its metabolites (M23, M27 and M31). The mean recovery values for dimethenamid-P and its metabolites were between 70% and 120%. The detailed results are given in Table 4.1.2-1.

Table 4.1.2-1: Validation results of a residue analytical method for dimethenamid-P (BAS 656 PH) and its metabolites Reg No 360715 (M23), Reg No 360714 (M27) and Reg No 360712 (M31) in soil and sediment.

Soil	Analyte	m/z	No of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA 2.2	Dimethenamid-P	276→244	5	0.05	99.4	1.6
		276→168	5	0.05	97.3	1.1
		276→244	5	0.005	98.2	1.4
		276→168	5	0.005	98.7	1.4
	Reg No 360715 (M23)	270→198	5	0.05	97.4	3.1
		270→166	5	0.05	91.4	4.4
		270→198	5	0.005	96.6	2.7
		270→166	5	0.005	101.5	3.4
	Reg No 360714 (M27)	320→121	5	0.05	96.5	3.1
		320→80	5	0.05	96.2	2.4
		320→121	5	0.005	95.9	3.1
		320→80	5	0.005	97.1	2.1
	Reg No 360712 (M31)	346→240	5	0.05	99.5	2.7
		346→198	5	0.05	98.1	5.0
		346→240	5	0.005	97.9	6.1
		346→198	5	0.005	96.2	5.1
LUFA 5M	Dimethenamid-P	276→244	5	0.05	106.2	1.0
		276→168	5	0.05	106.4	1.4
		276→244	5	0.005	99.9	1.8
		276→168	5	0.005	99.8	2.1
	Reg No 360715 (M23)	270→198	5	0.05	104.2	3.4
		270→166	5	0.05	100.1	9.5
		270→198	5	0.005	100.7	2.7
		270→166	5	0.005	96.4	9.7
	Reg No 360714 (M27)	320→121	5	0.05	101.9	2.6
		320→80	5	0.05	98.2	2.7
		320→121	5	0.005	101.0	3.9
		320→80	5	0.005	99.4	1.9
	Reg No 360712 (M31)	346→240	5	0.05	95.3	7.7
		346→198	5	0.05	96.8	5.4
		346→240	5	0.005	99.0	4.8
		346→198	5	0.005	97.0	5.1
Sediment	Dimethenamid-P	276→244	5	0.05	102.6	2.8
		276→168	5	0.05	101.0	3.3
		276→244	5	0.005	99.8	1.2
		276→168	5	0.005	100.5	1.3
	Reg No 360715 (M23)	270→198	5	0.05	104.1	3.4
		270→166	5	0.05	102.6	3.8
		270→198	5	0.005	99.0	4.7
		270→166	5	0.005	99.0	4.5
	Reg No 360714 (M27)	320→121	5	0.05	102.4	4.2
		320→80	5	0.05	100.3	2.6
		320→121	5	0.005	101.5	2.9
		320→80	5	0.005	99.0	2.1
	Reg No 360712 (M31)	346→240	5	0.05	95.4	6.5
		346→198	5	0.05	100.3	6.8
		346→240	5	0.005	97.1	1.8
		346→198	5	0.005	100.2	5.1

Linearity Good linearity (r >0.99) was observed in the range of 0.05 ng/mL to 1.0 ng/mL for the two mass transitions of each test item.

Specificity LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantitation The limit of quantitation (LOQ) was defined by the lowest fortification level successfully tested. LOQ is 0.005 mg/kg for all analytes, corresponding to a concentration of 0.25 ng/mL of the extract.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-1

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

Conclusion Method L0109/02 fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of Dimethenamid-P (BAS 656 PH) and its metabolites (M23, M27, M31) in both soil and sediment.

Soil

Report: CA 4.1.2/2
Obermann M., 2008a
Validation of analytical method L0109/01: Determination of Dimethenamid-P and its metabolites Reg.No. 360 714 and Reg.No. 360 715 in soil using HPLC/MS-MS
2008/1042152

Guidelines: EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, 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)

Principle of the methods Dimethenamid-P and its metabolite Reg No 360714 (M27) and Reg No 360715 (M23) were extracted from soil samples using methanol/water. Final determination of dimethenamid-P and its metabolites M23 and M27 was performed by using HPLC-MS/MS. For all analytes two parent-daughter ion transitions were used for quantitation and confirmation.

Recovery findings

Method L0109/01 was proved to be suitable for determining residues of dimethenamid-P and its metabolites M23 and M27 in soil and sediment using HPLC-MS/MS with a limit of quantitation (LOQ) of 0.005 mg/kg and a limit of detection (LOD) of 0.1 ng/mL. Validation experiments were conducted in two different soils for dimethenamid-P and its metabolites (Reg No 360714 and Reg No 360715). The mean recovery values for dimethenamid-P and its metabolites were between 70% and 120%. The detailed results are given in Table 4.1.2-2.

Table 4.1.2-2: Validation results of a residue analytical method for Dimethenamid-P and its metabolites Reg No 360714 and Reg No 360715 in two soils

Soil	Analyte	m/z	No of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA 5M	Dimethenamid-P	276→168	5	0.005	85.8	1.46
		276→244	5	0.005	85.8	1.46
		276→168	7	0.05	78.3	14.10
		276→244	7	0.05	78.5	13.58
	Reg No 360714 (M27)	320→80	5	0.005	88.8	3.46
		320→121	5	0.005	91.4	2.16
		320→80	6	0.05	94.1	6.31
		320→121	6	0.05	95.4	4.40
	Reg No360715 (M23)	270→166	5	0.005	91.3	11.91
			5	0.005	94.5	3.51
		270→198	7	0.05	98.1	7.51
			7	0.05	94.1	6.98
LUFA 2.2	Dimethenamid-P	276→168	5	0.005	96.4	2.01
		276→244	5	0.005	96.5	1.82
		276→168	5	0.05	94.2	4.22
		276→244	5	0.05	94.6	3.30
	Reg No 360714 (M27)	320→80	5	0.005	91.9	10.72
		320→121	5	0.005	101.5	6.27
		320→80	5	0.05	95.3	2.68
		320→121	5	0.05	102.2	4.38
	Reg No 360715 (M23)	270→166	5	0.005	102.8	11.84
		270→198	5	0.005	99.0	3.90
		270→166	5	0.05	97.7	15.65
		270→198	5	0.05	101.1	3.69

Linearity

Good linearity (regression coefficients ≥0.99) was observed in the range of 0.1 ng/mL to 1.0 ng/mL for dimethenamid-P and its metabolites M23 and M27.

Specificity

LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from soil components or from reagents, solvents and glassware used.

Limit of Quantitation The limit of quantitation was 0.005 mg/kg.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-2.

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

Conclusion Method L0109/01 fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of dimethenamid-P and its metabolites M23 and M27 in soil.

Water

Report: CA 4.1.2/3
Jooss S., 2012a
Determination of Dimethenamid-P and its metabolites M23, M27 and M31 in water
2012/1278546

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

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

Principle of the methods Drinking or surface water (1 mL of sample containing 1% of formic acid) was fortified with dimethenamid-P (BAS 656 PH) and its metabolites Reg No 360715 (M23), Reg No 360714 (M27) and Reg No 360712 (M31) to obtain the level of quantitation (0.03 µg/L) and 10x higher level (0.3 µg/L).
The samples were mixed and the analytes finally determined by LC-MS/MS. The limit of quantitation (LOQ) of all test items was 0.03 µg/L.

Recovery findings The method proved to be suitable to determine dimethenamid-P and its metabolites M23, M27 and M31 in water, with an LOQ of 0.03 µg/L and a limit of detection (LOD) of 0.009 µg/L. Validation experiments were conducted in drinking and surface water. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 120%. The detailed results are given in Table 4.1.2-3.

Table 4.1.2-3: Results of the method validation for the determination of dimethenamid-P and its metabolites in drinking water and surface water

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Dimethenamid-P	276→244	Drinking water	5	0.03	109	5
				0.30	110	4
		Surface water	5	0.03	109	7
				0.30	109	4
	276→168	Drinking water	5	0.03	108	6
				0.30	110	5
		Surface water	5	0.03	110	5
				0.30	110	5
Reg No 360715 (M23)	270→198	Drinking water	5	0.03	107	3
				0.30	98	6
		Surface water	5	0.03	108	7
				0.30	104	6
	270→166	Drinking water	5	0.03	103	8
				0.30	98	6
		Surface water	5	0.03	102	8
				0.30	103	6
Reg No 360714 (M27)	320→121	Drinking water	5	0.03	106	7
				0.30	96	6
		Surface water	5	0.03	110	3
				0.30	105	5
	320→80	Drinking water	5	0.03	110	6
				0.30	97	7
		Surface water	5	0.03	110	7
				0.30	106	6
Reg No 360712 (M31)	346→240	Drinking water	5	0.03	107	6
				0.30	97	5
		Surface water	5	0.03	110	8
				0.30	103	6
	346→198	Drinking water	5	0.03	97	12
				0.30	91	6
		Surface water	5	0.03	99	7
				0.30	103	5

Linearity Good linearity (r ≥0.99) was observed in the range of 0.009 ng/mL to 1 ng/mL for the two ion transitions of dimethenamid-P and its metabolites M23, M27 and M31 in the two different water types (drinking water and surface water).

Specificity LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from water components or from reagents, solvents and glassware used.

Limit of Quantitation The method has a limit of quantitation of 0.03 µg/L for dimethenamid-P and its metabolites M23, M27 and M31.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-3.

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

Conclusion The method fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine Dimethenamid-P and its metabolites M23, M27 and M31 in drinking water and surface water samples.

Water

Report: CA 4.1.2/4
Mewis A., 2013a
Validation of an analytical method for determination of metabolites of Dimethenamid-P in water
2013/1349800

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009

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

Principle of the methods The aim of the current study was the validation of an analytical method for the determination of metabolites of dimethenamid-P M3 (Reg No 360717), M656PH010 (Reg No 5931836), M23 (Reg No 360715), M27 (Reg No 360714), M31 (Reg No 360712), M656H032 (Reg No 395234), M656PH043 (Reg No 5917262), M656PH045 (Reg No 5917261), M656PH047 (Reg No 5917260) and M656PH054 (Reg No 5920718) in surface water. A 50 mL aliquot of a water sample was filled into 250 mL glass bottle together with 10 µL hydrochloric acid and the whole sample was extracted by SPE. The analytes were eluted with 2.5 mL methanol/acetonitrile/ammonium solution (28%) (50:50:2 v/v/v). An aliquot of 500 µL of the eluate was mixed with 450 µL water and 50 µL acetic acid. The residues of the metabolites of dimethenamid-P were determined by HPLC-MS/MS using two mass transitions.

The limit of quantitation (LOQ) of the method for the metabolites of dimethenamid-P (M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054) is 0.025 µg/L in surface water.

Recovery findings

The method proved to be suitable to determine the metabolites of dimethenamid-P in surface water with an LOQ of 0.025 µg/L. Validation experiments were conducted in surface water. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 120%. The detailed results are given in Table 4.1.2-4.

Table 4.1.2-4: Results of the method validation for the determination of the metabolites of dimethenamid-P in surface water

Substance	Mass transition	Replicates	Fortification level (µg/L)	Mean recovery [%]	RSD [%]
M656PH003 (Reg No 360717)	242→210	5	0.025	110	3.5
		5	0.25	98	1.7
	242→168	5	0.025	105	4.7
		5	0.25	98	3.5
M656PH010 (Reg No 5931836)	320→288	5	0.025	109	4.4
		5	0.25	101	2.1
	320→166	5	0.025	106	4.7
		5	0.25	104	2.1
M656PH023 (Reg No 360715)	270→198	5	0.025	99	16.4
		5	0.25	100	3.0
	270→166	5	0.025	99	14.1
		5	0.25	91	3.0
M656PH027 (Reg No 360714)	320→121	5	0.025	98	6.1
		5	0.25	97	3.2
	320→80	5	0.025	109	5.3
		5	0.25	102	0.4
M656PH031 (Reg No 360712)	346→240	5	0.025	104	9.8
		5	0.25	89	5.0
	346→198	5	0.025	110	12.6
		5	0.25	102	5.5
M656H032 (Reg No 395234)	330→240	5	0.025	97	15.9
		5	0.25	97	8.2
	330→198	5	0.025	110	11.3
		5	0.25	105	2.9
M656PH043 (Reg No 5917262)	286→242	5	0.025	110	10.8
		5	0.25	98	5.4
	286→210	5	0.025	103	6.1
		5	0.25	101	5.8
M656PH045 (Reg No 5917261)	300→184	5	0.025	110	9.5
		5	0.25	95	1.1
	300→228	5	0.025	110	4.6
		5	0.25	105	3.0
M656PH047 (Reg No 5917260)	350→306	5	0.025	89	15.0
		5	0.25	97	4.4
	350→121	5	0.025	109	10.5
		5	0.25	98	3.6
M656PH054 (Reg No 5920718)	320→198	5	0.025	100	19.1
		5	0.25	92	5.1
	320→121	5	0.025	110	18.2
		5	0.25	95	5.2

Linearity

For analysis of the metabolites of dimethenamid-P at the quantifier and qualifier mass transitions, the detector response was linear within the range from 0.075 ng/mL to 10 ng/mL (equivalent to 0.0075 µg/L to 1 µg/L sample concentration) with r ≥0.9957.

Specificity

LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from water components or from reagents, solvents and glassware used

Limit of Quantitation

The method has a limit of quantitation of 0.025 µg/L in surface water for dimethenamid-p metabolites M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-4.

Reproducibility

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

Conclusion

It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine the metabolites of dimethenamid-P in surface water samples.

Water

Report: CA 4.1.2/5
Schulz H.,Meyer M., 2007a
Determination of Dimethenamid-P and its metabolites M23 and M27 in tap and surface water - Validation of the method 519/0
2007/1054384

Guidelines: SANCO/825/00 rev. 7 (17 March 2004), OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, EU Guideline 8064/VI/97 rev. 4 15.12.1998, SANCO/3029/99 rev. 4 (11 July 2000), Residue analytical methods for post-registration control purposes (July 21 1998) Federal Biological Research Centre for Agriculture and Forestry Braunschweig Germany

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Principle of the method A 10.0 g aliquot of the water specimen was adjusted to pH 2 and extracted by SPE. The analytes were eluted with methanol. After evaporation to dryness the residues were dissolved in water/methanol (80 + 20, v+v). An aliquot of the final volume was measured using LC-MS/MS.
The limit of quantitation (LOQ) of the method for dimethenamid-P (Reg No 363851) and its metabolites M23 (Reg No 360715) and M27 (Reg No 360714) is 0.05 µg/kg in water.

Recovery findings Method No 519/0 proved to be suitable to determine dimethenamid-P and its metabolites M23 and M27 in water, with an LOQ of 0.05 µg/L. Validation experiments were conducted in surface and tap water. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 120%. The detailed results are given in Table 4.1.2-5.

Table 4.1.2-5: Results of the method validation for the determination of Dimethenamid-P and its metabolites in tap water and surface water

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Dimethenamid-P	276→244	Tap water	5	0.05	74.1	1.2
				0.5	76.8	3.8
		Surface water	5	0.05	87.6	1.8
				0.5	87.0	2.0
	276→168	Tap water	5	0.05	73.7	2.1
				0.5	77.3	3.7
		Surface water	5	0.05	86.5	2.8
				0.5	87.9	3.1
M23 (Reg No 360715)	270→198	Tap water	5	0.05	91.8	1.0
				0.5	89.3	3.7
		Surface water	5	0.05	106.6	0.8
				0.5	104.0	1.3
	270→166	Tap water	5	0.05	89.3	3.8
				0.5	89.5	2.6
		Surface water	5	0.05	106.0	1.8
				0.5	103.9	1.2
M27 (Reg No 360714)	320→121	Tap water	5	0.05	91.6	3.8
				0.5	89.2	3.5
		Surface water	5	0.05	103.5	4.5
				0.5	106.5	1.6
	320→80	Tap water	5	0.05	92.2	2.7
				0.5	89.9	2.9
		Surface water	5	0.05	109.0	1.5
				0.5	106.0	1.6

Linearity

Good linearity (r ≥0.99) was observed in the range of 0.1 ng/mL to 5 ng/mL for the two ion transitions of dimethenamid-P and its metabolites M23 and M27 in the two different water types (tap water and surface water).

Specificity

LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from water components or from reagents, solvents and glassware used.

Limit of Quantitation

The method has a limit of quantitation of 0.05 µg/kg for dimethenamid-P and its metabolites M23 and M27.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-5.

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

Conclusion It could be demonstrated that the method No 519/01 fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine dimethenamid-P and its metabolites M23 and M27 in tap water and surface water samples.

Air

Report: CA 4.1.2/6
Zangmeister W., 2010a
Validation of analytical method L0167/01: Determination of Dimethenamid-P in air
2010/1126085

Guidelines: SANCO/825/00 rev. 7 (17 March 2004), 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 Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Priniciple of the method Dimethenamid-P, collected on Tenax, was extracted with acetone and analyzed by HPLC-MS/MS. For all analytes two parent-daughter ion transitions were used for quantitation and confirmation.

Recovery findings Method No L0167/01 proved to be suitable to determine dimethenamid-P in air. The results from the fortification experiments at two spiking levels (0.0015 µg and 0.15 µg of dimethenamid-P per L air) resulted in recoveries between 70% and 120% (see Table 4.1.2-6).

Table 4.1.2-6: Results of method validation of Dimethenamid-P in air (uncorrected data)

Test system	Analyte	Replicates	Fortification level [µg/L]	m/z 276 →168			m/z 276 →244		
				Mean recovery [%]	SD [+/- %]	RSD [%]	Mean recovery [%]	SD [+/- %]	RSD [%]
Air	Dimethenamid-P	5	0.0015	87.9	10.8	12.3	87.9	10.7	12.1
		6	0.15	97.0	3.0	3.1	97.0	3.4	3.5

Linearity	Good linearity (r >0.99) was observed in the range of 0.1 ng/mL to 2 ng/mL for both mass transitions of dimethenamid-P.
Specificity	LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). Signals in blank values were ≤ 30% which proves that no interferences are observed at the given retention time.
Limit of Quantitation	The method has a limit of quantitation of 0.8 µg dimethenamid-P (lowest fortification level), corresponding with a concentration of 0.0015 µg dimethenamid-P / L air.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20% (see Table 4.1.2-6).
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	Method L0167/01 was validated and the method fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of dimethenamid-P in air.

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

Not relevant.

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

Since dimethenamid-P is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body tissues or fluids are not required.

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

Since no exposure studies were conducted with dimethenamid-P, such methods of analysis are not required.

(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 residue studies.

Plant

For plants, the proposed residue definition for MRL setting and risk assessment is parent dimethenamid-P plus metabolite M30 as a sum of all isomers calculated in parent equivalents. However, M23, M27 and M26 were included in addition tothe methods based on previous metabolism studies and the known metabolic pathways of chloroacetamide herbicides.

It should be noted that study validation of L0179/02 (2011/1182078) has been submitted to Germany previously in product dossiers but has never been fully reviewed. Therefore it is included below for completeness. The ILV of this method is new and is included in addition.

Report:	CA 4.1.2/7 Lehmann A., 2012b Validation of BASF method L0179/02: Method for the determination of Dimethenamid-P (BAS 656 H) and its metabolites M23, M26, M27 and M30 in plant matrices 2011/1182078
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), 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 Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This report includes the validation results obtained in maize, sugar beet, oilseed rape, strawberries, onions and dried beans. The principle of the BASF Method No L0179/02 is given below.

Principle of the method	Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) are extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant is diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings

Method No L0179/02 proved to be suitable for analysis of Dimethenamid-P (BAS 656 PH) and its metabolites M23, M26, M27 and M30 in maize, sugar beet, oilseed rape, strawberries, onions and dried beans. Plant samples were spiked with the analyte at the limit of quantitation and 10x higher. The recovery data were corrected for interferences from matrix compounds of the appropriate unfortified sample. The mean recovery values were between 70% and 120%. The detailed results are given in Table 4.1.2-7 to Table 4.1.2-11.

Table 4.1.2-7: Recovery results of Dimethenamid-P in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 276→244			Transition 276→168		
Maize	Whole plant	0.01	5	103.9	1.9	5	103.1	1.9
		0.1	5	104.1	1.2	5	103.3	0.7
Maize	Seeds	0.01	5	115.6	3.0	5	115.0	3.0
		0.1	5	107.6	2.6	5	107.0	3.0
Sugar beet	Leaves	0.01	5	105.3	1.4	5	105.4	0.8
		0.1	5	106.6	1.3	5	107.0	1.3
Sugar beet	Roots	0.01	5	103.7	0.5	5	104.3	1.6
		0.1	5	105.4	1.1	5	105.4	0.9
Oilseed rape	Seed	0.01	5	105.8	0.9	5	106.4	1.3
		0.1	5	105.9	1.3	5	106.2	1.7
Straw-berries	Fruit	0.01	5	102.1	1.2	5	101.4	0.2
		0.1	5	105.3	0.8	5	105.5	1.0
Onions	Bulb	0.01	5	104.6	1.0	5	104.1	1.0
		0.1	5	105.4	1.0	5	105.4	0.9
Dried beans	Seeds	0.01	5	106.6	2.6	5	105.9	1.4
		0.1	5	101.0	1.0	5	100.8	1.2

Table 4.1.2-8: Recovery results of metabolite M23 (Reg No 360715) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 270→198			Transition 270→166		
Maize	Whole plant	0.01	5	98.3	5.1	5	104.4	3.0
		0.1	5	105.2	1.5	5	105.9	2.0
Maize	Seeds	0.01	5	90.2	3.6	5	87.9	4.6
		0.1	5	88.4	3.1	5	87.3	3.0
Sugar beet	Leaves	0.01	5	104.2	1.9	5	105.5	4.9
		0.1	5	106.7	1.6	5	105.0	1.7
Sugar beet	Roots	0.01	5	103.0	2.3	5	91.3	4.3
		0.1	5	103.8	1.2	5	100.9	2.2
Oilseed rape	Seed	0.01	5	107.1	3.9	5	107.0	7.2
		0.1	5	106.6	1.8	5	105.4	1.8
Straw-berries	Fruit	0.01	5	99.0	2.7	5	99.8	5.7
		0.1	5	106.2	1.8	5	106.6	1.7
Onions	Bulb	0.01	5	106.1	4.8	5	105.4	5.5
		0.1	5	105.7	1.8	5	107.6	1.2
Dried beans	Seeds	0.01	5	99.3	3.5	5	99.4	5.7
		0.1	5	93.0	1.6	5	94.8	2.3

Table 4.1.2-9: Recovery results of metabolite M26 (Reg No 360716) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 360→272			Transition 360→142		
Maize	Whole plant	0.01	5	96.7	3.4	5	95.14	7.8
		0.1	5	105.2	1.5	5	105.9	3.2
Maize	Seeds	0.01	5	87.2	6.1	5	86.3	3.6
		0.1	5	94.9	1.6	5	95.2	2.6
Sugar beet	Leaves	0.01	5	104.8	1.7	5	103.2	4.1
		0.1	5	107.5	2.4	5	107.5	1.6
Sugar beet	Roots	0.01	5	103.0	2.3	5	91.3	4.3
		0.1	5	103.8	1.2	5	100.9	2.2
Oilseed rape	Seed	0.01	5	107.1	3.9	5	107.0	7.2
		0.1	5	106.6	1.8	5	105.4	1.8
Straw-berries	Fruit	0.01	5	99.0	2.7	5	99.8	5.7
		0.1	5	106.2	1.8	5	106.6	1.7
Onions	Bulb	0.01	5	106.1	4.8	5	105.4	5.5
		0.1	5	105.7	1.8	5	107.6	1.2
Dried beans	Seeds	0.01	5	99.3	3.5	5	99.4	5.7
		0.1	5	93.0	1.6	5	94.8	2.3

Table 4.1.2-10: Recovery results of metabolite M27 (Reg No 360714) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 320→121			Transition 320→80		
Maize	Whole plant	0.01	5	104.8	3.8	5	105.2	3.4
		0.1	5	104.5	1.1	5	105.6	0.7
Maize	Seeds	0.01	5	83.4	1.7	5	84.0	2.8
		0.1	5	82.6	2.0	5	82.2	2.2
Sugar beet	Leaves	0.01	5	106.2	1.9	5	104.6	2.4
		0.1	5	105.7	1.5	5	106.7	1.3
Sugar beet	Roots	0.01	5	101.8	1.1	5	105.0	1.8
		0.1	5	104.4	1.1	5	105.2	1.2
Oilseed rape	Seed	0.01	5	103.0	2.7	5	103.1	0.9
		0.1	5	101.7	1.9	5	101.5	1.8
Straw-berries	Fruit	0.01	5	102.9	2.0	5	101.7	1.8
		0.1	5	105.2	1.3	5	105.0	1.2
Onions	Bulb	0.01	5	103.3	2.3	5	104.0	2.0
		0.1	5	104.1	0.2	5	105.0	1.5
Dried beans	Seeds	0.01	5	99.2	2.5	5	98.9	2.6
		0.1	5	97.3	1.7	5	97.2	1.5

Table 4.1.2-11: Recovery results of metabolite M30 (Reg No 5296352) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 376→136			Transition 376→270		
Maize	Whole plant	0.01	5	108.2	8.5	5	98.5	7.4
		0.1	5	102.4	2.6	5	102.8	2.6
Maize	Seeds	0.01	5	83.5	5.4	5	89.0	7.2
		0.1	5	86.4	1.3	5	86.5	1.2
Sugar beet	Leaves	0.01	5	103.4	7.8	5	92.7	6.9
		0.1	5	103.3	0.9	5	104.6	1.8
Sugar beet	Roots	0.01	5	105.8	4.9	5	101.8	4.0
		0.1	5	104.6	1.8	5	104.3	2.0
Oilseed rape	Seed	0.01	5	96.5	10.6	5	100.6	11.1
		0.1	5	101.8	1.9	5	99.7	3.4
Straw-berries	Fruit	0.01	5	93.6	6.8	5	99.4	5.7
		0.1	5	105.6	2.5	5	105.6	1.8
Onions	Bulb	0.01	5	108.5	2.3	5	119.1	3.5
		0.1	5	104.0	1.9	5	102.4	2.1
Dried beans	Seeds	0.01	5	94.0	6.9	5	92.5	4.0
		0.1	5	93.6	2.2	5	91.6	2.4

Linearity

The linearity of the LC-MS/MS detector was tested using seven standard solutions at concentrations between 0.05 and 5 µg/L. Standards were injected in triplicate and the response plotted against concentration. Linear correlations with coefficients ≥0.9993 were obtained for dimethenamid-P and its metabolites M23, M26, M27 and M30.

Specificity LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantitation The limit of quantitation is 0.01 mg/kg for all analytes.

Repeatability The relative standard deviation for BAS 656 PH and all tested metabolite recoveries at each validated was <20%. The detailed values are shown in Table 4.1.2-7 to Table 4.1.2-11.

Reproducibility An independent laboratory validation of this method was successfully conducted and is reported below (see CA 4.1.2/8).

Conclusion The recovery data show that BASF method No L0179/02 is suitable to determine residues of BAS 656 PH and its metabolites M23, M26, M27 and M30 in plant matrices such as maize, sugar beet, oilseed rape, strawberries, onions and dried beans.

Report:	CA 4.1.2/8 Rogers P. et al., 2014a Independent laboratory validation of BASF analytical method L0179/02: Determination of Dimethenamid-P (BAS 656 H, Reg.No. 363851), M23 (Reg.No. 360715), M26 (Reg.No. 360716), M27 (Reg.No. 360714) and M30 (Reg.No. 5296352) in plant matrices 2013/7002656
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), 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 United States Environmental Protection Agency)

This report includes the independent laboratory validation results obtained in maize, oilseed rape, strawberries and dried beans. The principle of the BASF Method No L0179/02 is given below.

Principle of the method Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) are extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant is diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings Method No L0179/02 was successfully validated for BAS 656 PH and its metabolites M23, M26, M27 and M30 in maize (forage, grain), oilseed rape, strawberries and dried beans. Plant samples were spiked with the analyte at the limit of quantitation and 10x higher. The recovery data were corrected for interferences from matrix compounds of the appropriate unfortified sample. The mean recovery values were between 70% and 120%. For M26 in dried beans, at the fortification level 0.01 mg/kg the recovery at the confirmatory transition was 68%. However, the overall recovery was 70% considering the mean values of both fortification levels. For M30 in dried beans the primary mass transition resulted in recoveries slightly below 70% (66-68%, however the overall recoveries was 70%. The detailed results are given in Table 4.1.2-12 to Table 4.1.2-16.

Table 4.1.2-12: Recovery results of dimethenamid-P in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 276→244			Transition 276→168		
Straw-berries	Fruit	0.01	5	95	2.9	5	98	3.4
		0.1	5	96	2.2	5	97	2.8
Dried beans	Seed	0.01	5	85	2.3	5	87	3.3
		0.1	5	87	3.5	5	87	2.4
Oilseed rape	Seed	0.01	5	105	13.1	5	107	13.1
		0.1	5	103	12.0	5	103	11.7
Maize	Forage	0.01	5	108	2.3	5	106	2.0
		0.1	5	102	2.0	5	101	1.6
Maize	Grain	0.01	5	113	4.2	5	109	3.2
		0.1	5	104	1.5	5	105	1.6

Table 4.1.2-13: Recovery results of metabolite M23 (Reg No 360715) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 270→198			Transition 270→166		
Straw-berries	Fruit	0.01	5	102	2.1	5	101	7.1
		0.1	5	102	1.5	5	101	1.5
Dried beans	Seed	0.01	5	72	4.1	5	73	10.2
		0.1	5	74	4.3	5	75	6.0
Oilseed rape	Seed	0.01	5	104	4.6	5	109	2.4
		0.1	5	104	5.5	5	99	3.9
Maize	Forage	0.01	5	109	5.1	5	113	5.6
		0.1	5	107	1.7	5	105	3.8
Maize	Grain	0.01	5	101	3.2	5	105	3.4
		0.1	5	99	1.8	5	97	1.2

Table 4.1.2-14: Recovery results of metabolite M26 (Reg No 360716) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 360→272			Transition 360→142		
Straw-berries	Fruit	0.01	5	107	2.1	5	107	5.1
		0.1	5	101	1.0	5	99	1.4
Dried beans	Seed	0.01	5	71	5.9	5	68	8.8
		0.1	5	74	8.0	5	71	7.3
Oilseed rape	Seed	0.01	5	109	7.1	5	108	10.1
		0.1	5	107	9.0	5	106	9.4
Maize	Forage	0.01	5	103	5.6	5	103	4.9
		0.1	5	104	1.5	5	100	1.5
Maize	Grain	0.01	5	103	2.8	5	104	4.3
		0.1	5	99	2.5	5	99	1.9

Table 4.1.2-15: Recovery results of metabolite M27 (Reg No 360714) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 370→121			Transition 320→80		
Straw-berries	Fruit	0.01	5	102	2.0	5	103	1.7
		0.1	5	100	1.9	5	102	1.5
Dried beans	Seed	0.01	5	71	4.3	5	74	4.6
		0.1	5	74	6.4	5	74	7.1
Oilseed rape	Seed	0.01	5	99	1.9	5	102	2.9
		0.1	5	106	1.3	5	106	3.2
Maize	Forage	0.01	5	109	2.4	5	109	3.8
		0.1	5	111	0.8	5	112	0.6
Maize	Grain	0.01	5	102	1.5	5	102	3.2
		0.1	5	100	1.8	5	99	1.0

Table 4.1.2-16: Recovery results of metabolite M30 (Reg No 5296352) in plant matrices

Crop	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD (%)	No of tests	Average recovery (%)	RSD (%)
			Transition 376→136			Transition 376→270		
Straw-berries	Fruit	0.01	5	104	8.9	5	105	11.9
		0.1	5	101	2.4	5	106	0.5
Dried beans	Seed	0.01	5	66	9.8	5	73	7.5
		0.1	5	68	9.0	5	68	7.2
Oilseed rape	Seed	0.01	5	103	7.8	5	94	5.8
		0.1	5	108	3.9	5	104	5.0
Maize	Forage	0.01	5	111	4.9	5	115	8.5
		0.1	5	100	1.2	5	101	2.3
Maize	Grain	0.01	5	95	5.9	5	98	9.9
		0.1	5	94	4.3	5	93	2.9

Linearity

The linearity of the LC-MS/MS detector was tested using eight standard solutions at concentrations between 0.05 and 10 µg/L. Standards were injected in duplicate and the response plotted against concentration. Linear correlations with coefficients ≥0.9897 were obtained for dimethenamid-P and its metabolites M23, M26, M27 and M30.

Specificity

LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantitation

The limit of quantitation is 0.01 mg/kg for all analytes.

Repeatability The relative standard deviation of BAS 656 PH and all tested metabolites recoveries at each validated level and overall for each matrix was <20%. The detailed values are shown in Table 4.1.2-12 to Table 4.1.2-16.

Reproducibility The independent laboratory validation reproduced the results of the validation successfully.

Conclusion The recovery data show that BASF method No L0179/02 was successfully independently validated. The method is suitable to determine residues of BAS 656 PH and its metabolites M23, M26, M27 and M30 in plant matrices.

Animal

Since the animal methods are the methods proposed for enforcement, and no feeding studies have been conducted, they are included in 4.2.

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

Methods for concentration control are reported, where necessary, along with the respective ecotoxicological studies

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

Where necessary, these methods are reported along with the respective studies.

CA 4.2 Methods for post-approval control and monitoring purposes

(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

In the course of the MRL re-evaluation according to Article 12 of Regulation (EC) No 396/2005, it was concluded based on the overall availability of analytical methods that dimethenamid (sum of isomers) can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in dry, acidic, high water content and in high oil content commodities and an analytical method for enforcement of residues in food of animal origin was not necessary. However, based on the new proposed residue definition for monitoring as described in Document N, chapter 7.3 for products of plant and animal origin the following methods were valid for post-approval control and monitoring purposes of dimethenamid-P and its metabolites if necessary in these compartments. For **plants**, the proposed residue definition for MRL setting and risk assessment is **parent dimethenamid-P plus metabolite M30 as a sum of all isomers calculated in parent equivalents**. However, M23, M27 and M26 were included in addition to the methods based on previous metabolism studies and the known metabolic pathways of chloroacetamide herbicides. For **animals,** the proposed **residue definition for MRL setting** in liver and kidney of sheep, goats, cattle and other farm animals is **parent dimethenamid-P + M30**. For **risk assessmen**t in liver and kidney of sheep, goats, cattle and other farm animals is **parent dimethenamid-P plus metabolites M26 and M30 as a sum of all isomers calculated in parent equivalents**. The selection of M30 for MRL setting is based on the goat metabolism study where ^{14}C-M656PH030 was administered. Both M26 and M30 are the major metabolites observed in tissues, however, M26 is rapidly excreted in high amounts (~90% TRR). After feed burden calculations and extrapolations using the goat metabolism study, similar levels were seen in kidney for M26 and M30 (0.03-0.038 mg/kg) but liver was also above the LOQ (0.017 mg/kg) for M30. Therefore, for enforcement, M30 is the best candidate for a marker molecule. Since consumers may potentially be exposed to M26 and M30, they should be included for risk assessment. Parent dimethenamid is only included as a default in the residue definition. Based on plant and animal metabolism, as well as supervised residue trials, consumers will not be exposed to parent via plants or foods of animal origin.

Extractability

Extractability of various solvents was tested within three metabolism studies, Corn (2006), Soybean (2012) and Goat (dosed with ^{14}C-M656PH030). For Corn, methanol, water and acetonitrile were investigated in forage and straw. Grain could not be tested due to low radioactivity. Results showed good and similar extractability with all solvents. Therefore method L0197/02 using methanol is covered. For Soybean, methanol and water were tested in leaf, seed, hull, and rest of plant. Extractability results were impacted by significant bound residues in soybean seed and hull. Therefore, we have added the use of a high speed homogenizer (such as Mafia) to the method for high oil matrices to help release some of these bound residues. The extractability data in soybean supports L0179/02.

In the goat study, acetonitrile and water were tested. As usual with animal matrices a significant amount of bound residue was observed. For the extractable radioactive residue good recoveries were observed with acetonitrile and water mixtures. Therefore, method R0037/01 which is submitted for enforcement in animal matrices is suitable based on extractability.

These results are summarized in the tables below.

Table 4.2-1: Extractability of Corn Matrices in Study 2006/1024513

Matrix	DAT[1]	Solvents	Mixture (by vol)	ERR (%TRR)	RRR (%TRR)	TRR (calc)[2] Mg/kg
Corn Forage	30	Methanol 3X and water 2X	Consecutively	85.7	14.3	2.5
Corn Forage	30	Methanol/water	50/50	81.2	18.8	2.2
Corn Forage	30	Methanol/water/ HCL	50/45/5	82.3	17.7	2.6
Corn Forage	30	Methanol./Water/Ammonia	50/50/1	87.7	12.3	2.3
Corn Forage	30	Acetonitrile/Water	50/50	82.5	17.5	2.2
Corn Forage	30	Acetonitrile/Water/HCL	50/45/5	85.8	14.2	2.5
Corn Forage	30	Acetonitrile/water/ammonia	50/50/1	87	13	2.4
Corn Straw	119	Methanol 3X and water 2X	Consecutively	75.9	24.1	0.7
Corn Straw	119	Methanol/water	50/50	66.8	33.2	0.8
Corn Straw	119	Methanol/water/ HCL	50/45/5	68.8	31.2	0.7
Corn Straw	119	Methanol./Water/Ammonia	50/50/1	70.5	29.5	0.8
Corn Straw	119	Acetonitrile/Water	50/50	67	33	0.8
Corn Straw	119	Acetonitrile/Water/HCL	50/45/5	72.1	27.9	0.8
Corn Straw	119	Acetonitrile/water/ammonia	50/50/1	69.8	30.2	0.8

1 DAT= Days after last treatment
2 TRR was calculated as the sum of ERR + TRR
3 Grain could not be tested due to low radioactivity

Table 4.2-2: Extractability from Soybean Metabolism Study 2012/114379

Matrix	Solvent	ERR (%TRR)	RRR (% TRR)	TRR (calc)[1] mg/kg
Soybean leaf	Methanol	46.8	29.8	2.595
Soybean leaf	Water	23.4		
Soybean seed	Methanol	15.8	52.9	0.693
Soybean seed	Water	31.2		
Soybean Hull	Methanol	13	74.4	0.821
Soybean Hull	Water	12.6		
Soybean Rest of Plant	Methanol	29.9	62	0.629
Soybean Rest of Plant	Water	8		

1 TRR was calculated as the sum of ERR + RRR

Table 4.2-3: Extractability from Goat Metabolism Study 2013/7002636

Matrix	%TRR Recovered by Sequential Extractions[1]						Total ERR[2]
	Acetonitrile	Acetonitrile/ water (50:50)	Acetonitrile/ water (50:50)	Acetonitrile	Acetone	Methanol	
Liver	22.6	11.9	6	3.3	-[3]	-	43.8
Kidney	75.3	16.6	7	-	-	-	98.9
Loin Muscle	39.2	11.8	7.6	3.6	2.3	-	64.5
Renal Fat	12.2	30.3	7.8	4	-	4	58.3
Day 8 Milk	67.8	9.8	2	-	-	-	79.6

1 Solvents were tested sequentially. For example, Renal fat was extracted by acetonitrile, followed by acetonitrile/water followed by acetonitrile followed by methanol
2 As calculated as a sum of all recoveries from each solvent scheme
3 – not subjected to further extraction

Plant

BASF method L0179/02 used for data generation and enforcement for residue trials for plant and plant products for dimethenamid-P and its metabolites in accordance with the proposed residue definition for enforcement is presented below and also in CA 4.1.2. A summary of the independent laboratory validation is also presented below.

A new multi residue method (modified QuEChERS) was developed and validated to cover the 5 commodity categories with an LOQ of 0.01 mg/kg for parent dimethenamid-P, M26 and M30. This is in accordance with the residue definition for enforcement proposed for dimethenamid-P in plant and plant products, with the additional opportunity to cover M26 if necessary. The validation recovery results for dimethenamid-P were acceptable for all five matrix categories and for both monitored transitions. However, the method was only partially successful at recovering the metabolites M26 and M30, according to the guideline acceptability range of 70-120%. Modifications to the QuEChERS procedure were made to try and improve these recoveries without success. As per SANCO 825 the multi-residue method should be suitable for the determination of all compounds included in the residue definition. Method R0038/01 is not sufficient to be used as a multi-residue/enforcement method. In addition, as per SANCO 825 "Single residue methods should only be provided if data show and are reported that multi-residue methods involving GC as well as HPLC techniques cannot be used. Therefore, it is proposed that BASF method L0179/02 be the enforcement method of choice when monitoring dimethenamid-P and its metabolite M30 in plants and plant products.

Report:	CA 4.2/1 Lehmann A., 2012b Validation of BASF method L0179/02: Method for the determination of Dimethenamid-P (BAS 656 H) and its metabolites M23, M26, M27 and M30 in plant matrices 2011/1182078
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), 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 Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This report includes the validation results obtained in maize, sugar beet, oilseed rape, strawberries, onions and dried beans. The principle of the BASF Method No L0179/02 is given below.

Principle of the method	Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings

Method No L0179/02 proved to be suitable for analysis of dimethenamid-P (BAS 656 PH) and its metabolites M23, M26, M27 and M30 in maize, sugar beet, oilseed rape, strawberries, onions and dried beans. Plant samples were spiked with the analyte at the limit of quantitation and 10x higher. The recovery data were corrected for interferences from matrix compounds of the appropriate unfortified sample. The mean recovery values were between 70% and 120%. The detailed results were given in Table 4.2/1-1 to Table 4.2/1-5.

Table 4.2/1-1: Recovery results of dimethenamid-P in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 276→244			Transition 276→168		
Maize	Whole plant	0.01	5	103.9	1.9	5	103.1	1.9
		0.1	5	104.1	1.2	5	103.3	0.7
Maize	Seeds	0.01	5	115.6	3.0	5	115.0	3.0
		0.1	5	107.6	2.6	5	107.0	3.0
Sugar beet	Leaves	0.01	5	105.3	1.4	5	105.4	0.8
		0.1	5	106.6	1.3	5	107.0	1.3
Sugar beet	Roots	0.01	5	103.7	0.5	5	104.3	1.6
		0.1	5	105.4	1.1	5	105.4	0.9
Oilseed rape	Seed	0.01	5	105.8	0.9	5	106.4	1.3
		0.1	5	105.9	1.3	5	106.2	1.7
Straw-berries	Fruit	0.01	5	102.1	1.2	5	101.4	0.2
		0.1	5	105.3	0.8	5	105.5	1.0
Onions	Bulb	0.01	5	104.6	1.0	5	104.1	1.0
		0.1	5	105.4	1.0	5	105.4	0.9
Dried beans	Seeds	0.01	5	106.6	2.6	5	105.9	1.4
		0.1	5	101.0	1.0	5	100.8	1.2

Table 4.2/1-2: Recovery results of metabolite M23 (Reg No 360715) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 270→198			Transition 270→166		
Maize	Whole plant	0.01	5	98.3	5.1	5	104.4	3.0
		0.1	5	105.2	1.5	5	105.9	2.0
Maize	Seeds	0.01	5	90.2	3.6	5	87.9	4.6
		0.1	5	88.4	3.1	5	87.3	3.0
Sugar beet	Leaves	0.01	5	104.2	1.9	5	105.5	4.9
		0.1	5	106.7	1.6	5	105.0	1.7
Sugar beet	Roots	0.01	5	103.0	2.3	5	91.3	4.3
		0.1	5	103.8	1.2	5	100.9	2.2
Oilseed rape	Seed	0.01	5	107.1	3.9	5	107.0	7.2
		0.1	5	106.6	1.8	5	105.4	1.8
Straw-berries	Fruit	0.01	5	99.0	2.7	5	99.8	5.7
		0.1	5	106.2	1.8	5	106.6	1.7
Onions	Bulb	0.01	5	106.1	4.8	5	105.4	5.5
		0.1	5	105.7	1.8	5	107.6	1.2
Dried beans	Seeds	0.01	5	99.3	3.5	5	99.4	5.7
		0.1	5	93.0	1.6	5	94.8	2.3

Table 4.2/1-3: Recovery results of metabolite M26 (Reg No 360716) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 360→272			Transition 360→142		
Maize	Whole plant	0.01	5	96.7	3.4	5	95.14	7.8
		0.1	5	105.2	1.5	5	105.9	3.2
Maize	Seeds	0.01	5	87.2	6.1	5	86.3	3.6
		0.1	5	94.9	1.6	5	95.2	2.6
Sugar beet	Leaves	0.01	5	104.8	1.7	5	103.2	4.1
		0.1	5	107.5	2.4	5	107.5	1.6
Sugar beet	Roots	0.01	5	103.0	2.3	5	91.3	4.3
		0.1	5	103.8	1.2	5	100.9	2.2
Oilseed rape	Seed	0.01	5	107.1	3.9	5	107.0	7.2
		0.1	5	106.6	1.8	5	105.4	1.8
Straw-berries	Fruit	0.01	5	99.0	2.7	5	99.8	5.7
		0.1	5	106.2	1.8	5	106.6	1.7
Onions	Bulb	0.01	5	106.1	4.8	5	105.4	5.5
		0.1	5	105.7	1.8	5	107.6	1.2
Dried beans	Seeds	0.01	5	99.3	3.5	5	99.4	5.7
		0.1	5	93.0	1.6	5	94.8	2.3

Table 4.2/1-4: Recovery results of metabolite M27 (Reg No 360714) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 320→121			Transition 320→80		
Maize	Whole plant	0.01	5	104.8	3.8	5	105.2	3.4
		0.1	5	104.5	1.1	5	105.6	0.7
Maize	Seeds	0.01	5	83.4	1.7	5	84.0	2.8
		0.1	5	82.6	2.0	5	82.2	2.2
Sugar beet	Leaves	0.01	5	106.2	1.9	5	104.6	2.4
		0.1	5	105.7	1.5	5	106.7	1.3
Sugar beet	Roots	0.01	5	101.8	1.1	5	105.0	1.8
		0.1	5	104.4	1.1	5	105.2	1.2
Oilseed rape	Seed	0.01	5	103.0	2.7	5	103.1	0.9
		0.1	5	101.7	1.9	5	101.5	1.8
Straw-berries	Fruit	0.01	5	102.9	2.0	5	101.7	1.8
		0.1	5	105.2	1.3	5	105.0	1.2
Onions	Bulb	0.01	5	103.3	2.3	5	104.0	2.0
		0.1	5	104.1	0.2	5	105.0	1.5
Dried beans	Seeds	0.01	5	99.2	2.5	5	98.9	2.6
		0.1	5	97.3	1.7	5	97.2	1.5

Table 4.2/1-5: Recovery results of metabolite M30 (Reg No 5296352) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 376→136			Transition 376→270		
Maize	Whole plant	0.01	5	108.2	8.5	5	98.5	7.4
		0.1	5	102.4	2.6	5	102.8	2.6
Maize	Seeds	0.01	5	83.5	5.4	5	89.0	7.2
		0.1	5	86.4	1.3	5	86.5	1.2
Sugar beet	Leaves	0.01	5	103.4	7.8	5	92.7	6.9
		0.1	5	103.3	0.9	5	104.6	1.8
Sugar beet	Roots	0.01	5	105.8	4.9	5	101.8	4.0
		0.1	5	104.6	1.8	5	104.3	2.0
Oilseed rape	Seed	0.01	5	96.5	10.6	5	100.6	11.1
		0.1	5	101.8	1.9	5	99.7	3.4
Straw-berries	Fruit	0.01	5	93.6	6.8	5	99.4	5.7
		0.1	5	105.6	2.5	5	105.6	1.8
Onions	Bulb	0.01	5	108.5	2.3	5	119.1	3.5
		0.1	5	104.0	1.9	5	102.4	2.1
Dried beans	Seeds	0.01	5	94.0	6.9	5	92.5	4.0
		0.1	5	93.6	2.2	5	91.6	2.4

Linearity The linearity of the LC-MS/MS detector was tested using seven standard solutions at concentrations between 0.05 and 5 µg/L. Standards were injected in triplicate and the response plotted against concentration. Linear correlations with coefficients ≥0.9993 were obtained for dimethenamid-P and its metabolites M23, M26, M27 and M30.

Specificity LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glasswwere used.

Limit of Quantitation The limit of quantitation is 0.01 mg/kg for all analytes.

Repeatability The relative standard deviation for BAS 656 PH and all tested metabolite recoveries at each validated was <20%. The detailed values were shown in Table 4.2-1 to Table 4.2-5.

Reproducibility An independent laboratory validation of this method was successfully conducted and is reported below (see CA 4.1.2/8).

Conclusion

The recovery data show that BASF method No L0179/02 is suitable to determine residues of BAS 656 PH and its metabolites M23, M26, M27 and M30 in plant matrices such as maize, sugar beet, oilseed rape, strawberries, onions and dried beans. Therefore, it is proposed that BASF method L0179/02 be the enforcement method of choice when monitoring dimethenamid-P and its metabolites M26 and M30 in plants and plant products.

Report: CA 4.2/2
Rogers P. et al., 2014b
Independent laboratory validation of BASF analytical method L0179/02: Determination of Dimethenamid-P (BAS 656 H, Reg.No. 363851), M23 (Reg.No. 360715), M26 (Reg.No. 360716), M27 (Reg.No. 360714) and M30 (Reg.No. 5296352) in plant matrices
2013/7002656

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), 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 United States Environmental Protection Agency)

This report includes the independent laboratory validation results obtained in maize, oilseed rape, strawberries and dried beans. The principle of the BASF Method No L0179/02 is given below.

Principle of the method

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings

Method No L0179/02 was successfully validated for BAS 656 PH and its metabolites M23, M26, M27 and M30 in maize (forage, grain), oilseed rape, strawberries and dried beans. Plant samples were spiked with the analyte at the limit of quantitation and 10x higher. The recovery data were corrected for interferences from matrix compounds of the appropriate unfortified sample. The mean recovery values were between 70% and 120%. For M26 in dried beans, at the fortification level 0.01 mg/kg the recovery at the confirmatory transition was 68%. However, the overall recovery was 70% considering the mean values of both fortification levels. For M30 in dried beans the primary mass transition resulted in recoveries slightly below 70% (66-68%, however the overall recoveries was 70%. The detailed results were given in Table 4.2-1 to Table 4.2-5.

Table 4.2/2-1: Recovery results of dimethenamid-P in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 276→244			Transition 276→168		
Straw-berries	Fruit	0.01	5	95	2.9	5	98	3.4
		0.1	5	96	2.2	5	97	2.8
Dried beans	Seed	0.01	5	85	2.3	5	87	3.3
		0.1	5	87	3.5	5	87	2.4
Oilseed rape	Seed	0.01	5	105	13.1	5	107	13.1
		0.1	5	103	12.0	5	103	11.7
Maize	Forage	0.01	5	108	2.3	5	106	2.0
		0.1	5	102	2.0	5	101	1.6
Maize	Grain	0.01	5	113	4.2	5	109	3.2
		0.1	5	104	1.5	5	105	1.6

Table 4.2/2-2: Recovery results of metabolite M23 (Reg No 360715) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 270→198			Transition 270→166		
Straw-berries	Fruit	0.01	5	102	2.1	5	101	7.1
		0.1	5	102	1.5	5	101	1.5
Dried beans	Seed	0.01	5	72	4.1	5	73	10.2
		0.1	5	74	4.3	5	75	6.0
Oilseed rape	Seed	0.01	5	104	4.6	5	109	2.4
		0.1	5	104	5.5	5	99	3.9
Maize	Forage	0.01	5	109	5.1	5	113	5.6
		0.1	5	107	1.7	5	105	3.8
Maize	Grain	0.01	5	101	3.2	5	105	3.4
		0.1	5	99	1.8	5	97	1.2

Table 4.2/2-3: Recovery results of metabolite M26 (Reg No 360716) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 360→272			Transition 360→142		
Straw-berries	Fruit	0.01	5	107	2.1	5	107	5.1
		0.1	5	101	1.0	5	99	1.4
Dried beans	Seed	0.01	5	71	5.9	5	68	8.8
		0.1	5	74	8.0	5	71	7.3
Oilseed rape	Seed	0.01	5	109	7.1	5	108	10.1
		0.1	5	107	9.0	5	106	9.4
Maize	Forage	0.01	5	103	5.6	5	103	4.9
		0.1	5	104	1.5	5	100	1.5
Maize	Grain	0.01	5	103	2.8	5	104	4.3
		0.1	5	99	2.5	5	99	1.9

Table 4.2/2-4: Recovery results of metabolite M27 (Reg No 360714) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 370→121			Transition 320→80		
Straw-berries	Fruit	0.01	5	102	2.0	5	103	1.7
		0.1	5	100	1.9	5	102	1.5
Dried beans	Seed	0.01	5	71	4.3	5	74	4.6
		0.1	5	74	6.4	5	74	7.1
Oilseed rape	Seed	0.01	5	99	1.9	5	102	2.9
		0.1	5	106	1.3	5	106	3.2
Maize	Forage	0.01	5	109	2.4	5	109	3.8
		0.1	5	111	0.8	5	112	0.6
Maize	Grain	0.01	5	102	1.5	5	102	3.2
		0.1	5	100	1.8	5	99	1.0

Table 4.2/2-5: Recovery results of metabolite M30 (Reg No 5296352) in plant matrices

Crop	Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
			Transition 376→136			Transition 376→270		
Straw-berries	Fruit	0.01	5	104	8.9	5	105	11.9
		0.1	5	101	2.4	5	106	0.5
Dried beans	Seed	0.01	5	66	9.8	5	73	7.5
		0.1	5	68	9.0	5	68	7.2
Oilseed rape	Seed	0.01	5	103	7.8	5	94	5.8
		0.1	5	108	3.9	5	104	5.0
Maize	Forage	0.01	5	111	4.9	5	115	8.5
		0.1	5	100	1.2	5	101	2.3
Maize	Grain	0.01	5	95	5.9	5	98	9.9
		0.1	5	94	4.3	5	93	2.9

Linearity

The linearity of the LC-MS/MS detector was tested using eight standard solutions at concentrations between 0.05 and 10 µg/L. Standards were injected in duplicate and the response plotted against concentration. Linear correlations with coefficients ≥0.9897 were obtained for dimethenamid-P and its metabolites M23, M26, M27 and M30.

Specificity

LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glasswwere used.

Limit of Quantitation

The limit of quantitation is 0.01 mg/kg for all analytes.

Repeatability The relative standard deviation of BAS 656 PH and all tested metabolites recoveries at each validated level and overall for each matrix was <20%. The detailed values were shown in Table 4.2-1 to Table 4.2-5.

Reproducibility The independent laboratory validation reproduced the results of the validation successfully.

Conclusion The recovery data show that BASF method No L0179/02 was successfully independently validated. The method is suitable to determine residues of BAS 656 PH and its metabolites M23, M26, M27 and M30 in plant matrices. Therefore, it is proposed that BASF method L0179/02 be the enforcement method of choice when monitoring dimethenamid-P and its metabolites M26 and M30 in plants and plant products.

Report: CA 4.2/3
Diamaduros B., 2014a
Validation of BASF analytical method R0038/01: Modified QuEChERS for the determination of the residues of Dimethenamid-P and metabolites M26 (Reg.No. 360716) and M30 (Reg.No. 5296352) in plant matrices at a loq of 0.01 mg/kg using LC-MS/MS
2013/7002627

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

This report includes the validation results obtained in grape, lettuce, soybean seed, barley grain and dried beans. The principle of the BASF method No R0038/01 is given below.

Principle of the method Dimethenamid-P, M26, and M30 are extracted from plant commodities by shaking with acetonitrile, or for samples with low moisture content, acetonitrile:water (1:1, v/v). After sample extraction, a mixture of salts is added, and the extract is shaken and then centrifuged to separate the aqueous and organic phases. Residues in an aliquot of the organic layer are diluted with methanol:water (1:1, v/v) and then determined by high performance liquid chromatography (HPLC) electrospray ionization tandem mass spectrometry (MS/MS-ESI) monitoring ion transitions at m/z 276→244 (proposed as the primary transition for quantitation) and m/z 276→168 (typically for confirmatory purposes) for dimethenamid-P, m/z 360→272 (primary) and m/z 360→142 (secondary) for M26, and m/z 376→91 (primary) and m/z 376→136 (secondary) for M30. The results are calculated by direct comparison of the sample peak responses to those of external standards. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings The method validation was performed successfully for parent dimethenamid-P for each plant commodity and the LC/MS/MS ion transitions (primary and secondary) available for the method, using solvent-based standards. However, the method was only partially successful at recovering the metabolites M26 and M30, according to the guideline acceptability range of 70-120%.

Mean recoveries of **dimethenamid-P** from grape fruit, white navy bean dried seed, lettuce leaves, soybean seed, and barley grain fortified at 0.01 and 0.1 mg/kg were 81-93% (RSD, 1-5%) and 87-97% (RSD, 1-4%), respectively, for the primary transition. For the secondary transition, mean recoveries of dimethenamid-P at 0.01 mg/kg and 0.1 mg/kg were 78-95% (RSD, 2-4%) and 87-93% (RSD, 3-5%), respectively.

For metabolite **M26**, mean recoveries from grape fruit, lettuce leaves, and barley grain fortified at 0.01 and 0.1 mg/kg, ranged from 84 to 95% (RSD, 3-9%, both transitions); however, relatively low recoveries were obtained from the dried seed commodities of white navy bean and soybean, with mean recoveries for these matrices ranging from 55 to 68% (RSD, 3-11%, over both transitions).

Mean recoveries of **M30** from grape fruit, lettuce leaves, and barley grain fortified at 0.01 and 0.1 mg/kg, ranged from 66 to 81% (RSD, 3-14%, over both transitions), and were unacceptably low from white navy bean and soybean (dried seed), with mean recoveries ranging from 20 to 39% (RSD, 4-16%, over both transitions), with the exception of 74±11% from soybean dried seed at the 0.01 mg/kg fortification level for the primary transition.

The detailed results were given in Table 4.2/3-1 to Table 4.2/3-3.

Table 4.2/3-1: Recovery results of BAS 656 PH in plant matrices

Crop / matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD [%]	No of tests	Average recovery (%)	RSD [%]
		Transition 276→244			Transition 276→168		
Grape, Fruit	0.01	5	91	3	5	93	3
	0.1	5	93	3	5	92	5
White Navy Bean, Dried Seed	0.01	5	93	1	5	92	4
	0.1	5	97	2	5	91	3
Lettuce Leaves	0.01	5	91	5	5	90	2
	0.1	5	95	1	5	93	5
Soybean, Dried Seed	0.01	5	81	4	5	78	3
	0.1	5	87	4	5	87	5
Barley, Grain	0.01	5	89	3	5	95	2
	0.1	5	93	4	5	93	3

Table 4.2/3-2: Recovery results of metabolite M26 (Reg No 360716) in plant matrices

Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD [%]	No of tests	Average recovery (%)	RSD [%]
		Transition 360→272			Transition 360→142		
Grape, Fruit	0.01	5	86	4	5	88	3
	0.1	5	92	5	5	92	5
White Navy Bean, Dried Seed	0.01	5	68	4	5	66	3
	0.1	5	68	10	5	67	10
Lettuce Leaves	0.01	5	88	4	5	85	4
	0.1	5	95	7	5	92	8
Soybean, Dried Seed	0.01	5	55	11	5	58	10
	0.1	5	64	3	5	65	3
Barley, Grain	0.01	5	92	9	5	86	8
	0.1	5	88	8	5	84	7

Table 4.2/3-3: Recovery results of metabolite M30 (Reg No 5296352) in plant matrices

Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	RSD [%]	No of tests	Average recovery (%)	RSD [%]
		Transition 376→91			Transition 376→136		
Grape, Fruit	0.01	5	69	3	5	67	6
	0.1	5	78	4	5	78	4
White Navy Bean, Dried Seed	0.01	5	39	4	5	35	6
	0.1	5	31	13	5	29	13
Lettuce Leaves	0.01	5	73	6	5	73	6
	0.1	5	78	6	5	81	6
Soybean, Dried Seed	0.01	5	74	11	5	20	16
	0.1	5	32	12	5	23	9
Barley, Grain	0.01	5	68	14	5	66	14
	0.1	5	69	8	5	68	10

Linearity Acceptable LC-MS/MS detector linearity was observed for the standard range and the two mass transitions tested for each analyte: The method-detector response was linear over the 0.01-0.5 ng/mL range (r = ≥0.9946).

Specificity LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantitation The limit of quantitation is 0.01 mg/kg for all plant matrices.

Repeatability The relative standard deviation BAS 656 PH and all tested metabolites recoveries at each validated level and overall for each animal matrix was <20%. The detailed values were shown Table 4.2/3-1 to Table 4.2/3-3.

Reproducibility An independent laboratory validation of this method was not conducted. This method will not be proposed for enforcement.

Conclusion The results of this method validation study demonstrate that BASF Analytical Method No. R0038/01, a modified QuEChERS method, fulfils the requirements for parent dimethenamid-P with regard to specificity, repeatability, limit of quantitation and recoveries and is, therefore, applicable to correctly determine parent dimethenamid-P residues in plant commodities. However, the method was only partially successful at recovering the metabolites M26 and M30, according to the guideline acceptability range of 70-110%. Modifications to the QuEChERS procedure were made to try and improve these recoveries without success As per SANCO 825 the multi-residue method should be suitable for the

determination of all compounds included in the residue definition. Method R0038/01 is not sufficient to be used as a multi-residue/enforcement method. In addition, as per SANCO 825 "Single residue methods should only be provided if data show and were reported that multi-residue methods involving GC as well as HPLC techniques cannot be used. Therefore, it is proposed that BASF method L0179/02 be the enforcement method of choice when monitoring dimethenamid-P and its metabolites M26 and M30 in plants and plant products.

Animal

Method validation data of R0037/01 as well as results from an independent laboratory validation therefor was presented below for dimethenamid-P in animal matrices.

Report: CA 4.2/4
Gordon B., 2014a
Validation of BASF analytical method R0037/01: Determination of the residues of Dimethenamid-P (Reg.No. 363851) and metabolites M26 (Reg.No. 360716) and M30 (Reg.No. 5296352) in animal matrices at a LOQ of 0.01 mg/kg using LC-MS/MS
2013/7002631

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

This report includes the validation results obtained in bovine milk, liver, kidney, muscle, fat and chicken egg. The principle of the BASF method No R0037/01 is given below.

Principle of the method Dimethenamid-P, M26 (Reg No 360716) and M30 (Reg No 5296352) were extracted from livestock commodities (bovine muscle, kidney, liver, fat and milk, and poultry egg) by homogenization with acetonitrile:water (1:1, v/v). After sample extraction, the residues were centrifuged, diluted with methanol:water (1:1, v/v), and determined by high performance liquid chromatography (HPLC) positive (for dimethenamid-P) or negative (for M26 and M30) ion electrospray ionization tandem mass spectrometry (MS/MS-ESI) monitoring ion transitions at m/z 276→244 (proposed as the primary transition for quantitation) and m/z 276→168 (typically for confirmatory purposes) for dimethenamid-P, m/z 360→272 (primary) and m/z 360→142 (secondary) for M26, and m/z 376→91 (primary) and m/z 376→136 (secondary) for M30.

Recovery findings

For validation, untreated livestock samples (bovine muscle, kidney, liver, fat and milk, and poultry egg) were fortified with dimethenamid-P, M26, and M30 and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two controls, five replicates fortified with each analyte at the method limit of quantitation, 0.01 mg/kg (ppm), and five replicates fortified at a higher level, corresponding to 10x the limit of quantitation, 0.1 mg/kg. For each analyte, the two mass transitions described above were evaluated. The mean recovery values were between 70% and 120%. The detailed results were given in Table 4.2-14 to Table 4.2-16.

Table 4.2/4-1: Recovery results for dimethenamid-P in livestock commodities

Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
		Transition 276→244			Transition 276→168		
Bovine muscle	0.01	5	91	11	5	89	8
	0.1	5	91	8	5	91	9
Bovine kidney	0.01	5	96	5	5	87	15
	0.1	5	103	7	5	100	7
Bovine liver	0.01	5	88	8	5	94	8
	0.1	5	95	2	5	96	4
Bovine fat	0.01	5	100	2	5	98	3
	0.1	5	99	4	5	99	5
Bovine milk	0.01	5	83	3	5	81	7
	0.1	5	101	4	5	92	6
Poultry egg	0.01	5	98	3	5	103	6
	0.1	5	99	4	5	96	5

Table 4.2/4-2: Recovery results for M26 (Reg No 360716) in livestock commodities

Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
		Transition 360→272			Transition 360→142		
Bovine muscle	0.01	5	103	9	5	107	10
	0.1	5	100	8	5	97	8
Bovine kidney	0.01	5	102	10	5	99	16
	0.1	5	102	4	5	100	5
Bovine liver	0.01	5	103	8	5	101	9
	0.1	5	102	5	5	101	5
Bovine fat	0.01	5	100	3	5	97	6
	0.1	5	105	3	5	104	5
Bovine milk	0.01	5	97	3	5	94	3
	0.1	5	108	2	5	107	5
Poultry egg	0.01	5	102	2	5	102	3
	0.1	5	102	4	5	103	5

Table 4.2/4-3: Recovery results for M30 (Reg No 5296352) in livestock commodities

Matrix	Fortification level [mg/kg]	No of tests	Average recovery [%]	RSD [%]	No of tests	Average recovery [%]	RSD [%]
		Transition 376→91			Transition 376→136		
Bovine muscle	0.01	5	104	13	5	105	13
	0.1	5	99	5	5	96	8
Bovine kidney	0.01	5	108	9	5	104	12
	0.1	5	103	5	5	98	4
Bovine liver	0.01	5	98	8	5	97	9
	0.1	5	99	5	5	97	5
Bovine fat	0.01	5	99	3	5	106	5
	0.1	5	104	7	5	106	4
Bovine milk	0.01	5	107	5	5	116	3
	0.1	5	104	4	5	102	6
Poultry egg	0.01	5	111	4	5	106	2
	0.1	5	99	3	5	98	3

Linearity Acceptable LC-MS/MS detector linearity was observed for the standard range and the two mass transitions tested for each analyte: The method-detector response was linear over the 0.004-0.2 ng/mL range (r ≥0.9969).

Specificity LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from animal matrix components or from reagents, solvents and glassware used.

Limit of Quantitation The limit of quantitation is 0.01 mg/kg for all plant matrices.

Repeatability The relative standard deviation BAS 656 PH and all tested metabolites recoveries at each validated level and overall for each animal matrix was <20%. The detailed values were shown in Table 4.2-14to Table 4.2-16.

Reproducibility An independent laboratory validation of this method was successfully conducted.

Conclusion The results of this method validation study demonstrate that BASF Analytical method No R0037/01 fulfills the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is, therefore, applicable to correctly determine residues of the herbicide dimethenamid-P, including its metabolites M26 and M30, in livestock commodities.

Report: CA 4.2/5
Perez R., datea
Independent laboratory validation of BASF analytical method R0037/01: Analytical method for the determination of the residues of Dimethenamid-P (Reg. No. 363851) and metabolites M26 (Reg. No. 360716) and M30 (Reg. No. 5296352) in animal matrices at a LOQ of 0.01 mg/kg using LC-MS/MS
2013/7002632

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

This report includes the independent laboratory validation results obtained in bovine milk, liver, kidney, muscle, fat and chicken egg. The principle of the BASF Method No. R0037/01 is given below.

Principle of the method Dimethenamid-p, M26, and M30 are extracted from livestock commodities (bovine muscle, kidney, liver, fat and milk, and poultry egg) by homogenization with acetonitrile:water (1:1, v/v). After sample extraction, the residues are centrifuged, diluted with methanol:water (1:1, v/v), and determined by high performance liquid chromatography (HPLC) positive (for dimethenamid) or negative (for M26 and M30) ion electrospray ionization tandem mass spectrometry (MS/MS-ESI) monitoring ion transitions at m/z 276→244 (proposed as the primary transition for quantitation) and m/z 276→168 (typically for confirmatory purposes) for dimethenamid-p, m/z 360→272 (primary) and m/z 360→142 (secondary) for M26, and m/z 376→91 (primary) and m/z 376→136 (secondary) for M30. The limit of quantitation is 0.01 mg/kg for each analyte.

Recovery findings For validation, untreated livestock samples (bovine muscle, kidney, liver, fat and milk, and poultry egg) were fortified with dimethenamid-p, M26, and M30 and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two controls, five replicates fortified with each analyte at the method limit of quantitation, 0.01 mg/kg (ppm), and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 0.1 mg/kg. For each analyte, the two mass transitions described above were evaluated. The mean recovery values were between 70% and 110%. The detailed results are given in Table 4.2/4-1 to Table 4.2/4-3.

Table 4.2/5-1: Recovery results for Dimethenamid-p in livestock commodities

Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	Rel. standard deviation (%)	No of tests	Average Recovery (%)	Rel. standard deviation (%)
		Transition 276→244			Transition 276→168		
Bovine muscle	0.01	5	84	6	5	100	14
	0.1	5	79	6	5	79	4
Bovine kidney	0.01	5	76	4	5	92	24
	0.1	5	86	5	5	90	6
Bovine liver	0.01	5	91	13	5	87	19
	0.1	5	88	7	5	88	11
Bovine fat	0.01	5	78	4	5	83	9
	0.1	5	86	10	5	91	13
Bovine milk	0.01	5	92	9	5	100	12
	0.1	5	77	3	5	73	4
Poultry egg	0.01	5	95	14	5	118	4
	0.1	5	100	15	5	93	20

Table 4.2/5-2: Recovery results for M26 in livestock commodities

Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	Rel. standard deviation (%)	No of tests	Average Recovery (%)	Rel. standard deviation (%)
		Transition 360→272			Transition 360→142		
Bovine muscle	0.01	5	104	15	5	106	11
	0.1	5	109	11	5	91	20
Bovine kidney	0.01	5	93	8	5	81	15
	0.1	5	98	8	5	93	16
Bovine liver	0.01	5	93	10	5	99	12
	0.1	5	91	7	5	98	15
Bovine fat	0.01	5	110	19	5	100	7
	0.1	5	115	3	5	111	9
Bovine milk	0.01	5	105	11	5	110	7
	0.1	5	92	7	5	92	9
Poultry egg	0.01	5	77	9	5	93	19
	0.1	5	101	5	5	98	9

Table 4.2/5-3: Recovery results for M30 in livestock commodities

Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)	Rel. standard deviation (%)	No of tests	Average Recovery (%)	Rel. standard deviation (%)
		Transition 376→91			Transition 376→136		
Bovine muscle	0.01	5	115	4	5	100	9
	0.1	5	107	10	5	111	6
Bovine kidney	0.01	5	95	11	5	101	10
	0.1	5	99	5	5	97	2
Bovine liver	0.01	5	94	16	5	96	14
	0.1	5	98	2	5	93	6
Bovine fat	0.01	5	109	4	5	108	10
	0.1	5	109	6	5	114	7
Bovine milk	0.01	5	105	14	5	108	9
	0.1	5	103	2	5	103	8
Poultry egg	0.01	5	79	10	5	81	20
	0.1	5	102	4	5	100	5

Linearity	Acceptable LC-MS/MS detector linearity was observed for the standard range and the two mass transitions tested for each analyte: The method-detector response was linear over the 0.004-1.0 ng/mL range (r ≥0.9943).
Specificity	LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.
Limit of Quantitation	The limit of quantitation is 0.01 mg/kg for all animal matrices.
Repeatability	The relative standard deviation BAS 656 PH and all tested metabolites recoveries at each validated level and overall for each animal matrix was ≤20%. The detailed values are shown in Table 4.2/4-1 to Table 4.2/4-3.
Reproducibility	The successful completion of this independent laboratory validation demonstrates the reproducibility of this method.
Conclusion	The recovery data show that BASF method No R0037/01 is suitable to determine residues of BAS 656 PH and its metabolites M26 and M30 in animal matrices such as bovine milk, liver, kidney, muscle, fat and chicken egg.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Based on the proposed residue definitions for monitoring in soil, ground water, surface water and sediment as described in Document N, chapter 8.5 the following methods were valid for post-approval control and monitoring purposes of dimethenamid-P and its metabolites if necessary in these compartments.

Soil

Validated enforcement methods for dimethenamid-P and its metabolites (M23, M27 and M31) were presented in CA 4.1.2/1 and CA 4.1.2/2 according to the proposed residue definitions for monitoring in soil/sediment as described in Document N, chapter 8.5.

There were no independent laboratory validations for these soil methods as they were not required.

Water

Validated enforcement methods for dimethenamid-P and its metabolites (M23, M27 and M31) were presented in CA 4.1.2/3 and CA 4.1.2/4 according to the proposed residue definitions for monitoring in water as described in Document N, chapter 8.5.

Results from two independent laboratory validations were presented below for Dimethenamid-p and its metabolites in water matrices.

Report: CA 4.2/6
Shi Y.,Lui W., 2014a
Independent laboratory validation of BASF analytical method: Determination of Dimethenamid-P and its metabolites M23, M27 and M31 in water for Dimethenamid-P only
2014/7000491

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

Principle of the methods Drinking water containing 0.1% of formic acid was fortified with dimethenamid-P (BAS 656 PH) to obtain the level of quantification (0.03 µg/L) and higher level (0.3 µg/L).
The samples were mixed and the analytes finally determined by LC-MS/MS.
The limit of quantification (LOQ) of all test items was 0.03 µg/L.

Recovery findings The method proved to be suitable to determine dimethenamid-P in water, with an LOQ of 0.03 µg/L and a limit of detection (LOD) of 0.009 µg/L. Validation experiments were conducted in drinking water. All average recovery values (mean of 5 replicates per fortification level) were between 97% and 100%. The detailed results were given in Table 4.2/6-1.

Table 4.2/6-1: Results of the method validation for the determination of dimethenamid-P in drinking water

Analyte	Matrix	m/z	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Dimethenamid-P	Drinking water	**276→244**	5	0.03	97	5.5
			5	0.30	99	2.9
		276→168	5	0.03	97	2.9
			5	0.30	100	1.9

Linearity	Good linearity (r ≥0.99) was observed in the range of 0.009 ng/mL to 0.4 ng/mL for the two ion transitions of dimethenamid-P in drinking water.
Specificity	The method successfully determines dimethenamid-P in drinking water by using LC-MS/MS, and monitoring two ion transitions. No interferences (>30% of the limit of quantification) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.
Limit of Quantification	The method had a limit of quantification of 0.03 µg/L for dimethenamid-P.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values were shown in Table 4.2-20.
Reproducibility	In context of this ILV study, the reproducibility of this analytical method was estimated. As can be seen from the results, a high reproducibility was determined.
Conclusion	The report describes the independent validation of the analytical method "Determination of dimethenamid-P and its metabolites M23, M27 and M31 in Water" for dimethenamid-P only. The results confirm that this analytical method is suitable to determine dimethenamid-P in drinking water.

Report: CA 4.2/7
Yang J.,Michener P., 2014a
Independent laboratory validation of method MGeN0001/13: Method for the determination of Dimethenamid-P metabolites in water by LC-MS/MS
2013/7002762

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

Principle of the methods

The aim of the current study was the independent validation of an analytical method for the determination of metabolites of dimethenamid-P (M3, M10, M23, M27, M31, M32, M43/44, M45/46, M47/48 and M54/58) in tap water and surface water. 50 mL of a water sample was filled into 250 mL glass bottle together with 10 µL hydrochloric acid and the whole sample was extracted by SPE. The analytes were eluted with 2.5 mL methanol/acetonitrile/ammonium solution (28%) (50:50:2 v/v/v). An aliquot of 500 µL of the eluate was mixed with 450 µL water and 50 µL acetic acid. The residues of the metabolites of dimethenamid-P were determined by HPLC-MS/MS using two mass transitions.

The limit of quantitation (LOQ) of the method for the metabolites of dimethenamid-P (M3, M10, M23, M27, M31, M32, M43/44, M45/46, M47/48 and M54/58) is 0.025 µg/L in water.

Recovery findings

The method proved to be suitable to determine the metabolites of dimethenamid-P in tap and surface water with an LOQ of 0.025 µg/L. Validation experiments were conducted in tap water and surface water. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 120%. The detailed results were given in Table 4.2/7-1 and Table 4.2/7-2.

Table 4.2/7-1: Results of the method validation for the determination of the metabolites of dimethenamid-P in tap water

Substance	Mass transition	Replicates	Fortification level (µg/L)	Mean recovery [%]	RSD [%]
M3	242→210	5	0.025	97	5
		5	0.25	95	2
	242→168	5	0.025	97	6
		5	0.25	95	2
M10	320→288	5	0.025	100	6
		5	0.25	99	2
	320→166	5	0.025	96	6
		5	0.25	101	1
M23	270→198	5	0.025	105	3
		5	0.25	98	4
	270→166	5	0.025	102	6
		5	0.25	100	3
M27	320→121	5	0.025	105	4
		5	0.25	95	4
	320→80	5	0.025	106	4
		5	0.25	95	3
M31	346→240	5	0.025	105	5
		5	0.25	108	1
	346→198	5	0.025	104	8
		5	0.25	105	1
M32	330→240	5	0.025	107	2
		5	0.25	102	4
	330→198	5	0.025	106	5
		5	0.25	101	3
M43/44	286→242	5	0.025	92	7
		5	0.25	105	1
	286→210	5	0.025	103	11
		5	0.25	105	2
M45/46	300→184	5	0.025	93	5
		5	0.25	95	3
	300→228	5	0.025	95	7
		5	0.25	95	3
M47/48	350→306	5	0.025	94	4
		5	0.25	100	2
	350→121	5	0.025	93	17
		5	0.25	99	3
M54/58	320→198	5	0.025	105	8
		5	0.25	97	5
	320→121	5	0.025	100	7
		5	0.25	99	4

Table 4.2/7-2: Results of the method validation for the determination of the metabolites of dimethenamid-P in surface water

Substance	Mass transition	Replicates	Fortification level (µg/L)	Mean recovery [%]	RSD [%]
M3	242→210	5	0.025	105	5
		5	0.25	87	3
	242→168	5	0.025	105	4
		5	0.25	87	1
M10	320→288	5	0.025	107	5
		5	0.25	88	3
	320→166	5	0.025	105	6
		5	0.25	88	1
M23	270→198	5	0.025	107	5
		5	0.25	103	3
	270→166	5	0.025	100	10
		5	0.25	103	4
M27	320→121	5	0.025	109	8
		5	0.25	109	4
	320→80	5	0.025	108	6
		5	0.25	106	3
M31	346→240	5	0.025	107	9
		5	0.25	91	2
	346→198	5	0.025	103	4
		5	0.25	88	4
M32	330→240	5	0.025	103	8
		5	0.25	106	3
	330→198	5	0.025	103	6
		5	0.25	105	2
M43/44	286→242	5	0.025	98	4
		5	0.25	88	2
	286→210	5	0.025	102	17
		5	0.25	87	2
M45/46	300→184	5	0.025	99	8
		5	0.25	86	2
	300→228	5	0.025	100	13
		5	0.25	91	3
M47/48	350→306	5	0.025	103	5
		5	0.25	92	2
	350→121	5	0.025	99	15
		5	0.25	90	2
M54/58	320→198	5	0.025	105	9
		5	0.25	106	4
	320→121	5	0.025	107	8
		5	0.25	109	4

Linearity	Good linearity (r ≥0.99) was observed in the range of 0.075 ng/mL to 10.0 ng/mL for two ion transitions per analyte in tap water and surface water.
Specificity	The method successfully determines dimethenamid-P in drinking water by using LC-MS/MS, and monitoring two ion transitions. No interferences (>30% of the limit of quantification) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.
Limit of Quantification	The limit of quantitation (LOQ) of the method for the metabolites of dimethenamid-P (M3, M10, M23, M27, M31, M32, M43/44, M45/46, M47/48 and M54/58) is 0.025 µg/L in water.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values were shown in Table 4.2-21 and Table 4.2-22.
Reproducibility	In context of this ILV study, the reproducibility of this analytical method was estimated. As can be seen from the results, a high reproducibility was determined.
Conclusion	The report describes the independent validation of the analytical method MGeN0001/13. The results confirm that this analytical method is suitable to determine the metabolites of dimethenamid-P (M3, M10, M23, M27, M31, M32, M43/44, M45/46, M47/48 and M54/58) in tap water and drinking water.

(c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

A validated method for dimethenamid-P is presented in CA 4.1.2/6 according to the proposed residue definitions for monitoring in air as described in Document N, chapter 8.5.

(d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Since dimethenamid-P is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body tissues or fluids were not required.

BAS 656H

DOCUMENT M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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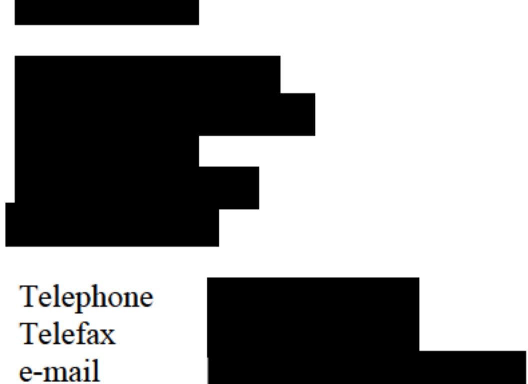

Version history[1]

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

[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

For the convenience of the reviewer information on the Annex I inclusion toxicological evaluation of dimethenamid-P are provided as extracted from the Monograph of dimethenamid-P are provided under the respective chapters.

Comparative toxicological assessment of dimethenamid-P and racemic dimethenamid – the Bridging concept

Dimethenamid is one of many organic substances that occur as “racemic” 50/50 mixtures of stereoisomers. It was discovered that only the S-isomer (dimethenamid-P) has useful herbicidal activity, while the other isomer (R) is inactive as a pesticide that was nearly completely removed in the synthesis of dimethenamid-P.

For the inclusion of dimethenamid-P in Annex I of Directive 91/414/EEC, the long-term and reproductive toxicity studies submitted were not performed with dimethenamid-P. Instead, the effects of racemic (R,S)-dimethenamid were tested in these extensive studies, which had been completed prior to discovery of the superior properties of the S-isomer (dimethenamid-P). The so-called “Bridging” concept was applied to avoid the additional conduct of the above mentioned studies with dimethenamid-P, and thus to save time and costs and avoid additional animal testing. By this Bridging approach, results from toxicological studies available for both racemic dimethenamid and dimethenamid-P were compared.

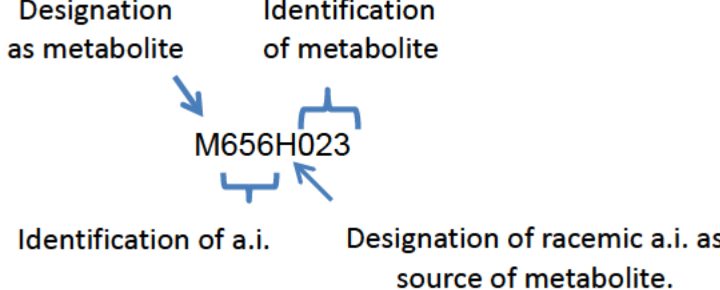
All in all, the Bridging studies that were available for assessment of acute toxicity, short-term toxicity, genotoxicity and developmental toxicity indicated that both test substances share the same toxicological profile, and that the effects established were observed at comparable dose levels. On this basis, it was the opinion that in principle the test substances racemic dimethenamid and dimethenamid-P are equivalent entities and that all studies available for racemic dimethenamid should be considered in the toxicological evaluation of dimethenamid-P. It is considered to be scientifically justifiable to accept relevant racemic dimethenamid studies for derivation of the ADI, ARfD and AOEL.

In conclusion the Bridging studies conducted with dimethenamid-P can be used in conjunction with the studies conducted with racemic dimethenamid to support a registration of the dimethenamid S-isomer (dimethenamid-P).

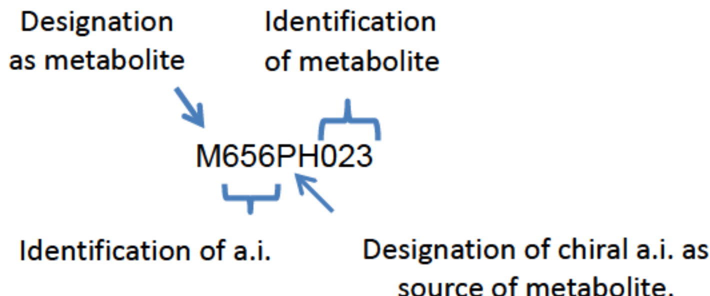
CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

General explanation on metabolite nomenclature in relation to stereoisomery.

Dimethenamid-P is the S-enantiomer of the racemic dimethenamid. For the active substance a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic pathway synthesis. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example M23:



Metabolites where the source of identification and the synthesis route could clearly be attributed to the chiral compound dimethenamid-P were assigned as follows:



The metabolic pathways in soil, water, mammals, and plants are equivalent for the racemic dimethenamid and dimethenamid-P (S-enantiomer). The metabolites derived from either racemic or enantio-enriched source are considered toxicologically equivalent and were taken into account for the assessment below.

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
BAS 656 H (275.8)	360720	-	87674-68-8	Rat	
BAS 656 PH	363851	-	163515-14-8	Rat	
M656H001	Not assigned	M1	Not assigned	Rat	
M656PH001	Not assigned	M1	Not assigned	Rat	
M656H002	Not assigned	M2	Not assigned	Rat	
M656PH002	Not assigned	M2	Not assigned	Rat	
M656H003	360717	M3	Not assigned	Rat	
M656PH003	5886782	M3	Not assigned	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H004	Not assigned	M4	Not assigned	Rat Rat Liver Slices	
M656PH004	Not assigned	M4	Not assigned	Rat Liver Slices	
M656H005	Not assigned	M5	Not assigned	Rat	
M656H006	Not assigned	M6	Not assigned	Rat	
M656H007	360718	M7	Not assigned	Rat	
M656PH007	5886783	M7	Not assigned	Rat Human Hepatocytes	
M656H008	Not assigned	M8	Not assigned	Rat	
M656H009	360719	M9	Not assigned	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H010	Not assigned	M10	Not assigned	Rat	
M656PH010	5931836	M10	Not assigned	Rat	
M656H011	403120	M11	Not assigned	Rat	
M656H012	Not assigned	M12	Not assigned	Rat	
M656H013	Not assigned	M13	Not assigned	Rat	
M656PH013	Not assigned	M13	Not assigned	Rat	
M656H014	Not assigned	M14	Not assigned	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH014	Not assigned	M14	Not assigned	Rat	
M656H015	360711	M15	Not assigned	Rat	
M656H016	Not assigned	M16	Not assigned	Rat	
M656PH016	Not assigned	M16	Not assigned	Rat	
M656H017	Not assigned	M17	Not assigned	Rat	
M656PH017	Not assigned	M17	Not assigned	Rat	
M656H018	Not assigned	M18	Not assigned	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H019	Not assigned	M19	Not assigned	Rat	
M656H020	360713	M20	Not assigned	Rat	
M656H021	Not assigned	M21	Not assigned	Rat	
M656H022	Not assigned	M22	Not assigned	Rat	
M656PH022	Not assigned	M22	Not assigned	Rat	
M656H023	360715	M23	Not assigned	Rat	
M656H025	Not assigned	M25	Not assigned	Rat Liver Slices	
M656PH025	Not assigned	M25	Not assigned	Rat Rat Liver Slices	
M656H026	360716	M26	Not assigned	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656H027	Not assigned	M27	Not assigned	Rat Mouse	
M656H030	Not assigned	M30	Not assigned	Rat	
M656H031	360712	M31	Not assigned	Rat Mouse	
M656H032	395234	M32	Not assigned	Rat	
M656H033	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656PH033	Not assigned	Not assigned	Not assigned	Rat Liver Slices	
M656PH034	Not assigned	Not assigned	Not assigned	Rat Human Hepatocytes	
M656H035	Not assigned	Not assigned	Not assigned	Rat Liver Slices	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH035	**Not assigned**	**Not assigned**	**Not assigned**	Rat Liver Slices	S, N, O, Cl, O, OH
M656H036	**Not assigned**	**Not assigned**	**Not assigned**	Rat Liver Slices	O, N, Cl, O, S, OGlA
M656PH036	**Not assigned**	**Not assigned**	**Not assigned**	Rat Rat Liver Slices	O, N, Cl, O, S, OGlA
M656PH067	**Not assigned**	**Not assigned**	**Not assigned**	Rat	O, N, S, O, S
M656PH080	**Not assigned**	**Not assigned**	**Not assigned**	Rat	O, N, SH, O, S
M656PH082	**Not assigned**	**Not assigned**	**Not assigned**	Rat	OH, N, SH, O, S, O
M656PH083	**Not assigned**	**Not assigned**	**Not assigned**	Rat	O, N, NH_2, O, S

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH085	Not assigned	Not assigned	Not assigned	Rat	OGlcA, NH, S
M656PH086	Not assigned	Not assigned	Not assigned	Rat	OGlcA, N, O, S
M656PH087	Not assigned	Not assigned	Not assigned	Rat	OH, O, N, OH, O, S
M656PH088	Not assigned	Not assigned	Not assigned	Rat	O, OH, O, S, O, N, S, O, O, S
M656PH091	Not assigned	Not assigned	Not assigned	Rat	OH, N, SH, O, S, OGlcA
M656PH092	Not assigned	Not assigned	Not assigned	Rat	O, N, S, O, O, O, S, OGlcA
M656PH093	Not assigned	Not assigned	Not assigned	Rat	O, O, HO, N, SH, O, S

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656PH095	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH096	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH097	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH098	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH099	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH100	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH101	**Not assigned**	**Not assigned**	**Not assigned**	Rat	
M656PH102	**Not assigned**	**Not assigned**	**Not assigned**	Rat	

Table 5-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	Reg. No	Metabolite code	CAS-No		
M656PH103	Not assigned	Not assigned	Not assigned	Rat	
M656PH105	Not assigned	Not assigned	Not assigned	Rat	
M656PH106	Not assigned	Not assigned	Not assigned	Rat	
M656PH107	Not assigned	Not assigned	Not assigned	Rat	
M656PH108	Not assigned	Not assigned	Not assigned	Rat	

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

The following was copied from the EFSA Conclusion 2005:

"Following oral intake, dimethenamid was slowly but nearly completely absorbed from the gastrointestinal tract. The test substance was widely distributed throughout the organism and rapidly eliminated via bile and urine (approx. 90% within 7 days). Only 1-2% of unchanged parent compound were detected in the excreta, over 40 metabolites were detected of which about 20 could be structurally identified. Metabolism occurred primarily via glutathione conjugation pathways. No evidence for accumulating potential."

Absorption, distribution, metabolism and excretion in rats

For the sake of completeness the following six already peer reviewed studies in rats and mice are briefly summarized below:

Table 5.1.1-1: Overview of summarized studies

Authors, year	Title	DocID
[redacted] 1992	Absorption, distribution, metabolism and excretion of (14C) SAN 582H in rats after single and multiple doses	1992/12428
[redacted] 1989	SAN 582 H - Metabolism in the rat	1989/11026
Dorobek F., Mueller F. 1993	Qualitative investigations of the in vitro (liver and kidney) metabolism of Dimethenamid (SAN 582 H)	1993/11765
[redacted] 1992	SAN 582 H: Determination of the presence of plant metabolites in rat	1992/12448
[redacted] 1992	SAN 582 H: Determination of the presence of sulfonate metabolite in mice	1992/12445
[redacted] 1992	SAN 582 H: Addendum to determine sulfoxide of thioglycolic acid conjugate in mouse excreta	1992/12446

A rat metabolism study was conducted using [3-thienyl-^{14}C] dimethenamid. The test compound was administered by intravenous injection (i.v.) or by oral gavage (p.o.). The study was conducted in 5 groups. Group 1 was administered an oral dose at 10 mg/kg bw. Group 2 received an intravenous dose at 10 mg/kg bw. Group 3 was administered an oral dose at 1,000 mg/kg bw. Group 4 was orally administered with unlabeled dimethenamid for 14 days at 10 mg/kg bw/day followed by a ^{14}C oral dose at 10 mg/kg bw. The rats in group 5 were bile duct cannulated and received an oral dose at 10 mg/kg bw. Groups 1 to 4 consisted of 6 males and 6 females while group 5 consisted of 3 rats per sex. Excreta from each group and bile from group 5 were collected periodically until sacrifice at 168 h. Additional rat experiments to collect blood and tissue samples were conducted at 1, 4, 24, 72 and 168 h. Two dose groups (10 and 1000 mg/kg) and three rats per sex per time point per dose group were used.

A summary of excretion in urine, feces and bile is presented in Table 5.1.1-2. The blood radioactivity level decreased slowly over the experimental period of 168 h. The radioactivity was mainly associated with red blood cells as radioactivity in plasma was much lower. Similar binding phenomenon was not observed in human blood as can be explained that the hemoglobin are different between rat and human. In general, tissue radioactivity levels were comparable in both sexes, showing a similar pattern of absorption, distribution and elimination. Radioactivity levels in kidney and liver were higher than brain and heart. Residue levels decreased steadily over time with the exception of blood. Overall, tissue levels were small by 168 h after treatment. For the low dose treated rats, the concentration was less than 0.5 mg/kg in all organs and tissues.

It appeared that there was no significant difference in absorption, distribution, and elimination for dimethenamid between sexes. There was only a slight difference in elimination rate between single and multiple doses. Residue levels in tissues were similar for single dose and multiple dose groups. These data showed that dimethenamid and its metabolites had no tendency to bioaccumulate in animal systems.

Table 5.1.1-2: Excretion of total radioactivity following administration of ^{14}C-dimethenamid

Exp. No		Route	Dose level	% of administered dose at 168 h after treatment				
				Urine	Feces	Bile	Carcass	Total
1	Male	p.o.	10 mg/kg	35.3	57.7	n.r.	6.7	99.7
	Female	p.o.	10 mg/kg	46.9	47.6	n.r.	8.0	102.5
2	Male	i.v.	10 mg/kg	31.2	56.4	n.r.	11.1	98.7
	Female	i.v.	10 mg/kg	49.4	36.6	n.r.	9.9	95.9
3	Male	p.o.	1,000 mg/kg	61.6	30.1	n.r.	3.4	95.1
	Female	p.o.	1,000 mg/kg	63.1	26.1	n.r.	3.7	92.9
4*	Male	Multiple p.o.	10 mg/kg	34.9	61.6	n.r.	4.4	100.9
	Female	Multiple p.o.	10 mg/kg	53.3	39.9	n.r.	3.6	96.8
5**	Male	p.o.	10 mg/kg	7.6	2.2	82.2	4.7	96.7
	Female	p.o.	10 mg/kg	12.4	3.7	75.1	5.3	96.5

* Rats received 10 mg/kg/day of unlabeled dimethenamid for 14 days, then received a ^{14}C dose at 10 mg/kg.
** Bile duct cannulated.
n.r. Not reported

Dimethenamid was well absorbed (>90%, then rapidly and extensively metabolized in the rat. Only 1-2% of unchanged dimethenamid was detected in excreta. Excretion was rapid primarily by bile. About 40 metabolites (20 identified) were found in organic extracts which were analyzed by TLC. Metabolism occurred primarily via the glutathione conjugation pathways. Dimethenamid was rapidly conjugated with glutathione and then through several steps to form cysteine conjugate (M25) and mercapturate (M17). M25 was further oxidized to form additional metabolites (M1, M2, M10, M13, M14, M16, M18, M19, M21, M22, M26, M27, M30, and M31). Although the glutathione adduct was not found in the rat study, it was identified in the *in vitro* study. Other metabolites qualitatively identified in the *in vitro* study included the cysteine conjugate (M25), the mercapturate (M17), the sulfonate (M27), the sulfoxide of thiolactic acid (M30), the sulfoxide of thioglycolic acid (M31), and the thioglycolic acid (M32).

Dimethenamid was also metabolized via reductive dechlorination (M3), oxidation (M4, M23), hydroxylation (M5, M11, M15), O-demethylation (M7, M12) and cyclization (M6, M8, M9, M15, M20).

Absorption, distribution, metabolism and excretion in mice

A mice metabolism study was conducted using [3-thienyl-^{14}C] dimethenamid. The study was conducted in 2 groups and each group (A and B) consisted of 5 males and 5 females mice. Groups A and B were administered a single oral dose at 1 and 100 mg/kg bw, respectively. Urine and feces samples were separately collected daily for 4 days and the animals were sacrificed at 96 h after dose administration.

A summary of radiocarbon in urine and feces is presented in Table 5.1.1-3. Dimethenamid was rapidly metabolized and excreted in urine and feces. The profile in mice was similar to the profile in rats.

Table 5.1.1-3: Summary of ^{14}C-dimethenamid material balance for dose groups A (1 mg/kg) and B (100 mg/kg) in mice administered orally

Dose group	Dose level	Sample	% of administered dose at 96 h after treatment*	
			Male	Female
A	1 mg/kg	Urine	43.99	46.25
		Feces	47.26	42.12
		Cage wash	1.68	2.92
		Total	92.93	91.29
B	100 mg/kg	Urine	59.60	59.89
		Feces	33.64	28.30
		Cage wash	0.99	0.62
		Total	94.23	88.81

* Data are the average of 5 animals per group and sex, except group B with 4 animals. Animals were sacrificed 96 h after ^{14}C-dimethenamid administration.

Table 5.1.1-4: Percentage of sulfonate in urine and feces for groups A (1 mg/kg) and B (100 mg/kg) mice administered with ^{14}C-dimethenamid orally

Dose group	Urine	Feces	Total
	% of sulfonate in urine and feces		
A	0.060	0.25	0.31
B	0.069	0.25	0.319
	% of sulfoxide of thioglycolic acid in urine and feces		
A	0.25	0.25	0.50
B	0.24	0.40	0.64

Dimethenamid was extensively metabolized and readily excreted by mice. Urinary radiocarbon accounted for approximately 44% to 60% while feces accounted for approximately 28% to 47%. Total recovery varied from 88.81 to 94.23%. Sulfonate was found to be 0.06% (group A) and 0.069% (group B) in urine and 0.25% (both groups) in feces. Sulfoxide of thioglycolic acid in urine accounted for 0.25% (both groups). In feces, this metabolite accounted for 0.25% in group A and 0.40% in group B.

The peer reviewed studies above were performed with racemic dimethenamid and metabolite identification was not performed using recent techniques. To augment these studies, a new rat metabolism (2012) was performed using dimethenamid-P and using state of the art identification. The study results of the new study confirm the absorption, distribution, metabolism and excretion of dimethenamid-P but there may be some slight differences in metabolite identification. Therefore the 2012 study expands upon the older studies for metabolite identification.

A new rat metabolism study was conducted with the isomer dimethenamid-P to further elucidate the detailed metabolism and excretion in male and female rats after oral administration.

Report:	CA 5.1.1/1 [redacted] 2014a Excretion and metabolism of 14C-Dimethenamid-P (BAS 656 H) after oral administration in rats 2012/1194996
Guidelines:	EPA 870.7485, EPA 860.1000, OECD 417, EEC 87/302
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.1.1/2 [redacted] 2012a 14C-BAS 656 H - Study on bile excretion in rats 2012/1021081
Guidelines:	OECD 417 (April 1984), (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 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	Dimethenamid-P (BAS 656 PH)		
Batch # / purity:	Radiolabeled (^{14}C):	824-6027	99.0%
	Radiolabeled (^{13}C):	1030-1004	100%
	Nonlabeled:	L74-120	96.5%
Stability of test compound:		Stable during dosing period	

2. **Vehicle and/or positive control:** Aqueos solution of carboymethylcellulose (0.5%) and Cremophore (1%)

3. **Test animals:**

Species: Rat
Strain: Sprague Dawley rats (Crl:CD(SD)) (Charles River Laboratories, Germany)
Age: About 8 weeks at start of acclimatization
Sex: Male and female
Number of animals: 16 (10 males+10 females, 6 males for bile excretion)
Weight at dosing: 223-456 g (prior to dosing)
Acclimation period: Not reported
Diet: Kliba lab diet for mouse and rat, *ad libitum*
Water: Tap water *ad libitum*
Housing: During acclimatization in groups in Macrolon cages, then individually in plexi-glass metabolism cages
Husbandry:
Environmental conditions:
Temperature: 21-24°C
Humidity: 45-75%
Photoperiod: Alternating 12-hour light and dark cycles

4. Preparation of dosing solutions:
For oral administration of the test item to animals of dose groups DXM and DXF, mixtures of ^{14}C-labeled, ^{13}C-labeled and unlabeled dimethenamid-P (in a ratio of 2:33:65) were prepared in 0.5% aqueous solution of carboxymethylcellulose, and 1% Cremophore was added.
In order to demonstrate the stability of the test item in the application formulations, confirm the identity and determine the isotope pattern, HPLC and FIA-MS analyses were performed. For both dose groups, diluted application solutions were LSC measured and the measured values were taken for calculation.

Aliquots of the formulated test item were administered to the rats by gavage. The actual dose applied was 251.9 mg/kg bw and day for dose group DXM and 250.2 mg/kg bw and day for dose group DXF, respectively.

For oral dosing of the RM and SM groups, mixtures of ^{14}C-labeled, ^{13}C-labeled and unlabeled BAS 656 H were prepared in 0.5% aqueous solution of carboxymethylcellulose, and 1% Cremophor EL was added. About 10 mL/kg body weight of the respective preparation was administered to the rats by gavage.

B. STUDY DESIGN AND METHODS

1. Dates of work: August 30, 2011 – January 9, 2013

The treated rats consisted of an oral single dose group (10 rats/sex, 250 mg/kg bw) and for bile excretion two oral single dose groups (10 mg/kg bw and 250 mg/kg bw male animals). All animals received the oral dose administered via gavage.

In the case of dose group DXM, urine was collected 6 h, 12 h and 24 h after administration and in further time intervals of 24 h up to 168 h, and feces were sampled 12 h and 24 h after administration and in further time intervals of 24 h up to 168 h (combined samples for 10 animals). After seven days, the animals were anesthetised and sacrificed.

In the case of dose group DXF, urine was collected 6 h, 12 h and 24 h after administration and in further time intervals of 24 h up to six days (last sampling 148 h after dosing; combined samples for 10 animals). A pooled urine sample (12 h to 48 h) was prepared for metabolite identification. Feces of dose group DXF were sampled 12 h and 24 h after administration and in further time intervals of 24 h up to six days (last sampling 148 h after dosing). The animals were sacrificed after six days.

For both dose groups DXM and DXF, blood was collected from the sacrificed animals in tubes containing the anticoagulant EDTA, and approximately half of each sample was stored in a Nalgene bottle, the remaining half was centrifuged to obtain plasma. Moreover, liver, kidney, spleen and carcass were taken from the sacrificed rats and stored in a freezer. Plasma, liver, kidney, spleen and carcass were not further investigated because the mass balance was acceptable. In addition, the cages were cleaned with water and methanol, and the cage wash samples were also stored frozen.

In the cases of dose groups RM and SM, bile was collected from each animal in three-hour time intervals, and urine and feces were sampled in time intervals of 24 h up to 72 h. The remaining samples of bile, urine and feces after determination of the radioactivity as well as aliquots of the application preparations were stored at -20°C prior to analyses.

Equal portions of the bile samples of the animals No 2, 5 and 6 (dose group RM) were combined from the time periods of 0-3 h, 3-6 h, 6-9 h, 9-12 h and 12-18 h, respectively.
Samples were analyzed for total ^{14}C-radioactivity by samples combustion and/or liquid scintillation counting (LSC).

II. RESULTS AND DISCUSSION

Excretion

The mean portions of excreted radioactive residues after single oral administration of ^{14}C-dimethenamid-P to rats at a dose level of 250 mg/kg body weight (dose groups DXM and DXF) are presented in Table 5.1.1-5. The mean recovery of radioactive residues in urine, feces and cage wash was found to be 89.35% and 89.44% of the administered dose for male (dose group DXM) and female rats (DXF), respectively. Within the observation period of seven days or 148 h (dose group DXM or DXF, respectively), total excretion of radioactive residues *via* urine was 40.89% of the dose for dose group DXM and 54.87% of the dose for dose group DXF. The portions of radioactive residues excreted *via* feces accounted for 46.41% of the dose for dose group DXM and 32.20% of the dose for dose group DXF. In the cage wash, 2.05% of the dose (dose group DXM) and 2.37% of the dose (DXF) were recovered. Excretion *via* urine was nearly complete after 120 h after dosing for both dose groups, and excretion *via* feces was nearly complete within 72 to 96 h after dosing.

A summary of the excretion of radioactive residues *via* bile by rats of these dose groups is presented in Table 5.1.1-6.

The bile excretion study showed high absorption of ^{14}C-dimethenamid-P after single oral administration of the test item to male, bile catheterized rats. Absorption was higher after administration of the low dose, with approximately 94% of the dose in the case of dose group RM compared to approximately 85% of the dose in the case of dose group SM. Mean excretion of radioactive residues *via* bile within 72 h was 79.62% and 50.34% of the administered dose (individual values ranging from 21.61 to 72.57% of the dose) for the dose levels of 10 and 250 mg/kg bw, respectively (see Table 5.1.1-6). Excretion *via* urine in the same observation period accounted for mean values of 13.12% and 30.29% of the dose for the low and the high dose, respectively. Smaller portions of radioactive residues were recovered in feces (4.36% of the dose for the low dose and 3.76% of the dose for the high dose).

In bile, excretion was almost complete after 9 to 12 h in the case of the low dose group RM or after 24 to 30 h in the case of the high dose group SM (besides the slower decrease, the % dose values in bile of dose group SM showed a second relative maximum at the end of Day 1). In urine and feces, excretion was nearly complete within 24 to 48 h in both dose groups RM and SM.

Table 5.1.1-5: Route of excretion and total recovery of dimethenamid-P in rat (percent of radioactive dose)

Group	Target dose [mg/kg bw]	Route of administration	Sex of animal*	Urine [%]	Feces [%]	Total [%]
Treated	250	Single oral	M	40.89	46.41	89.35
			F	54.87	32.20	89.44

* Mean of ten animals per group

Table 5.1.1-6: Route of excretion via bile and total recovery of dimethenamid-P in rat (percent of radioactive dose)

Group	Target dose [mg/kg bw]	Route of administration	Sex of animal	Bile [%]
Treated	10	Single oral	M*	79.62
	250		F1	21.61
			F2	56.84
			F3	72.57

* Mean of three animals

Table 5.1.1-7: Excretion and retention of radioactivity via urine and feces after single oral administration of ^{14}C-BAS 656 PH to male and female rats at a dose levels of nominal 250 mg/kg bw (group mean values, in percent of radioactive dose)

	[% of the administered radioactivity]	
	Male	**Female**
Urine (h)		
0-6	2.36	3.53
6-12	5.00	6.14
12-24	10.00	17.12
24-48	14.02	19.15
48-72	4.66	4.70
72-96	2.54	2.04
96-120	1.29	1.32
120-144	0.55	0.87
144-168	0.46	-
Subtotal Urine	**40.89**	**54.87**
Feces (h)		
6-12	0.45	0.09
12-24	18.56	9.63
24-48	18.33	17.38
48-72	5.46	3.65
72-96	1.81	0.74
96-120	0.93	0.46
120-144	0.54	0.26
144-168	0.32	-
Subtotal Feces	**46.41**	**32.20**
Other sources		
Cage wash	2.05	2.37
Total	**89.35**	**89.44**

Table 5.1.1-8: Excretion and retention of radioactivity via bile after single oral administration of ^{14}C-BAS 656 PH to male and female rats at a dose levels of nominal 250 mg/kg bw (group mean values, in percent of radioactive dose)

h	[% of the administered radioactivity]			
	Male	Female		
Bile				
0-3	68.36	2.71	9.79	17.44
3-6	6.94	6.27	7.83	8.18
6-9	2.66[1]	1.95	7.94	4.55
9-12	0.93	0.85	4.45	4.37
12-15	0.19	0.45	5.14	4.71
15-18	0.15	1.10	4.46	8.08
18-21	0.06	4.81	3.10	7.34
21-24	0.06	1.98	7.61	7.95
24-27	0.03	0.96	4.03	7.02
27-30	0.03	0.18	0.96	1.49
30-33	0.02	0.11	0.43	0.53
33-36	0.02	0.09	0.21	0.39
36-39	0.02	0.04	0.15	0.13
39-42	0.02	0.02	0.11	0.07
42-45	0.02	0.03	0.09	0.04
45-48	0.02	0.01	0.11	0.04
48-51	0.02	0.01	0.12	0.03
51-54	0.02	0.00	0.06	0.03
54-57	0.01[1]	n. r.	0.05	0.03
57-60	0.01[1]	n. r.	0.06	0.03
60-63	0.01[1]	0.00	0.03	0.03
63-66	0.01[1]	0.00	0.04	0.02
66-69	0.01[1]	0.01	0.05	0.02
69-72	0.01[1]	0.03	0.02	0.03
Total	**79.63**	**21.61**	**56.84**	**72.57**

n.r. not reported
1 For some time intervals, only bile samples of two animals were obtained (6-9 h: animals 5 and 6, 54-72 h: animals 2 and 6), and the mean values of two animals are given for the respective individual time intervals; the total was calculated as the mean value of the sums for the three animals of dose group RM

Metabolic pathway

Dimethenamid-P is extensively metabolized in the rat mainly by initial glutathione conjugation, enzymatic cleavage of the tripeptide intermediate (M656PH024, not detected in the investigated rat samples) and subsequent metabolic reactions on the resulting cysteine conjugate M656PH025 (iso), the predominant component identified in bile. Smaller portions of radioactive residues were identified as metabolites generated by direct transformation of the parent compound. The parent molecule dimethenamid-P was detected only in feces samples in portions below 2% of the applied dose after single oral administration. (The numbering in the codes "M656PH*xyz*" is equivalent to that in the former "M*x*" codes except for the addition of "zero" figures to obtain always a code with nine characters and the PH indicates the subject was tested with dimethenamid-P.)

One main route of further conversion of the cysteine conjugate M656PH025 (iso) is N-acetylation forming the mercapturic acid M656PH017 (iso) and its O-demethylated or oxidised derivatives M656PH097 (iso) and M656PH100 (iso).

Another main route of further transformation after conjugation with glutathione is hydrolysis of the S-conjugates to the mercaptan M656PH080 (rota) followed by S-methylation to the metabolite M656PH067 (rota), both basic intermediates for the formation of various further derivatives.

O-demethylation of the parent compound or of the S-methylated intermediate M656PH067 (rota) forms the metabolites M656PH007 and M656PH001 (rota), respectively, which are subsequently conjugated with glucuronic acid to form the prominent metabolites M656PH034 (iso) and M656PH095 (iso). The analogous reactions produce metabolite M656PH086 (iso) from metabolite M656PH003.

An additional metabolic reaction of metabolite M656PH067 (rota) is oxidation to the sulphoxide M656PH013 (iso) (analogous reactions convert M656PH001 (rota) to metabolite M656PH002 (iso), M656PH095 (iso) to M656PH098 (iso) (at least four isomers found) or M656PH107 (iso) to M656PH101 (iso)). Further oxidation of the sulphoxides results in the formation of the sulphone metabolites M656PH010, M656PH014 (rota), M656PH096 (iso) and M656PH092 (iso).

O-demethylation also occurs with the metabolites M656PH013 (iso), M656PH010 and M656PH080 (rota) to form metabolite M656PH002 (iso), metabolite M656PH014 (rota) and the putative precursor of the glucuronic acid conjugate M656PH091 (iso) and of the derivative M656PH082 (iso) (derived after oxidation of the sulphur in the thiophene ring to the sulphoxide), respectively. Conjugation of metabolites M656PH002 (iso) and M656PH014 (rota) leads to the glucuronides M656PH098 (iso) and M656PH096 (iso), respectively. Conjugation of M656PH002 (iso) with sulphuric acid produces the sulphate metabolite M656PH088 (iso).
The chlorine in dimethenamid-P can also be replaced by hydrogen through reductive dechlorination to the metabolite M656PH003 (and the analogous reaction leading from M656PH034 (iso) to metabolite M656PH086 (iso) or from M656PH036 (iso) to metabolite M656PH106 (iso)) or by a hydroxyl group through hydrolysis to the intermediate M656PH011 (not detected).

Oxidation of the 2-methyl group on the thiophene ring to the hydroxyl methyl and subsequent conjugation with glucuronic acid yields metabolite M656PH036 (iso) (*via* the intermediate M656PH005 which was not detected), and analogous reactions produce metabolite M656PH101 (iso) from metabolite M656PH013 (iso) (*via* M656PH016 (iso)), metabolite M656PH107 (iso) from M656PH067 (rota) and metabolite M656PH092 (iso) from M656PH010.
The methyl group on the methylethyl moiety of M656PH011 and M656PH086 (iso) is also oxidized to the hydroxyl methyl to form metabolites M656PH087 (rota) and M656PH108 (iso), respectively. The same reaction followed by conjugation with glucuronic acid produces metabolite M656PH099 (iso) from M656PH010. Two oxidation steps at the same methyl group of M656PH080 (rota) produce the carboxyl metabolite M656PH093 (rota).
Metabolite M656PH080 (rota) is also transformed to metabolite M656PH022 (iso) by dimerization, to the S-glucuronide M656PH105 (iso) by conjugation, or to metabolite M656PH102 (rota) by oxidation.

Metabolite M656PH103 (iso) is formed from M656PH002 (iso) by oxidation of the 2-hydroxy-1-methylethyl moiety and conjugation with glucuronic acid or from M656PH098 (iso) by oxidation of the conjugated 2-hydroxy-1-methylethyl moiety. Metabolite M656PH085 (iso) is formed from M656PH034 (iso) (or M656PH086 (iso)) by cleavage of the amide bond.
The amine derivative M656PH083 (rota) (two rotamers detected by HPLC-MS) observed as main component in the solubilizates released from the residual radioactive residues after solvent extraction of feces by reflux with water probably originated from dimethenamid-P by reaction with amines in feces.

The proposed metabolic pathway of dimethenamid-P in the rat is shown in Figure 5.1.1-1, Figure 5.1.1-2 and Figure 5.1.1-3. A summary of the identified components is given in Table 5.1.1-9.

Table 5.1.1-9: Summary of identified metabolites in urine, feces and bile of rats after oral administration of dimethenamid-P

Metabolite Designation (Code)	Molecular Mass	Urine	Feces	Bile
Dimethenamid-P (BAS 656 PH)	275.799		+	
M656PH001 (rota)	273.42		+	
M656PH002 (iso)	289.419	+		
M656PH003	241.354	+	(+)	
M656PH007	261.772			+ (n. q.)
M656PH010 (two rotamers)	319.446	+ (n. q.)		
M656PH013 (iso)	303.446	+ (n. q.)		
M656PH014 (rota) – 52.7 min	305.419		+	
M656PH014 (rota) – 54.0 min			+	
M656PH016 (iso)	319.446	+ (n. q.)		
M656PH017 (iso) – 73.0 min	402.535	+		+
M656PH017 (iso) – 73.5 min		+		+
M656PH022 (iso)	544.824		+	
M656PH025 (iso) – 47.9 min	360.498			+
M656PH025 (iso) – 48.9 min				+
M656PH034 (iso)	437.898	+		+
M656PH036 (iso)	467.924	+		
M656PH067 (rota) (former code PL 36-88)	287.447		+	
M656PH080 (rota) (formerly mercaptan)	273.42	+ (n. q.)		
M656PH082 (iso)	275.393		+	
M656PH083 (rota) (two rotamers)	256.369		+	
M656PH085 (iso)	361.372	+ (n. q.)		
M656PH086 (iso)	403.453	+ (n. q.)		
M656PH087 (rota)	273.353	+ (n. q.)		
M656PH088 (iso)	369.484	+ (n. q.)		
M656PH091 (iso)	451.518	+		
M656PH092 (iso) – 26.3 min	511.571	+ (n. q.)		
M656PH092 (iso) – 27.9 min		+ (n. q.)		
M656PH093 (rota) – 40.7 min	303.403	+ (n. q.)		
M656PH093 (rota) – 42.7 min		+		
M656PH095 (iso)	449.546	+		(+)
M656PH096 (iso) – 37.0 min	481.544	+		
M656PH096 (iso) – 38.9 min		+		
M656PH097 (iso) – 52.0 min	388.509	+ (n. q.)		
M656PH097 (iso) – 53.0 min		+ (n. q.)		

Table 5.1.1-9: Summary of identified metabolites in urine, feces and bile of rats after oral administration of dimethenamid-P

Metabolite Designation (Code)	Molecular Mass	Urine	Feces	Bile
M656PH098 (iso) – 22.8 min	465.545	+		
M656PH098 (iso) – 23.4 min, 23.8 min (two isomers)		+		
M656PH098 (iso) – 24.2 min		+		+
M656PH098 (iso) – 25.4 min		+		+
M656PH099 (iso)	511.571	+ (n. q.)		
M656PH100 (iso)	418.535	+		
M656PH101 (iso) – 20.9 min (two isomers)	495.571	+ (n. q.)		
M656PH101 (iso) – 21.8 min (two isomers)		+ (n. q.)		
M656PH102 (rota) (two rotamers)	289.419	+ (n. q.)		
M656PH103 (iso) (two isomers)	479.529	+ (n. q.)		
M656PH105 (iso)	449.546	+ (n. q.)		
M656PH106 (iso) (two isomers)	433.479	+ (n. q.)		
M656PH107 (iso)	479.572	+ (n. q.)		
M656PH108 (iso)	419.452	+ (n. q.)		

n. q. not quantified;
(+) tentatively assigned in feces or bile

III. CONCLUSION

The bile excretion study showed high absorption of dimethenamid-P after single oral administration of the test item to male, bile catheterized rats. Absorption was slightly higher after administration of the low dose (approximately 94% of the administered dose in the case of dose group RM) compared to the high dose (approximately 85% dose in the case of dose group SM). In the cases of the high dose groups DXM and DXF, similar portions were eliminated *via* urine and feces. Excretion *via* urine was nearly complete after 120 h after dosing, and excretion *via* feces was nearly complete within 72 to 96 h after dosing. In the cases of the dose groups RM and SM, mean excretion of radioactive residues *via* bile within 72 h was 79.62% and 50.34% dose for the dose levels of 10 and 250 mg/kg bw, respectively. Biliary excretion was almost complete after 9 to 12 h in the case of the low dose group RM or after 24 to 30 h in the case of the high dose group SM.

Dimethenamid-P was extensively metabolized in the rat. Transformation mainly proceeds *via* initial glutathione conjugation, enzymatic cleavage of the tripeptide intermediate and subsequent metabolic reactions on the resulting cysteine conjugate M656PH025 (iso), the predominant component identified in bile (20.69% dose in the low dose group RM, 3.22% to 17.91% dose in the high dose group SM). Important routes of further conversion are hydrolysis of the S-conjugates to the mercaptan M656PH080 (rota) followed by S-methylation to the metabolite M656PH067 (rota), both intermediates for the formation of various further derivatives (e.g. the dimer M656PH022 (iso) and the demethylated metabolite M656PH001 (rota), the two main metabolites in feces), or N-acetylation of the cysteine conjugate forming the mercapturic acid M656PH017 (iso) and its derivatives. Conjugation with glucuronic acid occurs at several positions, mainly after O-demethylation or hydroxylation, and the main components in urine are glucuronides. O-demethylation of the parent compound or of the S-methylated intermediate M656PH067 (rota) forms the metabolites M656PH007 and M656PH001 (rota), respectively,

which are subsequently conjugated with glucuronic acid to form the prominent metabolites M656PH034 (iso) and M656PH095 (iso). Oxidation of the sulphur atom in metabolite M656PH067 (rota) (and M656PH001 (rota)) forms sulphoxides and sulphones, e. g. M656PH098 (iso), M656PH014 (rota) and M656PH096 (iso), the latter representing the main component in urine. The unchanged parent molecule dimethenamid-P was detected only in feces samples in low portions in both dose groups DXM and DXF. The metabolite patterns were qualitatively similar for both sexes, with some variations in the relative portions and the exceptions that M656PH017 (iso) was detected in bile as well as in urine of female rats but not in urine of male rats and that metabolite M656PH082 (iso) was only detected in feces of male rats.

The main biotransformation steps of Dimethenamid-P in rats are:

- Conjugation with glutathione and enzymatic cleavage of the tripeptide to the cysteine conjugate
- N-acetylation of the cysteine moiety
- Hydrolysis of S-conjugates to the mercaptan (followed by S-methylation)
- Oxidation of the sulphur atom to form sulphoxides and sulphones
- O-demethylation
- Hydroxylation
- Conjugation with glucuronic acid
- Replacement of the chlorine atom by hydrogen (reduction) or by a hydroxyl group (hydrolysis)
- Dimerization of a mercaptan

Figure 5.1.1-1: Proposed metabolic pathway of dimethenamid-P in rats (part 1)

M656PH083 (rota)
(2 rotamers)
Dimethenamid-P
M656PH024
M656PH005
M656PH007
M656PH003
M656PH011
M656PH036 (iso)
M656PH034 (iso)
M656PH087 (rota)
or isomer
M656PH106 (iso)
(2 isomers)
M656PH085 (iso)
M656PH086 (iso)
M656PH108 (iso)

Figure 5.1.1-2: Proposed metabolic pathway of dimethenamid-P (BAS 656 H) in rats (Part 2: derivatives after conjugation with glutathione)

M656PH024

M656PH097 (iso)
(2 isomers)

M656PH025 (iso)
(2 rotamers)

M656PH017 (iso)
(2 rotamers)

M656PH100 (iso)

M656PH093 (rota)
(2 rotamers)

M656PH080 (rota)

M656PH022 (iso)

M656PH105 (iso)

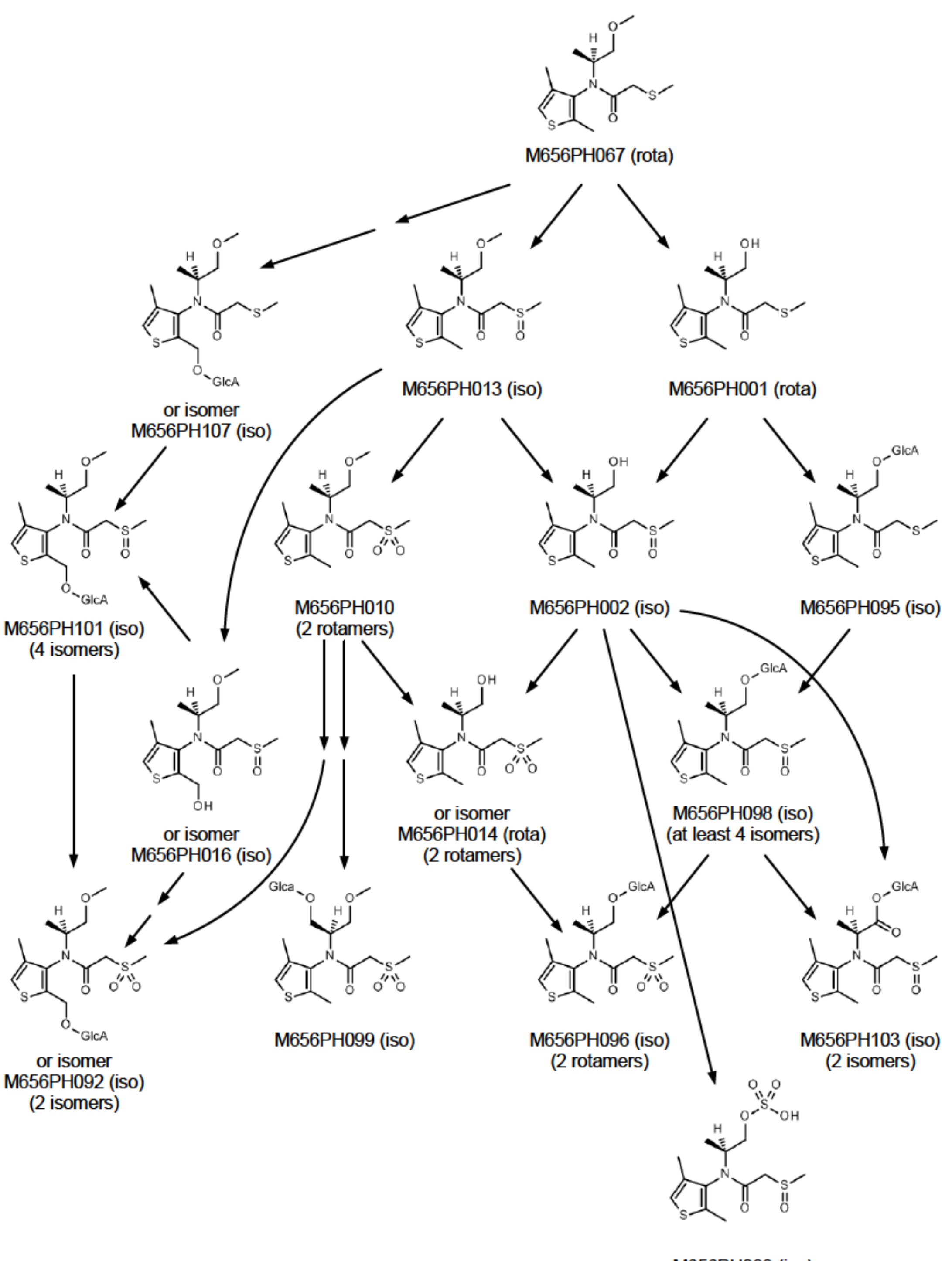
M656PH067 (rota)

M656PH102 (rota)
(2 rotamers)

or isomer
M656PH082 (iso)

or isomer
M656PH091 (iso)

Figure 5.1.1-3: Proposed metabolic pathway of dimethenamid-P (BAS 656 H) in rats (Part 3: Derivatives of the S-methyl metabolite M656PH067 (rota))



CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

To investigate any potential differences in metabolism between racemic dimethenamid and dimethenamid-P, an *in-vitro* metabolism study using rat liver slices was conducted. The results are presented below.

Report: CA 5.1.2/1
Fabian E., 2002a
Comparison of in vitro metabolism of enantiomers of BAS 656 H (Dimethenamid)
2002/1004042
Guidelines: <none>
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
Description: Racemic dimethenamid (BAS 656 H)
Dimethenamid-P (BAS 656 PH)
Batch # / purity: Radiolabeled (^{14}C):
Racemic dimethenamid: 1338-48G >97% 7.464 MBq/mg
Dimethenamid-P: 1338-48H >98% 6.783 MBq/mg
Nonlabeled:
Racemic dimethenamid: CP029795
Dimethenamid-P: 01311-220
Stability of test compound: Stable during testing

2. **Vehicle and/or positive control:**
Vehicle: liver slices
Positive control: liver slices were incubated with testosterone instead of the active ingredient to prove the metabolic activity of the liver slices

3. **Test animals:** Mammals
Species: Rat
Strain: Male Wistar rats (CrlGlxBrlHan:WI) (Charles River Laboratories, Germany)

B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany.

Test substance preparation
For the racemate and the S-isomer of BAS 656 H, the following test substance solutions were prepared separately: BAS 656 H was dissolved in DMSO at concentrations of about 50, 25, 18.75, 12.5 and 6.25 µM. Into each application, a solution of pure ^{14}C labeled test substance (50000 dpm/µL) was added. A total of 10 µL of test substance solution was added into 5 mL incubation medium (with a dilution factor of 500) resulting in a nominal concentration in the incubate of about 100, 50, 37.5, 25 and 12.5 µM. The radioactivity added to each incubation mixture is thus 500,000 dpm/5 mL.

Preparation and incubation of liver slices
For the preparation of tissue slices, the livers from freshly sacrificed rats were resected and were stored in ice cold Krebs Henseleit buffer, saturated with carbogen (95% oxygen, 5% nitrogen) for immediate use. For slicing with the Krumdieck slicer, tissue cores were prepared (diameter 8 mm), placed into and sliced in ice-cold, carbogen saturated Krebs Henseleit buffer. Slice thickness was adjusted to approx. 200 µm.
The incubation was performed at approx. 37°C in 6 well tissue plates, each well containing 5 mL Williams E Medium and two liver tissue slices. For heat denaturated control samples, the tissue slices were heated for about 10 min in boiling water. The plates were bidirectionally shaken and continuously gassed with 95% oxygen and 5% nitrogen. After a pre-incubation period of 30 min, the medium was substituted by test compound containing medium (10 µl DMSO test compound solution in 5 mL medium) and was incubated for approx. 24 h. After the incubation period the tissue slices were removed and samples were frozen in Falcon vials at -20°C until analysis.

HPLC-MS and NMR analysis
After the incubations the liver slices were removed from the plate. Incubates could be used directly for HPLC analysis and were injected without further purification. For LC-MS analysis the 100 µM incubation of racemate was used due to higher amounts of test compound for measurements. For LC-MS, purification was carried out by HPLC and collecting fractions during the time window between 11 and 25 min. For ^{1}H-NMR analysis approx. 100 µg of M4 were purified and concentrated by HPLC and a fraction collector. For the purification, different incubation media were used to generate a pooled sample of M4. The eluent was dissolved and the sample was diluted in $CDCl_3$ for analysis.

II. RESULTS AND DISCUSSION

Metabolic Pathway of BAS 656H in Rat Liver Slices
The present study was designed to investigate possible differences between the metabolism of stereoisomers of dimethenamid-P and racemic dimethenamid. For this purpose liver slices of male Wistar rats were prepared and incubated with the S-isomer and the racemate of ^{14}C-dimethenamid. First trials with dimethenamid were carried out to optimize the incubation conditions, mainly the concentration of the test compounds in the incubation medium. Thus, the S-isomer and the racemate of dimethenamid were incubated for 24 h in an atmosphere of 95% oxygen and 5% nitrogen at 37°C with nominal concentrations of 12.5, 25, 50, and 100 µM (concentrations of the test substances in the incubation medium). In addition, one control incubation was performed for each test concentration using heat denaturated rat liver slices.
In comparison with the active incubation, these control incubations allow the differentiation between enzymatic and non-enzymatic processes (abiotic processes could also occur in control

incubations) and allow the calculation of the turn-over (by calculating the relative decrease of parent compound in the active versus the inactive incubation using heat denaturated liver slices). For further series of incubations, the incubation concentration was selected by the turn-over of the metabolic reactions and by the received metabolite profile. Derived from these range finder studies, a nominal concentration of 37.5 µM was chosen (between 25 NM and 50 NM). This concentration was interpolated to guarantee a significant turn-over to make sure that the major part of the test substance will be metabolized but on the other hand to still have parent compound present after the incubation to allow the calculation of the turn-over of the enzymatic reactions.

Dimethenamid was extensively metabolized in liver slices: At a nominal concentration of 37.5 µM dimethenamid in the incubation medium, the turn-over was >50%. Up to 20 metabolites could be separated by HPLC. All metabolites are more polar than parent compound and have retention times between 11 min and 35 min. The main metabolite M4 has a retention time of about 22.5 min and has a relative amount of the total peak area of about 17-18% (37.5 µM incubation). The parent compound eluates at about 41 min.
To identify the major metabolic pathway of dimethenamid in rat liver slices, the 7 most intense peaks were analyzed by LC-MS and NMR. The identified metabolites are summarized in Table 5.1.2-2. The metabolism of BAS 656 H in rat liver slices occurred via various oxidation and conjugation reactions: It could be demonstrated by LC-MS and NMR-techniques that the main *in vitro* metabolite is identical to the *in vivo* metabolite M4. In the rat metabolism study, this metabolite was identified to be the sulfoxide of dimethenamid. Further metabolic reactions in rat liver slices are hydroxylations of the dimethylthiophene system with consequent glucuronidations to form the glucuronic acid conjugates M36. Oxidative desalkylation of the methoxy group results in the hydroxyl metabolite M7 that also undergoes a glucuronidation to form M34. Dimethenamid was conjugated with glutathione resulting in the formation of the GSH-adduct M33 and the cysteine-adduct M25 that was formed after a substitution of chlorine with GSH by consequent degradation of the tripeptide.

The *in vivo* situation in the rat is in analogy to the findings in this *in vitro*-study: Dimethenamid was rapidly and extensively metabolized in rats. The key steps of the proposed metabolic pathway of BAS 656 H in rats occured via glutathione conjugation, oxidation, hydroxylation, O-demethylation, reductive dechlorination, and cyclization. With respect to these results it has to be concluded that the *in vitro* metabolism of dimethenamid in rat liver slices follows in principle the same metabolic pathways that could be observed in rats in vivo. As described before, it could be demonstrated that the main *in vitro* metabolite is identical to the *in vivo* metabolite M4. In the rat metabolism study this metabolite was identified to be the sulfoxide of dimethenamid. The *in vitro* metabolites M7 and M25 are also identified rat metabolites resulting from oxidative demethylation and glutathione conjugation, respectively. Due to the applied MS technique in the rat *in vivo* study it can be postulated that it was not possible to identify glucuronic acid *in vivo*-conjugates. This explains why all glucuronic acid conjugates in the present study were not described in the rat study. In any case, the glucuronidation of Phase I hydroxy metabolites is a well-known metabolic reaction and is a consequent conjugation following the key step of hydroxylation. The glucuronidation is, therefore, a part of the same metabolic pathway.

Table 5.1.2-1: Turn-over of the metabolic reactions of dimethenamid-P and racemic dimethenamid[1]

Sample	Turn-over (%)	
	Dimethenamid-P	Racemic dimethenamid
1	76.00	88.22
2	52.27	87.66
3	55.37	92.99
4	59.00	96.50
5	70.50	67.42
6	53.40	90.20
7	72.99	87.11
8	46.03	56.75
9	76.54	88.06
10	70.30	88.87
Mean value	**63.24±11.21**	**84.38±12.35**

1 The turn-over was calculated as relative decrease of parent compound in the active incubation versus parent compound in the heat denaturated control incubation.

Metabolic pathway

The main steps of the metabolic pathway of BAS 656 H in rat liver slices are glutathion conjugation, oxidation reactions of the dimethylthiophene system, demethylation of the methoxy group, oxidation of the sulfur atom to form a sulfoxide, and consequent glucuronidation reactions of the hydroxy metabolites to form glucuronic acid conjugates. In principle these are the same metabolic reactions that are observed and described for the in vivo situation in the rat. Therefore the applied in vitro system is an appropriate model to investigate possible differences in the metabolism of the enantiomers of BAS 656 H. A summary of the identified components is given in Table 5.1.2-2.

Table 5.1.2-2: Summary of identified metabolites rat liver slices

Percentage of metabolites expressed as relative area [%][1]					
Racemic dimethenamid			Dimethenamid-P		
Metabolite designation (New)	Mean value	Standard deviation	Metabolite designation (New)	Mean value	Standard deviation
N/A	9.9	1.8	N/A	8.5	2.0
M656H004	28.4	6.0	M656PH004	31.9	3.0
M656H025	8.8	1.0	M656PH025	9.3	1.0
M656H033	23.0	4.7	M656PH033	15.7	4.5
M656H035 (iso)	6.7	1.0	M656PH035 (iso)	6.7	1.7
M656H035 (iso)	15.6	1.4	M656PH035 (iso)	20.3	1.8
M656H036	7.5	1.6	M656PH036	7.8	2.8

1 Calculated as the quotient of the peak area of the metabolite divided by the sum of peak areas of all regarded metabolites.

III. CONCLUSION

In vitro incubations of racemic and dimethenamid-P in rat liver slices showed that the metabolism of the racemate and the S-isomer of dimethenamid is qualitatively and quantitatively comparable.

In accordance with the requirements of Commission Regulation SANCO/11802/2010 and regulation (EC) No 1107/2009 an in **vitro comparative metabolism study** was performed and summarized below.

Report: CA 5.1.2/2
Funk D.,Glaessgen W.E., 2014b
Comparative in-vitro-metabolism with 14C-BAS 656-PH
2013/1337274
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: Dimethenamid (BAS 656 PH)
   Batch # / purity: <u>Radiolabeled (^{14}C):</u>
   Dimethenamid-P: 824-7101 >99.8% 7.58 MBq/mg
   <u>Nonlabeled:</u>
   Dimethenamid-P: BEAU201204
   Stability of test compound: Stable during testing

2. **Vehicle and/or positive control:**
   Vehicle: hepatocytes
   Positive control: hepatocytes were incubated with ethoxy coumarin and testosterone instead of the active ingredient to validate the metabolic activity of the hepatocytes

3. **Test animals:** Mammals
   Species: Dog, rat and human
   Strain: Female and male Sprague Dawley rats (SD), male and female Beagle Dog, human (Xenotech, Germany)

B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany.

Test substance preparation

The radiolabeled and non-radiolabeled test materials were prepared with the following specifications. The radiolabeled test item (solution in acetonitrile) was evaporated under nitrogen and taken up in an appropriate volume of DMSO. For this stock solution, a concentration of 0.236 mg/mL was determined. For the preparation of the stock solution of the unlabeled test item, 250 mg dimethenamid-P was dissolved in 20 mL acetonitrile to yield a concentration of 12.5 mg/mL.

The different application solutions were prepared by using specific amounts of unlabelled test item were concentrated to dryness, mixed with the desired amounts of radiolabelled test item and diluted with DMSO. The ratio of radiolabeled to unlabeled test item was approximately 8 : 92 for the application solutions for experiments with 10 µM dimethenamid-P, and approximately 0.8 : 99.2 for the application solutions for assays with 100 µM dimethenamid-P respectively. The purity of each application solution (and the retention time of dimethenamid-P) was confirmed by HPLC analysis.

Hepatocytes

Cryopreserved hepatocytes from dog, rat and human were stored in liquid nitrogen. On each incubation day, the cells were thawed according to a protocol provided by the supplier using appropriate kits. Aliquots of the resulting cell suspensions in hepatocyte incubation medium were diluted with phosphate-buffered saline (PBS), and the number of viable cells was measured using an automated cell counter. The cell suspensions were then adjusted to the desired cell density of 2×10^6 viable cells per mL with incubation medium. In the case of dog and rat hepatocytes, male and female cells were combined in this final step in a ratio of 1:1. The human hepatocytes were purchased as a mixture of male and female cells.

***In vitro* assays**

On each incubation day, the application solutions in DMSO (mix of ^{14}C-labeled and unlabeled test item in the case of dimethenamid-P) were diluted with hepatocyte incubation medium by a factor of 100 to prepare the respective application media. The application media were incubated at a final concentration of approximately 10 µM with rat, dog or human hepatocytes. In the case of dimethenamid-P, additional incubations were performed at approximately 100 µM for high dose conditions.

Each sample comprised of 0.3 mL of application medium and 0.3 mL of hepatocyte cell suspension in one of the wells of a 24-well cell culture plate. The reactions were performed for 180 minutes at approximately 37°C. In some cases, shorter incubations for 30 min or 60 min were also conducted. Incubation was terminated by pipetting the incubation mixture into a weighed tube containing cold ethanol and cell lysis was assisted by ultrasonication. In this stage, the samples were stored frozen prior to concentration.

In addition, two negative controls, two positive controls and a blank control (application medium with DMSO instead of test item) were performed for each species. Under these conditions no metabolization should occur. For the **"stability control"**, the application medium was mixed only with incubation medium instead of cell suspension. For the **"zero incubation control"** (t = 0 min), the reaction was stopped immediately after addition of the cell suspension. The stability control was only performed with the high dose of 100 µM and within the second experimental series with dog hepatocytes also with 10 µM.

In the **positive controls**, testosterone or ethoxycoumarin instead of the active substance was incubated with hepatocytes from the different species to validate the metabolic activity of the different hepatocytes.

In each experimental setup, the incubation of the substrates as well as all control assays was performed in triplicate.

II. RESULTS AND DISCUSSION

Control Experiments
The blank controls performed for each species without test item showed no significant amounts of radioactivity (LSC measurements), and no radioactive peaks were detected by HPLC analysis.

The triplicates of each negative control (stability control without cells and zero incubation control) were comparable and only showed unchanged dimethenamid-P. Hence, no metabolization or degradation of dimethenamid-P occurred without hepatocytes.

The positive controls showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. The portions of metabolized testosterone reached values above 70% of the radioactive residues recovered.

HPLC analysis of the positive controls with ethoxycoumarin revealed no unchanged ethoxycoumarin after incubation with dog and rat hepatocytes and portions of approximately 6% to 8% of the applied radioactivity (% AR) for ethoxycoumarin after incubation with human hepatocytes for 180 min. Low portions of 7-hydroxycoumarin were detected after incubation with dog and rat hepatocytes (2% to 7% AR). The portions of 7-hydroxycoumarin after incubation with human hepatocytes accounted for approximately 20% to 23% AR. All samples contained considerable portions of 7-hydroxycoumarin β-D-glucuronide and 7-hydroxycoumarin sulphate which indicates that 7-hydroxycoumarin was formed as a relevant O-dealkylated intermediate in hepatocytes of each species tested. The formation of the conjugated metabolites 7-hydroxycoumarin β-D-glucuronide and 7-hydroxycoumarin sulphate was a measure for the metabolic activity of the hepatocytes also with respect to Phase II reactions (conjugation). The concentrations of 7-hydroxycoumarin β-D-glucuronide accounted for approximately 18% to 37% AR after incubation with dog hepatocytes, 29% to 44% AR after incubation with rat hepatocytes and 17% to 23% AR after incubation with human hepatocytes. The portions of 7-hydroxycoumarin sulphate (indicating sulphotransferase activity) amounted to approximately 24% to 35% AR in the case of dog, 24% to 30% AR in the case of rat and 2% to 5% AR in the case of human hepatocytes.

Viability of the Hepatocytes

The hepatocyte suspensions were adjusted to a cell density of 2 x 10^6 viable cells per mL to achieve a final cell density of approximately 10^6 cells per mL in the incubation assays. After incubation for 180 min, the viability of the cells was determined using a luminescent cell viability assay.

The viability of the cells incubated with dimethenamid-P (measured in Relative Luminescence Units, RLU) was in the range of 77% to 109% of the viability of the cells incubated without test item.

Metabolites formed after incubation of dimethenamid-P with hepatocytes

Since the first experiments with 10 µM dimethenamid-P and dog hepatocytes (series 1) and all incubations with rat hepatocytes showed complete conversion of the active substance to more polar biotransformation products within 180 min; additional incubations were performed with 10 µM dimethenamid-P and dog hepatocytes for 30, 60 and 180 min (series 2).

HPLC-MS analysis of the samples after incubation with human hepatocytes allowed the assignment of four *m/z*-values to the relevant ^{14}C-peaks corresponding to the test item or its main conversion products.

After incubation with animal hepatocytes, dimethenamid-P was detected in all control samples with 0 min incubation and in the samples after incubation of 100 µM dimethenamid-P with dog hepatocytes for 180 min (19.96% AR). In the other samples after incubation with animal hepatocytes, no dimethenamid-P was detected any more. The transformation of dimethenamid-P thus proceeded faster in animal hepatocytes.

Based on the retention time (RT_{HPLC}) and the *m/z*-value of 276.082, the ^{14}C-peak at 31.1 min is assigned to dimethenamid-P. The respective peak representing the unchanged active substance is present in most of the samples after incubation with human hepatocytes (except for 10 µM dimethenamid-P, 180 min). The portion of dimethenamid-P in the concentrated supernatants continuously decreased from 81.27% AR to 22.73% AR after 60 min of incubation with 10 µM dimethenamid-P. At the high concentration of 100 µM dimethenamid-P, the portion of the active substance showed a slower decrease (Table 5.1.2-3). This lower conversion rate at the high concentration possibly indicates saturation of the metabolic capacity.

The peak at a retention time of approximately 23 min with a corresponding *m/z*-value of 262.066 was only detected in human hepatocyte samples (10 µM and 100 µM dimethenamid-P).
Therefore, HPLC-MS/MS spectra were evaluated for this peak which was identified as metabolite M656PH007 a derivative of the parent compound with the methyl ether cleft. This Phase I metabolite was also detected along with its glucuronic acid conjugate in a study on the metabolism of ^{14}C-dimethenamid-P in rats. HPLC-MS/MS analysis revealed that this component eluted as a double peak which results from the occurrence of two rotamers with hindered rotation. Therefore the AR's are presented as a sum of both peaks. In the 10 µM incubation, the AR for M656PH007 decreased over time from 27.33% AR to 11.98% AR. However, in the 100 µM incubation, the AR for M656PH007 increased over time from 7.63% to 25.30%

The peak at a retention time of approximately 18 min corresponds to the *m/z*-value of 438.098. Since this peak occurred in dog hepatocyte samples only in single replicates and in minor concentrations, HPLC-MS/MS spectra were evaluated for this peak as well. The MS/MS data allowed the identification of metabolite M656PH034 (iso). As expected, since it is a conjugated form of M656PH007, HPLC-MS/MS analysis revealed that this component also eluted as a double peak which results from the occurrence of two rotamers. It was detected after incubation of 10 µM dimethenamid-P with human hepatocytes for 180 min, as well as in dog hepatocytes. This M656PH034 was also detected in considerable concentrations in the study on the metabolism of ^{14}C-dimethenamid-P in rats (Doc ID 2012/1194996). M656PH034 was detected ranging from 9.03% AR in dog hepatocytes at 10µM to 22.99% AR in human hepatocytes at 100 µM (Table 5.1.2-3). The decrease of the portion of M656PH007 after 180 min goes parallel to the concomitant occurrence of the M656PH034.
The peak at a retention time of approximately 10 min with a corresponding *m/z*-value of 292.077 was detected after incubation of 10 µM dimethenamid-P with human hepatocytes for 60 min, (12.13% AR) for 180 min (20.72% AR), after incubation of 100 µM dimethenamid-P with human hepatocytes for 180 min (21.56% AR). In dog hepatocytes, it was detected incubation of 100 µM dimethenamid-P with dog hepatocytes for 180 min (15.63% AR) and after incubation of 10 µM dimethenamid-P with dog hepatocytes for 30 min (29.34% AR) and for 60 min (19.98% AR). Dogs are thus capable of forming the metabolite represented by the *m/z*-value of 292.077 as well.

A highly polar fraction with ^{14}C-peaks at retention times of 1.4 to 1.9 min occurred in many ^{14}C-chromatograms and represented the only or predominant radiosignals observed after incubation with rat hepatocytes and after incubation of 10 µM dimethenamid-P with dog hepatocytes for 180 min. In the other samples after incubation with dog hepatocytes, these highly polar peaks belonged to the most abundant ^{14}C-peaks. In the experiments with 10 µM dimethenamid-P and human hepatocytes, a highly polar fraction was detected after incubation times of 60 and 180 min. HPLC-MS analysis yielded no significant ions corresponding to these highly polar ^{14}C-peaks. Since the portions of those highly polar fractions are higher in the animal samples compared to the human hepatocyte samples, these results are sufficiently covered by the incubations with animal hepatocytes (Table 5.1.2-3).

Nevertheless, an additional HPLC method was applied for further investigation of this highly polar fraction. The HILIC chromatograms of the concentrated supernatants of the terminated incubation mixtures with human hepatocytes (10 µM, 180 min) show that the highly polar fraction was separated into several peaks with retention times (RT_{HILIC}) of 8 to 10 min (each below 10% AR). The highly polar fraction in the rat samples was separated into five to eight peaks with retention times of 8 to 21 min (RT_{HILIC}, each below 21% AR).

A minor ^{14}C-peak at approximately 35 min (RT_{HPLC}) was only detected in one single replicate of the incubation of 100 µM dimethenamid-P with human hepatocytes for 180 min and not detected again in the ^{14}C-chromatogram of the HPLC-MS analysis of the same sample. This minor peak was therefore considered not relevant.

Table 5.1.2-3: Comparison of metabolites of dimethenamid-P (10 µM or 100 µM) formed with human hepatocytes and formed with rat and dog hepatocytes

RT$_{HPLC}$ [min]	*m/z*	Human Mean % AR		Rat Mean % AR		Dog (Series 1) Mean % AR		Dog (Series 2) Mean % AR
		10 µM	100 µM	10 µM	100 µM	10 µM	100 µM	10 µM
0 min (Control)								
31.1	276.082	81.27	84.21	79.86	79.31	81.59	76.12	76.01
Incubation time: 30 min								
1.4		-	-					59.49
9.5	292.077	-	-					12.05*
9.9	292.077	-	-					29.34*
23.6	262.066	22.97	-	Not applied		Not applied		-
23.9	262.066	13.06*	7.63*					-
*23 min (sum)***		*27.33***	*7.63**					-
31.1	276.082	53.35	83.89					-
Incubation time: 60 min								
1.6		13.48	-					60.87
1.8		-	-					17.58*
10.0	292.077	12.13 *	-					19.98*
18.2	438.098	-	-	Not applied		Not applied		9.03*
23.5	262.066	21.37	-					-
23.7	262.066	15.26	12.18*					-
*23 min (sum)***		*36.63*	*12.18**					-
31.1	276.082	22.73	80.70					-
Incubation time: 180 min								
1.5		26.53	-	75.13	82.09	78.68	23.09	72.36
1.8		-	10.41*	-	-	-	-	11.90*
10.0	292.077	20.72	21.56*	-	-	-	15.63	-
18.6	438.098	22.99	-	-	-	-	6.71*	-
23.3	262.066	-	18.34*	-	-	-	-	-
23.6	262.066	11.98*	16.13*	-	-	-	-	-
*23 min (sum)***		11.98*	25.30**	-	-	-	-	-
31.1	276.082	-	54.86	-	-	-	19.96	-

* In the case of components which were only detected in one or two of the three replicate samples, the mean value of those samples in which the peak was detected is given to indicate a worst case situation; therefore, the sum of the components may exceed 100% of the applied radioactivity

** For the double peak at approximately 23 min representing rotamers of M656PH007 (*m/z* 262.066), a composite value (sum) is given in addition; if a double peak was not detected in each of the three replicate samples, the sum was calculated for the respective replicate prior to calculating the mean value of those samples in which the peak was detected

1 Calculated as the quotient of the peak area of the metabolite divided by the sum of peak areas of all regarded metabolites.

III. CONCLUSION

In summary the present study shows that ^{14}C-dimethenamid-P (Reg. No. 363581), which is the pure S-enantiomeric form of BAS 656 H, is extensively metabolised by hepatocytes from dogs, rats and humans under the investigated conditions.

Four ^{14}C-peaks and a highly polar fraction are present in human hepatocyte samples at a level above 5% AR (based on average of triplicates). For these four peaks, a retention time and *m/z*-value could be assigned. Three of these peaks with an assigned *m/z*-value, including the peak representing the unchanged active substance dimethenamid-P, also appear after incubation with animal hepatocytes, particularly in dog hepatocyte samples.

One of these components was determined as M656PH007, *m/z*-value of 262.066, and is a derivative of the parent compound with the methyl ether cleft. This Phase I metabolite was also detected in a study on the metabolism of ^{14}C-dimethenamid-P in rats.

Another of these components was determined as M656PH034, *m/z*-value of 438.098, is the glucuronic acid conjugate of metabolite M656PH007. Both metabolites M656PH007 and M656PH034 (iso), representing phase I and phase II of metabolic transformation, were also detected in a study on the metabolism of ^{14}C-dimethenamid-P in rats. As M656PH007 and M656PH034 are rotamers, they represented double peaks in the chromatograms.

No significant ions were detected by HPLC-MS analysis of the highly polar ^{14}C-fraction. These peaks were therefore further characterized using an additional HPLC method: HILIC chromatography separated the highly polar fraction in the samples of human hepatocytes into several peaks (each below 10% AR). Since the portion of this highly polar fraction is higher in the animal samples compared to the human hepatocyte samples, these results are sufficiently covered by the incubations with animal hepatocytes.

Thus, all metabolites detected after incubation with human hepatocytes were also present in animal hepatocyte samples, except for the metabolite M656PH007 which has already been described in the *in-vivo* rat metabolism study with dimethenamid-P.

CA 5.2 Acute Toxicity

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: The chiral biologically active Dimethenamid-P and the racemic dimethenamid have been tested in various species and via different routes of administration. All studies are scientifically valid; however, partially the studies have been conducted before the release of study guidelines and are without GLP according to the usual practice in those days. These studies have been evaluated by European authorities and Germany as Rapporteur Member State (European Commission Peer Review Program) and are, for the convenience of the reviewer, listed in Table 5.2-1.

Table 5.2-1: Summary of acute toxicity studies with Dimethenamid and Dimethenamid-P as available in the last evaluation

Study	Test substance/ Species/Sex	Dose range	Result	Reference
Acute toxicity Oral	Dimethenamid-P: Rat, Sprague-Dawley, m/f	350, 400 or 500 mg/kg bw	LD_{50}.(m): 429 mg/kg bw LD_{50} (f): 531 mg/kg bw	1996/11087
	Racemic dimethenamid: Rat, Sprague-Dawley, m/f	150, 300 or 600 mg/kg bw	LD_{50}.(m): 371 mg/kg bw LD_{50} (f): 427 mg/kg bw	1991/11940
Acute Toxicity Dermal	Dimethenamid-P: Rabbit, New Zealand White, m/f	2000 mg/kg bw	LD_{50} (m+f): > 2,000 mg/kg bw	1996/5395
	Racemic dimethenamid: Rabbit, New Zealand White m/f	2000 mg/kg bw	LD_{50} (m+f): > 2,000 mg/kg bw	1991/11938
Acute Toxicity Inhalation, 4h nose-only	Dimethenamid-P: Rat, Sprague Dawley, m/f	2.2 mg/l (4 h)	LC_{50} (m+f): > 2.2 mg/l	1996/5397
	Racemic dimethenamid: Rat, Wistar m/f	4.99 mg/l	LC_{50} (m+f): > 4.99 mg/l	1986/11166
Skin irritation	Dimethenamid-P: Rabbit, New Zealand White	0.5 ml/animal	Not irritating	1996/5406
	Racemic dimethenamid: Rabbit, New Zealand White	0.5 ml/animal	Not irritating	1988/11363
Eye irritation	Dimethenamid-P: Rabbit, New Zealand White	0.1 ml/animal	Not irritating	1996/5396
	Racemic dimethenamid: Rabbit, New Zealand White	0.1 ml/animal	Not irritating	1988/11364
Skin sensitization	Dimethenamid-P: Buehler test Guinea pig, Dunkin Hartley	Induction and challenge with undiluted material	Sensitizing	1996/11088
	Racemic dimethenamid: Magnusson-Kligman test Guinea pig, Dunkin Hartley	Intradermal induction: 5% Epidermal induction: 5% Challenge: 5%	Sensitizing	1987/11222

Based on the studies available at the time the EU agreed endpoints were:

Rat LD_{50} oral:	429 mg/kg bw	R 22
Rat LD_{50} dermal:	> 2000 mg/kg bw	
Rat LC_{50} inhalation:	> 2.2 mg/l (4-h, nose-only)	
Skin irritation:	Non-irritant	
Eye irritation:	Non-irritant	
Skin sensitization (Buehler-test)	Skin sensitiser	R 43

Submission of not yet peer-reviewed studies in this AIRIII-Dossier:
A new acute inhalation study according to current criteria has been performed with Dimethenamid-P for global registration as the former study was considered to have some limitations with regard to study design. This study is submitted within the AIR III process. In accordance with the data requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells has been performed and is given in detail under chapter 5.2.7. According to this study Dimethenamid-P does not have a phototoxic potential.
New data available is tabulated in Table 5.2-2

Table 5.2-2: Summary of newly available acute toxicity studies with Dimethenamid-P

Type of study	Test substance	Result classification	Reference
Inhalation route - rat	Dimethenamid-P	LC_{50} (m+f) > 5.16 mg/L EU classification not required GHS classification not required	2011/1171036
in vitro NRU-Phototoxicity study in Balb/c 3T3 cells	Dimethenamid-P	Not phototoxic	2013/1110119

Dimethenamid-P has moderate acute toxicity by the oral route and a low acute toxicity by the percutaneous, and inhalation routes of administration. Dimethenamid-P produces only slight reversible skin and eye irritation not requiring classification according to EU legislation. Dimethenamid-P is a skin sensitizer. Therefore, a classification with R22 and R43 according to EU Dir. 67/548/EEC and Acut Tox oral Cat. 4 and Skin Sens. 1B according to CLP Reg. EC 1272/2008 classification criteria is warranted.

With the newly available data, the relevant endpoints as proposed are:

Acute toxicity (SANCO/11802 data point 5.2)

Rat LD_{50} oral	429 mg/kg bw **H 302; Acut Tox Cat.4**
Rat LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	> 5.16 mg/l (4-h, head/nose-only)
Skin irritation	Not irritating to skin
Eye irritation	Not irritating to eyes
Skin sensitization (test method used and result)	Skin sensitiser (Buehler-test,) **Skin Sens. 1, H317**
Phototoxicity	Not phototoxic

The ECHA risk assessment committee on classification and labelling has evaluated the data and concluded that classification for Acute toxicity Category 4, H302 and skin sensitization Skin Sens. 1, H317 is warranted [see Committee for Risk Assessment RAC Opinion of Dimethenamid-P, adopted 4 June 2013]. Based on this proposal the EU commission has prepared a draft list entry [Follow up to the 13th meeting of competent authorities for REACH and CLP (CARACAL) 26-27-28 November 2013, Centre A. Borschetted, Brussels, Belgium of 12 February 2014] for inclusion into the 7th ATP to the CLP Regulation 1272/2008. Entry into force is expected by end of 2014 or beginning of 2015.

CA 5.2.1 Oral

The acute oral toxicity studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].

CA 5.2.2 Dermal

The acute dermal toxicity studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].

CA 5.2.3 Inhalation

The acute inhalation toxicity studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].
A new acute inhalation study according to current criteria has been performed with Dimethenamid-P for global registration as the former studies were considered to have some limitations with regard to study design.

Report:	CA 5.2.3/1 [redacted] 2012a BAS 656-P H - Acute inhalation toxicity (nose only) study in the rat 2011/1171036
Guidelines:	OECD 403 (2009), EPA 870.1300, (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
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In an acute inhalation toxicity study, groups of 5 male and 5 female Wistar rats were exposed to Dimethenamid-P as an aerosol (Batch COD-001509; Purity: 95.9%) at a concentration of 5.16 mg/L for 4 hours (limit test). The animals were observed for 14 days after exposure.
No mortality occurred at the tested concentration. Accordingly, the acute inhalation LC_{50} for Dimethenamid-P after aerosol inhalation exposure was determined to be

LC_{50} (male and female rats) > 5.16 mg/L

Clinical signs of toxicity consisted of wet fur, hunched posture, piloerection and increased respiratory rate. Findings were observed from hour 0 of exposure until study day 3. No clinical signs and findings were observed from study day 4 onward. Body weight development of the animals was not adversely affected throughout the study period. No gross pathological abnormalities were noted during the necropsy at termination of the post exposure observation period.
Cascade impactor measurements resulted in particle size distributions with a mass median aerodynamic diameters (MMAD) of 3.59 µm and a geometric standard deviation (GSD) of 2.39.

According to the EU and GHS classification criteria, no classification is warranted as to acute inhalation toxicity for Dimethenamid-P.

(DocID 2011/1171036)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 656-PH
Description:	Brown, liquid
Lot/Batch #:	COD-001509
Purity/content:	95.9% (tolerance ± 1.0 %)
Stability of test compound:	Stable (Expiry date: 01-Oct-2013)

2. Vehicle:	Test substance was applied unchanged.

3. Test animals:	
Species:	Rat
Strain:	Wistar / RccHan™:WIST
Sex:	male and female
Age:	approx. 8 - 12 weeks
Weight at dosing (mean):	Males: 249 - 278 g; females: 223 - 244 g
Source:	Harlan Laboratories UK Ltd, Oxon, UK
Acclimation period:	at least 5 days
Diet:	Harlan 2014C Rodent Diet, Harlan Laboratories UK Ltd, Oxon, UK, ad libitum
Water:	Drinking water, ad libitum
Housing:	In groups of five by sex in solid-floor polypropylene cages with stainless steel lids, furnished with softwood flakes.
Environmental conditions:	
Temperature:	19 - 25 °C
Humidity:	30 - 70%
Air changes:	at least 15/hour
Photo period:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 04-Jan-2012 - 28-Feb-2012

2. Animal assignment and treatment:

For determination of the acute inhalation toxicity (nose-only inhalation, 4-hour-exposure) groups of five male and five female rats were exposed to 5.16 mg/L of the test substance Dimethenamid-P, that was applied as an aerosol. After exposure, animals were observed for at least 14 days. Individual body weights were recorded on arrival, shortly before exposure (day 0) and on days 1, 3, 7 and 14. Detailed clinical observations were recorded for each animal separately hourly during exposure and immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days. At the end of the fourteen day observation period all animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

3. Statistics/calculations:

No statistics were performed.
The quantity of the test item collected by the filter was calculated as follows:

$$TI = \frac{A_{spl} \times N_{std} \times D_{spl}}{A_{std}}$$

Where: TI = amount of test item collected by the filter (mg), A_{spl} = mean peak area for sample solution, A_{std} = mean peak area of standard solution, corrected to nominal standard concentration, N_{std} = nominal standard concentration (mg/mL), D_{spl} = dilution factor for sample solution.

The concentration of the test item in the atmosphere was calculated as follows:

$$C_{atm} = \frac{TI_{ret}}{V}$$

Where: C_{atm} = concentration of the test item in test atmosphere (mg/L), TI_{ret} = amount of test item collected by the filter (mg), V = volume of test atmosphere sampled through the filter (L)

4. Generation of the test atmosphere and exposure:

The test item was aerosolised using a glass concentric jet nebuliser (Radleys, Saffron Waiden, Essex, UK) located at the top of the exposure chamber. The nebuliser was connected to a glass syringe attached to an infusion pump, which provided a continuous supply of test item formulation under pressure, and to a metered compressed air supply. Compressed air was supplied by means of an oil free compressor and passed through a water trap and respiratory quality filters before it was introduced to the nebuliser. The cylindrical exposure chamber had a volume of approximately 30 litres (dimensions: 28 cm diameter x 50 cm high). The concentration within the exposure chamber was controlled by adjusting the rate of the infusion pump. The extract from the exposure chamber passed through a 'scrubber' trap and was connected with a high efficiency filter to a metered exhaust system. The chamber was maintained under negative pressure. Homogeneity of the test atmosphere within the chamber was not specifically determined during the study.

Prior to the day of exposure each rat was acclimatised (for approximately 2 hours) to a tapered polycarbonate restraining tube. During the day of exposure, each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring to achive nose-only conditions for each animal to the test atmosphere during the exposure period of four hours. A target concentration of 5.0 mg/L was used for the exposure. As the mean achieved (analytical) concentration was 103% of target and no deaths occurred, no further levels were required.

5. Analytical investigation:

The test atmosphere was sampled nine times during the exposure period. The sampling procedure involved two litres of test atmosphere being drawn through a glass fibre filter. The actual chamber concentration was also measured gravimetrically seventeen times during the exposure period.

Each filter was weighed before and after sampling in order to calculate the weight of collected test item. The difference in the two weights, divided by the volume of atmosphere sampled, gave the actual chamber concentration. The nominal chamber concentration was calculated by dividing the mass of test item used by the total volume of air passed through the chamber.

6. Particle Size Analysis:
The particle size of the generated atmosphere inside the exposure chamber was determined three times during the exposure period using a Marple Personal Cascade Impactor (Westech IS Ltd, Beds., UK). This device consisted of six impactor stages (8.8, 5.8, 3.6, 1.9, 0.79 and 0.33 µm cut points) with stainless steel collection substrates and a back up glass fibre filter, housed in an aluminium sampler. The sampler was temporarily sealed in a sampling port in the animals' breathing zone and 0.67 L of exposure chamber air was drawn through it using a vacuum pump set at a flow rate of 2 L/min. The collection substrates and backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by difference. The mean amount for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 8.8, 5.8, 3.6, 1.9, 0.79 and 0.33 µm was calculated. The resulting values were converted to probits and plotted against Log_{10} cut-point size. From this plot, the Mass Median Aerodynamic Diameter (MMAD) was determined (as the 50% point) and the geometric standard deviation was calculated. In addition the proportion (%) of aerosol less than 4 µm (considered to be the inhalable fraction) was determined.

II. RESULTS AND DISCUSSION

A. MORTALITY

No lethality occurred at the tested concentration of 5.16 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} (male and female rats): > 5.16 mg/L

B. CLINICAL OBSERVATIONS

Wet fur, hunched posture, piloerection and increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one hour post-exposure. One day after exposure, all animals exhibited increased respiratory rate and hunched posture, two male and two female animals also exhibited piloerection. These observations were considered to be associated with the restraint procedure and not indicative of toxicity. All animals recovered quickly to appear normal on day 4 post-exposure. 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 Dimethenamid-P as an aerosol

Test group 1 (5.16 mg/L)	Males	Females
Wet fur	h0 – h1	h0 – h1
Hunched posture	h4 – d2	h4 – d2
Piloerection	h4 – d1	h4 – d1
Increased respiratory rate	h3 – d3	d3 – d3

hn: hour n of exposure; d0: post-exposure on the day of exposure; dn: day n after exposure

C. BODY WEIGHT

All males and four female animals exhibited slight body weight losses on the first day post-exposure. Reasonable bodyweight development was noted in all animals during the remainder of the recovery period, with the exception of one female animal which exhibited a slight bodyweight loss from days 3 to 7 post-exposure.

D. NECROPSY

No gross pathological abnormalities were detected in all animals during 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

Air flow (L/min)	Temperature (°C)	Relative humidity (%)	Oxygen concentration (%)
40	19-20	73-86	20.8

Test atmosphere concentrations are presented in Table 5.2.3-3.

Table 5.2.3-3: Atmosphere concentrations

Mean achieved (mg/L)	Standard deviation	Nominal (mg/L)
5.16	0.25	34.7

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) of 3.59 µm with a geometric standard deviation of 2.39 respectively (see Table 5.2.3-4).

Table 5.2.3-4: Particle size distribution

Mean achieved (analytical) atmosphere concentration (mg/L)	Mean mass median aerodynamic diameter (µm)	Inhalable fraction (% <4 µm)	Standard deviation
5.16	3.59	54.9	2.39

III. CONCLUSION

Under the conditions of this study the 4 hour inhalation LC_{50} of Dimethenamid-P for male and female rats was estimated to be > 5.16 mg/L. Based on the results of this study, Dimethenamid-P does not warrant classification as to acute inhalation toxicity according to EU (EC Directive on dangerous preparations 1999/45/EC, DPD) and (EC) No 1272/2008 Regulation on Classification, Labelling and Packaging of Substances and Mixtures (CLP).

CA 5.2.4 Skin irritation

The acute skin irritation studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].

CA 5.2.5 Eye irritation

The acute eye irritation studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].

CA 5.2.6 Skin sensitisation

The acute skin sensitization studies conducted for racemic dimethenamid and Dimethenamid-P have been evaluated during the Annex I listing of Dimethenamid-P [see Table 5.2-1 details].

CA 5.2.7 Phototoxicity

Report: CA 5.2.7/1
Cetto V.,Landsiedel R., 2013a
BAS 656-P H - In vitro 3T3 NRU phototoxicity test
2013/1110119

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

Dimethenamid-P (Batch COD-001509; purity 95.9%) 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 an UVA source. Vehicle and positive controls were included into the study.
Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in this study:

Without: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL
With: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL

Precipitation was seen in at the top dose of 1050 µg/mL with and without irradiation. In the absence and the presence of UVA irradiation the highest concentrations applied were clearly cytotoxic. On the basis from the results of the present study, the test substance was predicted to have no phototoxic potential (PIF= 0.7) indicated by Neutral Red Uptake method. The positive control chlorpromazine led to the expected cytotoxicity both with and without UVA irradiation (PIF: 29.8).
Thus, under the experimental conditions of this study, Dimethenamid-P is considered not to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

(BASF DocID 2013/1110119)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS 656-PH

Description: Liquid; brown, clear

Lot/Batch #: COD-001509

Purity: 95.9% (tolerance ± 1.0%) (see Certificate of Analysis, study code 346279_31)

Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Oct 2013 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by the high purity and by mixing prior to preparation of test substance solutions.
The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was determined analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control: The vehicle control cultures with and without irradiation only contained the vehicle used for the test substance at the same concentration and volume as used for the test substance and the positive control.

Solvent/final concentration: DMSO 1% (v/v) in PBS

Positive control compounds: Chlorpromazine (CPU) was dissolved in DMSO

A complete 96-well plate containing 8 concentrations was performed in parallel to demonstrate sensitivity of the test method.

Without irradiation	1.9-3.8-7.5-15-30-60-90-180 µg/mL
With irradiation	0.03-0.05-0.1-0.2-0.4-0.8-1.6-3.2 µg/mL

3. Test organisms:

The BaIb/c 3T3, clone A31, cell line was isolated from the muscle tissue of mouse embryo. This fibroblast cell line has a high proliferation rate (doubling time 16 - 20 hours) and a high plating efficiency (>70%)) of untreated cells both necessary for the appropriate performance of the study. 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 (date 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 wavelength > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest: Up to 1050 µg/mL with and without irradiation.

NRU test conditions: An appropriate amount of test article substance was taken up in the vehicle, shaken thoroughly and diluted in accordance with the planned doses under light protection conditions immediately before administration.

The experiment was performed in 96 well plates in one experiments (6 replicates per concentration with and without irradiation; two plates per substance (test substance or positive control) were prepared.) The test substance concentrations were:

Without:	4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL
With:	4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1050.0 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 18-Mar-2013 - 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 in parallel for 1 hour in the dark (5% (v/v) CO_2, ≥90% humidity; 37° C). Then, one microtiterplate per substance was irradiated for 50 minutes with UVA (UV 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. After test substance removal and washing step (100 µL PBS) the cells were incubated in culture medium overnight. The medium was removed after 24 hours, the cells washed again, 100 µL medium containing 50 µg/mL Neutral red was added and the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Afterwards, the cells were washed and the dye was extracted by Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake using 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

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor 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 the special case of absence of cytotoxicity in the presence and absence of light for substances obviously showing no phototoxic potential (see below).
- The Mean Photo Effect prediction model which is used if no equi-effective concentrations (EC_{50}) are obtained in the absence and presence of UV light.

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 arbitrariliy set at 100 %.

$$Cytotoxicity\,[\%] = \frac{\text{Absorbance}_{\text{mean}}\text{ of the test group}}{\text{Absorbance}_{\text{mean}}\text{ of the vehicle control}} \times 100$$

In case of cytotoxicity, an EC_{50} value (Inhibition concentration 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve).

3.2 Photo-Irritancy-Factor

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 UVA irradiation.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥5	phototoxic potential predicted
If 2 < PIF < 5:	probable phototoxic potential predicted
If PIF ≤ 2:	no phototoxic potential predicted

If cytotoxicity occurs only after irradiation a C PIF has to be calculated using the highest test concentration (Cmax) applied in the experimental part in the absence of UV light (-UVA):

$$C\ PIF = \frac{C\max\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

C PIF > 1	probable phototoxic potential predicted
If C PIF ≤ 1	no phototoxic potential predicted

If no cytotoxicity occurs in the concurrently performed experiments in the absence and presence of UV light up to the highest applied test concentration it has to be considered that the test substance has no phototoxic potential.

In this case, a formal PIF = *1 is used to characterize the result:

$$PIF = {*1} = \frac{C\max\ (-UVA)}{C\max\ (+UVA)}$$ resulting in the following classification rule:

PIF = *1	no phototoxic potential predicted

3.3 Mean Photo Effect

The Mean Photo Effect is calculated based on a comparison of the +UVA and -UVA concentration response curves on a grid of concentrations ci (i=1,..., N) chosen from the common concentration range of the (-UVA) and (+UVA) experiments. The photo effect (PEi) at concentration ci is calculated as the product of the concentration effect (CEi) and the response effect (REi). The mean photo effect (MPE) is defined as a weighted averaging across all PEi values, with a weighting factor defined by the highest response value.
The resulting classification rules are:

If MPE ≥ 0.1	phototoxic potential predicted
If MPE < 0.1	no phototoxic potential predicted

3.4 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.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.

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 UVA 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

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was determined analytically.
Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation precipitation in culture medium was observed at test substance concentrations of 1050 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 Experiment in the absence and the presence of UVA irradiation at least in the highest applied concentrations (see Table 5.2.7-1). Thus an EC_{50} was calculated for all experimental parts.
Without UVA irradiation, there was a decrease in the cell number from 215.4 µg/mL (EC_{50}: 206.3 µg/mL) onward.
With UVA irradiation, there was a decrease in the cell number at 464.2 µg/mL (EC_{50}: 311.9 µg/mL) and above.
Based on the EC_{50} values a PIF of 0.7 (no phototoxic potential) was obtained.

Table 5.2.7-1: Mean relative cytotoxicity of Dimethenamid-P with and without UVA irradiation in Balb 3T3 cells

Test group	UVA irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Cytotoxicity [% of control]
Vehicle control (1% DMSO)	-	-	0.413	100.0
Dimethenamid-P				
4.6 µg/mL	-	-	0.416	100.6
10.0 µg/mL	-	-	0.433	104.7
21.5 µg/mL	-	-	0.462	111.8
46.4 µg/mL	-	-	0.433	104.7
100.0 µg/mL	-	-	0.411	99.3
215.4 µg/mL	-	-	0.189	45.8
464.2 µg/mL	-	-	0.181	43.9
1050.0 µg/mL	-	+	0.034	8.2
Vehicle control (1% DMSO)	-	-	0.489	100.0
Dimethenamid-P				
4.6 µg/mL	+	-	0.456	93.3
10.0 µg/mL	+	-	0.495	101.3
21.5 µg/mL	+	-	0.493	100.9
46.4 µg/mL	+	-	0.492	100.6
100.0 µg/mL	+	-	0.455	93.2
215.4 µg/mL	+	-	0.371	75.9
464.2 µg/mL	+	-	0.045	9.1
1050.0 µg/mL	+	+	0.049	10.0

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx.. 5 J/cm^2)
**: 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 UVA irradiation at least in the highest applied concentrations.
Without UVA irradiation, there was a decrease in the cell number from 30.0 µg/mL (EC_{50}: 21.2 µg/mL) onward.
With UVA irradiation, there was a decrease in the cell number at 0.8 µg/mL (EC_{50}: 0.7 µg/mL) and above.
Based on the EC_{50} values a PIF of 29.8 (phototoxic potential) was obtained (see Table 5.2.7-2).

Table 5.2.7-2: Mean relative cytotoxicity of Chlorpromazine with and without UVA irradiation in Balb/c 3T3 cells

Test group	UVA irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative Cytotoxicity [% of control]	Standard deviation [%]
Blank	-	0.038	-	-	-
Vehicle control 1	-	0.429	0.392	-	9.6
Vehicle control 2	-	0.524	0.486	-	3.5
Vehicle control mean (1% DMSO)	-	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 control mean (1% DMSO)	+	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. CONCLUSIONS

According to the results of the present study, the test substance Dimethenamid-P is considered not to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells under the experimental conditions.

CA 5.3 Short-Term Toxicity

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: Short-term toxicity studies (28 - 90 days) with oral administration are available from three different species (rats, mice, dogs) for racemic Dimethenamid. In addition a 1-year dog study is available for racemic Dimethenamid. Furthermore 28 – 90 day studies in rat are available for Dimethenamid-P. Short-term toxicity following dermal exposure was determined in a 21-day study in rabbits conducted with racemic Dimethenamid. These studies have been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.3-1: Summary of short-term toxicity studies conducted with Dimethenamid-P and racemic Dimethenamid

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference
Dimethenamid-P 4-week, diet, range-finder, Sprague-Dawley rat (constant diet concentrations: 0, "150", 500, 1500 and 3000 ppm)	M: 12, 50, 155 and 306 F: 12, 52, 143 and 290	Not established due to limited investigations performed	Decreased bw and bw gain; liver: weight increase LOAEL: 50 mg/kg bw/d	1996/11147
Racemic dimethenamid 5-week, diet, range-finder, Wistar rat (constant diet concentrations: 0, 30, 100, 300, 1000 and 3000 ppm)	M: 2.92, 9.5, 28.8, 95.6 and 285 F: 3.32, 10.8, 35.7, 109 and 328	29	Decreased bw and bw gain; liver: γ-GT increase, cholesterol increase, weight increase, hepatocellular cytoplasmic swelling LOAEL: 96 mg/kg bw/day	1987/11227
Dimethenamid-P 13-week, diet, Sprague-Dawley rat (constant diet 0, 500, 1500 and 3000 ppm)	M: 37, 110 and 222 F. 40, 125 and 256	37	Decreased bw and bw gain, liver: γ-GT increase, cholesterol increase, weight increase, hepatocellular hypertrophy LOAEL: 110 mg/kg bw/day	1996/5420 1999/10270 (
Racemic dimethenamid 13-week + 4- week recovery , diet, Sprague-Dawley rat (constant diet 0, 50, 150, 500, 1500 and 3000 ppm)	M: 3.5, 10, 33.5, 98 and 204 F. 3.9, 11.8, 40,1, 119 and 238	33.5	Decreased bw and bw gain, decreased FC Liver: γ-GT increase, Cholesterol increase, protein increase Liver weight increase, centrilobular hypertrophy LOAEL: 98 mg/kg bw/day	1986/11183 1995/11323 1999/10270
Racemic dimethenamid 13-week, diet, CD-1 mice (constant diet concentrations: 0, 300, 700, 2000, 5000 ppm)	M: 46, 105, 301 and 805 F: 60, 137, 383 and 972	46	Subdued behaviour, decreased bw gain, decreased FC, Liver: weight increase Kidney: rel. weight increase LOAEL: 105 mg/kg bw/day	1988/11360
Racemic dimethenamid 13-week, diet, Beagle dog (constant diet concentration 9, 91.5, 750, 2000 ppm)	M: 4.3, 34 and 90 F: 4.6, 40 and 87	4.3	Decreased bw gain Liver: AP increase, cholesterol increase, weight increase, hepatocyte vacuolation, dilatation of sinusoids LOAEL: 35 mg/kg bw/day	1986/11159 1986/11178
Racemic dimethenamid 52-week, diet, Beagle dog (constant diet concentration 0, 50, 250, 1500 ppm)	2, 10, 49	2	Decreased bw gain Liver: AP increase, cholesterol increase, weight increase, hepatocyte enlargement, hepatocyte vacuolation, LOAEL: 10 mg/kg bw/day	1988/11361 1988/11362
Racemic Dimethenamid 3-week, dermal, New Zealand White rabbit (6 h/day / 5 days a week) Limit-test 1000 µl/kg	0, 1190	Systemic NOAEL: 1190	Dermal irritation, no systemic findings.	1990/11144

After oral treatment, the signs of toxicity observed in rats, mice and dogs were overall similar with the liver as the target organ. The effects observed typically included the increase in one or more serum liver enzymes and changes in cholesterol levels. Increased liver weights were observed in all three species. Histologically, hepatocyte hypertrophy was observed in rats and hepatocyte vacuolation and dilatation of liver sinusoids occurred in dogs.

Feeding of racemic Dimethenamid to dogs for 1 year resulted in decreased body weight gain and changes indicative of liver alteration at the high dose. Liver changes included increased alkaline phosphatase and cholesterol, increased liver weight and hepatocyte enlargement and vacuolation.

In a 3-wk dermal toxicity study in rabbits no substance-related systemic findings were detected up to the highest dose level tested (1190 mg/kg bw/day)

In order to assess the validity of the "Bridging concept", the toxicological effects observed in the 90-day oral studies conducted with either dimethenamid-P or racemic dimethenamid revealed only marginal differences between the two studies. The NOAELs and LOAELs were the same irrespective of the test substance administered. Therefore, on the basis of the available data at the time of Annex I inclusion of Dimethenamid-P, the requirements were considered to have been met for a scientifically-based justification of the "Bridging concept" for Dimethenamid-P / racemic Dimethenamid.

Based on the available studies, the following endpoints were determined in the Annex I listing of Dimethenamid-P:

Target / critical effect:	Liver (biochemical and histopathological changes), decreased body weight gain
Lowest relevant oral NOAEL/NOEL	90-d dog: 100 ppm (4.3 mg/kg bw/d) based on study with racemic dimethenamid
Lowest relevant dermal NOAEL/NOEL	21-d rabbit: 1190 mg/kg bw/d (systemic toxicity) based on study with racemic dimethenamid
Lowest relevant inhalation NOAEL/NOEL	No data, not required

The only new studies to consider whether they could affect the short-term relevant NOAEL/NOEL would be the 90-day neurotoxicity study in rats [please refer to section MCA 5.7.1/2 of this dossier] and the 28-day immunotoxicity study in mice [please refer to section MCA 5.8.2/1 of this dossier]. However the determined study NOAELs were clearly above the derived lowest relevant oral NOAEL of the 90-day dog study and no additional targets / critical effects were determined. Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Target / critical effect	Liver (weight increases, biochemical and histopathological changes), decreased body weight gain No classification required
Lowest relevant oral NOAEL / NOEL	90-d, dog: 100 ppm (4.3 mg/kg bw/d) *
Lowest relevant dermal NOAEL / NOEL	21-d, rabbit: 1190 mg/kg bw/d (systemic toxicity) *
Lowest relevant inhalation NOAEL / NOEL	No data; not required

* based on studies performed with racemic dimethenamid

The ECHA risk assessment committee on classification and labelling has evaluated the data and concluded that no classification for systemic toxicity is warranted. Based on this proposal the EU commission has prepared a draft list entry [Follow up to the 13th meeting of competent authorities for REACH and CLP (CARACAL) 26-27-28 November 2013, Centre A. Borschetted, Brussels, Belgium of 12 February 2014] for inclusion into the 7th ATP to the CLP Regulation 1272/2008. Entry into force is expected by end of 2014 or beginning of 2015.

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph are provided under the respective chapters.

CA 5.3.1 Oral 28-day study

Dimethenamid-P, Rat 4-week study

Groups of 5 male and 5 female Sprague-Dawley rats received 94.7% Dimethenamid-P in the diet at doses of 0, 50 (increased to 150 after 1 week), 500, 1500 and 3000 ppm for 28 consecutive days. No histopathological investigations were conducted in this range finding study.

There were no treatment-related clinical signs. Reduced body weight (males) and body weight change were observed at high dose without an effect on food consumption. The increase in serum γ-glutamyltransferase in the high dose animals is in agreement with the increased liver weights, and indicates the possibility of either frank liver toxicity or an adaptive response to handling the chemical. There were no gross pathological findings considered related to treatment.

Conclusion
The liver was identified as target organ. The examinations performed were limited. Therefore, it is not considered appropriate to derive a general NOAEL for risk assessment purposes.

Racemic Dimethenamid, 5-week study

Groups of 8 male and 8 female Wistar rats received 99% racemic dimethenamid in the diet at doses of 0, 30, 100, 300, 1000 or 3000 ppm for 5 consecutive weeks.

No mortality or clinical symptoms that could be related to substance intake were observed. Body weight gain was reduced significantly at 3000 ppm in males only which was accompanied by a significantly reduced food consumption during the first study week. Over the whole study, food intake was only slightly reduced. In 2000 ppm females, anon-significant reduction of body weight gains was observed; food consumption was not significantly affected.

Assessment of hematology did not reveal biologically relevant differences between control and dose groups. The only clinical chemistry parameter to reach statistical significance was cholesterol in males with a dose related increase already at 300 ppm and above. In females cholesterol was increased only in the high dose group. γ-GT activity values were increased at 3000 ppm in both male and female groups.

Absolute liver weights were increased among both 3000 ppm males and females. Relative liver weights of this group were significantly increased for both sexes. Histopathological findings regarded to be related to treatment were cytoplasmic swelling of predominantly centrolobular hepatocytes at 3000 ppm in both sexes.

Conclusion
Following administration of racemic Dimethenamid for 5 weeks a LOAEL was established at 1000 ppm. At 300 ppm, the only change observed was a slight increase in cholesterol levels, which was found in males only and not considered to represent an adverse effect; therefore 300 ppm, equivalent to 29 mg/kg bw/d, is considered to be the NOAEL.

CA 5.3.2 Oral 90-day study

Dimethenamid-P, 90-day rat study

Groups of 10 male and 10 female Sprague-Dawley CD rats per dose group received 91.1% Dimethenamid-P in the diet at doses of 0, 500, 1500 and 3000 ppm for 90 consecutive days.

There were no overt signs of clinical toxicity observed at any treatment level during the course of the study. A slight non-significant decrease of terminal body weight was observed in high dose males and females.

The only change noted in hematology was an increase in clotting time. Serum γ-GT was increased in both sexes at the high dose in males of the mid dose. There was a trend towards increased cholesterol values in the treated animals (statistically increased for high-dose group males only).

Liver weights were increased in males of all treatment groups and in females at dose levels of 1500 ppm and above being statistically significant in male mid and high dose and in female high dose group. At 500 ppm relative liver weights were slightly increased in males only. A slight increase of absolute and relative kidney weights for males receiving 3000 ppm was not associated with morphological or clinicochemical changes and was not considered treatment-related. Histopathological examination revealed hepatocellular hypertrophy in both sexes at 3000 and 1500 ppm and in females only at 500 ppm. There were no corresponding histological liver changes in males of the 500 ppm group. The localization of hypertrophy appeared to be sex-dependent (centrilobular in females, periportal in males) Furthermore few periportal eonsinophilic inclusions were observed in mid and high dose group males.

Conclusion
The slight changes at 500 ppm are not considered to represent adverse effects and, therefore 500 ppm (37 mg/kg bw/day) is considered a NOAEL.

Racemic dimethenamid, 90-day rat study

Groups of 10 male and 10 female Sprague-Dawley CD rats per dose group received 91.5% racemic dimethenamid in the diet at doses of 0, 50, 150, 500, 1500 and 3000 ppm for 90 consecutive days. An additional 10 rats per sex were used in the control and high dose groups to determine effects after 4-week recovery from treatment

There were no overt signs of clinical toxicity observed at any treatment level during the course of the study. The mean body weights and body weight gains of the animal treated with 1500 and 3000 ppm were lower than control. The body weight change at 500 ppm males was only marginally reduced. During the recovery period, males and females given 3000 ppm showed a higher body weight gain than controls. Food consumption was marginally lower in 1500 and 3000 ppm animals.

At the end of 13-week treatment increased levels of serum γ-GT were observed in males and possibly in females at 3000 ppm. Cholesterol levels were increased in both sexes at 3000 ppm and in females at 1500 ppm. The statistically significant increase in females at 500 ppm is slight and not considered to be of biological significance. At 1500 ppm and above, total protein levels, in some cases in conjunction with albumin and/or globulin were slightly increased over control levels. Liver enzyme, protein and cholesterol levels at 3000 ppm were generally comparable to control following the recovery period.

A dose-related increase of liver weights compared to control levels was found for female rats given 1500 or 3000 ppm, for both absolute and relative weights. Microscopically, minimal to moderate enlargement of centrilobular hepatocytes was observed in females at 1500 and 3000 ppm, the centrilobular hepatocyte enlargement was still present in 2 of 10 females at 3000 ppm following the 4-week recovery. In the histopathological re-assessment conducted blindly by BASF the results obtained for females were comparable to the original findings. However, in the review, in males treated with 3000 ppm, a substance-related effect was evident. A minimal to slight periportal hepatocellular hypertrophy was observed in five males. Also in six males, eosinophilic inclusions were visible in the cytoplasm of few periportal hepatocytes. These findings were comparable to those seen after treatment with Dimethenamid-P.

Conclusion
In summary, the only effect observed at 500 ppm was a marginal decrease in body weight gain. Therefore, the NOAEL is considered to be 500 ppm (34 mg/kg bw/day).

Racemic Dimethenamid, 90-day mouse study

Groups of 12 male and 12 female CD-1 mice per dose group received 91.5% racemic dimethenamid in the diet at doses of 0, 300, 700, 2000 and 5000 ppm for 90 consecutive days. Being a range-finder no examinations were performed regarding ophthalmology, hematology and clinical chemistry. Histopathology was only conduced in liver and kidney tissue (control, low and high dose group).

Clinical signs of subdued behavior (not specified in the report) were observed in 10 male and 10 female mice at 5000 ppm and in 1 male animal at 2000 ppm. Significant body weight gain depression accompanied by only a slight decrease in mean weekly food consumption was observed in high dose males. There was no statistically significant effect on body weight at other dose levels in males or at any dose level in females.

Absolute and relative liver weights and relative kidney weights were increased in both male and female animals at 2000 and 5000 ppm. Absolute and relative liver weight were also slightly increased in males at 700 ppm. No treatment related histopathological changes wf the liver or kidney were recorded for any dose group.

Conclusion
In a 13-week oral toxicity study with mice that was limited with regard to the extent of examinations performed a NOAEL of 300 ppm (equivalent to 46 mg/kg bw/d) was found.

Racemic dimethenamid, 90-day dog study

Groups of 4 male and 4 female Beagle dogs per dose group received 91.4% racemic dimethenamid in the diet at doses of 0, 100, 750 and 2000 ppm for 13 consecutive weeks.

No overt clinical signs of toxicity were observed in the study with the possible exception of thin appearance in 1 to 2 high dose group dogs. Body weight gains were reduced in both sexes at 2000 ppm and in females at 750 ppm.

In clinicochemical examinations of animal of the highest dose group serum alkaline phosphatase was increased in 7 out of 8 dogs. Elevated plasma ALAT levels were recorded for 2 of 4 high-dos dogs at the last time-point of investigation.

Pronounced organ weight increases occurred in the livers of high- , and to a lesser degree in mid-dose animals of both sexes. Relative thyroid weights were statistically increased in high-dose males; a similar increase in high-dose females was statistically non-significant. Slight effects on organ weight were also noted for the adrenals, heart kidney pituitary and thymus, which were regarded to be of doubtful biological significance. The histopathological assessment revealed treatment-related changes were confined to the livers. Periportal hepatocellular vacuolation was found in livers of all high-dose dogs, and in 1 male and 1 female dog of the mid-dose group. Sinusoidal dilation was observed at increased incidences in the high-dose group only.

Conclusion
The liver proved to be the target organ following 13-week dietary exposure to the test substance. Based on the reduced body weight gains, increased liver weights and histopathological changes at 750 ppm, the NOAEL was found to be 91.5 ppm (equivalent to 4.3 mg/kg bw/day).

Racemic dimethenamid, 1-year dog study

Groups of 4 male and 4 female Beagle dogs per dose group received 91.3% racemic dimethenamid in the diet at doses of 0, 50, 250 and 1500 ppm for 12 consecutive month.

No overt clinical signs of toxicity were observed in the study. A significant body weight gain decrease was observed at the high dose. At the mid dose of 250 ppm, the mean body weight gain in males was decreased, however, due to one animal only. As the mean weight gain of the other three animals is very similar to control or even exceeding control, the mean weight gain decrease is considered to be spurious.

In clinicochemical examinations of animals of the high dose group revealed serum alkaline phosphatase and cholesterol levels at some time-points.

Increased liver weights were observed in high dose group animals of both sex and in addition in females at 250 and 50 ppm. Histopathological findings that were related to the treatment were confined to the liver of high dose animals. Periportal hepatocyte vacuolation was present in two of four males and in all females of the high dose group. In the absence of any evidence for lipid or glycogen accumulation, this finding is most likely to indicate a degenerative change of a hydrophobic type, supported by the corresponding AP serum activity levels.

Conclusion
Following a 52-week dietary administration of racemic dimethenamid to beagle dogs, adverse effects were observed at feed concentrations of 1500 ppm. Increased liver weights were also observed in females at 250 and 50 ppm. Therefore, the NOAEL was established at 50 ppm equivalent to 2 mg/kg bw/day.

CA 5.3.3 Other routes

Racemic dimethenamid, 3-week rabbit dermal limit test

Groups of 5 male and 5 female New Zealand White rabbits were administered dermally 91.6% racemic dimethenamid 6 hours/day, 5 days/week covered by a semi-occlusive dressing at a dose level of 1000 µl/kg bw/day (equivalent to 1190 mg/kg bw/day). The test material was applied undiluted.

Skin edema and erythema were observed in treated animals. This effect reached a maximum during the first week of treatment followed by a gradual recovery which was nearly complete by the end of the study.

No overt clinical signs of systemic toxicity were observed in the study. Body weights were decreased in the treated group during the first week of treatment. However, this change was transient and overall body weight gain for the treatment periods were similar between control and treated group. The slight and transient change in body weight is not considered toxicologically significant. Food consumption was not affected by treatment nor were any alterations of hematology or clinical chemistry parameters observed. There were no treatment-related effects on organ weight and macroscopic findings. Histologically, the only change observed was a minimal to slight hyperkeratosis and acanthosis of the skin with inflammatory cell infiltration.

Conclusion
Toxicologically significant signs of systemic toxicity were not observed in treated animals under the study conditions. The systemic NOAEL was found to be 1190 mg/kg bw/day.

CA 5.4 Genotoxicity Testing

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000 and in the addendum 1 to the monograph of rapporteur member state Germany of July 03, 2001: An extended data-package of in vitro genotoxicity studies in bacterial and mammalian cell systems and of in vivo genotoxicity studies conducted with racemic dimethenamid and Dimethenamid-P is available. These studies as listed in Table 5.4.1-1 below have been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph including addenda.

Based on the available data the following assessment was drawn in the Annex I listing of Dimethenamid-P:

Genotoxicity	Weight of evidence suggests no genotoxic concern

The different types of mutagenicity assays and the test results obtained in the various genotoxicity endpoints are presented in tabular form in Table 5.4.1-2 and Table 5.4.1-3

Dimethenamid-P
Dimethenamid-P was evaluated for its potential genotoxicity *in vitro* using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis test. The mutagenicity tests were negative, with the exception of a single positive result obtained in the Ames Test with *S.typhimurium* strain TA-100 in the absense of an exogenous metabolic activation system. This result could not be reproduced in several repeat assays. The *in vitro* chromosome aberration study gave equivocal test results both in the presence and absence of an exogenous metabolic activation system. However, the result of the corresponding *in vivo* assay for chromosomal aberration, *i.e.* the mouse micronucleus test, gave a clearly negative result, indicating that dimethenamid-P has no chromosome-damaging potential. The results of the toxicokinetic studies [see section MCA 5.01 of this dossier] confirmed that the test compound reached the bone marrow after oral treatment.

Racemic dimethenamid
In addition to the studies mentioned above, additional genotoxicity studies conducted with racemic dimethenamid were submitted for comparative evaluation. The test results obtained in bacterial and mammalian mutagenicity testing were negative. An *in vitro* chromosome aberration assay with racemic dimethenamid was submitted but not performed according to currently accepted guidelines. Three *in vitro* assays for unscheduled DNA synthesis conducted with racemic dimethenamid were submitted. One study gave a positive test result; the other two tests (one of which was not acceptable) gave inconclusive results due to poor experimental design or reporting. An in vivo UDS assay with rats and an in vivo micronucleus test with mice gave negative results.

Overall, the results do not indicate that dimethenamid-P or racemic dimethenamid possess a genotoxic potential.

The database has been extended with an in vitro mouse lymphoma assay to fulfill the new data requirement and with an in vivo micronucleus test in mice in order to demonstrate toxicological equivalence of the proposed specification. Both studies did not provide any evidence for genotoxicity of Dimethenamid-P and thus clearly support the weight of evidence approach that Dimethenamid-P is not genotoxic.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

In vitro studies	Weight of evidence suggests no genotoxic concern
In vivo studies in somatic cells	Weight of evidence suggests no genotoxic concern
In vivo studies in germ cells	No data, not required

CA 5.4.1 *In vitro* studies

A summary of the in vitro genotoxicity studies evaluated for Annex I inclusion of Dimethenamid-P is summarized in Table 5.4.1-1 below.

Table 5.4.1-1: Summary of genotoxicity studies in vitro conducted with Dimethenamid-P and racemic Dimethenamid and evaluated for Annex I inclusion of Dimethenamid-P

Study type	Test System	Test material / Purity	With S-9 mix	Result	Reference
In vitro Mutagenicity in bacterial cells (Ames test)	*Salmonella thyphimurium* (TA 1535, 100, 1537, 98); *Escherichia coli* (WP2 uvrA)	Dimethenamid-P / 93.3% (total dimethenamid), 91.1% (S-isomer)	No Yes	Positive with TA 100 Negative all strains	1996/5403
	Salmonella thyphimurium (TA 1535, 100, 1537, 98); *Escherichia coli* (WP2 uvrA)	Dimethenamid-P / 91.1%	No Yes	Negative Negative	1997/10622
	Salmonella thyphimurium (TA 1535, 100, 1537, 98); *Escherichia coli* (WP2 uvrA)	Dimethenamid-P / 99.4%	No Yes	Negative Negative	1997/10621
	Salmonella thyphimurium (TA 100)	Dimethenamid-P / 91.1%	No	Negative	1997/5271
	Salmonella thyphimurium (TA 1535, 1537, 1538, 98, 100)	Racemic dimethenamid (91.4%)	No Yes	Negative Negative	1989/11032
In vitro Mutagenicity in mammalian cells	CHO/HGPRT	Dimethenamid-P / 96.3% (total dimethenamid technical); 91.1% (S-dimethenamid)	No Yes	Negative Negative	1996/5404
	V79/HGPRT	Racemic dimethenamid / 92%	No Yes	Negative Negative	1986/11167
In vitro Cytogenicity	Chromosome aberration in CHO cells	Dimethenamid-P / 96.3% (total dimethenamid technical); 91.1% (S-dimethenamid)	No Yes	Equivocal Equivocal	1996/5400
In vitro DNA damage and repair	UDS, rat primary hepatocytes	Dimethenamid-P / 96.3% (total dimethenamid technical); 91.1% (S-dimethenamid)	No	Negative	1996/5399
	UDS, rat primary hepatocytes	Racemic dimethenamid / not specified	No	Inconclusive	1986/11169
	UDS, rat primary hepatocytes	Racemic dimethenamid / 91.4%	No	Positive	1989/11033

Additionally a mouse lymphoma assay has been conducted with Dimethenamid-P to fulfill the data-requirements as presented below.

Report:	CA 5.4.1/1 Schulz M.,Landsiedel R., 2013b BAS 656-PH - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK +/- locus assay, microwell version) 2013/1003738
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. B.17, OECD 476 (1997), EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

BAS 656-PH (Batch: COD-001509, Purity: 95.9%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Three independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 400 and 200 µg/mL were used in the main experiments without and with metabolic activation, respectively. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h in all three experiments. After treatment, cells were cultured for an expression period of 48 hours and then cultured in selection medium for another approximately 10 days. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxicity was observed in all experiments with the strongest effects observed at the highest applied concentration. Precipitation at the end of treatment was not observed. Based on the results of the present study, the test substance did not cause any biologically relevant increase in the mutant frequencies either without S9 mix and/or after the addition of a metabolizing system in three independently performed experiments. However, a single statistically significant increase was observed in the 2nd experiment in the presence of S9 mix. This finding occurred at a clearly cytotoxic concentration and it was not corroborated in a confirmatory experiment. Therefore, this finding has to be regarded as biologically irrelevant. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test BAS 656-PH does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1003738)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS 656-PH

Description: Liquid; brown, clear

Lot/Batch #: COD-001509

Purity: 95.9% (tolerance ± 1.0%)

Stability of test compound: Stable in DMSO

The stability of the test substance under storage conditions over the test period was guaranteed until 01 Oct 2013 by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study.

Solvent control: DMSO

Positive control -S9: Methyl methanesulfonate (MMS): 15 µg/mL (4-hour exposure period) and 5 µg/mL (24-hour exposure period)

Positive control +S9: Cyclophosphamide (CPA) 2.5 µg/mL

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3) parts followed by centrifugation at 9000 g.

An appropriate quantity of S9 supernatant was mixed with an equal volume of S9 cofactor solution. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
Phosphate buffer (pH 7.4)	15 mM

4. Test organism: The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 9 - 10 h in stock cultures) and a high cloning efficiency of about 90%. The cells have a stable karyotype with a near diploid number of 40 ± 1 chromosomes. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in “THMG" medium (pretreatment medium A), and for the following 3 days in “THG" medium (pretreatment medium B).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 1% (v/v) penicillin/streptomycin (10 000 IU / 10 000 µg/mL) 1% (v/v) sodium pyruvate (10 mM) (RPMI-0); fetal calf serum was added with respect to the respective treatment conditions: 5% for the 4-hour exposure interval (RPMI-5), 10% for the 24-hour exposure interval (RPMI-10), 20% for cloning efficiency and selection (RPMI-20)

Selection medium: RPMI-20 (complete culture medium) by addition of 4 µg/mL TFT

Pretreatment medium A ("THMG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), methotrexate (0.1 µg/mL), glycine (7.5 µg/mL)

Pretreatment medium B ("THG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), glycine (7.5 µg/mL)

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 11.3 to 2900 µg/mL

b) Mutation assay:

1st experiment: 6.25, 12.5, 25.0, 50.0, 100. 0, 200.0 and 400.0 µg/mL without metabolic activation

3.13, 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 µg/mL with metabolic activation

2nd experiment: 3.13, 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 µg/mL without metabolic activation

4.69, 9.38, 18.75, 37.50, 75.0, 150.0 and 200.0 µg/mL with metabolic activation

A slight increase in the mutation frequency was observed in the 2nd Experiment in the presence of metabolic activation. To corroborate this finding a further experiment was performed designated 3rd Experiment.

3rd experiment: 6.25, 12.5, 25.0, 50.0, 100. 0, 150.0 and 200.0 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Jul-2012 to 16-May-2013

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the purity and the molecular weight of the test substance 2900 µg/mL (approx. 10 mM) BAS 656-PH was used as top concentration both with and without S9 mix at 4 hour exposure time and without S9 mix at 24 hour exposure time. The pretest was performed following the method described for the main experiment. The relative suspension growth (RSG) was determined as toxicity indicator for dose selection and as well as precipitation, pH value and osmolarity.

3. Mutation Assay:

Cell treatment and expression: For each test group, about 1x10^7 cells per flask were seeded into 75 cm^2 flasks. Two cultures were treated in parallel for each test group. Subsequently the treatment medium was added. The cultures were incubated for the respective exposure period. In case of experiments without metabolic activation the treatment medium consisted of 19.8 mL RPMI-5/10 plus 0.2 mL positive control, test substance preparation or vehicle, respectively. In case of metabolic activation the treatment medium consisted of 19 mL RPMI-5/10 medium, 0.2 mL positive control, test substance preparation or vehicle and 0.8 mL S9-mix, respectively. Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity. At the end of the exposure period, the cells were transferred in tubes, centrifuged for 5 minutes at 1000 rpm and resuspended in RPMI-5 medium. The washing of the cells was repeated at least once. Then the cells were centrifuged and resuspended in RPMI-10 medium. From each test group a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) were pipetted in 75 cm² flasks and were incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium.

Selection: For the selection of the mutants, 5 x 10^5 cells from each test group were resuspended in 50 mL selection medium (“TFT" medium; 1 x 10^4 cells/mL). Per test group 200 µL were dispensed in each well of two 96-well plates (2000 cells/well). After incubation for at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF). The viability (cloning efficiency) was determined after the expression period, 2 days after end of exposure. The cells were centrifuged and 400 cells from each test group were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per test group 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well) and evaluated after at least 9 days of incubation. For calculation of the suspension growth (SG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of

4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used.

Size distribution of the colonies: The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.

Calculations:

Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{-\ln(\textit{total number of empty wells / total number of seeded wells}(96))}{\textit{number of seeded cells}(2000)} \, x\, 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_x = \frac{-\ln(\textit{total number of empty wells / total number of seeded wells}(96))}{\textit{number of seeded cells per well}(1.6)} x100$$

relative, in comparison to control:

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative / vehicle control}} x100$$

Relative total growth (RTG):

$$RTG = \frac{RSG \times RCE_2}{100}$$

$$RSG = \frac{\text{Suspension Growth of the test group}}{\text{Suspensiongrowthofthenegativ / vhiclecontrol}} x100$$

4. Statistics:

An appropriate statistical trend test (SAS procedure PROC REG; 9) was performed to assess a possible dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective negative/vehicle control groups. A trend was judged as statistically significant whenever the p-value was below 0.10 and the slope was greater than 0. However, both, biological and statistical significance has been considered together.

5. Evaluation criteria:

The test item is considered mutagenic if all of the following criteria are met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures of one experiment.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls of this study were taken into consideration.
Results of test groups have been rejected if the relative total growth (RTG) and/or the cloning efficiency 1 (CE_1) were less than 10% of the respective negative/vehicle control.
Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose-related shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (BASF study code 01Y0442/08Y014).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 11.3 µg/mL and 2900 µg/mL (equal to a molar concentration of approximately 10 mM) were used. After 4 hours treatment in the absence of S9 mix cytotoxicity indicated by reduced relative suspension growth of about or below 20% was observed at 362.5 µg/mL and above. In addition, in the presence of S9 mix, clearly reduced relative suspension growth was observed after treatment with 90.6 µg/mL and above. After 24 hours treatment in the absence of S9 mix reduced relative suspension growth of below 20% was observed after treatment with 90.6 µg/mL and above. In culture medium test substance precipitation occurred at 725 µg/mL and above after 4 hours treatment in the absence and the presence of S9 mix, and from 1450 µg/mL onward after 24 hours treatment in the absence of S9 mix. There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test item. The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 400.0 and 200.0 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

In this study, in the absence and the presence of S9 mix no precipitation in culture medium was observed up to the highest applied test substance concentration in the main experiments.

In the absence of S9 mix in the 1st experiment there was a strong decrease in the number of colonies at 400 µg/mL after an exposure period of 4 hours. In the 2nd experiment after an exposure period of 24 hours the relative total growth was strongly reduced from 100 µg/mL onward. In both experimental parts the cell densities were distinctly reduced at the highest applied concentrations each. Thus, these test groups were discontinued within the expression period.

In addition, in the presence of metabolic activation, a clear reduction of the relative total growth was observed at 100 µg/mL in the 1st experiment and at 200 µg/mL in the 2nd experiment. In the 3rd experiment, a reduction of the relative total growth was observed at 200 µg/mL. In the 1st and 3rd experiment the cell densities were distinctly reduced at the highest applied concentration of 200 µg/mL each. Thus, these test groups were discontinued within the expression period.

No biologically relevant increase in the number of mutant colonies was observed without S9 mix. In detail, in the 1st experiment after 4 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and clearly within the range of the historical negative control data. In addition, in the 2nd experiment after 24 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and nearby the range of historical negative control data. The corrected mutant frequencies obtained for both experiments were always below the calculated threshold.

In the presence of S9 mix a statistically significant dose-related increase of mutant frequencies was observed in the 1st and 2nd experiment. However, these results were not confirmed in a 3rd experiment. In detail, in the 1st experiment after 4 hours treatment the values for the corrected mutation frequencies were close to the respective vehicle control value and nearby the range of the historical negative control data. In the 2nd experiment the corrected mutant frequencies were clearly increased at higher concentrations showing dose-related cytotoxicity as indicated by relative total growth below 50% of control. The values of the test groups from 37.5 µg/mL onward were either clearly above the respective vehicle control value or clearly above the range of the historical negative control data. At 75 and 200 µg/mL, strong growth depression and mutation frequencies clearly exceeding the mutation frequency threshold were observed. In the confirmatory 3rd experiment in the presence of S9 mix the values for the corrected mutation frequencies were close to the respective vehicle control values and nearby the historical negative control range data. In this experimental part, the highest applied concentration of 200 µg/mL was not scorable for mutation potency due to strong cytotoxicity [see Table 5.4.1-2 and Table 5.4.1-3].
The statistical analyses of all data sets by testing for linear trend led to a negative finding for the 2nd experiment in the absence of S9 mix and for the 3rd experiment in the presence of S9 mix. In the 1st experiment in the absence and presence of S9 mix a statistically significant dose-related increase of mutant frequencies was obtained. However, in this experiment all values were clearly below the respective mutant frequency threshold and, therefore, the statistical finding was regarded as biologically irrelevant. In addition, in the 2nd experiment in the presence of S9 mix the linear trend analyses led to a statistically significant dose-related increase of mutant frequencies. Two values were clearly above the respective mutant frequency threshold. However, this finding occurred at a clearly cytotoxic concentration and it was not corroborated in a confirmatory experiment. Therefore, this finding has to be regarded as biologically irrelevant.

The positive control substances MMS and CPP induced clearly increased mutant frequencies as expected. The values of the corrected mutant frequencies clearly exceeded the respective calculated thresholds for a mutagenic effect based on the global evaluation factor (GEF: 126 plus the mutant frequency of the respective negative control). In addition, the corrected mutant frequencies were clearly within the historical positive control data range.

Table 5.4.1-2: Gene mutation in mammalian cells – experimental parts without S9 mix

				Cytotoxicity		Genotoxicity (colonies per 10^6 cells)	
	Con. µg/mL	S9 mix	Prec.*	Relative cloning efficiency 1 (RCE_1, %)	Relative total growth (RTG, %)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold**
Experiment I / 4 h treatment							
Vehicle control[1]		-	n.d.	100.0	100.0	64	190
Test item	6.25	-	-	99.2	93.7	47	190
Test item	12.50	-	-	95.6	73.3	58	190
Test item	25.00	-	-	101.5	68.2	54	190
Test item	50.00	-	-	103.1	68.0	57	190
Test item	100.00	-	-	90.7	60.0	70	190
Test item	200.00	-	-	77.4	37.9	66	190
Test item	400.00	-	-	2.2	n.c.	n.c.	190
Positive ontrol[2]		-	n.d.	62.2	34.2	1078	190
Experiment II / 24 h treatment							
Vehicle control[1]		-	n.d.	100.0	100.0	36	162
Test item	3.13	-	-	131.5	77.8	51	162
Test item	6.25	-	-	125.7	73.2	42	162
Test item	12.50	-	-	100.8	75.2	39	162
Test item	25.00	-	-	117.3	54.4	49	162
Test item	50.00	-	-	101.5	42.9	50	162
Test item	100.00	-	-	40.3	7.9	69	162
Test item	200.00	-	-	3.5	n.c.	n.c.	162
Positive ontrol[3]		-	n.d.	69.5	43.9	397	162

* : Precipitation in culture medium at the end of exposure period
** : Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle plus 126
n.c. : Culture was not continued due to strong cytotoxicity
1 : DMSO 1% (v/v)
2 : MMS 15.0 µg/mL
3 : MMS 5.0 µg/mL

Table 5.4.1-3: Gene mutation in mammalian cells – experimental parts with S9

				Cytotoxicity		Genotoxicity (colonies per 10^6 cells)	
	Con. µg/mL	S9 mix	Prec.*	Relative cloning efficiency 1 (RCE_1, %)	Relative total growth (RTG, %)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold**
Experiment I / 4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	49	175
Test item	3.13	+	-	124.9	81.0	51	175
Test item	6.25	+	-	99.3	70.1	46	175
Test item	12.50	+	-	103.0	55.4	59	175
Test item	25.00	+	-	96.4	34.0	53	175
Test item	50.00	+	-	92.3	21.7	99	175
Test item	100.00	+	-	85.9	15.4	97	175
Test item	200.00	+	-	59.7	n.c.	n.c.	175
Positive ontrol[2]		+	n.d.	82.9	37.1	499	175
Experiment II / 4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	59	185
Test item	4.69	+	-	105.3	109.1	66	185
Test item	9.38	+	-	101.5	82.5	95	185
Test item	18.75	+	-	84.8	78.7	74	185
Test item	37.50	+	-	61.1	43.4	140	185
Test item	75.00	+	-	56.7	32.0	191	185
Test item	150.00	+	-	56.7	26.2	156	185
Test item	200.00	+	-	45.0	14.1	270	185
Positive ontrol[2]		+	n.d.	48.7	28.1	922	185
Experiment III / 4 h treatment							
Vehicle control[1]		+	n.d.	100.0	100.0	61	187
Test item	6.25	+	-	100.8	83.8	77	187
Test item	12.50	+	-	101.6	81.9	70	187
Test item	25.00	+	-	89.8	64.0	68	187
Test item	50.00	+	-	86.5	49.3	84	187
Test item	100.00	+	-	79.3	45.1	118	187
Test item	150.00	+	-	65.4	28.1	75	187
Test item	200.00	+	-	41.6	n.c.	n.c.	187
Positive ontrol[2]		+	n.d.	61.8	48.4	685	187

* : Precipitation in culture medium at the end of exposure period
** : Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle plus 126
n.c. : Culture was not continued due to strong cytotoxicity
1 : DMSO 1% (v/v)
2 : CPP 2.5 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test BAS 656-PH does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

CA 5.4.2 *In vivo* studies in somatic cells

Table 5.4.2-1: Summary of genotoxicity studies in vivo conducted with Dimethenamid-P and racemic Dimethenamid and evaluated for Annex I inclusion of Dimethenamid-P

Study type	Test System	Test material / Purity	With S-9 mix	Result	Reference
In vivo Clastogenicity	Mouse Micronucleus test (103 – 205 – 410 mg/kg bw (i.p. injection)	Dimethenamid-P / 96.3% (total dimethenamid technical); 91.1% (S-dimethenamid)	N.A.	Negative	1996/5401
	Mouse micronucleus test (1000 mg/kg bw, oral gavage)	Racemic dimethenamid / not specified	N.A.	Negative	1986/11168
	Mouse micronucleus test (710 mg/kg bw/d, 2 d, oral gavage)	Racemic dimethenamid / 97.6%	N.A.	Negative	1993/11758
	In vivo UDS, rat primary hepatocytes (500 and 158 mg/kg bw, oral gavage)	Racemic dimethenamid / 97.6%	N.A.	Negative	1993/11757

Additionally an in vivo mouse micronucleus has been conducted with Dimethenamid-P according to current guidelines to demonstrate toxicological equivalence of the proposed specification.

Report: CA 5.4.2/1
[redacted] 2014e
BAS 656-PH - Micronucleus assay in bone marrow cells of the mouse
2014/1038343

Guidelines: OECD 474 (1997), (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.12, EPA 870.5395, EPA 712-C-98-226

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.4.2/2
Grauert E.,Kamp H., 2014b
Analytical report - BAS 656-PH Concentration control analyses in corn oil
2014/1104188

Guidelines:

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

BAS 656 PH (Dimethenamid-P; batch: 0258B01BH, purity: 97.6%) was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. Based on the result of a pre-tests, the test substance dissolved in corn oil was administered once orally to groups of 7 male mice at dose levels of 125, 250, and 500 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 animals/group). The animals were sacrificed 24 or 48 hours (additional high dose and vehicle group) after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei (6000 in one animal of the 125 mg/kg bw dose group (24 h treatment)). The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.
Oral administration of the test substance did not lead to any biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used. The rate of micronuclei were below the value of the negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Clinical signs were noted only on the application day and comprised ruffled fur, reduction of spontaneous activity, abdominal position, eyelid closure, hunchback, whitely lacrimation, salivation, excitement and hyperemia at ≥125 mg/kg bw as well as rapid breathing at 500 mg/kg bw. The positive control led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.
According to the results of the study the test substance BAS 656 PH did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and is therefore considered non-mutagenic.

(BASF DocID 2014/1038343)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	BAS 656 PH (Dimethenamid-P)
Description:	Liquid, brown
Lot/Batch #:	0258B01BH
Purity:	97.6%
Stability of test compound:	stable
Solvent used:	Corn oil

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	Corn oil
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw

3. Test animals:

Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks
Weight at dosing:	mean value 35.4 g (SD ± 1.6)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound concentration:

Range finding test:	500, 1000 mg/kg bw
Micronucleus assay:	125, 250 and 500 mg/kg bw (doses were corrected for purity with a correction factor of 1.02. Test item dose levels as is were 127.5, 255 and 510 mg/kg bw) The test substance was administered once orally using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 06-Feb-2014 to 05-Mar-2014

2. Preliminary cytotoxicity assay:

Male and female NMRI mice were administered the test substance once by oral administration at a dose of 500 and 1000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling: Groups of male mice were treated once with either the vehicle, positive control substance or 125, 250 and 500 mg test substance / kg bw by oral administration. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study.

Twenty-four or 48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet re-suspended.

Slide preparation: A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide evaluation: In general, 2000 (6000 in one animal of the 125 mg/kg bw dose group (24 h treatment)) 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 and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

5. Evaluation criteria:

A test item is considered as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y0442/08Y018).

B. PRELIMINARY RANGE FINDING TEST

In the 1000 mg/kg bw dose group reduction of spontaneous activity, abdominal position, eyelid closure, tremor, shortness of breath, hunchback, salivation, uncoordinated movements and whitely lacrimation were observed within one hour after application. Due to the severe clinical signs, all animals were euthanized one hour after application. In the 500 mg/kg bw dose group, reduction of spontaneous activity, eyelid closure, excitement, rapid breathing, hunchback, whitely lacrimation, salivation, incontinence and hyperemia were observed in animals of both sexes starting after application of the test substance until hour 6 post-application. No animals died. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Clinical signs were noted only on the application day and comprised ruffled fur, reduction of spontaneous activity, abdominal position, eyelid closure, hunchback, whitely lacrimation, salivation, excitement and hyperemia at ≥125 mg/kg bw as well as rapid breathing at 500 mg/kg bw.

After treatment with the test item at 24h and 48h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range [see Table 5.4.2-2].

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.25%), thereby demonstrating the sensitivity of the test system.

Table 5.4.2-2: Micronucleus test in mice administered BAS 656 PH

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Corn oil	24	0.190	2-6	1250
BAS 656 PH				
125 mg/kg bw	24	0.171	1-6	1231
250 mg/kg bw	24	0.186	1-6	1277
500 mg/kg bw	24	0.171	1-7	1219
Positive control				
Cyclophosphamide	24	2.250	23-61	1186
48 h sampling				
Corn oil	48	0.110	1-5	1194
BAS 656 PH				
500 mg/kg bw	48	0.093	0-4	1187

III. CONCLUSION

Based on the results of this study, BAS 656 PH does not induce the formation of micronuclei in mouse polychromatic erythrocytes under in vivo conditions.

CA 5.4.3 *In vivo* studies in germ cells

Not a data requirement

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: Chronic toxicity and oncogenicity studies were only conducted with racemic dimethenamid. Long-term toxicity feeding studies (94 - 104 weeks) are available from two different species (rats, mices) for racemic dimethenamid. These studies have been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.5-1: Summary of long-term toxicity/carcinogenicity studies conducted with and racemic dimethenamid

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference and year
Racemic dimethenamid 104-week, diet, Sprague-Dawley rat (diet concentrations: 0, 100, 700 and 1500 ppm)	M: 5, 36 and 80 F: 7, 49 and 109	5	36	Systemic toxicity: 1500 ppm: decreased food consumption and bw gain, lenticular opacities; increased serum γ-GGT (m), cholesterol (f) and urinary ketones (m); increased rel. liver wt (f), epithelial hyperplasia of the stomach (m), altered eosinophilic hepatocytes (m), bile duct hyperplasia (f), cystically dilated bile ducts (f), hyperplasia of parathyroid (m) 700 ppm: decreased food consumption and bw gain (f); increased rel. liver wt; bile duct hyperplasia (f), hyperplasia of parathyroid (m) Oncogenicity: no evidence of carcinogenictity	1990/1117 1993/11798
Racemic dimethenamid 94-week, diet, CD-1 mice (diet concentrations: 0, 30, 300, 1500 and 3000 ppm)	M: 3.8, 41, 205 and 431 F: 4.1, 40, 200 and 411	40	200	Systemic toxicity: ≥1500 ppm: reduced bw gain, increased rel. liver wt and rel. kidney wt (f) and enlarged hepatocytes 3000 ppm: increased incidence of stomach hyperkeratosis Oncogenicity: no evidence of carcinogenictity	1990/11139

The results of a 2-yr chronic/oncogenicity study in rats indicated that a maximum tolerated dose was clearly met at the high dose of 1500 ppm (ca. 80 mg/kg bw/d males; 109 mg/kg bw/d females). This is demonstrated by a body weight gain depression for the first 80 wk of treatment in males and females. The liver was a target organ for dimethenamid in the rat. Observations included an increase in serum γ-glutamyltransferase (γ-GGT) and cholesterol, an increase in liver weight and liver pathology including altered eosinophilic hepatocytes, bile duct hyperplasia and cystically dilated bile ducts. Other effects noted in high dose males were an increase in epithelial hyperplasia of the limiting ridge of the stomach and hyperplasia in the parathyroid. There was no evidence of a treatment-related increase in neoplasms.

A carcinogenicity study in mice was conducted up to 3000 ppm, which represented the maximum tolerated dose as evidenced by significant body weight gain depression. As with the rat, the liver was the apparent target organ in mice. Liver weights were increased and hepatocyte enlargement was observed at the 2 highest dose levels. An additional finding in mice was hyperkeratosis of the limiting ridge of the stomach. There was no evidence of a treatment-related increase in neoplasms.

In summary, long-term feeding studies with racemic dimethenamid in rats and mice demonstrated that the primary target organ was the liver. No evidence of a carcinogenic potential could be established.

Based on the available studies, the following endpoints were determined in the Annex I listing of Dimethenamid-P:

Target/critical effect:	Liver
Lowest relevant NOAEL	104-wks, rat: 5 mg/kg bw/d (Racemic dimethenamid)
Carcinogenicity:	No evidence of carcinogenicity

There are no new studies available with either racemic dimethenamid or dimethenamid-P that could affect the overall evaluation for long-term toxicity and carcinogenicity. Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Target/critical effect	Liver (weight increases, biochemical and histopathological changes), decreased body weight gain No classification required
Lowest relevant NOAEL / NOEL	105-week, rat: 100 ppm (5 mg/kg bw/d) *
Carcinogenicity	No evidence for carcinogenicity* No classification required

* based on studies performed with racemic dimethenamid

The ECHA risk assessment committee on classification and labelling has evaluated the data and concluded that no classification for systemic toxicity or carcinogenicity is warranted. Based on this proposal the EU commission has prepared a draft list entry [Follow up to the 13th meeting of competent authorities for REACH and CLP (CARACAL) 26-27-28 November 2013, Centre A. Borschetted, Brussels, Belgium of 12 February 2014] for inclusion into the 7th ATP to the CLP Regulation 1272/2008. Entry into force is expected by end of 2014 or beginning of 2015.

For convenience of the reviewer brief summaries the respective studies as extracted from the monograph of are provided below.

Racemic dimethenamid, Rat 104-week oral study

Racemic dimethenamid (batch No. 8605; purity: 91.3%) was administered to groups of 70 male and 70 female Sprague-Dawley rats at dietary concentrations of 0, 100, 700 and 1500 ppm. 50 animals/sex/group were treated for 24 mo. Satellite animals of 20/sex/group were used in the chronic toxicity evaluations and sacrificed after 12 months of treatment.

Survival rates were below 50% for male controls and for low-dose group males and females.
The maximum tolerated dose (MTD) was clearly met at the high dose in both sexes as demonstrated by a body weight gain depression of 16% in males and 31% in females (week 104). A body weight gain decrease (13%) was also observed in females at 700 ppm (week 0- 10 and 10-80). The liver was a target organ for dimethenamid in the rat. Observations included an increase in serum γ-glutamyltransferase and cholesterol, an increase in liver weight and liver pathology including altered eosinophilic hepatocytes, bile duct hyperplasia and cystically dilated bile ducts. Other effects noted in high dose males were an increase in epithelial hyperplasia of the limiting ridge of the stomach and hyperplasia in the parathyroid. The mid dose of 700 ppm produced body weight gain decreases and liver alterations in females. A slight increase in liver tumors was noted at the high dose. The incidence of carcinomas was not statistically different from controls and was within historical control range. The incidence of adenomas was also not statistically different from controls but was just slightly outside of historical control range at the conducting laboratory. The slight increase in adenomas was most likely due to a considerably increased survival at the high dose compared to control. The increased survival allowed for more old age animals to develop the spontaneously occurring adenoma which increases in incidence with age. In addition, the incidence for dimethenamid in high dose males was well within the historical control range for Sprague-Dawley rats as compiled by the Registry of Industry Toxicology Animals (RITA).
The original report indicated a slight increase in ovarian tubular adenomas. In view of the borderline nature of the ovarian findings, and of recent advances in diagnostic criteria for rodent ovarian neoplasia, a pathology peer review was conducted by two reviewers following the issue of the final report. The final analysis demonstrates that there is no statistical or biologically significant evidence to indicate that dimethenamid causes ovarian tumors. The incidence at the high dose is within historical control range, and the difference in incidence from control is not statistically significant. Sertoliform tubular hyperplasia and adenoma are mainly found in Sprague-Dawley rat. These lesions are rarely found in other strains of rat, and are not found in man or domestic animals. They have therefore only very limited relevance for man.

Conclusion:
The maximum tolerated dose (MTD) was clearly met at the high dose of 1500 ppm as evidenced by significant body weight gain depression and liver alterations in both sexes. Histopathological changes were noted at the high dose in the liver, stomach and parathyroid. The mid dose of 700 ppm produced body weight gain decreases and liver alterations in females. Dimethenamid did not produce a carcinogenic response. The NOAEL was found to be 100 ppm (ca. 5 mg/kg bw/d).

Racemic dimethenamid, Mice 94-week oral study

Racemic dimethenamid (batch No. 8605; purity: 91.4%) was administered to groups of 52 male and female CD-1 mice at dietary concentrations of 0, 30, 300, 1500 and 3000 ppm for 94 wks. Satellite groups of 16 animals/sex received 0 or 3000 ppm dimethenamid for 65 wk.

There were no adverse treatment-related effects on survival or clinical observations. Body weight changes were reduced in males and females at 1500 and 3000 ppm. The body weight change impairment at the high dose demonstrated that a Maximum Tolerated Dose (MTD) was attained.

Relative liver weights were increased in females by 25% at 1500 ppm and by 18% at 3000 ppm. Kidney weights were also increased in females at 1500 and 3000 ppm, but considering the lack of any histopathological findings in the kidney, the toxicological significance of this finding is equivocal. There was no other organ weight changes considered related to treatment.

Effects on the liver were also noted with histopathology. The incidence of enlarged hepatocytes was increased in a dose-related manner at doses of 300 to 3000 ppm. However, at 300 ppm, enlarged hepatocytes were observed in only 1 male and 2 females and the severity was only minimal. The minimal enlargement of hepatocytes in the absence of any other toxicity at this dose is not considered an adverse effect. Also, the incidence of hyperkeratosis of the limiting ridge of the stomach was increased at the high dose, but only minimally at the interim sacrifice. By the terminal sacrifice, this effect was not noted indicating a recovery had occurred. This effect may have been due to an irritating effect of the chemical.

There were no test substance-related findings at 30 ppm. In addition, there was no evidence that dimethenamid caused a treatment-related increase in tumors at any dose level.

Conclusion:
The NOAEL was found to be 300 ppm (ca. 40 mg/kg bw/d). There was no evidence that dimethenamid produced a carcinogenic effect in mice.

CA 5.6 Reproductive Toxicity

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: A two-generation study is available for racemic dimethenamid in rats. Furthermore, prenatal toxicity studies in rats are available for both dimethenamid-P and racemic dimethenamid, and a prenatal toxicity study in rabbits with racemic dimethenamid. These studies have been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.6-1: Summary of reproduction toxicity studies conducted with and racemic dimethenamid and dimethenamid-P

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference and year
Racemic dimethenamid 2-Generation, oral, feed Wistar rats (constant diet concentrations: 0, 100, 500 and 2000 ppm)	7. 5, 37.5 and 151 (average without lactation values)	Parental toxicity: ca. 50 Developmental toxicity: ca. 50 Reproduction: ca.150	Parental toxicity: ca. 150	Parental toxicity: 2000 ppm: decreased food intake and bw gain (m), increased liver wt Developmental toxicity: 2000 ppm: decreased bw gain during lactation	1990/11140
Dimethenamid-P Developmental toxicity, gavage, Sprague-Dawley rats	0, 25, 150 and 300 mg/kg bw/d	Maternal toxicity: <25 Developmental toxicity: 25	Maternal toxicity: 25	Maternal toxicity: 300 mg/kg bw/d: decreased bw gain and food consumption; clinical signs, increased liver wt 150 mg/kg bw/d: decreased bw gain and food consumption 25 mg/kg bw/d: decreased body weight gain and food consumption Developmental toxicity: ≥150 mg/kg bw/d: slightly lower fetal body weights, increased delayed skeletal ossifications (considered spurious)	1997/5274
Racemic dimethenamid Developmental toxicity, gavage, CD rats	0, 50, 215 and 425 mg/kg bw/d	Maternal toxicity: 50 Developmental toxicity: 50	Maternal toxicity: 215	Maternal toxicity: ≥ 215 mg/kg bw/d: decrased bw gain and feed consumption, clinical signs, increased liver wt Developmental toxicity: ≥ 215 mg/kg bw/d: increased early resorptions 425 mg/kg bw/d: decreased live litter size	1987/11225
Racemic dimethenamid, Developmental toxicity, gavage, New-Zealand-White rabbits	0, 37.5, 75 and 150 mg/kg bw/d	Maternal toxicity: 37.5 Developmental toxicity: 75	Maternal toxicity: 75	Maternal toxicity: ≥ 75 mg/kg bw/d: decreased bw gain, clinical signs 150 mg/kg bw/d: decreased food intake, bw loss Developmental toxicity: 150 mg/kg bw/d: abortions in 2 animals	1988/11376

Reproductive function was not affected in the 2-generation study, so the NOAEL for reproductive function is the highest dose tested (2000 ppm, ca. 150 mg/kg bw/d). The NOAEL concerning systemic toxicity for the parental animals in the 2-generation study was 500 ppm (ca. 50 mg/kg bw/d). The only pup effect noted was a decreased body weight gain during lactation at the high dose. The NOAEL for developmental toxicity in the F1 and F2 litters was 500 ppm (ca. 50 mg/kg bw/d).

In the prenatal toxicity study in rats using dimethenamid-P, developmental toxicity was observed at the two highest doses tested (150 and 300 mg/kg bw/d). The only treatment-related developmental effects were reduced fetal weights. The NOAEL for developmental toxicity was 25 mg/kg bw/d. Maternal toxicity was observed in all dose groups characterized by decreased body weight gain, and food consumption in all dose levels and clinical signs and liver weight increases in the high 300 mg/kg bw/d dose group. The NOAEL for maternal toxicity was < 25 mg/kg bw/d.

In the prenatal toxicity study in rats using racemic dimethenamid maternal toxicity and developmental toxicity were observed at the two highest doses tested (215 and 425 mg/kg bw/d). Therefore, the NOAEL for maternal and developmental toxicity is 50 mg/kg bw/d. the NOAEL`s for maternal toxicity and developmental toxicity were 50 mg/kg bw/d.

In the rabbit prenatal toxicity study, significant maternal toxicity (reduced food consumption, body weight loss, clinical signs) was observed at the high dose and less severe effects were noted at the mid dose. Abortions in 2 high-dose animals were considered treatment-related, but attributed to the marked maternal toxicity and the thus induced animal stress, rabbits are well-known to be susceptible for. The NOAEL for maternal toxicity was 37.5 mg/kg bw/d and the developmental toxicity NOAEL was 75 mg/kg bw/d.

The lowest NOAEL for developmental toxicity was 25 mg/kg bw/d (rat prenatal toxicity study, dimethenamid-p). Given the dose spacing in that study and the only slight effects at the next 6-fold higher dose level of 150 mg/kg bw/d and taking into consideration the results of the other rat studies conducted with racemic dimethenamid and overall NOAEL in the rat of 50 mg/kg bw/day was derived.

Based on the available data, the following endpoints were determined during the last Annex I listing of dimethenamid-P:

Target / critical effect - Reproduction:	Pup body weight gain reduced during lactation at parental toxic dose level
Lowest relevant reproductive NOAEL / NOEL:	Rat: 50 mg/kg bw/d (racemic dimethenamid)
Target / critical effect - Developmental toxicity:	Fetal body weight slightly decreased at maternal toxic dose level
Lowest relevant developmental NOAEL / NOEL:	Rat: 25 mg/kg bw/day (Dimethenamid-P)

There are no new studies available with either racemic dimethenamid or dimethenamid-P that could affect the overall evaluation for reproduction and developmental toxicity. Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Reproductive toxicity (SANCO/11802 data point 5.6)

Reproduction target / critical effect	No effects on reproduction **No classification required**
Lowest relevant reproductive NOAEL / NOEL	**1500 ppm (150 mg/kg bw/d)***
Developmental target / critical effect	Pup body weight gain reduced during lactation at parental toxic dose level, Early resorptions increased at maternal toxic dose level, not teratogenic **No classification required**
Lowest relevant developmental NOAEL / NOEL	Overall NOAEL Rat: 50 mg/kg bw/d**

* based on studies performed with racemic dimethenamid

** based on studies performed with racemic dimethenamid and dimethenamid-P

The ECHA risk assessment committee on classification and labelling has evaluated the data and concluded that no classification for reproduction toxicity or developmental toxicity is warranted. Based on this proposal the EU commission has prepared a draft list entry [Follow up to the 13th meeting of competent authorities for REACH and CLP (CARACAL) 26-27-28 November 2013, Centre A. Borschetted, Brussels, Belgium of 12 February 2014] for inclusion into the 7th ATP to the CLP Regulation 1272/2008. Entry into force is expected by end of 2014 or beginning of 2015.

For convenience of the reviewer brief summaries the respective studies as extracted from the monograph of are provided under the respective chapters.

CA 5.6.1 Generational studies

Racemic dimethenamid, 2-generation study, rat

Dimethenamid (batch No. 8710; purity: 92.6%) was administered to groups of 25 male and 25 female sexually immature Wistar rats (F0 parental generation) in the diet at concentrations of 0; 100; 500 or 2000 ppm. 10 weeks after the beginning of treatment, F0 animals were mated to produce a litter (F1). Groups of 25 males and 25 females selected from F1 pups as the F1 parental generation were offered diets containing 0; 100; 500 and 2000 ppm of the test substance post weaning for 101 d, and the breeding program was repeated to produce an F2 litter. The study was terminated with the sacrifice of the F2 weanlings and F1 adult animals.
There were no effects due to treatment on any reproductive parameter in the parent animals of the F0 and F1 generation. Clear signs of general, systemic toxicity occurred in both parental generations at 2000 ppm. Toxicity was characterized by decreased food consumption and increased liver weight in both sexes and impaired body weight gain in males. At 500 ppm the increase of liver weight was very slight (F0 males 4%, females 10%; F1 males 3%, females 4%), and therefore considered not to represent an adverse effect. There were no effects on F1 and F2 pup survival. At 2000 ppm, pup body weight gains were reduced during the lactation period for both the F1 and F2 generations. There was no effect on pup body weights at 500 or 100 ppm.

Conclusion:
There were no adverse effects on reproductive parameters of the parental animals at any dose level. Clear signs of general, systemic toxicity occurred in both parental generations at 2000 ppm. The only substance-related effect on pups was a decreased pup weight gain during lactation at 2000 ppm. Therefore, the NOAEL for reproductive function is 2000 ppm (about 150 mg/kg bw/d). The NOAEL for parental systemic toxicity and developmental toxicity is 500 ppm (about 50 mg/kg bw/d).

CA 5.6.2 Developmental toxicity studies

Dimethenamid-P, developmental toxicity, rat

Dimethenamid-P (batch No. 6663-50-1; purity: total dimethenamid: 96.3%; S-dimethenamid: 91.1%) was tested for its prenatal toxicity in Sprague-Dawley rats. The test substance or the vehicle was administered to 25 pregnant female rats/group by stomach tube at dosages of 0, 25, 150 and 300 mg/kg bw on days 6–15 *post coitum* (p.c.).
No mortalities, abortions or premature deliveries occurred during the study. The 300 mg/kg bw/d group had increased incidences of excess lacrimation, piloerection, excess salivation, decreased motor activity, orange substance on fur, swollen ocular membrane, ptosis, dark pink skin, urine-stained abdominal fur and coldness to touch. There were no signs of clinical toxicity related to treatment observed in mid or low dose animals. Body weight gains and feed consumption were reduced in all treatment groups. Relative liver weights were increased at 300 mg/kg bw/d. There were no necropsy observations considered treatment-related. There were no treatment-related effects on pre- or postimplantation loss, on the number of resorptions or number of viable fetuses, or on the sex distribution of fetuses. Mean fetal weight was slightly reduced at 300 mg/kg bw/d (-3%) and 150 mg/kg bw/d (-2%) compared to the control value (statistically not significant). No treatment-related findings occurred in relation to external, soft tissue or skeletal malformations. Distended ureters were seen in 7 high dose fetuses in 3 litters compared to 3 control group fetuses in 2 litters. Because the litter incidence did not differ significantly from control, this increase was not considered treatment related. At 300 and 150 mg/kg bw/d there was an increase in incidence of 2 retarded ossifications, sternal centra and pelvic pubes. Further evaluation of the delayed ossifications indicated that these differences were spurious, primarily due to unusually low control values, and not related to treatment.

Conclusion:
The administration of dimethenamid-P to pregnant Sprague-Dawley rats during organogenesis produced distinct signs of maternal toxicity at the high and mid dose of 300 and 150 mg/kg bw/d. Slight fetal weight decreases were observed at 150 and 300 mg/kg bw/d. The only differences noted from control at 25 mg/kg bw/d were a slight and transient decrease in maternal body weight gain and reduced food consumption during the first three days of treatment. Therefore, the NOAEL for maternal toxicity is <25 mg/kg bw/d and the NOAEL for developmental toxicity is 25 mg/kg bw/d.

Racemic dimethenamid, developmental toxicity, rat

The teratogenic potential and developmental toxicity of racemic dimethenamid (batch and purity not specified) was studied in CD rats. Groups of 25 pregnant female rats were administered the test substance as an aqueous suspension or the vehicle at dose levels of 0, 50, 215 and 425 mg/kg bw/day by gavage from days 6–15 of gestation.
Two control dams died during the study. No other deaths or abortions occurred in the dams during the study. Clinical signs which occurred included excess salivation at 425 and 215 mg/kg bw/d and urine-stained abdominal fur at 425 mg/kg bw/d. Body weight loss was confined to dams at 425 mg/kg bw/day during the first 3 days of treatment, reduced body weight gain also occurred in dams at 215 mg/kg bw/d during this time period. Body weight development continued to be delayed for high-dose group rats during days 9–12 p.c. but was no longer observed during days 12–16 p.c. or thereafter. Based on these early effects on body weight, significant decreases in body weight gain were observed for the overall treatment period (-16% at 215 mg/kg bw/day and -35% at 425 mg/kg bw/day). A slight decrease in body weight gain (11%) was noted in 50 mg/kg bw/day dams during the first 3 days of treatment. This was transient in that no differences in body weight were noted on subsequent days of treatment. However, this resulted in a 9% decrease in body weight gain during the overall treatment period. Because the effect was slight and transient, this difference is not considered to be of toxicological significance. Relative feed consumption was significantly reduced in the mid dose group during the first three days of treatment, and in the high dose group from days 6-12. Liver weights were found to be significantly increased in mid- and high-dose group rats. Relative liver weight was statistically significantly increased in dams at 50, 215 and 425 mg/kg bw/d by 6, 8 and 19%, respectively.
While the resorption incidence at 50 mg/kg bw/d did not appear to be affected by treatment, a small dose-related increase in early resorptions was observed in groups administered 215 and 425 mg/kg bw/d, which in the high-dose group resulted in a minimal decrease in the average live litter size. Neither of these observations were significantly different from concurrent control values upon statistical data analysis. However, based on historical control data, the increased incidences of early resorptions observed at 215 and 425 mg/kg bw/d are regarded to be related to treatment. At doses of 215 and 425 mg/kg bw/d, a small dose-dependent increase in the average percentage of resorbed conceptuses per litter was observed. Although not statistically significant, the high-group value exceeded the historical control range. No other Caesarean delivery parameter was affected.
Administration of the test substance to the dams, as compared with the vehicle, did not affect the incidence of fetuses with alterations or the percentage of fetuses with alterations per litter. Difference among the four groups were neither dose-dependent nor statistically significant. Fetal body weights were marginally decreased at 215 (-1%) and at 425 (-2%) mg/kg bw/d and not considered to be of toxicologically relevance. The same effect was observed with the p isomer (see above). Fetal sex ratio was unaffected by treatment. At the high dose, 2 fetuses in 2 litters had incompletely ossified manubria. This small incidence was not considered related to treatment. There were no other increased incidences of fetal gross, soft tissue or skeletal variations or malformations.

Conclusion
Maternal toxicity was observed at the 425 and 215 mg/kg bw/d dose levels. Marginal fetal body weight decreases were observed at 215 and 425 mg/kg bw/day. An increase in early resorptions occurred at the high dose and to a lesser extent at the mid dose. Slight and transient decreases in body weight gain and food consumption during the first three days of treatment at 50 mg/kg bw/d were considered not to be of toxicological significance. Therefore, the NOAEL for maternal and developmental toxicity is 50 mg/kg bw/day bw. There were no teratogenic effects observed which were considered treatment-related.

Racemic dimethenamid, developmental toxicity, rabbit

Racemic dimethenamid (batch No. 8605; purity: 92%) was tested for its prenatal toxicity in New Zealand White rabbits. The test substance was combined with equal amounts of HiSil and suspended in aqueous 0.5% carboxylmethyl-cellulose. 20 pregnant female rabbits/group were administered the test substance or vehicle by stomach tube at doses of 0, 37.5, 75 and 150 mg/kg bw/d on days 6–18 post insemination (p.i.).
At the high dose, 2 animals aborted and this is considered a treatment-related effect. Clinical signs considered related to treatment were localized alopecia at the high dose and reduced feces at the mid and high doses. Maternal feed consumption was reduced in the middle and high dose animals especially during the second half of the treatment period (days 12-19). Statistically significant reductions in feed consumption were obtained only in the high-dose group when based on body weight. During the treatment period, a body weight loss occurred in high dose animals and body weight gain was reduced at the mid dose. There were no gross pathological findings which were related to treatment. There were no treatment-related effects on implantation, live litter size, fetal sex ratio or fetal body weight. Likewise, there were no effects on external, soft tissue or skeletal variations or malformations.

Conclusion:
Racemic dimethenamid produced clear signs of maternal toxicity at 150 mg/kg bw/d and less severe signs of toxicity also at the mid dose of 75 mg/kg bw/d. Although two abortions occurred in the high-dose group, this finding must be seen in connection with the accompanied clear maternal toxicity, especially for rabbits. Therefore, the no observed adverse effect level (NOAEL) for maternal toxicity was 37.5 mg/kg bw/d, and the developmental toxicity NOAEL was 75 mg/kg bw/d for New Zealand White rabbits.

CA 5.7 Neurotoxicity Studies

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: There were not studies available and no studies considered necessary for dimethenamid-P as the data package on acute studies in rats and subchronic and chronic exposure studies in three species conducted with either dimethenamid-P or racemic dimethenamid gave no evidence of a neurotoxic effect. A study on delayed neurotoxicity in hens being only required for organophosphorous or carbamate compounds was also not considered warranted as neither dimethenamid nor any of the metabolites are belonging to these chemical classes.

Thus, the following conclusion was given in the list of endpoints for Annex I listing of Dimethenamid-P:

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

	No data submitted, no concern from other studies

In order to satisfy an US EPA requirement an acute neurotoxicity study and a 90-day neurotoxicity study were conducted and are thus now presented as supplementary information. The NOAELs for neurotoxicity in these studies are summarized in see **Table 5.7-1**.

Table 5.7-1: Summary of neurotoxicity studies with dimethenamid-P

Study Dose levels (Batch / purity)		NOAEL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Adverse effects at LOAEL	Reference
Acute neurotoxicity study Rat (Wistar, Crl:WI(Han), 0, 60, 200 and 600 mg/kg bw/d (COD-001509 / 95.9%)	M	600	not obtained	no signs of neurotoxicity observed	2013/1028330
	F	600	not obtained	no signs of neurotoxicity observed	
Subchronic (90-day) neurotoxicity study Rat (Wistar, Crl:WI(Han), 0, 300, 1000 and 4500 ppm (COD-001509 / 95.9%)	M	323 (4500)	not obtained	no signs of neurotoxicity observed	2013/1165818
	F	390 (4500)	not obtained	no signs of neurotoxicity observed	

In the acute neurotoxicity single animals (1 or 2 out of 10) of all male dose groups showed strongly contracted pupils under incidence of light There was however no dose-response relationship and it was not seen in females who showed treatment related toxicity while no other toxicity effects were noticed in males. The effect was noted as a transient effect only on the day of administration. Additionally, no treatment-related neuropathological findings were determined, i.e. no brain weight changes or neurohistopathological findings were observed. Therefore, the observed pupillary reaction under light was considered to represent a transient neuropharmacological effect rather than to be indicative for adverse neurotoxicity.
The observed systemic toxicity in females at 600 mg/kg bw/day is in line with the toxicity observed in other acute oral studies in rats conducted with either dimethenamid-P or racemic dimethenamid.

Oral administration of dimethenamid-P to rats over 3 month revealed no adverse neurobehavioral effects and did not show any alterations in neuropathology investigations. Effects indicating a certain level of systemic toxicity were given at least for the high concentration of 4500 ppm, i.e. impairment of body weight development in male and female Wistar rats accompanied by an increase of liver (males and females) and kidney (males only) weights and were in line with findings in other repeated dose administrations of either dimethenamid-P or racemic dimethenamid to rats.

Based on these studies the conclusion for relevant endpoints for the current re-registration is as follows:

Neurotoxicity / Delayed neurotoxicity (SANCO/11802 data point 5.7)

Acute neurotoxicity	Not neurotoxic **No classification required**
Subchronic neurotoxicity	Not neurotoxic **No classification required**

CA 5.7.1 Neurotoxicity studies in rodents

Report:	CA 5.7.1/1 [redacted] 2013a BAS 656-PH: Acute oral neurotoxicity study in Wistar rats - Administration via gavage 2013/1028330
Guidelines:	OECD 424, EPA 870.6200, (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 acute neurotoxicity of Dimethenamid-P (Batch COD-001509, Purity: 95.9%) was investigated in groups of 10 male and female Wistar rats (Crl:WI(Han) after a single administration by gavage at dose levels of 0, 60, 200, and 600 mg/kg bw/d.

Signs of general systemic toxicity changes were observed in the high dose group female animals on the day of application during home-cage observations and open field observations. Clinical signs comprised of piloerection, half-closed and/or permanently closed eyelids, clear lacrimation, slight salivation, reddish discharge of the nose, accelerated or irregular respiration, reduced exploration of the area, unsteady gait, slight tremors and reduced rearing. There was no indication of general systemic toxicity in males. No treatment-related effects on these parameters were observed on study days 7 and 14.

On the day of application strongly contracted pupils under incidence of light (pupillary reflex were note as an isolated finding in individual male animals (2 high-dose, 2 mid-dose and 1 low-dose animal out of 10) considered to be possibly treatment-related. There was however no dose-response relationship and it was not seen in females who showed treatment related toxicity while no other toxicity effects were noticed in males. The effect was noted as a transient effect only on the day of administration. Additionally, no treatment-related neuropathological findings were noted, i.e. no brain weight changes or neurohistopathological findings were observed. Therefore, the observed pupillary reaction under light was considered to represent a transient neuropharmacological adaptive effect rather than to be indicative for adverse neurotoxicity.

Based on the afore mentioned findings the NOAEL for neurotoxicity was 600 mg/kg bw in males and female rats. The NOAEL for systemic toxicity was 200 mg/kg bw in females and 600 mg/kg bw in males.

(DocID 2013/1028330)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimethenamid-P
Description: liquid/brown, clear
Batch/purity #: COD-001509, Dimethenamid-P: 95.9%
Stability of test compound: The test substance was stable over the study period under storage conditions (ambient, room temperature; protect from temperatures below 0°C; protect from temperatures above 40°C); Expiry date 01-Oct-2013

2. Vehicle: 1% aqueous carboxymethylcellulose (CMC)
Positive control: Neostigmin Bromide
R-(-)-Apomorphine hydrochloride
Methylphenidate hydrochloride
Diazepam
Tritethyltin bromide
Carbaryl (1-Naphthylmethylcarbamate)
Acrylamide
Trimethyltinchloride
Nomifensin
3,3'-Iminodipropionitrile

3. Test animals:
Species: Rat
Strain: Crl:WI(Han)
Sex: Male and female
Age: Males: 35-37 (Section A), 33-35 (Section B)
Females 34-36 (Section A), 32-34 (Section B)
49 days at administration
Source:

B. STUDY DESIGN AND METHODS

1. Dates of work: 16-Oct-2012 - 25-Apr-2013
(In life dates: 22-Oct-2012 (first day of FOB and MA determinations on day -7) to 15-Nov-2012 (necropsy of last subset)

2. Animal assignment and treatment:

Dimethenamid-P was administered once to groups of 10 male and 10 female Wistar rats by oral gavage at dose levels of 0, 60 (low dose), 200 (mid dose) and 600 mg/kg (high dose). The application volume was 10 ml/kg bw.
Each group per sex was subdivided into 2 subsets (Section A males and Section A females = first 5 animals of each dose group and Section B males and Section B females = second 5 animals of each dose group) in order to balance the groups for FOB and motor activity measurements. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:

The dosing suspensions were prepared by mixing weighed amounts of test substance with appropriate amounts of the vehicle (1% aqueous CMC) with 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.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in 1% aqueous CMC for 4 days when stored at room temperature.

Homogeneity analyses of suspension preparation were performed for the lowest and the highest concentrations. For this - as laid down in the SOP - samples were taken from the top, middle and bottom of the storage containers. The homogeneity samples were also used for concentration control analysis. According to the SOP three samples were taken from the top middle and bottom of the beaker at the end of the administration period for the high and the low dose. Furthermore a single sample of the mid dose (200 mg/kg bw/day) was taken to confirm the correctness of the concentration.

Table 5.7.1-1: Analysis of diet preparations for homogeneity and test-item content

Concentration [g/100 ml]	Sampling	Concentration [g/100 ml]	% of nominal concentration	Relative standard deviation
0.6	29.10.12	0.605 ± 0.006	100.8	1.1
2	29.10.12	1.867	93.4	n.a.
6	29.10.12	5.711 ± 0.157	95.2	2.8

values may not calculate exactly due to rounding of values
n.a. = not applicable

Relative standard deviations in the range of 1.1 to 2.8% indicate the homogenous distribution of Dimethenamid-P in the suspensions. The actual average test-substance concentrations were in the range of 93.4 to 100.8% of the nominal concentrations.

4. Statistics:

Means, medians, and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table 5.7.1-2: Statistics of clinical examinations

Parameter	Statistical test
Food consumption, 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
Feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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

Table 5.7.1-3: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. Methods

1. Clinical observations:

The animals were checked daily for evident signs of toxicity. Abnormalities and changes were recorded for each animal individually.
A check for moribund and dead animals was made twice on each working day and once on Saturdays, Sundays, and public holidays. Animals that were in a moribund state were sacrificed and necropsied.

2. Body weight:

Body weight was determined before the first neurofunctional tests in order to randomize the animals. During the study body weights were determined on the days FOBs were conducted, i.e. 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. On study day 15 the body weights of the fasted animals were used for determination of the relative organ weights.

3. Food consumption, food efficiency, and compound intake:
Individual food consumption was checked daily by visual inspection. No food consumption data were recorded.

4. Water consumption:
Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. No water consumption data were recorded.

5. Ophthalmoscopy:
Not performed in this study

6. Functional observation battery (FOB):
FOBs were performed in all animals prior to administration (day -7) and on study days 0, 7 and 14. The FOBs were performed starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 being not aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation attention was paid to posture, tremors, convulsions, abnormal movements, impairment of gait and (if applicable) other findings.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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 (no. fecal pellets/appearance/consistency) within 2 minutes
8. posture	17. urine (appearance/quantity) within two minutes (Q)
9. palpebral closure	18. number of rearings within two minutes (Q)

(Q) quantitative parameter	

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 (Q)
5. pinna reflex	12. grip strength of hind limbs (Q)
6. audition ("startle response")	13. landing foot-splay test (Q)
7. coordination of movements ("righting response")	14. other findings
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(Q) quantitative parameter	

7. Motor activity measurement:

Motor activity examinations (MA) were performed in all animals prior to administration (day -7) and on study days 0, 7 and 14 with the exception of the female section B subset where MA were conducted on day 8 instead of day 7 due to a technical error. MA was performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Neuropathology:

The first five surviving animals per sex and test group were selected for neuropathology evaluation. These animals were sacrificed by perfusion fixation under deep Isoflurane anesthesia. SOERENSEN's phosphate buffer was used as the rinsing solution and fixation was performed with a solution according to KARNOVSKY.

The remaining animals were sacrificed using CO_2 and discharged without further examination.

The sacrificed animals were necropsied and the visible organs or organ sections were assessed by gross pathology as accurately as it is possible for perfused animals. The weight of the brain (without olfactory bulb) was determined in all perfused animals after removal of the brain but before any other preparation. For determination of the relative brain weights the terminal body weights were uses

Additionally to organ/tissues listed in paragraphs below, the following organs/tissues were preserved in neutral buffered 4% formaldehyde:

Brain (remaining material after trimming)
Spinal cord (parts of cervical and lumbar cord)
Gross lesions

Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were embedded and histologically examined. The remaining organ material and the animal bodies were stored in neutrally buffered 4% formaldehyde solution. Details are given below:

	The following organ samples were embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E) and assessed by light microscopy. (✓: all dose groups; # only control and high dose - low and mid dose organs were stored in 4% formaldehyde)
#	Brain (cross sections): - Frontal lobe - Parietal lobe with diencephalon and hippocampus - Midbrain with occipital and temporal lobe - Pons - Cerebellum - Medulla oblongata
#	Brain-associated organs/tissues - Eyes with retina and optical nerve
#	Spinal cord (cross and longitudinal sections): - Cervical cord (C3-C6) - Lumbar cord (L1-L4)
#	Peripheral nervous system: - Gasserian ganglia with nerve - Gastrocnemius muscle
	The following nerves were embedded in an epoxy resin, semi thin sectioned and stained with Azure II - Methylene blue basic Fuchsine (AMbf) and assessed by light microscopy. (✓: all dose groups; # only control and high dose, - low and mid dose organs/tissues were stored in buffer solution)
#	Dorsal root ganglion, 3 of (C3-C6)
#	Dorsal root fiber (C3-C6)
#	Ventral root fiber (C3-C6)
#	Dorsal root ganglion, 3 of (L1-L4)
#	Dorsal root fiber (L1-L4)
#	Ventral root fiber (L1-L4)
#	Proximal sciatic nerve
#	Proximal tibial nerve (at knee)
#	Distal tibial nerve (at lower leg)

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical observations were noted during the daily standard clinical observation.

2. Mortality

No mortality was observed in this study.

3. Ophthalmoscopy

No ophthalmoscopy examinations were performed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

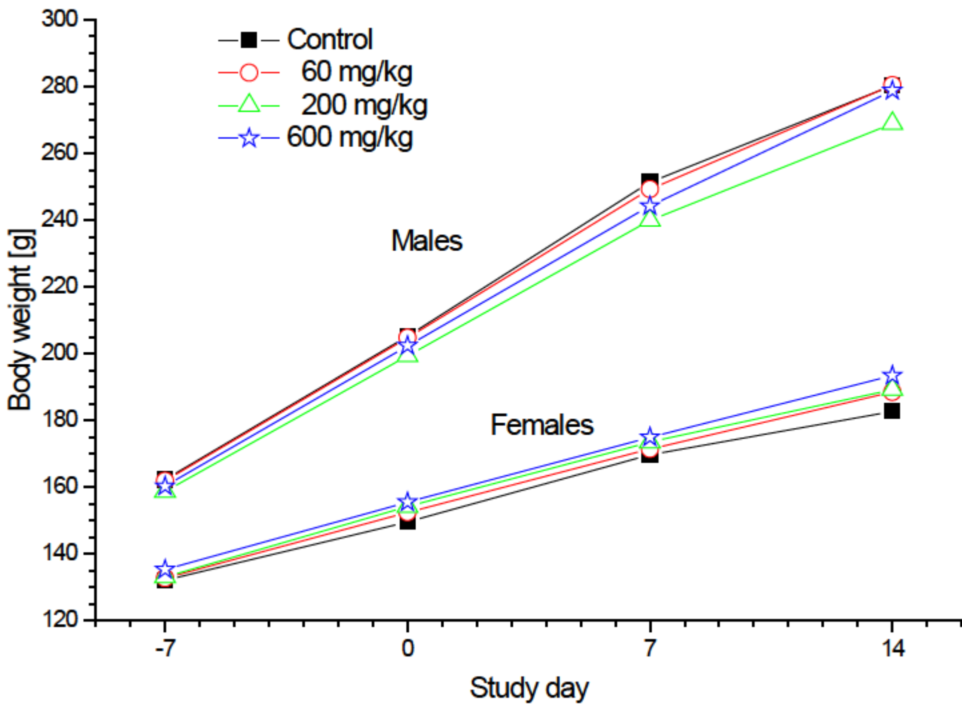
No treatment-related effects on body weight were observed [**Figure 5.7.1-1**, **Table 5.7.1-4**].

Table 5.7.1-4: Mean body weights and body weight gain of rats administered Dimethenamid-P once and observed for 14 days

	Males				Females			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
Body weight [g]								
- Day 0	205.2	204.8	199.3	202.4	149.6	152.5	154.2	155.5
- Day 14	280.3	280.4	268.9	278.8	182.7	188.5	189.2	193.4
Δ% (compared to control) #		0.0	-4.1	-0.6		3.2	3.6	5.8
Overall body weight gain [g]	75.2	75.7	69.5	76.3	33.2	36.0	35.1	37.9
Δ% (compared to control)#		0.7	-7.5	1.6		8.5	5.8	14.2

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.7.1-1: Body weight development of rats administered Dimethenamid-P once and observed for 14 days



C. FOOD CONSUMPTION

Not determined quantitatively for this study. No test-substance related effects on food consumption were observed

D. FUNCTIONAL OBSERVATION BATTERY

Deviations from (rank) "zero values" were obtained in several animals. However, most findings were either equally distributed between treated groups and controls or displayed no dose-response relationship or occurred in single animals only, thus these observations were considered incidental.

1. Home cage observations

On the day of application (study day 0), clinical findings during home cage observation were only observed in female animals of test group 3 (600 mg/kg body weight), i.e. piloerection in 6 females (Nos. 47, 52, 64, 66, 81, 87), half-closed eyelids in 3 females (Nos. 66, 81, 87) as well as permanently closed eyelids and slight salivation in 1 female animal (No. 52), and clear lacrimation in 2 females (Nos. 52, 87) [see **Table 5.7.1-5**]. These findings were assessed as being treatment-related. They were not observed on study days 7 and 14.

Table 5.7.1-5: Clinical findings during home cage observation after single dose of Dimethenamid-P on day of administration (d0)

	Males				Females			
Dose level [mg/kg bw]	**0**	**60**	**200**	**600**	**0**	**60**	**200**	**600**
Piloerection	0	0	0	0	1	0	0	**6**
Salivation, **- slight**	0	0	0	0	0	0	0	**1**
Nose discharge, **- reddish**	0	0	0	0	0	0	0	**2**
Lacrimation, **- clear**	0	0	0	0	0	0	0	**2**
Eyelids, **- half-closed** **- permanently closed**	 0 0	 0 0	 0 0	 0 0	 0 0	 0 0	 0 0	 **3** **1**

During home cage observation, no treatment-related findings were observed for male animals of test group 3 (600 mg/kg body weight) as well as for male and female animals of the other test groups.

2. Open-field observations

Clinical findings in female animals of the high dose group comprised piloerection, slight salivation, reddish discharge of the nose, slight lacrimation, half-closed eyelids, accelerated or irregular respiration, slight tremors, unsteady gate and reduced exploration of the area [see**Table 5.7.1-6**]. These findings were observed on the day of administration only and were not observed in any of the other dose groups. Oppose to females were the slight tremors observed in 2 females were accompanied by other clinical findings and thus considered possibly treatment-related, the slight tremors observed in individual males of all dose groups are considered incidental as not accompanied by other clinical findings and not showing a clear dose-response relationship. The observed isolated findings of reduced exploration of the area in male and female animals of the low (60 mg/kg bw) and mid dose group (200 mg/kg bw) are well known to be occasionally observed during open field observation, were also observed in the same incidence in male control group animals and thus considered to be incidental.

Table 5.7.1-6: Clinical findings during open field observation after single dose of Dimethenamid-P on day of administration (d0)

	Males				Females			
Dose level [mg/kg bw]	**0**	**60**	**200**	**600**	**0**	**60**	**200**	**600**
Piloerection	0	0	0	0	1	0	0	**7**
Salivation, - slight	0	0	0	0	0	0	0	**2**
Nose discharge, - reddish	0	0	0	0	0	0	0	**2**
Lacrimation, - slight	0	0	0	0	0	0	0	**2**
Eyelids, - half-closed	0	0	0	0	0	0	0	**4**
Respiration, - accelerated	0	0	0	0	0	0	0	**5**
- irregular	0	0	0	0	1	0	0	1
Tremors, - slight	0	1	2	1	1	0	0	**2**
Impairment of gait, - slight	0	0	0	0	0	0	0	**3**
Exploration of area, - reduced	2	2	2	0	0	1	1	**4**
Contracted pupil, - strongly	0	0	0	1	0	0	0	**0**

The only clinical finding considered as potentially treatment-related observed in male animals during open field observation were strongly contracted pupils in one animal of the high dose group (600 mg/kg bw) [see**Table 5.7.1-6**]. This isolated finding was supported by findings in the sensorimotor testing [see **Table 5.7.1-6** below].

A treatment-related decrease in the frequency of rearings was observed in high dose females on day 0 [see **Table 5.7.1-5**]. At the following FOBs (days 7 and 14) this value was comparable to the controls. No effect was observed in males.

All findings in females were assessed as being related to an impairment of the overall condition of the animals rather than being related to a neurotoxic mode of action.

Table 5.7.1-5: Frequency of rearing in rats administered Dimethenamid-P once and observed for 14 days

	Males				Females			
Dose level [mg/kg bw]	0	60	200	600	0	60	200	600
- Day -7	3 ± 4	3 ± 2	3 ± 3	4 ± 3	10 ± 5	8 ± 7	7 ± 4	9 ± 6
- Day 0	2 ± 2	3 ± 3	1 ± 1	4 ± 3	12 ± 7	8 ± 6	6 ± 7	**2 ± 3****
- Day 7	3 ± 2	4 ± 4	2 ± 3	4 ± 5	13 ± 8	13 ± 8	10 ± 8	15 ± 7
- Day 14	2 ± 2	4 ± 3	4 ± 3	4 ± 4	17 ± 9	12 ± 8	15 ± 5	13 ± 7

** $p \leq 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

During open field observation, no treatment-related findings were observed in any dose group on day 7 and day 14 after administration.

3. Sensorimotor tests / reflexes

A potentially treatment-related incidence of strong contracted pupil under incidence of light was observed in single animals of all male dose groups [see **Table 5.7.1-6**]. There was however, no clear dose response relationship and it was observed in a small subset of male animals only. Moreover, this finding was not observed in high dose group females who generally showed more pronounced toxicity effects than males. This finding was neither observed at 7, nor at 14-days after administration.

Deviations from "zero values" for non-quantitative parameters were obtained in several animals. However, as all findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered incidental.

Table 5.7.1-6: Pupillary contraction determined in sensorimotor tests/ reflexes after single dose of Dimethenamid-P on day of administration (d0)

	Males				**Females**			
Dose level [mg/kg bw]	**0**	**60**	**200**	**600**	**0**	**60**	**200**	**600**
Pupillary reflex **- strong contracted pupil under incidence of light**	0	**1**	**2**	**2**	0	0	0	0

F. MOTOR ACTIVITY

Regarding the overall motor activity as well as single intervals, no relevant deviations were observed on study days -7, 0, 7 as well as study day 14 for male and female rats of test groups 1-3 (60, 200 and 600 mg/kg bw) when compared to the control group. The significantly lower value of interval No. 6 for male animals of test group 3 (600 mg/kg bw) on study day 14 was assessed as being incidental.

G. NECROPSY

1. Terminal body and brain weight

No treatment-related changes of terminal body weights or absolute and relative brain weights were noted [see **Table 5.7.1-9**]. The obvious differences in female brain weights of the low and mid dose group (60 and 200 mg/kg bw) are artificial differences due to technical reasons of brain preparation, that deviates for the control group and the high dose group (weights of trimmed brains for control and high dose group were compared to total brain weights for the low and mid dose group).

Table 5.7.1-9: Mean terminal body weights and absolute and relative brain weights of rats administered Dimethenamid-P once and observed for 14 days

Sex		Males				Females			
	Dose [mg/kg bw]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
Terminal body weight	0	263.76				176.16			
	60	262.56	(0)			177.52	(1)		
	200	243.38	(-8)			176.60	(0)		
	600	258.52	(-2)			179.96	(2)		
Brain	0	1.972		0.749		1.744		0.991	
	60	1.966	(0)	0.750	(0)	1.816**	(4)	1.025	(3)
	200	2.028	(3)	0.839	(12)	1.850*	(6)	1.051	(6)
	600	1.984	(1)	0.769	(3)	1.738	(0)	0.969	(-2)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
Values may not calculate exactly due to rounding of figures

2. Gross and neuro-histopathology (Perfusion fixed animals)

No treatment related gross pathology or neuro-histopathological findings were observed.

H. POSITIVE CONTROLS

No concurrent positive control was employed in this study.

However, in several positive control studies, behavioral and neuro-pathological sequelae of substances with nervous system effects were evaluated using Functional Observational Batteries (FOB), Motor Activity Measurements and Neuropathology. Clinical signs of peripheral neuropathy (e.g. ataxia, limb weakness), central neuropathy (e.g. tremors) and autonomic signs (e.g. salivation) could be shown. Histopathologically, changes in the peripheral nervous system (e.g. Wallerian-like degeneration) and central nervous system (e.g. neuronal necrosis) were seen. The motor activity device was able to show both increased and decreased activity. The inter-observer reliability of the technicians performing FOBs was proven. Thus, the ability of the methods used to detect signs of neurotoxicity was demonstrated.

Positive control studies employed single or repeated administration of the following neurotoxicants: 3,3-Iminodipropionitrile, Carbaryl, Nomifensin, Diazepam, Acrylamide and Trimethyltinchloride, Triethyltin bromide, Methylphenidate hydrochloride, Neostigmine Bromide, R-(-)-Apomorphine hydrochloride. Study summaries are attached to the report.

III. CONCLUSIONS

Single oral gavage of Dimethenamid-P to rats at dose levels of 0, 60, 200 and 600 mg/kg bw did result in signs of general systemic toxicity in female animals of the high group observed during home cage and open field observations on the day of administration only. All findings were assessed as being related to an impairment of the overall condition of the animals rather than being related to a neurotoxic mode of action.

The observed neuro-behavioral effect of strongly contracted pupils under incidence of light that was observed on the day of administration in a small subsets (1 or 2 out of 10 animals) of all male dose-groups might possibly be treatment-related. There was however no dose-response relationship and it was not seen in females who showed treatment related toxicity while no other toxicity effects were noticed in males. The effect was noted as a transient effect only on the day of administration. Additionally, no treatment-related neuropathological findings were noted, i.e. no brain weight changes or neurohistopathological findings were observed. Therefore, the observed pupillary reaction under light was considered to represent a transient neuropharmacological adaptive effect rather than to be indicative for adverse neurotoxicity.

Based on the afore mentioned findings the NOAEL for neurotoxicity was 600 mg/kg bw in males and female rats. The NOAEL for systemic toxicity was 200 mg/kg bw in females and 600 mg/kg bw in males.

Report: CA 5.7.1/2
[redacted] 2013b
BAS 656-PH - Repeated dose 90-day oral neurotoxicity study in Wistar rats - Administration via the diet
2013/1165818

Guidelines: OECD 424, EPA 870.6200, (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

Dietary administration of dimethenamid-P (Batch COD-001509; Purity: 95.9%) to groups of 10 male and female Wistar Crl:WI(Han) rats at dietary dose levels of 0, 300, 1000, and 4500 ppm for 3 months did not result in any clinical (general clinical observation, FOB and motor activity) or neurohistopathological indication of neurotoxicity.

Signs of systemic toxicity observed in this study were in line with those observed in other rat studies employing repeated administration of dimethenamid-P. Effects indicating a certain level of systemic toxicity were given at least for the high concentration of 4500 ppm, i.e. impairment of body weight development in male and female Wistar rats accompanied by an increase of liver (males and females) and kidney (males only) weights (histopathological examinations were not performed in these organs).

Under the conditions of the present study the no observed adverse effect level (NOAEL) for neurotoxicity was at least 4500 ppm, which is equivalent to about 323 mg/kg bw/d in males and 390 mg/kg bw/d in females. -The NOAEL for systemic toxicity was 1000 ppm corresponding to 63 mg/kg bw/day in males and 72 mg/kg bw/day in females.

(DocID 2013/1165818)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Dimethenamid-P
   Description: liquid/brown, clear
   Batch/purity #: COD-001509: 95.9%
   Stability of test compound: Stability under storage conditions (ambient temperature +5 to +30 °C) guaranteed by the sponsor; Expiry date: 01-Oct-2013

2. **Vehicle and/or positive control:** None

3. Test animals:

Species:	Rat
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	34-36 days (Section A) and 33-35 days (Section B) at delivery, 48-50 days at start of treatment
Weight at dosing:	males: 196.5 ± 9.6 g; females: 150.6 ± 7.6
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld
Acclimation period:	13 to 16 days
Diet:	Kliba maintenance diet for mouse/rat "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water from water bottles, ad libitum
Housing:	Group housing (5 animals per cage) polysulfonate cages from Tecniplast, Hohenpeißenberg, Germany (floor area about 2065 cm^2); with dust free bedding (SSNIFF, Soest, Germany) enriched with wooden gnawing blocks (TYP NGM E-022; Abedd® Lab. and Vet. Service GmbH, Vienna, Austria). Motor activity (MA) measurements were conducted in polycarbonate cages (Tecniplast, Hohenpeißenberg, Germany; floor area about 800 cm^2 and small amounts of absorbent material).
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 6.00 am to 6.00 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 08-Jan-2013 - 03-Jul-2013
(In life dates:14-Jan-2013 (first day of pre-treatment FOB and MA determinations) to 26-April 2013 (necropsy of last subset of animals)

2. Animal assignment and treatment:

Dimethenamid-P was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 300 (low dose), 1000 (mid dose) and 4500 ppm (high dose) for at least 90 days. Each group per sex was subdivided into 2 subsets (Section A males and Section A females = first 5 animals of each dose group and Section B males and Section B females = second 5 animals of each dose group) in order to balance the groups for FOB and motor activity measurements. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, corresponding amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Three diet preparations were performed for this study and were mixed at least every 32 days.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 32 days when stored at room temperature.
According to the SOP three samples were taken from the top middle and bottom of the beaker at the end of the administration period for the high and the low dose. Furthermore a single sample of the mid dose (1000 mg/kg bw/day) was taken to confirm the correctness of the concentration.

Table 5.7.1-10: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration# [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
300 ppm	21.01.2013	294.7 ± 3.8	98.2	1.3
1000 ppm	21.01.2013	985.3	98.5	n.a.
4500 ppm	21.01.2013	4116.5 ± 90.4	91.5	2.2

based on mean values of the three individual samples; values may not calculate exactly due to rounding of values.
n.a.: not applicable

No test-article was determined in control diets. Relative standard deviations in the range of 1.3 to 2.2% indicate the homogenous distribution of Dimethenamid-P in the diet preparations. The actual average test-substance concentrations were in the range of 91.5 to 98.5% of the nominal concentrations.

4. Statistics:

Means, medians, and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table 5.7.1-11: Statistics of clinical examinations

Parameter	Statistical test
Food consumption, 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
Feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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

Table 5.7.1-12: Statistics of clinical pathology

Parameter	Statistical test
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 Wilcoxon-test (two-sided) for the equal medians

Table 5.7.1-13: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. Methods

1. Clinical observations:

The animals were checked daily for evident signs of toxicity. Abnormalities and changes were documented for each animal individually.
A check for moribund and dead animals was made twice on each working day and once on Saturdays, Sundays, and public holidays. Animals that were in a moribund state were sacrificed and necropsied.
Detailed clinical observations (DCO) in all animals was performed prior to the administration period and in weekly intervals thereafter. For DCO animals were transferred to a standard arena (50 x 37.5 cm with sides 25 cm high) and the following parameters were analyzed:

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, on day 0 (start of the administration period) and weekly thereafter as well as on days of FOB performance. 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 consumption, food efficiency, and compound intake:

Individual food consumption was determined weekly and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal based on individual values for body weight and average food consumption for animals per cage:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y :body weight [g] on day x and day y (last weighing date before day x),
$FC_{y\ to\ x}$: 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 from 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.

$$\text{Compound intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x : mean daily food consumption (in g/day) on day x,
C: concentration in the food on day x [mg/kg] equivalent to dose in ppm on day x
BW_x : body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. No water consumption data were recorded.

5. Ophthalmoscopy:

Not performed in this study

6. Functional observation battery (FOB):

FOBs were performed in all animals prior to administration (day -7) and on study days 1, 22, 50 and 85. The FOBs were performed starting at about 10.00 a.m.. The FOB started with passive observations without disturbing the animals (home cage observations), 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 being not aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation attention was paid to posture, tremors, convulsions, abnormal movements, impairment of gait and (if applicable) other findings.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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 (no. fecal pellets/appearance/consistency) within 2 minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Clinical chemistry:

Not performed in this study

9. Urinalysis:

Not performed in this study.

10. Pathology:

10.1 Neuropathology

Five animals per sex and test group were selected for neuropathology evaluation. These animals were sacrificed by perfusion fixation under deep Isoflurane anesthesia. SOERENSEN's phosphate buffer was used as the rinsing solution and fixation was performed with a solution according to KARNOVSKY.

The sacrificed animals were necropsied and the visible organs or organ sections were assessed by gross pathology as accurately as it is possible for perfused animals.

10.2 General pathology

Animals not selected for perfusion fixation were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

10.3 Weight parameters

The following weights were determined for animals sacrificed on schedule:

✓	Anesthetized animals
#	Brain (without olfactory bulb)[1]
✓	Liver
✓	Kidneys

✓: all animals/dose group sacrificed on schedule
#: only animals/dose group scheduled for perfusion fixation
[1] The weight of the brain was determined in all perfused animals after removal of the brain but before any other preparation.

10.4 Organ/tissue fixation

Additionally to organ/ tissues listed in paragraphs below, the following organs/tissues were preserved in neutral buffered 4% formaldehyde:

#	Brain (remaining material after trimming)
#	Spinal cord (parts of cervical and lumbar cord)
✓	Gross lesions
○	Kidneys
○	Liver
○	Remaining organ material and animal body

✓: all animals/dose group sacrificed on schedule
#: only animals/dose group scheduled for perfusion fixation
○: only animals/dose group not scheduled for perfusion fixation

10.5 Histotechnical processing and histopathological evaluation

Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were embedded and histologically examined. The remaining organ material and the animal bodies were stored in neutrally buffered 4% formaldehyde solution. Details are given in **Table 5.7.1-14** below:

Table 5.7.1-14: Histotechnical processing and histopathological evaluation

	Dose level [ppm]	0		300		1000		4500	
		M	F	M	F	M	F	M	F
#	Brain (cross sections)			FT	FT	FT	FT		
#	- Frontal lobe	P	P					P	P
#	- Parietal lobe with diencephalon and hippocampus	P	P					P	P
#	- Midbrain with occipital and temporal lobe	P	P					P	P
#	- Pons	P	P					P	P
#	- Cerebellum	P	P					P	P
#	- Medulla oblongata	P	P					P	P
#	Brain-associated organs/tissues - Eyes with retina and optical nerve	P	P	F	F	F	F	P	P
#	Spinal cord (cross and longitudinal sections): - Cervical swelling (C3-C6) - Lumbar swelling (L1-L4)	P P	P P	F F	F F	F F	F F	P P	P P
#	Peripheral nervous system: - Gasserian ganglia with nerve - Gastrocnemius muscle	P P	P P	F F	F F	F F	F F	P P	P P
°	Gross-lesions	P	P	P	P	P	P	P	P
#	Peripheral nervous system								
#	- Dorsal root ganglion, (3 of C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root fiber (C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Ventral root fiber (C3-C6)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root ganglion, (3 of L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Dorsal root fiber (L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Ventral root fiber (L1-L4)	PL	PL	S	S	S	S	PL	PL
#	- Proximal sciatic nerve (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL
#	- Proximal tibial nerve (at knee) (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL
#	- Distal tibial nerve (at lower leg) (cross and longitudinal sections)	PL	PL	S	S	S	S	PL	PL

#: only animals / test group scheduled for perfusion fixation
°: only affected animals / test group scheduled for perfusion fixation

P: Paraffin embedding (paraplast), sectioning, staining with hematoxylin-eosin (HE) and histopathological evaluation
PL: Plastic embedding (epoxy resin), semi thin sectioning and staining with Azure II Methylene blue-basic Fuchsine (AMbf) and histopathological evaluation
S: Storage of fixed specimen in buffer solution
F: Preservation in 4% formaldehyde solution
FT: Preservation in 4% formaldehyde solution in total

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No findings of toxicological concern were seen in male and female animals of all test groups. A tooth anomaly was observed in one female control group animal from study day 0 until study day 27. This finding occurred only temporarily in a single animal and was assessed as being incidental and not related to treatment.

2. Mortality

No mortality was observed throughout the study period.

3. Ophthalmoscopy

No ophthalmoscopy examinations were performed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

On study day 1, the mean body weight of female animals before performing the FOB was significantly lower in all test groups (300, 1000 and 4500 ppm). This initial finding might be related to palatability problems before the animals got used to the test material. Therefore, the effect was not assessed as being averse.

Treatment-related effects on body weight development were observed for male and female animals of the 4500 ppm dose group. Although not significantly altered, mean body weights of male animals were lower during the entire study period with a maximum of -5.9% on study day 28. In addition, on study day 1 the mean body weight of male animals before performing the FOB was significantly lower. Mean body weight change values were significantly lower in this group from study day 0 to 42 (maximum of -24.5% on study day 7). Significant differences occurred only during the first half of the study: However, the mean body weight change value of these animals was still -7.8% lower at the end of the administration period. Therefore, the changes were assessed as being test substance-related and adverse. In female animals of the high dose group as well as the mean body weight change values were significantly lower from study days 49 onwards (maximum of -7.2% mean body weight and -15.1% mean body weight change on study day 77) including study day 85 when body weight was determined before performing the FOB. This effect was regarded to be related to treatment and adverse.

During the weekly performed body weight determination no relevant changes were observed for male and female animals of the low and mid dose group (300 and 1000 ppm).

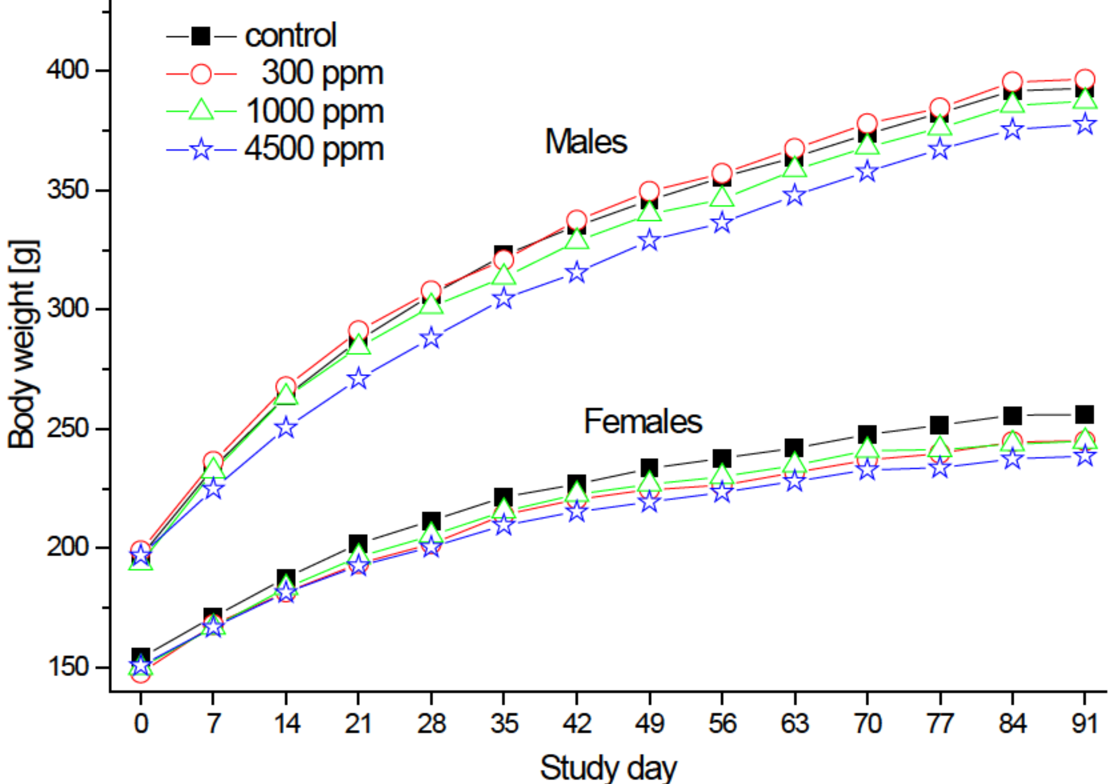
Table 5.7.1-15: Mean body weight, body weight gain and cumulative food consumption data of rats administered dimethenamid-P for at least 91 days

	Males				Females			
Dose level [ppm]	0	100	1000	4500	0	100	1000	4500
Body weight [g]								
- Day 0	196.6	198.9	193.7	196.9	154.1	147.7	149.9	150.8
- Day 91	392.7	396.5	387.4	377.7	256.1	245	244.8	238.7
Δ% (compared to control) #		1.0	-1.3	-3.8		-4.3	-4.4	-6.8
Overall body weight gain [g]	196.1	197.6	193.7	180.8	102	97.3	94.9	**87.9***
Δ% (compared to control)#		0.8	-1.2	-7.8		-4.6	-7.0	-**13.8**
Cumulative food consumption [g/animal]§								
- Day 0 to 91	1828	1879	1860	1996	1609	1499	1404	1687
Δ% (compared to control)		2.8	1.8	9.2		-6.8	-12.8	4.9

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

§ Values calculated based on group mean daily food consumption

Figure 5.7.1-2: Body weight development of rats administered Dimethenamid-P for at least 91 days



C. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed.
Note: Food spilling was observed in one male and one female of the 4500 ppm group on several days during the administration period. Some values had to be declared as outliers and were not used for calculation of the mean test substance intake.

The following intake of test substance was calculated for the 90-day administration of dimethenamid-P as follows:

Table 5.7.1-16: Calculated intake of dimethenamid-P administered for at least 90-days

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	300	19	23
2	1000	63	71
3	4500	323	390

D. CLINICAL PATHOLOGY

No hematology, clinical chemistry investigations or urinalysis were conducted in this study.

E. FUNCTIONAL OBSERVATION BATTERY

There were no findings of toxicological concern in any of the treatment groups applied 300, 1000 or 4500 ppm to male and female rats.
Some deviations from (rank) "zero values" were obtained in several animals.

1. Home cage observations

No treatment-related findings were observed.

Teeth anomaly was detected in a female control group animal on study days 1 and 22. This finding was assessed as being incidental.

2. Open-field observations

No treatment-related findings were observed.

Deviations from (rank) "zero values" were obtained in some animals. However, all findings were either equally distributed between treated groups and controls or displayed no dose-response relationship or occurred in single animals only, thus these observations were considered incidental.

3. Sensorimotor tests / reflexes

No treatment-related findings were observed.

Very frequent vocalization when touched was detected in one female animal of the (300 ppm) on study day 22. This finding was assessed as being incidental and not related to treatment.

The assessment of quantitative parameters revealed an isolated and not dose-dependent difference, which was considered to be of incidental nature. Landing foot-splay test was significantly higher in male animals of the 300 ppm dose group on study day 50 (+11.8%).

F. MOTOR ACTIVITY

No treatment-related changes of motor activity were noted in treated groups.

There were some statistically significant differences between control and treated groups,, however, these changes were neither dose related nor consistent over time. Therefore, these changes were considered incidental. These changes consisted of:

- significantly decreased activity in low dose females at interval 9 on study day -7.
- significantly decreased activity in high dose males at interval 4 and significantly increased activity in female high dose animals at intervals 5 and 6 on day 1.
- significantly decreased activity in male animals of the low dose group at intervals 1 and 2 and significantly increased activity in female low dose animals at interval 11 on study day 22.
- significantly decreased activity in male low dose animals at interval 1 and significantly increased activity in female animals of the low dose group at single interval 5 was and significantly increased activity in female high dose animals at intervals 4 and 5 on study day 50.
- significantly increased activity of female low dose animals at single interval 10 on study day 85

No statistically significant differences on overall motor activity were observed at any dose or any time.

G. NECROPSY

1. Terminal body and brain weight (Perfusion fixed animals)

Brain weights in treated animals were not significantly altered from control group animals.

2. Terminal body and organ weights (Animals not subjected to perfusion fixation)

Terminal body weight was significantly altered in any of the dose groups. However, in both sexes the reduced body weight development of the high dose group [see **Table 5.7.1-15** and **Figure 5.7.1-2**] was reflected by the in tendency reduced terminal body weight.

The increase of kidney weights in male animals of the high dose group as well as the increase of absolute and relative liver weight in male and female animals in the mid and high dose group and relative liver weight in female animals in the low dose group was regarded to be test substance related [see **Table 5.7.1-17**].

Table 5.7.1-17: Mean terminal body weights and selected absolute and relative organ weights of rats administered dimethenamid-P for at least 91 days

Sex	Males				Females			
Dose [ppm]	0	300	1000	4500	0	300	1000	4500
[mg/kg bw/day]		19	63	323		23	71	390
Terminal bodyweight [g]	381.62	375.94	369.54	362.0	237.84	231.7	229.5	225.42
[% of control]	100	99	97	95	100	97	96	95
Liver, absolute [g]	8.164	8.798	8.814	**10.844****	5.406	5.77	6.08	**7.3****
[% of control]	100	108	108	**133**	100	107	112	**135**
Liver, relative [%]	2.139	2.342	2.385	**2.996****	2.269	**2.494***	**2.647****	**3.243****
[% of control]	100	110	112	**140**	100	**110**	**117**	**143**
Kidneys, absolute [g]	2.246	2.336	2.276	**2.58***	1.596	1.584	1.686	1.686
[% of control]	100	104	101	**115**	100	99	106	106
Kidneys, relative [%]	0.589	0.622	0.618	**0.712****	0.673	0.686	0.734	0.748
[% of control]	100	106	105	**121**				

*** p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)**
Values may not calculate exactly due to rounding of figures

No histopathological examination of the liver and kidneys was performed in this study. From the dimethenamid-P subchronic 90-day study in rats [see DocID 1996/5420 and1999/10270], and the racemic dimethenamid 90-day study [see 1986/11183, 1995/11323 and 1999/10270] which were performed at comparable dose levels, as well as other rat studies with dimethenamid-P or racemic dimethenamid it is known that liver weight increases were accompanied at higher dose levels ≥ 100 mg/kg bw/day only by hepatocellular hypertrophy and altered clinical chemistry parameters (increase in γ-GT and cholesterol) indicative for liver enzyme induction. There was no indication for adverse histopathological findings (e.g. necrosis, fatty change, degeneration) in these studies.

3. Gross and neurohistopathology (Perfusion fixed animals)

No gross pathology or neurohistopathological findings were observed.

4. Gross and histopathology (Animals not subjected to perfusion fixation)

Four female animals of the high 4500 ppm dose group revealed an enlargement of the liver. This finding was seen in relation to the above noted liver weight increases and regarded to be test substance-related.

For the dilation of renal pelvis in male animals of all test groups a test substance-related effect could not be excluded. However, no such treatment relation has been determined in any of the other rat toxicity studies conducted with dimethenamid-P or racemic dimethenamid. As no histopathological examination was performed, no further judgment could be made.

H. POSITIVE CONTROLS

No concurrent positive control was employed in this study.

However, in several positive control studies, behavioral and neuropathological sequelae of substances with nervous system effects were evaluated using Functional Observational Batteries (FOB), Motor Activity Measurements and Neuropathology. Clinical signs of peripheral neuropathy (e.g. ataxia, limb weakness), central neuropathy (e.g. tremors) and autonomic signs (e.g. salivation) could be shown. Histopathologically, changes in the peripheral nervous system (e.g. Wallerian-like degeneration) and central nervous system (e.g. neuronal necrosis) were seen. The motor activity device was able to show both increased and decreased activity. The inter-observer reliability of the technicians performing FOBs was proven. Thus, the ability of the methods used to detect signs of neurotoxicity was demonstrated.

Positive control studies employed single or repeated administration of the following neurotoxicants: 3,3'-Iminodipriopionitril, Carbaryl, Nomifensin, Diazepam, Acrylamide, Trimethyltinchloride, Triethyltin bromide, Methylphenidate hydrochloride, Neostigmin bromide and R-(-)-Apomorphine hydrochloride. Study summaries are attached to the report.

III. CONCLUSIONS

In conclusion, the oral administration of dimethenamid-P to Wistar rats over a period of 3 months revealed no adverse neurobehavioral effects in male and female Wistar rats at any concentration. In addition, no test substance-related effects were observed in the neurohistopathology investigation. Under the conditions of this study the NOAEL for neurotoxicity was 4500 ppm for male (323 mg/kg bw/d) and female animals (390 mg/kg bw/d).

Effects indicating a certain level of systemic toxicity were given at least for the high concentration of 4500 ppm, i.e. impairment of body weight development in male and female Wistar rats accompanied by an increase of liver (males and females) and kidney (males only) weights (histopathological examinations were not performed in these organs). Thus, the NOAEL for systemic toxicity was 1000 ppm corresponding to 63 mg/kg bw/day in males and 72 mg/kg bw/day in females.

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 Dimethenamid-P does not belong to a chemical class suspected to induce delayed neuropathies, no study was considered necessary and thus no study was conducted.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000 and in the addendum 1 to the monograph of rapporteur member state Germany of July 03, 2001: Studies (Acute oral toxicity, ames test and micronucleus test in vitro) on plant metabolites M656H023 (former assigned M23) and sodium salt of M656H037 (former assigned M27) have already been evaluated for Annex I inclusion of dimethenamid-P. For the convenience of the reviewer, these are summarized below as extracted from the monograph including addenda together with the extended studies conducted meanwhile for these metabolites.

Based on the available data the following assessment was drawn in the Annex I listing of Dimethenamid-P:

Toxicity studies of plant metabolites M23 and M27	- LD50 of both metabolites > 5000 mg/kg bw - no evidence for mutagenic potential in vitro and in vivo

Dimethenamid-P as is the racemic Dimethenamid are extensively metabolised in all matrices (mammal, plant and soil/water) resulting in numerous metabolites identified. Meanwhile further insights into behavior of dimethenamid-P in plants and in the environment has been obtained. Consequently the database for the above mentioned metabolites now considered as significant ground-water metabolites has been extended and additional metabolites that were determined at significant levels in plants or ground-water were included into the evaluation

With regard to toxicological relevance the following metabolites have to be taken into consideration and are addressed in this dossier section.

Table 5.8.1-1: Dimethenamid-P metabolites considered for potential toxicological relevance

Metabolite	Structure	Reason for relevance assessment
M656PH023		Ground water metabolite Human exposure: 0.1 µg/l < M656PH023 ≤ 0.75 µg/l
M656PH026		Plant metabolite determined in edible commodities Human exposure: 0.0025 µg/kg bw/day < M656PH026

Table 5.8.1-1: Dimethenamid-P metabolites considered for potential toxicological relevance

		≤ 1.5 µg/kg bw/day
M656PH027		Ground water metabolite Human exposure: 0.75 µg/l < M656PH027 ≤ 4.5 µg/l
M656PH030		Plant metabolite determined in edible commodities Human exposure: 0.0025 µg/kg bw/day < M656PH030 ≤ 1.5 µg/kg bw/day
M656PH031		Ground water metabolite Human exposure: M656PH031 ≤ 0.1 µg/l
M656PH032		Ground water metabolite Human exposure: 0.75 µg/l < M656PH032 ≤ 4.5 µg/l
M656PH043		Ground water metabolite Human exposure: 0.1 µg/l < M656PH043 ≤ 0.75 µg/l
M656PH045		Ground water metabolite Human exposure: 0.75 µg/l < M656PH045 ≤ 4.5 µg/l
M656PH047		Ground water metabolite Human exposure: 0.1 µg/l < M656PH047 ≤ 0.75 µg/l

Table 5.8.1-1: Dimethenamid-P metabolites considered for potential toxicological relevance

M656PH049		Ground water metabolite Human exposure: 0.1 µg/l < M656PH049 ≤ 0.75 µg/l
M656PH050		Ground water metabolite Human exposure: 0.1 µg/l < M656PH050 ≤ 0.75 µg/l
M656PH051		Ground water metabolite Human exposure: 0.1 µg/l < M656PH051 ≤ 0.75 µg/l
M656PH052		Ground water metabolite Human exposure: 0.1 µg/l < M656PH052 ≤ 0.75 µg/l
M656PH053		Ground water metabolite Human exposure: 0.75 µg/l < M656PH053 (2 isomers) ≤ 4.5 µg/l
M656PH054		Ground water metabolite Human exposure: 0.75 µg/l < M656PH054 ≤ 4.5 µg/l
M656H055		Ground water metabolite Human exposure: 0.1 µg/l < M656H055 ≤ 0.75 µg/l

Table 5.8.1-1: Dimethenamid-P metabolites considered for potential toxicological relevance

M656PH059		Ground water metabolite Human exposure: 0.1 µg/l < M656PH059 (2 isomers) ≤ 0.75 µg/l < M656PH059 (1 isomer) ≤ 4.5 µg/l
M656PH062		Ground water metabolite Human exposure: 0.75 µg/l < M656PH062 ≤ 4.5 µg/l

Thus, the conclusion for relevant endpoints for the current re-registration was drawn as follows:

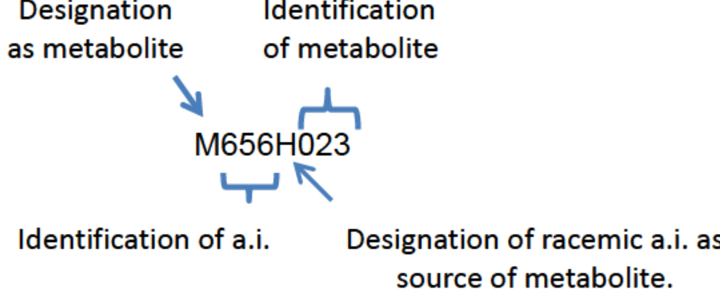
Other toxicological studies (SANCO/11802 data point 5.8)

Toxicity studies of metabolites as referred to in the introduction	<u>**M31-group:**</u> <u>**M656PH030**</u> (plant metabolite) - by weight of evidence not genotoxic in vitro and in vivo **Conclusion: not toxicologically relevant** <u>**M656PH031**</u> (non-significant ground-water metabolite, toxicologically evaluated) - no evidence for genotoxicity in vitro - 28-day rat study: no adverse effects up to limit dose NOAEL males: 1068 mg/kg bw/day Females: 1140 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH032**</u> (ground-water metabolite) - not mutagenic in Ames-test, not toxicologically **Conclusion: not toxicologically relevant based on grouping approach** <u>**M656PH051**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach** <u>**M11-group:**</u> <u>**M656PH043**</u> (ground-water metabolite) - by weight of evidence not genotoxic in vitro and in vivo **Conclusion: not toxicologically relevant** <u>**M19-group:**</u> <u>**M656PH054**</u> (ground-water metabolite) - by weight of evidence not genotoxic in vitro and in vivo - 28-day rat study: Effects on food consumption and body weight at limit dose NOAEL: males: 346 mg/kg bw/day Females: 472 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH055**</u> (ground-water metabolite) - no evidence for genotoxicity in vitro and in vivo **Conclusion: not toxicologically relevant** <u>**M62-group:**</u> <u>**M656PH062**</u> (ground-water metabolite) tested as ethylester derivate - by weight of evidence not genotoxic in vitro and in vivo - 28-day rat study: Effects on food consumption and body weight development; target organs liver and thyroid indicative for enzyme induction at limit dose NOAEL: males: 323 mg/kg bw/day Females: 385 mg/kg bw/day **Conclusion: not toxicologically relevant**

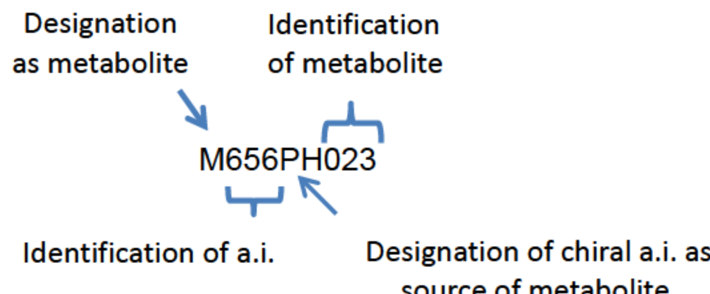
	<u>**M23-group:**</u> <u>**M656PH023**</u> (ground-water metabolite) - LD_{50} > 5000 mg/kg bw - no evidence for genotoxicity in vitro and in vivo - 28-day rat study: no adverse effects up to limit dose NOAEL males: 1388 mg/kg bw/day Females: 1057 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH045**</u> (ground-water metabolite) - no evidence for genotoxicity in vitro - 28-day rat study: no adverse effects up to limit dose NOAEL males: 1174 mg/kg bw/day Females: 1298 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH049**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach** <u>**M656PH050**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach** <u>**M27-group:**</u> <u>**M656PH027**</u> (ground-water metabolite) tested as sodium-salt - LD_{50} > 5000 mg/kg bw - no evidence for genotoxicity in vitro and in vivo - 28-day rat study: no adverse effects up to limit dose NOAEL males: 1064 mg/kg bw/day Females: 1247 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH047**</u> (ground-water metabolite) - by weight of evidence not genotoxic in vitro and in vivo - 28-day rat study: no adverse effects up to limit dose NOAEL males: 1161 mg/kg bw/day Females: 967 mg/kg bw/day **Conclusion: not toxicologically relevant** <u>**M656PH052**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach** <u>**M656PH053**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach** <u>**M656PH059**</u> (ground-water metabolite) **Conclusion: not toxicologically relevant based on grouping approach**

General explanation on metabolite nomenclature in relation to stereoisomery.

Dimethenamid-P is the S-enantiomere of the racemic Dimethenamid. For the active ingredient a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of Dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic pathway synthesis. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example M23:



Metabolites were the source of identification and the synthesis route could clearly be attributed to the chiral compound Dimethenamid-P were assigned



The metabolic pathways in soil,water, mammals, and plants are equivalent for the racemic Dimethenamid and Dimethenamid-P (S-enantiomer). The metabolites derived from either racemic or enantio-enriched source are considered toxicologically equivalent and were taken into account for the assessment below.

QSAR evaluation of metabolites

For all metabolites identified with potential relevance or as corresponding group members (see Doc N4) presence for potential structural alerts was evaluated with different SAR/QSAR models. Models used, were the OECD toolbox, OASIS TIMES, DEREK (partly) and VEGA. These evaluations were in particular taken into account for those metabolites in the grouping approach presented in Doc N4 where toxicological data are not available. However, the QSAR predictions obtained are limited by the reliability as most of the structures evaluated were not in the prediction domain. Thus, given the structural relationship of the metabolites evaluated inter alia and in relation to the parent molecule dimethenamid-p, the predicted alerts were compared to those for the parent and those metabolites were toxicological data were available in order to overcome the limitations of the predictions made.
Moreover, the systems used do not distinguish chiral structures. Thus, any prediction made applies generally to the racemic molecules as well as to the S-enantiomere metabolites considered for Dimethenamid-P.

OECD Toolbox (Profiling module)

The OECD toolbox version 3.2 as downloadable via link of the ECHA webpage [http://www.qsartoolbox.org/download.html] was used for the evaluation. The outcome of the OECD toolbox profiling conducted for the metabolites was exported and collected [see DocID 2014/1089828] as the report function of the current OECD toolbox version did not work properly. The profiling module provided structural alerts for different endpoints. Of particular interest were the modules dealing with protein- or DNA-binding capacity as well as genotoxicity and/or carcinogenicity predictions. It should be noticed 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. The current version of the OECD toolbox does not allow to generate reports out of the conducted evaluations, instead the toxicological profiles obtained with the different modules were exported to EXCEL. These exported profiles however, do not include the explanation that are available for the different alerts identified, these can be obtained when running the evaluation with the OECD toolbox as available and have been included for evaluation of the individual compounds below.

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 biotransformations 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 DocIDs 2014/1088460, 2014/1088478, 2014/1088461 and 2014/1088479. Q(SAR) Model Reporting Formats (QMRF) for both endpoints are provided in DocIDs 2013/1414242 and 2013/1414460.

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.

DEREK

DEREK is a predictive computer program, which is an expert system for the identification of toxic potential from chemical structure. The evaluation conducted for metabolites of Dimethenamid-P is presented in DocID 2012/1107265. Further details of the system can be found on the supplier's web site http://www.lhasalimited.org/ under the section for DEREK. The QMRFs for mutagenicity, chromosomal damage and carcinogenicity are available on the ECP-JMPR homepage [http://qsardb.jrc.it/qmrf/]. *DEREK Nexus* uses a knowledge base, which contains alerts describing structure-toxicity relationships, with an emphasis on the understanding of mechanisms of toxicity and metabolism. During an interactive session, *DEREK Nexus* identifies any toxophores or substructures associated with toxicity, and highlights these to the user with a brief statement about the hazard it represents. The user can access additional information concerning the structure-toxicity relationship including literature references and supporting examples. It is well known that the physicochemical properties of a compound play an important role in determining potential toxicity. *DEREK Nexus* calculates Log Kp (by the Potts & Guy equation) Log P (by the Moriguchi estimation) and Molecular weight –by LPS). These values are used in the DEREK assessment where appropriate (e.g. when skin penetration is a factor in assessing the significance of a finding). The knowledge base covers a wide variety of important toxicological end points, which include carcinogenicity, mutagenicity, skin sensitisation, reproductive toxicity, irritation, and respiratory sensitisation.

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/] only the two independent statistical prediction models for mutagenicity (Ames) were selected. The data obtained for Dimethenamid-P and its metabolite can be found in DocIDs 2014/1088457 and 2014/1088458. The first one is an implementation of CAESAR, which makes predictions based on the comparison of the structure of interest to the CAESAR database of mutagenicity data of substances in the structure database. A score is provided for the match of the structures, and the mutagenicity data of the closest related substances compared to the structure of interest. Consequently, if a structure is not adequately presented in the database, the prediction is only of very limited validity. It is important to note, that although the chemical space of any moiety of chloroacetanilid herbicides is covered no significant match to dimethenamid-P was identified.

The second algorithm SarPy searches for isolated structural alerts of substructures in the molecule. Again this is based on the mutagenicity data provided in the structure database.

CAVEAT on reliability of QSAR modules implied

With regard to the QSAR evaluations as implied in the OECD toolbox, in OECD TIMES, in DEREK and in VEGA it should be noted that for nearly all analysis the algorithm reported are out of structural domain error. 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, DEREK) taking into account possible functional group interaction and stereochemical hindrance. It is well acknowledged that these structural activity predictions are therefore of limited validity. To overcome these limitations the evaluation was conducted mainly in comparison to the parent compound Dimethenamid-P or metabolites with available toxicological data, in order to assess whether same or other predictions than for the compared compound were made.

1. Metabolite M656PH023 former assigned M23

M656PH023 (Reg. No. 5886780) is a metabolite of dimethenamid-P that was determined in soil, surface water, ground-water and plants. The predicted exposure level in ground-water is 0.1 µg/l < M656PH023 ≤ 0.75 µg/l. In plants it was not determined in edible commodities.

Acute toxicity and genotoxicity studies as already submitted for Annex I inclusion of dimethenamid-P and presented below have been conducted with M656H023 (Reg.No. 360715) representing the toxocilogically considered equivalent surrogate of the racemic dimethenamid metabolism pathway. The short-term toxicity study in rats has been conducted with M656PH023.

A Structural alerts for M656PH023

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times [see molecule 21 of report DocID 2014/1088460] predicted M656PH023 to be not mutagenic in the Ames test neither without nor with metabolic activation but with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 21 of report DocID 2014/1088461] the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure similar to M656PH049. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by interaction with topoisomerases / proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (12 in total) gave no alert for chromosomal aberration.

In the DEREK analysis conducted structural alerts for M656PH023 were the thiophene alert for hepatotoxicity and nephrotoxicity which was also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (molecule 6) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for chromosomal aberration in vitro with metabolic activation considered of low relevance for the in vivo situation.

B Acute toxicity studies for M656PH023

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: An acute oral toxicity study in rats demonstrating a low toxicity (LD_{50} > 5000 mg/kg bw) has been conducted with M656H023 (Reg. No. 360 715; metabolite of Dimethenamid) and was already evaluated during Annex I inclusion of Dimethenamid-P [see Monograph of the Rapporteur Member State: Germany of Sep, 12, 2000; DocID 1995/11340]

C Genotoxicity studies for M656PH023

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: An Ames test [DocID 1995/11336] and a mouse micronucleus test [DocID 1998/10169] have been conducted with M656H023 (Reg. No. 360 715 / former assigned M23; metabolite of BAS 656 H) and were already evaluated during Annex I inclusion of Dimethenamid-P [see Monograph of the Rapporteur Member State: Germany of Sep, 12, 2000]. M656H023 was negative in the Ames both without and with metabolic activation and gave no evidence for induction of micronuclei in the mouse.

Report:	CA 5.8.1/1 Wollny H.-E., 2000a Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) with Reg.-No. 360 715/M23 2000/1000178
Guidelines:	EEC 87/302, OECD 476
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Executive Summary

M656H031 (Reg. No. 360 715, metabolite of Dimethenamid; Batch: L59-52, Purity: 99.83%) 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 experiments were conducted in the presence and absence of metabolic activation. Based on the results of a pre-test, concentrations of up to 2700 µg/mL were used in the original and the confirmatory experiment. The treatment intervals in the absence and presence of metabolic activation were 4 hours in the first and second experiment, respectively. Ethylmethanesulfonate (EMS) and 7,12-dimethylbenz(a)-anthracene (DMBA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects and precipitation of the test substance were not observed in any of the experiments irrespective of treatment interval and the presence or absence of metabolic activation.
Neither in the original nor in the confirmatory studies was a relevant increase in the mutant frequency observed. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656H023 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2000/1000178)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg. No. 360 715/M23
Description:	Solid (powder), white
Lot/Batch #:	L59-52
Purity:	99.83%
Stability of test compound:	The storage stability of the test substance covering the period of the study was guaranteed by the sponsor.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: Untreated cells

Solvent control: 0.5% (v/v) DMSO in culture medium

Positive control -S9: Ethylmethane sulfonate (EMS) 300 µg/mL (dissolved in nutrient medium)

Positive control +S9: 7,12-dimethylbenz(a)anthracene (DMBA) 2.5 µg/mL (dissolved in DMSO)

3. Activation:

S9 was produced from the livers of induced male Wistar [HanIbm] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix.

4. Test organism:

V79 cells with a doubling time of 12-16h, a good cloning efficiency (as a rule more than 50%) and a modal chromosome number of 22. Stocks of the cell line were maintained in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability and spontaneous mutant frequency.

5. Culture media:

Culture medium:	Minimal essential medium (MEM) supplemented with 10% (v/v) fetal calf serum (FCS).
Treatment medium:	Serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with “saline G” (containing NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x7H_2O$ 290 mg, KH_2PO_4 150 mg; pH 7.2).
Selection medium:	no data given

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay:	Nine concentrations ranging from 26 to 3333 µg/mL
b) Mutation assay:	
1st and 2nd experiment:	84.4, 168.8, 337.5, 675.0, 1350.0 and 2700.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 05-Oct-1999 to 22-Dec-1999

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range for the main experiments under the same culturing and experimental conditions as described for the main test. The colony forming ability of approximately 500 single cells (dublicate cultures per concentration level) after treatment with the test substance was observed and compared to the controls. Toxicity of the test substance was indicated by a reduction of the cloning efficiency (CE). PH and osmolarity were determined in the solvent control and in the maximal concentration in the experiment without metabolic activation.

3. Mutation Assay:

Pretreatment and seeding of cells: Three days old exponentially grown stock cultures (more than 50% confluent) were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. A 0.2% trypsin concentration in Ca-Mg-free salt solution was used which contained NaCl 8000 mg, KCl 400 mg, Glucose 1000 mg; $NaHCO_3$ 350 mg. Prior to trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/mL EDTA. Approximately $1.5x10^6$ (single culture) and $5x10^2$ cells (in duplicate) were seeded in culture medium for the determination of mutation rate and toxicity, respectively.

Cell treatment: 24 hours after seeding, the medium was replaced with serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with “saline G” (containing NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x7H_2O$ 290 mg, KH_2PO_4 150 mg; pH 7.2).

Colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope.

Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 4.5% CO_2, 37°C.

4. Statistics:

Since the distribution of mutant cells did not follow known statistical models, an adequate statistical method is not available.

5. Evaluation criteria:

The test chemical is considered positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.
The test substance producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

A significant response is described as follows:

- Test substance is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- The test substance is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding negative control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.5 - 33.1 mutants per 10^6 cells) a concentration-related increase of the mutations within this range has to be discussed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The storage stability of the test substance covering the period of the study was guaranteed by the sponsor. The stability of the test substance in solvent was not determined analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment, neither precipitation nor toxicity was observed up to the maximal concentration of 3333 µg/mL.

Based on these data the highest concentration tested in the mutagenicity experiments was 2700 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Up to the highest investigated concentration, no relevant increase in mutant colony numbers was observed in both independent experiments. No cytotoxicity and no precipitation was observed up to the maximal concentration of 2700 µg/mL (see **Table 5.8.1-2** and **Table 5.8.1-3**). Low solvent control counts in both cultures of the first and culture I of the second experiment are the reason that the threshold of three times the colony count of the corresponding solvent control was exceeded at some concentrations. The absolute numbers of colonies however, were low and remained well within the range of the historical negative and solvent controls. Therefore, this effect was judged incidental without any biological relevance.

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.

Table 5.8.1-2: Gene mutation in mammalian cells - 1st experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE_1 (survival), relative		CE_2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	8.0	7.2	100.0	100.0	0.70	0.81
Vehicle control (DMSO)	-	2.0	0.6	100.0	100.0	0.52	0.86
M656H023							
84.4 µg/mL	-	n.c.	n.c.	100.6	105.2	n.c.	n.c.
168.8 µg/mL	-	4.3	5.3	105.6	94.3	0.74	0.84
337.5 µg/mL	-	7.9	6.6	101.4	93.6	0.70	0.67
675.0 µg/mL	-	2.2	1.2	99.7	96.4	0.48	0.85
1350.0 µg/mL	-	4.0	1.2	103.0	95.8	0.62	0.90
2700.0 µg/mL	-	6.4	4.0	100.8	97.3	0.64	0.88
Positive control EMS							
300.0 µg/mL	-	536.3	158.1	75.6	97.9	0.68	0.93
4-hour exposure period							
Negative control	+	1.1	10.5	100.0	100.0	0.87	0.82
Vehicle control (DMSO)	+	8.7	12.1	100.0	100.0	0.86	0.76
M656H023							
84.4 µg/mL	+	n.c.	n.c.	100.6	n.c.	n.c.	n.c.
168.8 µg/mL	+	8.4	2.9	96.2	88.0	0.77	0.71
337.5 µg/mL	+	16.3	5.8	97.3	83.6	0.75	0.81
675.0 µg/mL	+	4.6	3.6	106.5	79.2	0.75	0.64
1350.0 µg/mL	+	4.4	4.7	104.7	91.3	0.82	0.71
2700.0 µg/mL	+	4.3	4.9	108.2	92.9	0.87	0.63
Positive control DMBA							
2.5 µg/mL	+	849.4	974.2	32.4	37.2	0.51	0.61

CE_1 : (mean number of found number of cells per flask/corresponding control) x 100
CE_2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Table 5.8.1-3: Gene mutation in mammalian cells – 2nd experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE_1 (survival), relative		CE_2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	3.9	0.5	100.0	100.0	0.58	0.77
Vehicle control (DMSO)	-	1.9	7.9	100.0	100.0	0.98	0.87
M656H023							
84.4 µg/mL	-	n.c.	n.c.	103.6	114.6	n.c.	n.c.
168.8 µg/mL	-	16.4	1.3	98.8	100.9	0.69	0.79
337.5 µg/mL	-	1.3	5.2	105.5	104.9	0.82	0.91
675.0 µg/mL	-	0.7	5.4	100.0	100.6	0.78	0.78
1350.0 µg/mL	-	7.3	5.0	104.5	103.0	0.59	0.81
2700.0 µg/mL	-	5.3	2.6	96.7	96.7	0.83	0.78
Positive control EMS							
300.0 µg/mL	-	208.6	208.7	83.3	95.4	0.75	0.78
4-hour exposure period							
Negative control	+	1.6	0.6	100.0	100.0	0.86	0.74
Vehicle control (DMSO)	+	6.0	0.7	100.0	100.0	0.82	0.66
M656H023							
84.4 µg/mL	+	n.c.	n.c.	97.7	114.7	n.c.	n.c.
168.8 µg/mL	+	7.6	4.7	97.7	100.3	0.72	0.72
337.5 µg/mL	+	6.0	5.4	102.5	106.9	0.87	0.56
675.0 µg/mL	+	2.3	2.6	98.8	100.5	0.84	0.53
1350.0 µg/mL	+	4.6	2.6	96.7	112.6	0.64	0.81
2700.0 µg/mL	+	3.3	7.0	101.7	103.6	0.83	0.53
Positive control DMBA							
2.5 µg/mL	+	494.0	673.5	102.0	63.9	0.64	0.55

CE_1 : (mean number of found number of cells per flask/corresponding control) x 100
CE_2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656H023 does not induce forward mutations in the HPRT locus in V79 cells in vitro.

Conclusion on genotoxicity of M656PH023

Overall there is no evidence for genotoxicity of M656PH023 based on these study results available.

D Short-term toxicity of M656PH023

Report:	CA 5.8.1/2 [redacted] 2014b Reg.No. 5886780 (metabolite of BAS 656-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats administration via the diet 2013/1342918
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of M656PH023 (Reg.No. 5886780, Dimethenamid-P; Batch: L82-104; Purity: 99.9%) to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm for at least 28 days did not cause any test substance-related adverse signs of toxicity.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1388 mg/kg bw/d) and in female (1057 mg/kg bw/d) Wistar rats.

(DocID 2013/1342918)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 5886780, metabolite of BAS 656-PH (Dimethenamid-P)
Description:	Solid/white
Batch/purity #:	L82-104,
Purity:	99.9%
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor. Expiry date: Jun. 01, 2015

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar Crl:WI (Han) Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 153.3 ± 6.1 g, ♀ 129.3 ± 6.8 g
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
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:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 11-Jun-2013 - 13-Jan-2014
(In life dates: 20-Jun-2013 (start of administration) to 19-Jul-2013 (necropsy))

2. Animal assignment and treatment:

M656PH023 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 31 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level a single sample was analysed. No test-article was determined in control diets.

Table 5.8.1-4: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1200 ppm	17-Jun-13	1291 ± 26[#]	107.6	2.0
4000 ppm	17-Jun-13	4589, 4577[¥]	114,7, 114.4[¥]	n.a.
12000 ppm	17-Jun-13	12607 ± 607[#]	105.1	4.8

n.a.: not applicable;
[#] based on mean values of the three individual samples
[¥] values for sample and retain sample
Values may not calculate exactly due to rounding of figures

Considering the low relative standard deviation in the homogeneity analysis, it can be concluded that M656PH023 was distributed homogeneously in ground Kliba maintenance diet/mouse rat „GLP“ meal.

Generally the mean values of M656PH023 in ground Kliba maintenance diet mouse/rat “GLP” meal were found to be in the range of 90-110% of the nominal concentrations demonstrating the correctness of the concentrations of M656PH023 in the vehicle. There was however a single deviation for the mid dose (4000 ppm) sample as confirmed by the retain sample were the determined concentrations were in the range of 114.4 to 114.7% of the nominal concentration. These values still in the range of ± 15% of the target concentration can be regarded as acceptable for analysis of complex matrices like diet also considering that generally all determined concentrations for all dose levels were above 100%.

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 5.8.1-5: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-6: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except pH, volume, color, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table 5.8.1-7: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y \text{ to } x}} \times 100$$

BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), $FC_{y \text{ to } x}$ 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted.

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from 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 hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓	✓	#	seminal vesicles with coagulating glans
✓		#	aorta	✓			lachrymal glands, extraorbital	✓			skin
✓		#	bone marrow§	✓		#	larynx	✓		#	spinal cord (3 levels)@
✓	✓	#	brain	✓	✓	✓	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓		#	sternum w. marrow
✓		#	colon	✓		#	lymph nodes#	✓		#	stomach (fore- & glandular)
✓		#	duodenum	✓		#	mammary gland (♂ and ♀)	✓	✓	#	testes
✓	✓	#	epididymides¥	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	eyes (with optic nerve)	✓		#	nose/nasal cavity‡	✓		#	trachea
✓			femur (with joint)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
			gall bladder	✓		#	pancreas	✓	✓	#	uterus with cervix
✓		✓	gross lesions	✓			pharynx	✓		#	vagina
✓			Harderian glands	✓		#	pituitary				
✓	✓	#	heart	✓	✓	#	prostate				
✓		#	ileum	✓		#	rectum		✓		body (anesthetized animals)
✓		#	jejunum (w. Payer's plaque)	✓			salivary glands*				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; ‡1 histopathology at level III, ¥left epididymidis collected for histopathology

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina. A correlation between gross lesions and histopathological findings was attempted.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Regarding the overall motor activity as well as single intervals, no test substance-related deviations were noted for male and female animals.

There were some statistically significant differences between control and treated groups, however, these changes were isolated findings and not clearly dose related. Therefore, these changes were considered incidental. These changes consisted of

- Decreased activity at single interval 8 of male animals of the high dose group (12000 ppm)
- Decreased activity at single interval 8 of female animals of the mid dose group (4000 ppm)

No statistically significant differences on overall motor activity were observed at any dose.

C. BODY WEIGHT AND BODY WEIGHT GAIN

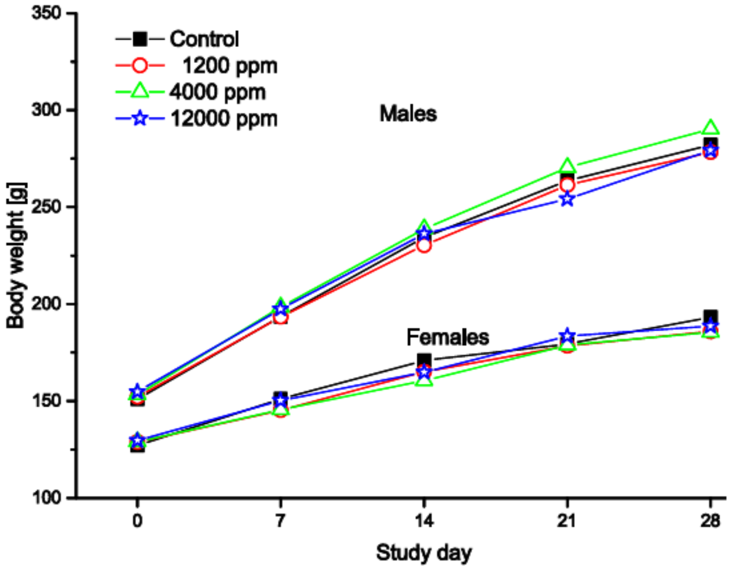
No treatment-related differences of absolute body weights or body weight gain were noted [see **Table 5.8.1-8** and **Figure 5.8.1-1**]. Body weight change values of female animals of all groups dosed with M656PH023 were significantly lower on study day 14, irrespective of the concentration in the diet. However, no significant differences were observed after 21 and 28 days of treatment. A relation to treatment was not assumed.

Table 5.8.1-8: Mean body weight of rats administered M656PH023 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
Body weight [g]								
- day 0	151.0	152.1	153.3	154.7	127	128.8	129	129.6
- day 28	282.2	278.3	290.2	279.2	193.3	185.9	185.4	188.6
Δ% (compared to control)[#]		-1.4	2.8	-1.1		3.8	-4.1	-2.4
Body weight gain [g]								
- day 0 → day 7	42.5	41.4	45.1	42.9	24.1	16.5	16.5	20.6
- day 0 → day 14	83.4	78.2	85.4	81.6	43.9	**36.3 ***	**31.5 ****	**35.1 ***
- day 0 → day 21	112.6	109.3	117.1	99.5	52.5	49.7	49.8	53.9
Overall body weight gain - day 0 → day 28	131.2	126.2	136.9	124.5	66.3	57.1	56.4	59
Δ% (compared to control)[#]		-3.8	4.3	-5.1		-13.9	-15	-11

* $P \leq 0.05$, ** $p \leq 0.01$ (Dunnett test - two sided)
[#] Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-1: Body weight development of rats administered M656PH023 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats.
Note: Some values i.e. for male control group animals from day 25 to 28 and for males of the high dose group (12000 ppm) between study days 11 to 14, 18 to 21 as well as 25 to 28 were declared as outlier and thus was not taken into consideration. Increased food spilling was observed for these animals.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following table:

Table 5.8.1-9: Calculated intake of sodium salt of M656PH023

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1200	106	106
2	4000	357	349
3	12000	1388	1057

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes among hematological parameters were observed.

2. Clinical chemistry findings

No treatment-related adverse changes among clinical chemistry parameters were observed.

At the end of the study in males of the high dose group (12000 ppm), triglyceride levels were increased [see **Table 5.8.1-10**], but as this was the only changed clinical pathology parameter in these individuals, this alteration was regarded as possibly treatment-related but not adverse following the criteria laid down in the ECETOC Technical Report No. 85, 2002 [DocID 2002/1027057].

Table 5.8.1-10: Selected clinical chemistry findings in rats administered M656PH023 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		106	357	1388		106	349	1057
Triglycerides [mmol/l]	0.84	0.85	1.01	1.35*	0.37	0.39	0.36	0.41

*p ≤ 0.05 ,**p≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed.

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls [see **Table 5.8.1-11**].

Regarding pathology, the only finding was a significant increase (+14%) of the mean relative liver weight in males of the high dose group (12000 ppm). As there was no histopathological correlate, the liver weight increase was considered to be treatment-related but adaptive.
Because there was no dose-response relationship, the increased mean relative kidney weight in males of the mid dose group (4000 ppm) was regarded to be incidental. No other statistically significant changes of absolute or relative organ weights were observed.

Table 5.8.1-11: Selected mean absolute and relative organ weights of rats administered M656PH023 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		106	357	1388		106	349	1057
Terminal bodyweight [g] [% of control]	254.5 100	252.74 99	266.62 105	251.96 99	175.74 100	168.34 96	169.24 96	174.02 99
Liver, absolute [g] [% of control]	6.606 100	6.574 100	7.092 107	7.45 113	4.838 100	4.69 97	4.754 98	4.616 95
Liver, relative [%] [% of control]	2.599 100	2.599 100	2.66 102	**2.955**** **114**	2.751 100	2.789 101	2.811 102	2.653 96
Kidneys, absolute [g] [% of control]	1.87 100	1.75 94	2.096 112	1.944 104	1.386 100	1.344 97	1.328 96	1.328 96
Kidneys, relative [%] [% of control]	0.735 100	0.691 94	**0.786**** **107**	0.773 105	0.788 100	0.8 102	0.783 99	0.764 97

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross and histopathology

No treatment-related macroscopic or microscopic alterations were observed. All gross lesions and all histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered incidental or spontaneous in origin.

III. CONCLUSIONS

The administration of M656PH023 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance-related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1388 mg/kg bw/d) and in female (1057 mg/kg bw/d) Wistar rats.

E Toxicological evaluation of metabolite M656PH023

The limited toxicological alert for chromosomal aberration in vitro identified for M656PH023 was not confirmed by the genotoxicological testing conducted. There was no evidence for genotoxicity of M656PH023 in the in vitro and in vivo genotoxicity studies conducted fulfilling the requirements for evaluation of ground-water metabolites. The available data on systemic toxicity – acute oral toxicity and short-term toxicity study in rats - clearly demonstrated that the compound is of low toxicity and thus less toxic than the parent molecule Dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.

Thus, M656PH023 is considered to be of no toxicological relevance.

2. Metabolite M656PH026 former assigned M26

M656PH026 is a metabolite of dimethenamid-P determined in rat, goat (dosed with M565PH030), plant and soil. The determined levels of M656H026 in rats and mice were at trace levels but M656PH026 was up to 68% of the applied dose in urine when dosed with M656PH030. In plants, the only human consumable it was measured in was bulb onions at a level near the LOQ. It was measured in animal feed items. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment. Exposure estimates for consumer were 0.0025 µg/kg bw/day (threshold for genotoxic compounds) < M656PH026 ≤ 1.5 µg/kg bw/day (threshold for non-genotoxic Cramer Class III compounds).

A Structural alerts for M656PH026

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered to be of relevance.
OASIS-Times (Molecule 1 of Report Amendment) predicted M656PH026 to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (20 in total) gave no alert for chromosomal aberration.

The Vega prediction (Molecule 7) for Ames mutagenicity in both modules CAESAR and SarPy was negative. However, the reliability of this prediction was low, a no similar compounds with known experimental data were in the database, similar molecules found in the training set disagree with the prediction and the accuracy of the prediction was not optimal. In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

In conclusion there was a limited alert for chromosomal aberration in vitro with metabolic activation in one of the structure activity evaluation tools employed considered of no relevance based on related alert for M656PH031 not confirmed by genotoxicity testing.

B Toxicological evaluation of metabolite M656PH026

The limited alert for chromosomal aberration in vitro identified for M656PH026 was considered to be of no relevance in vivo in comparison to the parent molecule dimethenamid-P and the closely related metabolites M656PH030, M656PH031 and M656PH032 for which genotoxicity data are available leading to the conclusion M656PH026 not to be genotoxic. Furthermore the determined exposure levels in plant are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 1.5 µg/kg bw/day.
Thus M656PH026 is considered to be of no toxicological relevance.

3. Metabolite M656PH027 former assigned M27

M656PH027 is a metabolite of dimethenamid-P determined in rat, hen, goat, mice, plant and ground-water and surface water. The determined levels of M656H023 in rats, mice, and goat were at trace levels and were previously reviewed under Annex I.
The predicted exposure levels in ground water is 0.75 µg/l < M656PH027 ≤ 4.5 µg/l.

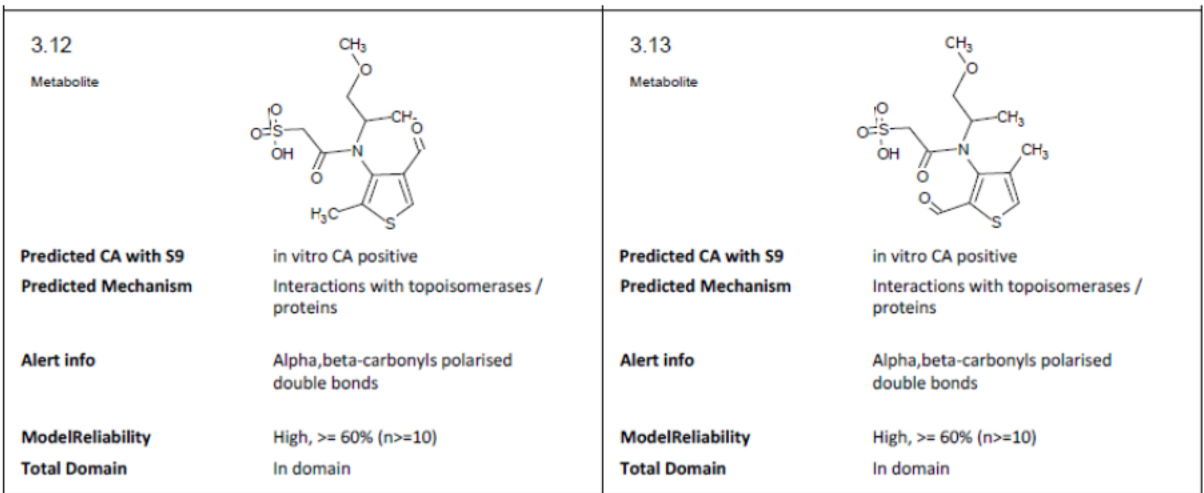
Acute toxicity, genotoxicity studies and short-term toxicity study in rat as already submitted for Annex I inclusion of dimethenamid-P and/or presented below have been conducted with the sodium salt of M656H027 (Reg.No. 360714) representing the stable and considered toxocilogically equivalent surrogate of the racemic dimethenamid metabolism pathway.

C Structural alerts for M656PH027

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

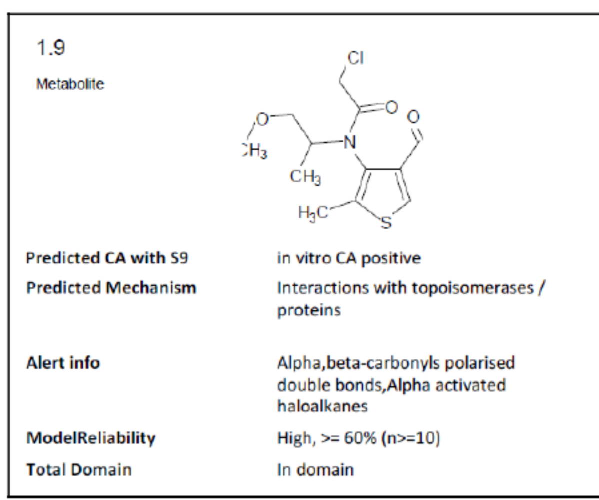
OASIS-Times (Molecule 3) predicted M656PH027 to be not mutagenic in the Ames test neither without nor with metabolic activation but with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into structures forming an alpha-beta carbonyl with polarized double bond [see **Figure 5.8.1-2**]. For these alpha-beta unsaturated carbonyls was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins (2 transformation products). In this case the prediction was in the domain for this model. Thus the alert was considered principally relevant. Similar structures with the same alerts were also identified for dimethenamid-P. All other presumed transformation products (16 in total) gave no alert for chromosomal aberration.

Figure 5.8.1-2: Presumed mammalian transformation products of M656PH027 and related structures of dimethenamid-P with structural alert for chromosomal aberration in vitro

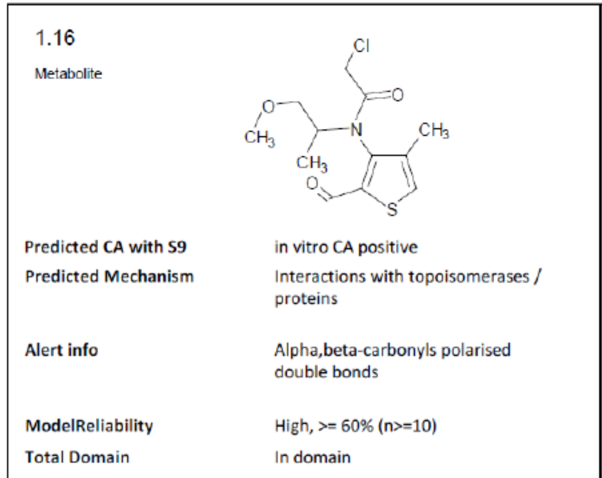


3.12 Metabolite		3.13 Metabolite	
Predicted CA with S9	in vitro CA positive	**Predicted CA with S9**	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins	**Predicted Mechanism**	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds	**Alert info**	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)	**ModelReliability**	High, >= 60% (n>=10)
Total Domain	In domain	**Total Domain**	In domain

Presumed metabolite of M656PH027 Presumed metabolite of M656PH027



Presumed metabolite of Dimethenamid-P



Presumed metabolite of Dimethenamid-P

In the DEREK analysis conducted structural alerts for M656PH027 were the thiophene alert for hepatotoxicity and nephrotoxicity which was also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (Molecule 8) for Ames mutagenicity in both modules CAESAR and SarPy was inconclusive. While the CAESAR module predicted M656PH027 to be mutagenic the prediction of the SarPy module was non-mutagenic. However the reliability of these predictions was low.

In conclusion a structural alert for chromosomal aberration in vitro after metabolic activation was identified. The genotoxicity testing conducted [see section CA 5.8.1 3 E] did however not confirm this alert.

D Acute toxicity studies of M656PH027

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: An acute oral toxicity study in rats conducted with the sodium salt of M656H027 (Reg. No. 360 714; metabolite of Dimethenamid) demonstrating a low toxicity (LD_{50} > 5000 mg/kg bw) has been conducted and was already evaluated during Annex I inclusion of Dimethenamid-P [see Monograph of the Rapporteur Member State: Germany of Sep, 12, 2000; DocID 1992/12507]

E Genotoxicity studies of M656PH027

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: An Ames test [DocID 1995/11338] and a mouse micronucleus test [DocID 1998/10168] have been conducted with the sodium salt of M656H027 (Reg. No. 360 714, metabolite of Dimethenamid) and were already evaluated during Annex I inclusion of Dimethenamid-P [see Monograph of the Rapporteur Member State: Germany of Sep, 12, 2000].

Report: CA 5.8.1/3
Wollny H.-E., 2000b
Gene mutation assay in Chinese Hamster V79 cells in vitro (V79/HPRT) with Reg.-No. 360 714/M27
2000/1000179
Guidelines: EEC 87/302, OECD 476
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Executive Summary

Sodium salt of M656H027 (Reg. No. 360 714, metabolite of Dimethenamid; Batch: L59-50, Purity: 97.2%) 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 experiments were conducted in the presence and absence of metabolic activation. Based on the results of a pre-test, concentrations of up to 3400 µg/mL were used in the original and the confirmatory experiment. The treatment intervals in the absence and presence of metabolic activation were 4 hours in the first and second experiment, respectively. Ethylmethanesulfonate (EMS) and 7,12-dimethylbenz(a)-anthracene (DMBA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects and precipitation of the test substance were not observed in any of the experiments irrespective of treatment interval and the presence or absence of metabolic activation.
Neither in the original nor in the confirmatory study was a relevant increase in the mutant frequency observed. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test sodium salt of M656H027 does not induce forward mutations in mammalian cells in vitro.
(BASF DocID 2000/1000179)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**	**Reg. No. 360 714 (sodium salt of metabolite of BAS 656 H, dimethenamid)**
Description:	Solid (powder), white
Lot/Batch #:	L59-50
Purity:	97.2%
Stability of test compound:	The storage stability of the test substance covering the period of the study was guaranteed by the sponsor.
Solvent used:	deionized water

2. Control Materials:

Negative control: Untreated cells

Solvent control: 10 % v/v deionized water, DMSO

Positive control -S9: Ethylmethane sulfonate (EMS) 300 µg/mL (dissolved in nutrient medium)

Positive control +S9: 7,12-dimethylbenz(a)anthracene (DMBA) 2.5 µg/mL (dissolved in DMSO)

3. Activation:

S9 was produced from the livers of induced male Wistar [HanIbm] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

The ratio of S9 supernatant to cofactor solution was 1:2.0 in the first and 1:9.0 in the second experiment based upon the total volume of S9 mix.

4. Test organism:

V79 cells with a doubling time of 12-16h, a good cloning efficiency (as a rule more than 50%) and a modal chromosome number of 22. Stocks of the cell line were maintained in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability and spontaneous mutant frequency.

5. Culture media:

Culture medium: Minimal essential medium (MEM) supplemented with 10% (v/v) fetal calf serum (FCS).

Treatment medium: Serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with “saline G” (containing NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4 x 7H_2O$ 290 mg, KH_2PO_4 150 mg; pH 7.2).

Selection medium: no data given

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 26.6 to 3400 µg/mL

b) Mutation assay:
1st and 2nd experiment: 106.3, 212.5, 425.0, 850.0, 1700.0 and 3400.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 12-Oct-1999 to 30-Dec-1999

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range for the main experiments under the same culturing and experimental conditions as described for the main test. The colony forming ability of approximately 500 single cells (dublicate cultures per concentration level) after treatment with the test substance was observed and compared to the controls. Toxicity of the test substance is indicated by a reduction of the cloning efficiency (CE). PH and osmolarity were determined in the solvent control and in the maximal concentration in the experiment without metabolic activation.

3. Mutation Assay:

Pretreatment and seeding of cells: Three days old exponentially grown stock cultures (more than 50% confluent) were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. A 0.2% trypsin concentration in Ca-Mg-free salt solution was used which contained NaCl 8000 mg, KCl 400 mg, Glucose 1000 mg; $NaHCO_3$ 350 mg. Prior to trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/mL EDTA. Approximately $1.5x10^6$ (single culture) and $5x10^2$ cells (in duplicate) were seeded in culture medium for the determination of mutation rate and toxicity, respectively.

Cell treatment:	24 hours after seeding, the medium was replaced with serum-free medium containing the test substance either without S9 mix or with 50 µL/mL S9 mix. After 4 hours this medium was replaced with culture medium following two washing steps with "saline G" (containing NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4 x 7H_2O$ 290 mg, KH_2PO_4 150 mg; pH 7.2). Colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope. Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 4.5% CO_2, 37°C.

4. Statistics:

Since the distribution of mutant cells did not follow known statistical models, an adequate statistical method is not available.

5. Evaluation criteria:

The test chemical is considered positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.
The test substance producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

A significant response is described as follows:

- Test substance is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- The test substance is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding negative control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.5 - 33.1 mutants per 10^6 cells) a concentration-related increase of the mutations within this range has to be discussed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The storage stability of the test substance covering the period of the study was guaranteed by the sponsor. The stability of the test substance in solvent was not determined analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment, neither precipitation nor toxicity was observed up to the maximal concentration of 3400 µg/mL.

Based on these data the highest concentration tested in the mutagenicity experiments was 3400 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Up to the highest investigated concentration, no relevant increase in mutant colony numbers was observed in both independent experiments. No cytotoxicity and no precipitation was observed up to the maximal concentration of 3400 µg/mL (see **Table 5.8.1-12**, **Table 5.8.1-13**).

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.

Table 5.8.1-12: Gene mutation in mammalian cells - 1st experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE₁ (survival), relative		CE₂ (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	8.1	6.2	100.0	100.0	0.75	0.75
Vehicle control (deio.water)	-	11.5	5.0	100.0	100.0	0.77	0.79
Sodium salt of M656H027							
106.3 µg/mL	-	n.c.	n.c.	95.8	99.0	n.c.	n.c.
212.5 µg/mL	-	13.6	0.7	83.2	108.9	0.73	0.75
425.0 µg/mL	-	3.8	8.7	88.6	104.4	0.77	0.79
850.0 µg/mL	-	5.8	11.8	80.6	89.5	0.84	0.80
1700.0 µg/mL	-	5.0	3.9	85.5	106.5	0.71	0.69
3400.0 µg/mL	-	5.8	6.5	78.6	81.6	0.76	0.76
Positive control EMS							
300.0 µg/mL	-	143.7	163.9	85.4	74.7	0.70	0.67
4-hour exposure period							
Negative control	+	6.8	3.1	100.0	100.0	0.65	0.67
Vehicle control (deio.water)	+	9.0	3.1	100.0	100.0	0.77	0.67
Vehicle control (DMSO)	+	4.3	5.3	96.2	103.9	0.67	0.72
Sodium salt of M656H027							
106.3 µg/mL	+	n.c.	n.c.	98.8	n.c.	n.c.	n.c.
212.5 µg/mL	+	7.8	4.9	98.5	110.7	0.76	0.76
425.0 µg/mL	+	2.8	8.0	100.8	104.1	0.79	0.76
850.0 µg/mL	+	11.1	4.3	97.7	109.8	0.62	0.63
1700.0 µg/mL	+	17.2	1.5	100.8	108.8	0.58	0.58
3400.0 µg/mL	+	2.3	5.5	97.3	107.9	0.67	0.68
Positive control DMBA							
2.5 µg/mL	+	1102.7	1063.3	49.3	57.0	0.46	0.47

CE_1 : (mean number of found number of cells per flask/corresponding control) x 100
CE_2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

Table 5.8.1-13: Gene mutation in mammalian cells – 2nd experiment

Test group	Metabolic activation	Mutant frequency (per 10^6 cells)		CE_1 (survival), relative		CE_2 (viability), relative	
		Culture I	Culture II	Culture I	Culture II	Culture I	Culture II
4-hour exposure period							
Negative control	-	2.9	3.3	100.0	100.0	0.68	0.75
Vehicle control (deio.water)	-	5.7	6.8	100.0	100.0	0.77	0.86
Sodium salt of M656H027							
106.3 µg/mL	-	n.c.	n.c.	98.1	82.0	n.c.	n.c.
212.5 µg/mL	-	3.2	3.6	90.2	83.3	0.74	0.72
425.0 µg/mL	-	3.8	1.3	99.5	92.0	0.82	0.71
850.0 µg/mL	-	6.9	3.7	81.7	80.5	0.68	0.79
1700.0 µg/mL	-	11.0	3.1	91.0	68.0	0.70	0.79
3400.0 µg/mL	-	1.2	3.6	81.2	66.6	0.80	0.83
Positive control EMS							
300.0 µg/mL	-	224.7	330.8	75.2	104.2	0.72	0.56
4-hour exposure period							
Negative control	+	6.9	1.4	100.0	100.0	0.79	1.03
Vehicle control (deio.water)	+	3.6	4.7	100.0	100.0	0.92	1.00
Vehicle control (DMSO)	+	6.0	1.0	109.2	101.2	0.85	0.97
Sodium salt of M656H027							
106.3 µg/mL	+	n.c.	n.c.	100.6	94.8	n.c.	n.c.
212.5 µg/mL	+	4.9	7.2	111.9	99.7	0.82	0.86
425.0 µg/mL	+	3.1	1.0	106.2	87.0	0.79	0.86
850.0 µg/mL	+	1.6	3.6	118.9	93.0	0.93	0.88
1700.0 µg/mL	+	1.3	1.2	108.6	103.0	0.78	0.89
3400.0 µg/mL	+	1.9	2.2	113.7	101.5	0.89	0.94
Positive control DMBA							
2.5 µg/mL	+	321.0	322.6	89.7	94.6	0.87	0.91

CE_1 : (mean number of found number of cells per flask/corresponding control) x 100
CE_2 : (mean number of found number of cells per flask/seeded cells) x 100
n.c.: not continued because five concentrations ranges were selected for evaluation

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test Sodium salt of M656H027 does not induce forward mutations in the HPRT locus in V79 cells in vitro.

Conclusion on genotoxicity of M656PH027

Overall there is no evidence for genotoxicity of M656PH027 based on the study results available.

F Short-term toxicity of metabolite M656PH027

Report:	CA 5.8.1/4 [redacted] 2014a Reg.No. 360714 (metabolite of BAS 656 H, Dimethenamid) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2013/1342917
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.1/5 Class T., 2013b Analytical report - Homogeneity and concentration control of Dimethenamid metabolite M27 (Reg.No. 360714) in vehicle 2013/1413980
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

Administration of the sodium salt of M656H027 (Reg.No. 360 714, metabolite of Dimethenamid; Batch: L81-46; Purity: 98.7%) to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm for at least 28 days did not cause any test substance-related adverse signs of toxicity.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1064 mg/kg bw/d) and in female (1247 mg/kg bw/d) Wistar rats.

(DocID 2013/1342917)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** **Reg. No. 360 714 (sodium salt of metabolite of BAS 656 H, dimethenamid)**
   Description: Solid/white
   Batch/purity #: L82-97,
   Purity: 92.3%
   Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.
   Expiry date: Sep. 09, 2014

2. **Vehicle and/or positive control:** Rodent diet

3. **Test animals:**
   Species: Rat
   Strain: Wistar Crl:WI (Han)
   Male and female
   Age: 42 ± 1 day at start of administration
   Weight at dosing: ♂: 155.0 ± 6.8 g, ♀ 125.2 ± 5.7 g
   Source: Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
   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: Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm^2 with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment
   Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm^2) and small amounts of absorbent material
   Environmental conditions:
   Temperature: 20 - 24 °C
   Humidity: 30 - 70 %
   Air changes: 15 air changes 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 - 09-Jan-2014
(In life dates: 04-Jul-2013 (start of administration) to 02-Aug-2013 (necropsy))

2. Animal assignment and treatment:

Sodium salt of M656H027 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 31 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level a single sample was analysed. No test-article was determined in control diets.

Table 5.8.1-14: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1200 ppm	03-Jul-13	1235 ± 152[#]	103.3	12.3
4000 ppm	03-Jul-13	4170	104.0	n.a.
12000 ppm	03-Jul-13	11765 ± 878[#]	98.3	7.5

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Homogeneity tested for the lower level of 1200 µg/g (ppm) indicated a slightly lower concentration of about 1064 ppm or about 11 % lower than nominal in the sample aliquot collected from the lower portion of the vessel and slightly higher concentrations (≤ 13% higher than nominal) in sample aliquots collected from the middle and upper portions of the vessel.

Homogeneity tested for the highest level of 12000 µg/g (ppm) indicated a slightly lower concentration of about 10755 ppm or about 10 % lower than nominal in the sample aliquot collected from the upper portion of the vessel.
Relative standard deviations (RSD) were found to be in the range of 12 % to 7 % for the lowest (1200 ppm) and highest concentration (12000 ppm). In general, a RSD of ≤ 10% can be regarded as acceptable for diet analysis. This very slight deviation for the lowest concentration (RSD = 12 %) from the acceptance criterion is, however, considered acceptable taking into account that the highest concentration was determined in the mid sample and thus not indicating a systematical inhomogeneity.

The mean values of sodium salt of M656H027 in ground Kliba maintenance diet mouse/rat "GLP" meal were found to be in the range of 90-110% of the nominal concentrations. These results demonstrated the correctness of the concentrations of sodium salt of M656H027 in the vehicle.

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 5.8.1-15: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-16: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except pH, volume, color, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table 5.8.1-17: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ \text{to}\ x}} \times 100$$

BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), $FC_{y\ \text{to}\ x}$ 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from 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 hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓	✓	#	seminal vesicles with coagulating glans
✓		#	aorta	✓			lachrymal glands, extraorbital	✓			skin
✓		#	bone marrow[§]	✓		#	larynx	✓		#	spinal cord (3 levels)[@]
✓	✓	#	brain	✓	✓	✓	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓		#	sternum w. marrow
✓		#	colon	✓		#	lymph nodes[#]	✓		#	stomach (fore- & glandular)
✓		#	duodenum	✓		#	mammary gland (♂ and ♀)	✓	✓	#	testes
✓	✓	#	epididymides[¥]	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	eyes (with optic nerve)	✓		#	nose/nasal cavity[‡]	✓		#	trachea
✓			femur (with joint)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
			gall bladder	✓		#	pancreas	✓	✓	#	uterus with cervix
✓		✓	gross lesions	✓			pharynx	✓		#	vagina
✓			Harderian glands	✓		#	pituitary				
✓	✓	#	heart	✓	✓	#	prostate				
✓		#	ileum	✓		#	rectum		✓		body (anesthetized animals)
✓		#	jejunum (w. Payer's plaque)	✓			salivary glands*				
[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology											

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
No statistically significant differences of overall motor activity between control and treated animals were observed for males of the other test groups (1200 and 4000 ppm) as well as for the female animals of all test groups.

C. BODY WEIGHT AND BODY WEIGHT GAIN

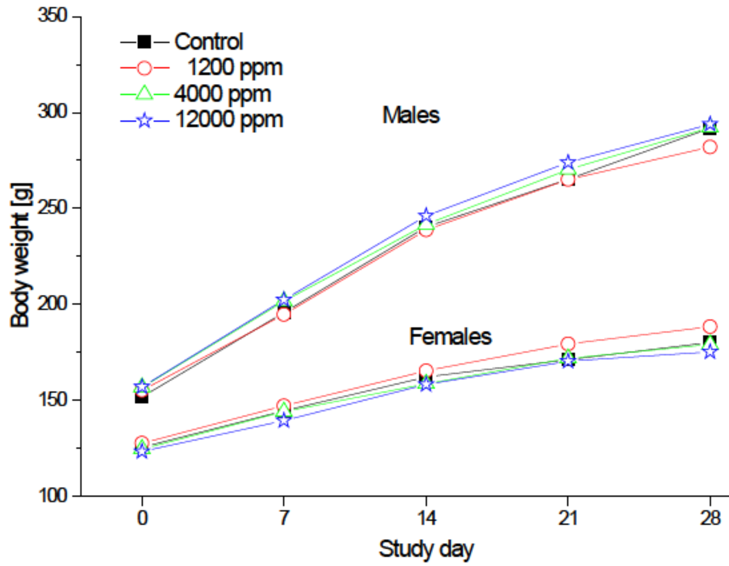
No statistically significant differences of absolute body weights or body weight gain were noted [see **Table 5.8.1-18** and **Figure 5.8.1-3**].

Table 5.8.1-18: Mean body weight of rats administered sodium salt M656H027 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
Body weight [g]								
- Day 0	151.7	155.2	156.8	157.0	125.5	127.6	124.6	123.3
- Day 28	291.8	282.0	292.2	293.9	180.1	188.4	179.1	175.2
Δ% (compared to control)#		-3.3	0.2	0.7		4.6	-0.5	-2.7
Overall body weight gain [g]	138.8	126.9	135.5	136.9	54.7	60.8	54.6	51.9
Δ% (compared to control)#		-8.6	-2.4	-1.3		11.2	-0.1	-5

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-3: Body weight development of rats administered sodium salt of M656H027 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats.
Note: The value for male animals of the high dose group (12000 ppm) between study days 11 to 14 was declared as outlier and thus was not taken into consideration. Increased food spilling was observed for these animals.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following table:

Table 5.8.1-19: Calculated intake of sodium salt of M656H027

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1200	99	144
2	4000	364	341
3	12000	1064	1247

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes among hematological parameters were observed.

2. Clinical chemistry findings

No treatment-related changes among clinical chemistry parameters were observed.

At the end of the study in females of test groups 1, 2 and 3 (1200, 4000 and 12000 ppm), ALP activities were decreased (in test group 2 not significantly). However, all means were within the historical control range [**Table 5.8.1-20**]. Therefore, this alteration was regarded as incidental and not treatment-related.

Table 5.8.1-20: Selected clinical chemistry findings in rats administered sodium salt of M656H027 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day]		99	364	1064		144	341	1247
ALP [µkat/l]	2.48	2.64	2.38	2.54	1.43	**1.25***	**1.18**	**1.10****
Historical control range [µkat/l]					0,71 - 2.01 (mean 1.25)			

*p ≤ 0.05 ,**p≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed.

The lower specific gravity of the urine in males of the 4000 ppm group [see **Table 5.8.1-21**] was not considered to be incidental as there was no dose-response relationship.

Table 5.8.1-21: Selected findings in urinalysis in rats administered sodium salt of M656H027 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day		99	364	1064		144	341	1247
Specific gravity [g/l]	1,054	1,052	**1,039***	1,056	1,064	1,056	1,077	1,073

*p ≤ 0.05 ,**p≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls [see **Table 5.8.1-22**].

When compared to control group the mean absolute thymus weights of in the male 1200 ppm group and the mean absolute ovary weights in the 12000 ppm group were significantly decreased. In the male 12000 ppm group the mean relative heart weights were significantly decreased. The significant thymus weight decrease in males of test group 1 (1200 ppm) occurred without any dose-dependency and was regarded as incidental. The significant weight decrease of the ovaries in females of the 12000 ppm group and the significant relative heart weight decreases in males of the 12000 ppm group occurred without any histopathological findings and, therefore, were considered to be incidental.

Table 5.8.1-22: Selected mean absolute and relative organ weights of rats administered sodium salt of M656H027 for at least 28 days

Sex	**Males**				**Females**			
Dose [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
[mg/kg bw/day]		**99**	**364**	**1064**		**144**	**341**	**1247**
Terminal bodyweight [g]	264.84	258.66	264.66	268.26	164.2	169.1	163.34	160.02
[% of control]	100	98	100	101	100	103	99	97
Thymus, absolute [mg]	470	**403.6****	534.8	514.6	456.8	398.2	430.4	374.8
[% of control]	100	**86**	114	109	100	87	94	82
Thymus, relative [%]	0.178	0.156	0.202	0.192	0.277	0.235	0.265	0.234
[% of control]	100	88	114	108	100	85	96	85
Ovaries, absolute [g]					95.2	96.4	92	**81***
[% of control]					100	101	97	**85**
Ovaries, relative [%]					0.058	0.057	0.056	0.051
[% of control					100	99	97	88
Heart, absolute [g]	0.886	0.864	0.886	0.818	0.592	0.620	0.598	0.612
[% of control]	100	98	100	92	100	102	96	93
Heart, relative [%]	0.334	0.334	0.334	**0.305***	0.360	0.366	0.367	0.382
[% of control	100	100	100	**91**	100	102	102	106

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No other statistically significant changes of absolute or relative organ weights were observed.

2. Gross and histopathology

No treatment-related macroscopic or microscopic alterations were observed. All gross lesions and all histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered incidential or spontaneous in origin.

III. CONCLUSIONS

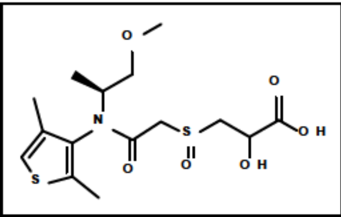
The administration of sodium salt of M656H027 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance-related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1064 mg/kg bw/d) and in female (1247 mg/kg bw/d) Wistar rats.

G Toxicological evaluation of metabolite M656PH027

The toxicological alert for chromosomal aberration in vitro identified for M656PH027 was not confirmed by the genotoxicological testing conducted. There was no evidence for genotoxicity of M656PH027 in the studies conducted fulfilling the requirements for evaluation of ground-water metabolites. The available data on general toxicity – acute oral toxicity and short-term toxicity study in rats - clearly demonstrated that the compound is of low toxicity and thus less toxic than the parent molecule Dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
Thus M656PH027 is considered to be of no toxicological relevance.

4. Metabolite M656PH030 former assigned M30

M656PH030 a metabolite identified in rat, hen, and goats dosed with M656PH030. The determined levels of M656H030 in rats and mice were at trace levels but M656PH030 reached 4% of the applied dose in goats that were dosed with 12 mg/kg of M656PH030. In plants it was only determined in edible commodities of bulb and spring onions, kale, Chinese cabbage, and head cabbage as well as several animal feed items. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment. Exposure estimates for consumer were 0.0025 µg/kg bw/day (threshold for genotoxic compounds) < M656PH030 ≤ 1.5 µg/kg bw/day (threshold for non-genotoxic Cramer Class III compounds)



A Structural alerts for M656PH030

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times (molecule 5) predicted M656PH030 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. There was no structural alert for in vitro chromosomal aberration for the structure itself and the only structural alert identified for a transformation products was predicted not become active. Again the reliability of the predictions was limited as the molecule was out of the prediction domain.

The Vega prediction (molecule 10) of both modules CAESAR and SarPy was non-mutagenic. However the reliability of these predictions was low as no similar compounds with known experimental data were in the database, as some similar molecules found have experimental values that disagree with the predicted value, as the accuracy of prediction for similar molecules is not optimal and as some atom centered fragments of the compound have not been found or ar rare fragments in the database.

Overall no conclusive, relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

B Genotoxicity studies of M656PH030

Report:	CA 5.8.1/6 Woitkowiak C., 2014d Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2014/1018061
Guidelines:	OECD 471, (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656H030 (Reg.No. 5296352, metabolite of Dimethenamid; Batch: L82-138; Purity: 98.7%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation assays as well as in the preincubation test, M656H030 was tested up to 5000 µg/plate. In detail, concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate were used. A weak bacteriotoxic effect was observed in the standard plate test from about 2500 µg/plate onward only in tester strain TA 1537 without S9 mix and in the preincubation assay depending on the strain and test conditions from about 2500 µg/plate onward. Test substance precipitation was found in the standard plate test at 5000 µg/plate without S9 mix and from about 2500 µg/plate onward after adding the metabolizing system.
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 M656H030 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2014/1018061)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5296352 (Metabolite of BAS 656 PH, Dimethenamid-P)

Description: Solid, white to slightly off gray

Lot/Batch #: L82-138

Purity: 98.7% (tolerance +/- 1.0%, (see Certificate of Analysis, study code ASAP13_228))

Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate. The optical density of the fresh thawed bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 10^9 cells per mL).

5. Test concentrations:

Plate incorporation assay (1st experiment):	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay (2nd experiment):	The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Jan-2014 to 07-Feb-2014

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

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 dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically (see separate BASF study no. 01Y0009/13Y055).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A weak bacteriotoxic effect (slight decrease in the number of his^+ revertants) was observed in the standard plate test from about 2500 µg/plate onward only in tester strain TA 1537 without S9 mix. In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 2500 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table 5.8.1-23).

Test substance precipitation was found in the standard plate test at 5000 µg/plate without S9 mix and from about 2500 µg/plate onward after adding the metabolizing system.

Table 5.8.1-23: Bacterial gene mutation assay with M656H030 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	23.3	19.3	55.3	36.7	11.0	14.0	8.0	7.3	49.3	57.7
M656H030										
33 µg/plate	23.7	14.0	48.3	47.3	8.7	9.3	6.3	7.7	57.3	55.0
100 µg/plate	24.3	17.0	59.7	33.3	13.3	9.7	5.7	5.7	52.7	57.3
333 µg/plate	27.0	21.3	49.0	40.0	12.0	10.7	7.7	5.7	49.0	51.3
1000 µg/plate	25.0	12.7	52.0	48.3	11.0	9.3	6.3	9.7	58.3	64.3
2500 µg/plate	25.3 P	15.0 P	62.0 P	43.7 P	9.0 P	10.0 P	8.0 P	4.7 P	67.7 P	45.7 P
5000 µg/plate	29.0 P	16.0	56.3 P	40.7	9.3 P	8.0	6.7 P	3.7	93.3 P	58.7
Pos. control§	2254.3	383.0	3148.7	4949.3	220.3	4110.7	250.3	2023.3	253.7	817.0
2nd experiment: Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	19.3	17.0	47.0	34.3	10.3	9.3	8.7	7.7	46.7	58.7
M656H030										
33 µg/plate	22.3	17.0	44.3	32.0	10.7	8.7	7.3	6.7	44.7	54.7
100 µg/plate	19.7	12.3	48.3	29.7	9.0	7.3	7.3	5.3	49.7	48.3
333 µg/plate	20.0	12.7	53.0	36.3	7.7	7.7	7.0	6.0	49.7	55.7
1000 µg/plate	20.3	16.0	33.0	43.3	10.0	7.7	7.0	6.0	52.7	53.7
2500 µg/plate	12.7 B	18.7 B	0.0 B	22.0 B	8.7 B	5.7 B	4.3 B	6.0 B	45.7 B	59.7 B
5000 µg/plate	0.0 B	0.0 B	0.0 B	0.0 B	0.0 B	1.3 B	0.0 B	0.0 B	14.0 B	0.0 B
Pos. control§	473.0	404.7	349.7	922.3	197.3	1497.7	259.3	1172.0	232.3	951.3

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B= reduced background growth
P= Precipitation

III. CONCLUSION

According to the results of the present study, the test substance M656H030 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/7
Wollny H.-E., 2014b
Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P): In vitro cell mutation assay at the Thimidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells
2014/1018062

Guidelines: OECD 476 (1997), (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.17, EPA 870.5300, EPA 712-C-98-221

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH030 (Reg.No. 5296352, metabolite of Dimethenamid-P; Batch: L82-138, Purity: 98.7%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations between 119.4 and 3820 µg/mL were used in the main experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects indicated by a relative total growth below 50% in both parallel cultures solely occurred at the highest concentration of the second experiment without metabolic activation. No visible precipitation of the test item in the culture medium was observed. No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656PH030 does not induce forward mutations in mammalian cells in-vitro.
(BASF DocID 2014/1018062)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No. 5296352 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, white to slightly off gray
Lot/Batch #:	L82-138
Purity:	98.7% (tolerance ± 1.0%) for details see Certificate of Analysis ASAP13_228 (dose calculation adjusted to purity)
Stability of test compound:	Stable in DMSO over 4 hours
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration of the S9 preparation was 29.8 mg/mL (Lot. No.: 050913) in the pre-experiment and experiments I and II. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism: The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}M$), Aminopterin ($2.0x10^{-5}M$), Thymidine ($1.6x10^{-3}M$) and Glycin ($5.0x10^{-3}M$) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}M$) and Thymidine ($1.6x10^{-3}M$). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium:	RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.
Selection medium:	RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT
Saline G solution:	Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 192 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay:	Eight concentrations ranging from 29.8 to 3820 µg/mL
b) Mutation assay:	
1st and 2nd experiment:	119.4, 238.8, 477.5, 955.0, 1910.0, 3820.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 02-Dez-2013 to 21-Jan-2014

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.

$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression:	In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection: After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies: Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test

total suspension growth (4 h treatment):

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

relative suspension growth:

total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control
relative total growth:
relative suspension growth × relative cloning efficiency / 100
cloning efficiency (viability):
ln(mean number of empty wells per plate / 96) / cells seeded per well
relative cloning efficiency:
cloning efficiency × 100 / cloning efficiency of corresponding control
cells survived:
cloning efficiency × cell number seeded in TFT medium
mutant colonies / 106 cells:
small mutant colonies + large mutant colonies
threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_228).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 29.8 µg/mL and 3820 µg/mL were chosen with regard to the molecular weight (377.5 g/mol) and the purity of the test item (according to the preliminary information concerning the purity of the test item (98.7%, tolerance ± 1.0%). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.

Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. Only a minor shift of the pH-value and no shift of the osmolarity occurred. The osmolarity was generally high as compared to the normal physiological value of approximately 300 mOsm. This effect was based on DMSO in the culture medium. As the osmolarity is measured by freezing point reduction, a final concentration of 1% DMSO has a substantial impact on the freezing point of the medium.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3820 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Relevant and reproducible cytotoxic effects indicated by a relative total growth below 50% in both parallel cultures solely occurred at the highest concentration of the second experiment without metabolic activation. No precipitation was noted up to the maximum concentration with and without metabolic activation

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached (see **Table 5.8.1-24**, **Table 5.8.1-25**).

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls. The relative total growth of the MMS control of the first experiment, culture I fell just short of the lower limit of 10% (9.5%). The data are acceptable however, as the deviation was minor and the MMS control of the parallel culture remained above the 10% limit (17.0%).

Table 5.8.1-24: Gene mutation in mammalian cells - 1st experiment

	Conc. µg/mL	S9 mix	relative total growth	mutant colonies/ 10^6 cells	threshold	relative total growth	mutant colonies/ 10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	110	236	100.0	74	200
Pos. Control MMS	19.5	-	9.5	1407	236	17.0	377	200
M656PH030								
	119.4	-	culture was not continued#			culture was not continued#		
	238.8	-	128.0	36	236	99.1	92	200
	477.5	-	96.2	52	236	98.8	85	200
	955.0	-	101.2	56	236	106.3	84	200
	1910.0	-	79.0	65	236	85.6	72	200
	3820.0	-	141.8	42	236	94.8	79	200
Solv. Control DMSO		+	100.0	83	209	100.0	71	197
Pos. Control CPA	3.0	+	38.7	254	209	44.0	329	197
Pos. Control CPA	4.5	+	25.9	523	209	29.7	498	197
M656PH030								
	119.4	+	culture was not continued#			culture was not continued#		
	238.8	+	100.8	44	209	76.4	119	197
	477.5	+	107.0	69	209	86.8	69	197
	955.0	+	111.3	73	209	86.0	93	197
	1910.0	+	82.3	127	209	90.5	78	197
	3820.0	+	76.8	122	209	117.4	79	197

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-25: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/ 10^6 cells	threshold	relative total growth	mutant colonies/ 10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	132	258	100.0	117	243
Pos. Control MMS	13.0	-	22.8	359	258	28.6	388	243
M656PH030								
	119.4	-	culture was not continued#			culture was not continued#		
	238.8	-	78.2	126	258	140.1	95	243
	477.5	-	100.8	94	258	122.2	93	243
	955.0	-	78.5	75	258	116.3	83	243
	1910.0	-	50.9	88	258	86.1	77	243
	3820.0	-	25.4	92	258	27.9	126	243
Experiment II / 4 h treatment								
Solv. Control DMSO		+	100.0	109	235	100.0	63	189
Pos. Control CPA	3.0	+	83.7	272	235	49.2	167	189
Pos. Control CPA	4.5	+	36.5	458	235	26.4	258	189
M656PH030								
	119.4	+	culture was not continued#			culture was not continued#		
	238.8	+	111.0	100	235	102.7	61	189
	477.5	+	218.1	65	235	106.6	39	189
	955.0	+	144.7	80	235	92.0	66	189
	1910.0	+	176.0	70	235	102.1	47	189
	3820.0	+	187.7	83	235	100.3	61	189

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656PH030 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/8 Bohnenberger S., 2014b Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P): Micronucleus test in chinese hamster V79 cells in vitro 2014/1018063
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH030 (Reg. No. 5296352, metabolite of BAS 656-PH, Dimethenamid-P; Batch: L82-138; Purity: 98.8%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations of 7.5 to 3820 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment IA.
In Experiment IB the exposure period was 4 hours with and without metabolic activation and in experiment II 4 hours with and 24 h without metabolic activation. Concentrations of up to 3820 µg/mL were used for the experiments and the concentrations used for evaluation ranged between 955 and 3820 µg/mL. Cytotoxicity as well as precipitation were not observed in any experiment.
In Experiment IA in the absence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 3820.0 µg/mL, clearly exceeding the range of the laboratory historical control data. In Experiment IB in the absence of S9 mix this finding could not be confirmed. Although, all evaluated concentrations showed statistical significance due to the low solvent control value. These values were clearly within the historical control range. In Experiment IA in the presence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 1910.0 µg/mL. The value was within the range of the laboratory historical control data and is therefore considered being biologically irrelevant.
In Experiment II in the presence of S9 mix no relevant increase in micronucleated cells was observed. In Experiment II in the absence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 1910.0 and 3820.0 µg/mL. The second value clearly exceeded the range of the laboratory historical control data.
Based on the results of this study, M656PH030 is considered to induce micronuclei in vitro in V79 cells in the absence of metabolic activation.

(BASF DocID 2014/1018063)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 5296352 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, white to slightly off gray
Lot/Batch #:	L82-138
Purity:	98.7 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in DMSO over 4 hours
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive controls, -S9:	Mitomycin C (MMC, 0.1 and 0.3 µg/mL, dissolved in deionised water) Griseofulvin (8.0 µg/mL, dissolved in DMSO)
Positive control, +S9:	Cyclophosphamide (CPA, 10.0 µg/mL, dissolved in saline)
3. Activation:	S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice. The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥ 70 %).

5. Culture medium/conditions:

About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay: 7.5 - 3820.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment IA. Due to the positive findings in Experiment IA without S9 mix a confirmatory experiment (Exp. IB) was performed. This experimental part was repeated again due to invalid positive controls. (see Table 5.8.1-26).

b) Cytogenicity assay: 7.5 – 3820.0 µg/mL without metabolic activation

119.4 - 3820.0 µg/mL with metabolic activation

Table 5.8.1-26: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with M656PH030

Exp.	Prep. interval	Exposure period	Concentrations in µg/mL									
Without S9 mix												
IA	24 hrs	4 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	**955.0**	**1910.0**	**3820.0**
IB*	24 hrs	4 hrs	59.7	119.4	238.8	477.5	955.0	1910.0	2387.5	2865.0	3342.5	3820.0
IB	24 hrs	4 hrs	59.7	119.4	238.8	477.5	955.0	1910.0	2387.5	**2865.0**	**3342.5**	**3820.0**
II	24 hrs	24 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	**955.0**	**1910.0**	**3820.0**
With S9 mix												
IA	24 hrs	4 hrs	7.5	14.9	29.8	59.7	119.4	238.8	477.5	**955.0**	**1910.0**	**3820.0**
II	24 hrs	4 hrs					119.4	238.8	477.5	**955.0**	**1910.0**	**3820.0**

Evaluated experimental points are shown in bold characters

* Was repeated due to invalid positive controls

B. TEST PERFORMANCE:

1. Dates of experimental work: 27-Nov-2013 - 17-Feb-2014

2. Preliminary cytotoxicity assay: With respect to the molecular weight and the purity (98.8 %, preliminary information at the start of the experiment) of the test item, 3820.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 7.5 and 3820.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose-related and reproducible increase in the number of cells containing micronuclei is observed
- if the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_228).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix.

C. CYTOGENICITY ASSAYS:

In Experiment I and II in the absence and presence of S9 mix no clear cytotoxicity was observed up to the highest applied concentration. In addition, no visible precipitation of the test item in the culture medium was observed.

In Experiment IA in the absence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 3820.0 µg/mL (3.25 %), clearly exceeding the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells). In Experiment IB in the absence of S9 mix this finding could not be confirmed. However, all evaluated concentrations showed statistical significance due to the low solvent control value. These values were clearly within the historical control range (0.15 - 1.50 % micronucleated cells).
In Experiment IA in the presence of S9 mix one statistically significant increase in micronucleated cells was observed after treatment with 1910.0 µg/mL (1.33 %). The value was within the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) and is therefore biologically irrelevant.
In Experiment II in the presence of S9 mix no relevant increase in micronucleated cells was observed. In Experiment II in the absence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 1910.0 and 3820.0 µg/mL (1.15 and 4.35 %, respectively). The second value clearly exceeded the range of the laboratory historical control data (0.05 - 1.50 % micronucleated cells) (see **Table 5.8.1-27**).

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-27: Summary of results of the micronucleus test with M656PH030

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
IA	24 hrs	Solvent control[1]	2.88	0.60
		Positive control[2]	2.71	**4.20**[S]
		955.0	2.79	0.20
		1910.0	2.83	0.60
		3820.0	2.42	**3.25**[S]
IB	24 hrs	Solvent control[1]	2.69	0.05
		Positive control[3]	2.48	**14.95**[S]
		955.0	2.85	**0.40**[S]
		1910.0	2.90	**0.70**[S]
		3820.0	2.87	**0.60**[S]
Exposure period 24 hrs without S9 mix				
II	24 hrs	Solvent control[1]	2.49	0.10
		Positive control[4]	2.38	**14.60**[S]
		955.0	2.43	0.40
		1910.0	2.19	**1.15**[S]
		3820.0	1.58	**4.35**[S]
Exposure period 4 hrs with S9 mix				
IA	24 hrs	Solvent control[1]	2.32	0.65
		Positive control[5]	1.53	**13.25**[S]
		955.0	2.36	0.65
		1910.0**	2.31	**1.33**[S]
		3820.0	2.29	0.85
II	24 hrs	Solvent control[1]	2.43	0.95
		Positive control[5]	1.72	**17.45**[S]
		955.0	2.50	1.30
		1910.0	2.46	0.80
		3820.0	2.20	1.00

* The total number of micronucleated cells was determined in a sample of 2000 cells
** The total number of micronucleated cells was determined in a sample of 4000 cells
[S] Number of micronucleated cells statistically significantly higher than corresponding control values
[1] DMSO 1.0 % (v/v)
[2] Mitomycin C 0.03 µg/mL
[3] Mitomycin C 0.3 µg/mL
[4] Griseofulvin 8.0 µg/mL
[5] CPA 10.0 µg/mL

III. CONCLUSIONS

Based on the results of the study, M656PH030 is considered as mutagenic in this in vitro micronucleus test, when tested up to the highest required concentrations.

Report: CA 5.8.1/9
[redacted] 2014g
Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1094090

Guidelines: OECD 474 (1997), (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.12, EPA 870.5395, EPA 712-C-98-226

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.8.1/10
Becker M.,Landsiedel R., 2014d
Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P) - Plasma analysis for externa studies
2014/1092435

Guidelines: <none>

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.8.1/11
Grauert E.,Kamp H., 2014c
Analytical report - Reg.No. 5296352 (metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in dimethylsulfoxide / polyethylenglycol (3+7, v/v)
2014/1101994

Guidelines: <none>

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656PH030 (Reg.No. 5296352; Metabolite of BAS 656 PH, Dimethenamid-P; batch: L82-138, purity: 98.7%) was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. Based on the result of a pre-tests, the test substance dissolved in DMSO/PEG 400 was administered once orally to groups of 7 male mice at dose levels of 500, 1000, and 2000 mg/kg body weight in a volume of 10 mL/kg body weight. Due to a wrong dilution of the test item, which was confirmed by the results of the formulation analysis after the administration phase, mice of the high dose group did not receive the initially planned dose of 2000 mg/kg bw, but similar to the medium-dose mice a nominal dose of 1000 mg/kg bw. Therefore, the results of all animals treated with 1000 mg/kg bw (14 animals for the 24 h treatment, 7 animals for the 48 h treatment) were included in the evaluation and reporting of the medium dose level. The high dose level of 2000 mg/kg bw was investigated in a second main experiment.
The vehicle served as negative and cyclophosphamide as positive control (5 animals/group). The animals were sacrificed 24 or 48 hours after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were

evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.
Oral administration of the test substance did not lead to any biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used. The rate of micronuclei were below or near to the value of the negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Ruffled fur was observed in some animals treated with test item concentrations of 1000 and 2000 mg/kg bw, respectively. Furthermore, loose feces were observed in two animals treated with 2000 mg/kg bw. The animals treated with the low dose level did not exhibit any clinical symptoms. The systemic availability of M656PH030 was confirmed analytics in blood plasma. The positive control led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.
According to the results of the study the test substance M656PH030 did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and is therefore considered non-mutagenic.

(BASF DocID 2014/1094090)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 5296352 (metabolite of BAS 656 PH, Dimethenamid-P)

Description:	Solid, white to slightly off gray
Lot/Batch #:	L82-138
Purity:	98.7% (tolerance ± 1.0%)
Stability of test compound:	stable in solvent (Confirmed indirectly by dose formulation analytics (see BASF study 04Y009/13Y086)
Solvent used:	DMSO / PEG 400 (3/7)

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	DMSO / PEG 400 (3/7) 30% DMSO, 70% PEG 400
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw

3. Test animals:

Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks
Weight at dosing:	mean value 35.0 g (SD ± 1.4 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/500 and 2000 mg/kg bw; 14 males/1000 mg/kg bw (24 treatment); 7 males/1000 mg/kg bw (48 treatment); 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound concentration:

Range finding test:	2000 (per os) mg/kg bw
Micronucleus assay:	500, 1000 and 2000 mg/kg bw (doses were corrected for purity with a correction factor of 1.01. Test item dose levels as is were 505, 1010 and 2020 mg/kg bw.) The test substance was administered once orally using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 21-Jan-2014 – 26-Mar-2014

2. Preliminary cytotoxicity assay:

Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once with either the vehicle, positive control substance or 500, 1000 or 2000 mg test substance / kg bw by oral administration. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four or 48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet re-suspended.
Slide preparation:	A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:	In general, 2000 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 and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

5. Evaluation criteria:

A test item is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y009/13Y086).

B. PRELIMINARY RANGE FINDING TEST

None of the male or female mice died after single oral dosing of 2000 mg/kg bw. Ruffled fur was observed in one male after 30 hours post-treatment. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Ruffled fur was observed in some animals treated with test item concentrations of 1000 and 2000 mg/kg bw, respectively. Furthermore, loose feces were observed in two animals treated with 2000 mg/kg bw. The animals treated with the low dose level did not exhibit any clinical symptoms. The systemic availability of M656PH030 was confirmed by plasma-analytics [see DocID 2014/1092435]

After treatment with the test item at 24h and 48h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below or near to the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range [see **Table 5.8.1-28**].

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (1.93%), thereby demonstrating the sensitivity of the test system.

Table 5.8.1-28: Micronucleus test in mice administered M656PH030

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
DMSO/PEG 400 (3/7)	24	0.080	0-5	1196
M656PH030 *				
500 mg/kg bw	24	0.121	0-7	1211
1000 mg/kg bw	24	0.171	1-8	1238
Positive control				
Cyclophosphamide	24	1.930	20-63	1283
48 h sampling				
DMSO/PEG 400 (3/7)	48	0.100	0-4	1139
M656PH030 *				
1000 mg/kg bw	48	0.064	0-4	1183

* Doses were corrected for purity with a correction factor of 1.01. Test item dose levels as actually were 505, 1010 and 2020 mg/kg bw.

III. CONCLUSION

Based on the results of this study, M656PH030 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under in vivo conditions.

Conclusion on genotoxicity of M656PH030

By weight of evidence M656PH030 is not considered to be genotoxic based on the study results available.

C Toxicological evaluation of metabolite M656PH030

No relevant toxicological alert was identified for M656PH030. By weight of evidence M656PH030 is not considered to be genotoxic based on the in the in vitro and in vivo studies conducted.

Thus, M656PH030 is considered to be of <u>no</u> toxicological relevance.

5. Metabolite M656PH031 former assigned M31

M656PH031 is a metabolite of dimethenamid-P determined in maize and soybean metabolism studies but not observed edible commodities as well as in soil, surface water and ground-water. The exposure estimates for ground-water are M656PH031 < 0.1 µg/l.

A Structural alerts for M656PH031

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times (molecule 6) predicted M656PH031 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure [see section N4 3.3 of this dossier]. For this alpha-beta unsaturated carbonyl (transformation predicted by 2 different pathways) was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (14 in total) gave no alert for chromosomal aberration.

In the DEREK analysis conducted structural alerts for M656PH031 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction in both modules (CAESAR and SarPy) was non-mutagenic, however the reliability of this prediction was low and thus not taken into further consideration.

In conclusion there was a limited alert for chromosomal aberration in vitro with metabolic activation in one of the structure activity evaluation tools employed considered of no relevance as not confirmed by the genotoxicity testing conducted.

B Genotoxicity of M656PH031

M656H031 was used for toxicological testing and considered toxicologically equivalent to M656PH031.

Report:	CA 5.8.1/11 Schulz M.,Landsiedel R., 2008a Reg.No. 360 712 (metabolite M31 of BAS 656 H) - Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) 2008/1064992
Guidelines:	OECD 471, EEC 2000/32 B.13/B.14, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656H031 (Reg.No. 360 712, metabolite of Diemethenamid; Batch: L81-46; Purity: 98.7%) 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 plate incorporation assay as well as in the preincubation test, M656H031 was tested up to the limit concentration of 5000 µg/plate. Concentrations of 20, 100, 500, 2500 and 5000 µg/plate were used in the plate incorporation and 312.5, 625, 1250, 2500 and 5000 µg/plate in the preincubation assay with and without metabolic activation, respectively. A bacteriotoxic effect was occasionally observed in the standard plate test depending on the strain from about 2500 µg/plate onward. In the preincubation assay, bacteriotoxicity was observed from about 2500 µg/plate onward in all strains irrespective from metabolic activation. Precipitation of the test substance did not occur up to the highest tested concentration.

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 M656H031 is not mutagenic in the Salmonella typhimurium/ Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2008/1064992)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 360 712 (Metabolite M31 of BAS 656 H)

Description: Solid, white

Lot/Batch #: L81-46

Purity: 98.7% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed as indicated by the sponsor and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	Triplicate plates were prepared for each concentration (neg. control; 20, 100, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay:	The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 312.5, 625, 1250, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-Jul-2008 to 07-Aug-2008

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (slight decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was occasionally observed in the standard plate test depending on the strain from about 2500 µg/plate onward. In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed from about 2500 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see **Table 5.8.1-29**].

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-29: Bacterial gene mutation assay with M656H031 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	35	25	107	99	14	16	9	8	42	33
M656H031										
20 µg/plate	34	23	94	94	13	15	9	7	39	31
100 µg/plate	31	23	95	92	16	13	10	7	44	33
500 µg/plate	30	26	98	100	13	15	7	6	36	39
2500 µg/plate	23	21	84	94	12	13	6	5	32	32
5000 µg/plate	17	10	67	62	11	12	4	3	18	22
Pos. control§	578	490	640	671	183	620	126	507	283	777
Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	29	25	102	101	15	14	8	8	34	29
M656H031										
312.5 µg/plate	32	29	96	93	15	13	8	6	39	35
625 µg/plate	34	24	93	108	14	13	7	7	33	30
1250 µg/plate	31	25	100	94	14	14	6	5	55	32
2500 µg/plate	16	17	71	99	5	15	5	7	33	34
5000 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	0B	0B
Pos. control§	560	481	806	608	151	689	143	142	219	581

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B= reduced background growth

III. CONCLUSION

According to the results of the present study, the test substance M656H031 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/13 Schulz M.,Landsiedel R., 2008b Reg.No. 360 712 (metabolite M31 of BAS 656 H) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2008/1051510
Guidelines:	OECD 476, (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, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656H031 (Reg.No. 360712, metabolite of Dimethenamid-P; Batch: L81-46; Purity: 98.7%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 3500 µg/mL (approx. 10 mM) were used in the original and the confirmatory experiment. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiments, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Ethylmethanesulfonate (EMS) and methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments after the incubation period treatment media were replaced by culture medium and the cells were incubated for 6-8 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.
Cytotoxic effects indicated by reduced cloning efficiencies of below 20 % of the respective vehicle control were not observed in any of the experiments irrespective of treatment interval and the presence of metabolic activation. In addition, no precipitation was observed.
Neither in the original nor in the confirmatory studies was a relevant increase in the mutant frequency observed. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656H031 does not induce forward mutations in mammalian cells in-vitro.
(BASF DocID 2008/1051510)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 360712 (Metabolite M31 of BAS 656 H)

Description: Solid, white

Lot/Batch #: L81-46

Purity: 98.7% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2018 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

The stability of the test substance at room temperature in the vehicle DMSO and in aqueous formulations was determined analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: 1% (v/v) DMSO in culture medium

Positive control -S9: Ethyl methanesulfonate (EMS) 300 µg/mL

Positive control +S9: Methylcholantrene (MCA) 10 µg/mL

3. Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organism: Chinese hamster CHO cells (sub-strain K3). Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL
All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericin B (250 µg/mL)

During pulse exposure (4 hours) to the test substance, Ham's F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham's F12 medium with FCS supplementation was used.

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 13.7 to 3500 µg/mL

b) Mutation assay:

1st experiment: 218.8, 437.5, 875, 1750 and 3500 µg/mL with and without metabolic activation

2nd experiment: 218.8, 437.5, 875, 1750 and 3500 µg/mL without metabolic activation

250, 500, 1000, 2000 and 3500 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-Jul-2008 to 08-Sep-2008

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation) after an attachment period of 20-24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells:	Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.
Cell treatment:	For each test group, about $1x10^6$ cells per flask were seeded into 175 cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 - 24 hours with 5% CO_2 at 37°C and > 90% humidity for cell attachment. 2 flasks were used for each test group. After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive control or test substance. For the vehicle control group 20 mL of the treatment medium was supplemented with 0.2 mL of the vehicle. In case of metabolic activation the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL positive control or test substance preparation and 4 mL S9-mix. Analogously, for the vehicle control group 16 mL of medium was supplemented with 0.2 mL vehicle and 4 mL S9-mix. For the exposure period of more than 4 hours Hams´s F12 medium with 10% FCS was used.

	Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity.
Expression:	After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).
Selection:	For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10 % FCS. After a 20-24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the treatment medium was replaced by Ham's F12 medium with 10% FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted. Cloning efficiency 2 (viability): The viability (cloning efficiency 2; CE_2) was determined in parallel to the selection of mutants after the expression period under the same conditions as described for cloning efficiency 1.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{\text{total number of colonies in the test group}}{\text{total number of seeded cells in the test group}} \times 100$$

relative, in comparison to control:

$$CE_{relative} = \frac{\text{CE of the dose group}}{\text{CE of the vehicle control}} \times 100$$

4. Statistics:
Due to the negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. The stability of the test substance at room temperature in the vehicle DMSO and in water for 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of relative cloning efficiency below 20% was not observed. The relative cloning efficiency ($CE_{1\ relative}$) after treatment with the highest concentration (3500 µg/mL; approx. 10 mM) ranged between 88.9 to 106.5% depending on the treatment interval and metabolic activation.

Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. No marked effect on osmolarity was observed. The pH of the stock solution was adjusted prior to application to physiological levels using 2 N NaOH.

Based on these data the highest concentration tested in the mutagenicity experiments was 3500 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

A significant cytotoxic effect was not observed in both experiments up to the highest tested concentration irrespective of treatment interval and presence of metabolic activation. The obtained relative cloning efficiency did not drop below 86.3% under any of the tested conditions.

A relevant increase in the number of mutant colonies was not observed in the original and confirmatory experiments with and without metabolic activation [see **Table 5.8.1-30** and Table 5.8.1-31].

The mutant frequencies obtained at any tested concentration with or without metabolic activation were within the range of the concurrent vehicle control as well as within the range of the historical negative control data.

The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 3500 µg/mL.

Treatment with the positive controls EMS and MCA 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.

Table 5.8.1-30: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded)		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	11	3.06	3.80	85.3	100.0	81.6	100.0
M656H031							
218.8 µg/mL	5	1.39	1.73	75.7	88.7	80.9	99.1
437.5 µg/mL	1	0.28	0.34	76.4	89.6	80.3	98.4
875.0 µg/mL	3	0.84	1.11	77.0	90.3	75.7	92.8
1750.0 µg/mL	1	0.28	0.35	80.2	94.0	78.9	96.7
3500.0 µg/mL	3	0.84	1.02	81.7	95.8	81.8	100.2
Positive control EMS							
300.0 µg/mL	325	90.28	137.00	77.7	91.1	65.7	80.5
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	6	1.67	1.98	85.1	100.0	84.3	100.0
M656H031							
218.8 µg/mL	4	1.11	1.37	83.3	97.9	82.2	97.5
437.5 µg/mL	3	0.84	1.03	83.3	97.9	78.0	92.5
875.0 µg/mL	5	1.39	1.84	81.9	96.2	77.8	92.3
1750.0 µg/mL	2	0.56	0.74	80.2	94.2	76.4	90.6
3500.0 µg/mL	1	0.28	0.36	84.0	98.7	78.8	93.5
Positive control MCA							
10.0 µg/mL	228	63.34	107.83	74.0	87.0	58.8	69.8

[a] number of colonies approx 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

Table 5.8.1-31: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded) Cloning efficiency (%)		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded) Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 24-hour exposure period							
Vehicle (DMSO)	7	1.95	2.60	81.9	100.0	75.1	100.0
M656H031							
218.8 µg/mL	8	2.23	3.13	76.3	93.2	72.3	96.3
437.5 µg/mL	0	0.00	0.00	73.8	90.1	75.1	100.0
875.0 µg/mL	6	1.67	2.39	72.2	88.2	70.6	94.0
1750.0 µg/mL	5	1.39	1.99	73.4	89.6	73.5	97.9
3500.0 µg/mL	7	1.95	2.65	70.7	86.3	72.8	96.9
Positive control EMS							
300.0 µg/mL	529	146.95	245.20	59.5	72.6	60.1	80.0
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	7	1.95	2.50	83.4	100.0	75.5	100.0
M656H031							
250 µg/mL	0	0.00	0.00	75.3	90.3	83.3	110.3
500 µg/mL	2	0.56	0.78	79.7	95.6	72.3	95.8
1000 µg/mL	5	1.39	1.75	78.0	93.5	73.2	97.0
2000 µg/mL	1	0.28	0.34	76.7	92.0	75.2	99.6
3500 µg/mL	3	0.84	0.99	75.3	90.3	82.8	109.7
Positive control MCA							
10.0 µg/mL	283	78.61	118.42	62.9	75.4	67.9	89.9

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656H031 does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report:	CA 5.8.1/14 Schulz M.,Landsiedel R., 2008c Reg.No. 360 712 (metabolite M31 of BAS 656 H) - In vitro chromosome aberration assay in V79 cells 2008/1063692
Guidelines:	OECD 473, (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, EPA 870.5375
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.1/15 Schulz M., 2008a Amendment No. 1 to the report: Reg.No. 360 712 (metabolite M31 of BAS 656 H) - In vitro chromosome aberration assay in V79 cells 2008/1070759
Guidelines:	OECD 473, (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, EPA 870.5375
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656H031 (Reg.No. 360 712, metabolite of Dimethenamid-P; Batch: L81-46; Purity: 98.7%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation. Based on the results of a preliminary toxicity test, concentrations of 218.8 to 3500 µg/mL (approx. 10 mM at the top dose) were tested for clastogenic effects with and without metabolic activation in experiments with a pulse treatment of 4 hours or continuous treatment of 18 hours. The preparation intervals used were 18 h or 28 h post treatment-begin. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) 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 200 well spread metaphases per dose and treatment condition were analyzed for chromosomal aberrations, except for the positive control cultures where only 50 metaphases were scored due to clearly increased aberration rates.
The highest concentration scored was 3500 µg/mL. Cytotoxicity (mitotic index of 36.3%) was observed in the 2nd Experiment after 18 hours treatment at 28 hours preparation interval in the absence of S9 mix at 3500 µg/mL only. No precipitation was observed. The test substance did not cause any biologically relevant increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S9 mix and/or after adding a metabolizing system in two experiments performed independently of each other. No biologically relevant increase in the frequency of cells containing numerical chromosome aberrations was demonstrated either.

Based on the results of this study M656H031 is considered not to have a clastogenic potential in vitro in Chinese hamster V79 cells in the presence or absence of metabolic activation.
(BASF DocID 2008/1063692)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 360712 (Metabolite M31 of BAS 656 H)

Description: Solid, white

Lot/Batch #: L81-46

Purity: 98.7% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jul 2018 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
The stability of the test substance dissolved in the vehicle DMSO and in aqueous solution over a period of 4 hours was verified analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: DMSO

Positive control, -S9: Ethylmethanesulfonate 500 µg/mL

Positive control, +S9: Cyclophosphamide 0.5 µg/mL

3. Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

Chinese hamster V79 cells with high proliferation rate (doubling time of about 12 - 16 hours), a high plating efficiency (> 90%) and a stable karyotype (modal number of 22 chromosomes). Stocks of the V79 cell line were maintained at -196°C in liquid nitrogen. Each batch used for the cytogenetic experiments was checked for mycoplasma contamination, karyotype stability and plating efficiency incl. vital staining.

5. Culture medium:

MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation. For exposure periods of more than 4 hours MEM medium with 10 % (v/v) FCS was used.

5. Test concentrations:

a) Preliminary toxicity assay: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL with and without metabolic activation (4- and 18-hour exposure, (18 h preparation interval)

b) Mutation assay:

1st experiment: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL with and without metabolic activation

2nd experiment: 218.8, 437.5, 875.0, 1750.0 and 3500.0 µg/mL without metabolic activation (18 h preparation interval) as well as with metabolic activation (28 h preparation interval)

875, 1750 and 3500 µg/mL without metabolic activation (28 h preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 21-Jul-2008 to 09-Sep-2008

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by mitotic index, cell attachment (morphology) and quality of the slides. The cells were prepared at a sampling time of 18 hours (about 1.5-fold cell cycle time) after 4 and 18 hours exposure time without S9 mix and after 4 hours exposure time with S9 mix. The pretest was performed following the method described for the main experiment. In addition to the mitotic index, the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Cytogenicity Assay:

Cell treatment: Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours or continuous treatment experiments for 18 hours. The preparation intervals were 18 h and 28 h post treatment-begin. Duplicate cultures were run for each dose and condition. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration.

For determination of cytotoxicity, additional cell cultures (using 25 cm^2 plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.

Spindle inhibition: 100 µL colcemid was added to each chamber 2 - 3 hours prior to harvesting.

Cell harvest: At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37 °C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.

Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.
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. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced from the planned 200 mitoses/test group A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related, and reproducible increase in the number of structural chromosomal aberrations (excl. gaps).
- The proportion of aberrations (excl. gaps) exceeded both the concurrent negative control range and the historical negative control range.

A test substance is generally considered negative in this test system if:

- The number of cells with structural aberrations (excl. gaps) in the dose groups is not statistically significant increased above the concurrent negative/vehicle control value and is within the historical negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of at least one of the toxicity parameters to more than 50% of the respective control was not observed. The overall lowest mitotic index observed after treatment with the highest concentration (3500 µg/mL; approx. 10 mM) regardless any test condition was 78.8%.

Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. No marked effect on osmolarity was observed. The pH of the stock solution was adjusted prior to application to physiological levels using 2 N NaOH.

Based on these data the highest concentration tested in the mutagenicity experiments was 3500 µg/mL without and with metabolic activation.

C. CYTOGENICITY ASSAYS:

In this study treated cells could be evaluated up to the highest used test substance concentration (3500 µg/mL). Cytotoxicity (mitotic index of 36.3%) as described by a drop in the relative mitotic index below 50% was observed in the 2nd Experiment after 18 hours treatment at 28 hours preparation interval in the absence of S9 mix at 3500 µg/mL only.

A relevant increase in cells with structural chromosomal aberrations was not observed in any of the performed experiments neither with nor without metabolic activation. For all tested treatment or preparation intervals the number of aberrant cells was within the historical control range and close to the value obtained for the concurrent control group. In the 1st Experiment after 4 hours treatment in the absence of S9 mix a dose-related increase of structural chromosome aberrations was observed (1 - 2.5%). However, the values were clearly within the historical negative control data range (0.0% - 5.5% aberrant metaphase cells, exclusive gaps) and, therefore, this observation was regarded as biologically irrelevant [see **Table 5.8.1-32**, **Table 5.8.1-33**]. The frequency of cells containing numerical aberrations was not affected by treatment with the test substance.

The osmolarity of the incubations were not altered by the addition of the test-substance. The pH was adjusted to physiological conditions prior to treatment. Precipitation of the test substance did not occur up to the highest tested concentration.

Vehicle and positive controls were all in a range to ensure the validity of the test.

Table 5.8.1-32: Chromosome aberration test with M656H031 – without metabolic activation

Exp.	Exposure/ Preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Aberrant cells [%]					
					incl. gaps[#]	**excl. gaps[#]**	with exchanges	Polyploid cells [%]	Cell number [%]	Mitotic index [%]
1	4/18 hrs	Vehicle control[1]	-	-	2.5	**1.0**	0.5	0.0	100.0	100.0
		M656H031								
		218.8 µg/mL	-	-	-	-	-	-	103.2	-
		437.5 µg/mL	-	-	-	-	-	-	101.2	-
		875.0 µg/mL	-	-	3.0	**1.0**	1.0	0.0	105.2	76.7
		1750.0 µg/mL	-	-	2.5	**1.5**	1.5	1.5	96.0	81.9
		3500.0 µg/mL	-	-	3.0	**2.5**	2.0	0.0	91.6	91.7
		Positive control[2]	-	-	23.0[S]	**21.0[S]**	9.0[S]	0.0	n.t.	122.3
2	18/18 hrs	Vehicle control[1]	-	-	5.0	**2.0**	0.5	2.4	100.0	100.0
		M656H031								
		218.8 µg/mL	-	-	7.0	**3.0**	1.0	3.4	100.6	113.7
		437.5 µg/mL	-	-	5.0	**2.0**	0.0	3.8	100.0	101.6
		875.0 µg/mL	-	-	7.0	**2.0**	1.0	2.9	98.8	96.2
		1750.0 µg/mL	-	-	n.s.	**n.s.**	n.s.	n.s.	91.9	-
		3500.0 µg/mL	-	-	n.s.	**n.s.**	n.s.	n.s.	79.7	-
		Positive control[2]	-	-	37.0[S]	**33.0[S]**	19.0[S]	0.0	n.t.	96.7
2	18/28 hrs	Vehicle control[1]	-	-	3.0	**2.0**	0.5	1.0	100.0	100.0
		M656H031								
		875.0 µg/mL	-	-	-	-	-	-	90.8	-
		1750.0 µg/mL	-	-	-	-	-	-	101.8	79.3
		3500.0 µg/mL	-	-	3.5	**2.0**	1.5	0.0	73.8	36.3
		Positive control[2]	-	-	24.0[S]	**24.0[S]**	19.0[S]	0.0	n.t.	65.8

P: Precipitation determined at the end of exposure period
*: Relative values compared with the respective vehicle control
#: Inclusive cells carrying exchanges
n.t.: Not tested
n.s.: Not scorable due to low metaphase numbers and/or poor metaphase quality
-: Not scored
S: Aberration frequency statistically significant higher than corresponding control values
1: DMSO 1% (v/v)
2: EMS 500 µg/mL

Table 5.8.1-33: Chromosome aberration test with M656H031 – with metabolic activation

Exp.	Exposure/Preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Aberrant cells [%]				Cell number [%]	Mitotic index [%]
					incl. gaps#	**excl. gaps#**	with exchanges	Polyploid cells [%]		
1	4/18 hrs	Vehicle control[1]	+	-	3.5	**1.5**	1.0	0.0	100.0	100.0
		M656H031								
		218.8 µg/mL	+	-	-	-	-	-	96.6	-
		437.5 µg/mL	+	-	-	-	-	-	97.7	-
		875.0 µg/mL	+	-	2.0	**1.5**	1.0	0.0	111.8	108.5
		1750.0 µg/mL	+	-	3.5	**1.5**	0.5	0.0	101.9	107.6
		3500.0 µg/mL	+	-	4.5	**1.5**	1.5	0.5	82.1	100.0
		Positive control[2]	+	-	20.0^S	**18.0^S**	11.0^S	0.0	n.t.	89.6
2	18/18 hrs	Vehicle control[1]	+	-	3.5	**2.5**	2.0	1.0	100.0	100.0
		M656H031								
		218.8 µg/mL	+	-	-	-	-	-	102.5	-
		437.5 µg/mL	+	-	-	-	-	-	93.4	-
		875.0 µg/mL	+	-	4.0	**2.5**	1.0	0.5	91.0	142.5
		1750.0 µg/mL	+	-	5.0	**3.5**	2.5	0.0	91.5	101.4
		3500.0 µg/mL	+	-	3.0	**2.0**	2.0	0.0	99.2	128.8
		Positive control[2]	+	-	11.0^S	**11.0^S**	6.0^S	1.0	n.t.	125.6

P: Precipitation determined at the end of exposure period
*: Relative values compared with the respective vehicle control
#: Inclusive cells carrying exchanges
n.t.: Not tested
-: Not scored
S: Aberration frequency statistically significant higher than corresponding control values
1: DMSO 1% (v/v)
2: CPP 0.5 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that M656H031 does not have a clastogenic potential in vitro in the presence or absence of metabolic activation.

Conclusion on genotoxicity of M656PH031

There is no evidence for genotoxicity of M656PH031 based on the studies conducted.

C Short-term toxicity of M656PH031

Report: CA 5.8.1/16
[redacted] 2013c
Reg.No. 360712 (metabolite of BAS 656 H) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet
2013/1042165

Guidelines: OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of M656H031 (Reg.No. 360 712, metabolite of Dimethenamid; Batch: L81-46; Purity: 98.7%) to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm for at least 28 days did not cause any test substance-related adverse signs of toxicity.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1068 mg/kg bw/d) and in female (1140 mg/kg bw/d) Wistar rats.

(DocID 2013/1042165)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Reg.No. 360712 (Metabolite of BAS 656 H)
   Description: Solid/white
   Batch/purity #: L81-132, 100.0%
   Stability of test compound: Stable until 01 Jul 2019. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. **Vehicle and/or positive control:** Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 166.5 ± 9.5 g, ♀ 132.4 ± 7.9 g
Source:	Charles River, Germany
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:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 28-Jun-2012 - 29-Jan-2013
(In life dates: 28-Jun-2012 (start of administration) to 27-Jul-2012 (necropsy))

2. Animal assignment and treatment:

M656H031 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 32 days

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level single samples were analysed. No test-article was determined in control diets.

Table 5.8.1-34: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1200 ppm	28.06.12	1157.3 ± 19.7#	96.4	1.7
4000 ppm	28.06.12	3967.5	99.2	n.a.
12000 ppm	28.06.12	11882.9 ± 468.5#	99.0	4.0

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 1.7 to 4% indicate the homogenous distribution of M656H031 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 96.4 to 99.2% of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H031 in the vehicle.

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 5.8.1-35: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-36: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except reticulocytes and differential blood count, urine pH, urine volume, urine specific gravity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urinalysis, except volume, color, turbidity and specific gravity	Pair wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Table 5.8.1-37: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once weekly and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y body weight [g] at 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:

Not performed in this study.

6. Functional observation battery (FOB):

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane anesthetized animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides[¥]	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetized animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.

Comparing the single intervals with the control groups, no significant deviations were measured with the exception of a decreased value at interval 1 in males of test group 3 (12000 ppm). As a consequence, the overall motor activity measurement in male animals of test group 3 (12000 ppm) was significantly decreased. As no FOB parameter was changed and no findings occurred during the detailed clinical observation, the change was assessed as being spontaneous and not related to treatment. No statistically significant differences of overall motor activity between control and treated animals were observed for males of the other test groups (1200 and 4000 ppm) as well as for the female animals of all test groups.

C. BODY WEIGHT AND BODY WEIGHT GAIN

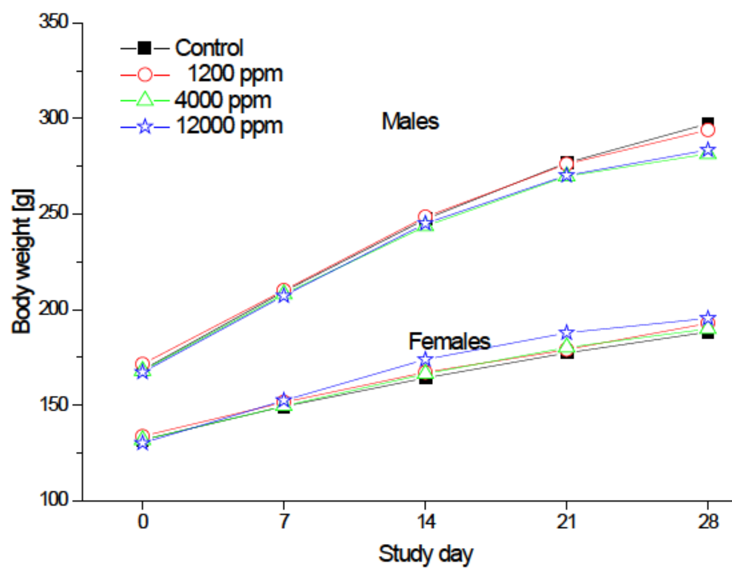
No statistically significant differences of absolute body weights or body weight gain were noted [see **Table 5.8.1-38** and **Figure 5.8.1-4**].

Table 5.8.1-38: Mean body weight of rats administered M656H031 for at least 28 days

	Males				Females			
Dose level [ppm]	0	1200	4000	12000	0	1200	4000	12000
Body weight [g]								
- Day 0	168.4	171.4	167.9	167.2	131.7	133.6	131.6	130.0
- Day 28	297.4	194.0	281.5	283.6	188.2	192.8	189.8	195.4
Δ% (compared to control)#		-1.1	-5.3	-4.6		2.4	0.9	3.8
Overall body weight gain [g]	129	122.6	113.7	116.5	56.5	59.2	58.2	65.4
Δ% (compared to control)#		-4.9	-11.9	-9.7		4.8	3.1	15.8

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-4: Body weight development of rats administered M656H031 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following table:

Table 5.8.1-39: Calculated intake of M656H031

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1200	108	111
2	4000	342	352
3	12000	1068	1140

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes among hematological parameters were observed.

In female animals of test group 1 (1200 ppm), relative basophil counts were lower compared to controls [see **Table 5.8.1-40**]. However, the alteration was not dose-dependent and, therefore, this change was regarded as incidental and not treatment-related.

Table 5.8.1-40: Selected hematology findings in rats administered M656H031 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day		108	342	1068		111	352	1140
Basophilic leucocytes [giga/l]	0.02	0.03	0.03	0.03	0.02	0.01	0.02	0.02
[%]	0.4	0.4	0.5	0.4	0.5	**0.2***	0.5	0.5

*$p \leq 0.05$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Clinical chemistry findings

No treatment-related changes among clinical chemistry parameters were observed.

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed.

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls [see **Table 5.8.1-41**].

When compared to the control group 0, the mean absolute organ weights of treated animals showed no significant deviations. A non-significant decrease of -47% was recorded for the mean uterus weight of females of test group 3 (12000 ppm).
In relation to terminal body weight the liver weights in female animals were significantly increased.

Table 5.8.1-41: Selected mean absolute and relative organ weights of rats administered M656H031 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000	0	1200	4000	12000
[mg/kg bw/day		108	342	1068		111	352	1140
Terminal bodyweight	268.74	266.96	257.64	256.44	171.58	175.94	170.64	179.94
[% of control]	100	99	96	95	100	103	99	105
Uterus weight, absolute [g]					0.712	0.604	0.588	0.380
[% of control]					100	85	83	53
Uterus weight, relative [%]					0.414	0.344	0.346	0.212
[% of control]					100	83	84	51
Liver weight, absolute [g]	7.210	7.120	7.132	7.556	4.840	4.808	4.688	4.825
[% of control]	100	99	99	105	100	99	97	100
Liver weight, relative [%]	2.681	2.671	2.761	**2.946***	2.822	2.735	2.746	2.680
[% of control]	100	100	103	**110**	100	97	97	95

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

No other statistically significant changes of absolute or relative organ weights were observed.

2. Gross and histopathology

A single macroscopic finding (pelvic dilation of the kidney) recorded in males of test group 3 (12000 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.

This difference in uterus weights was consistent with the different sexual cycle state observed histologically in each test group. Thus the uterus weight decrease in females of test group 3 (12000 ppm) [see **Table 5.8.1-41**] reflects a physiologic uterus state and not a treatment-related effect.

No histopathological correlate was found for the liver weight increase. Furthermore, this increase was of low magnitude and weak statistical significance. Therefore, the relative liver weight increase in males of test group 3 (12000 ppm) was considered to be incidental and not related to treatment.

All other histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental.

III. CONCLUSIONS

The administration of M656H031 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance-related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1068 mg/kg bw/d) and in female (1140 mg/kg bw/d) Wistar rats.

D Toxicological evaluation of metabolite M656PH031

The toxicological evaluation of M656PH031 was based on studies conducted with M656H031. No conclusive, relevant toxicological alerts were identified for M656PH031. There was no evidence for genotoxicity of M656PH031 in the in vitro genotoxicity studies conducted fulfilling the requirements for evaluation of ground-water metabolites. The available data on systemic toxicity – short-term toxicity study in rats - clearly demonstrated that the compound is of low toxicity and thus less toxic than the parent molecule dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water and also below the threshold of 0.75 µg/l of the EU DG Sanco guidance document for relevance assessment of groundwater metabolites.
In conclusion M656PH031 is considered to be of no toxicological relevance.

6. Metabolite M656PH032 formerly assigned M32

M656PH032 is a metabolite of dimethenamid-P determined in hen and ground-water. The exposure estimates for ground water are 0.75 µg/l < M656PH032 ≤ 4.5 µg/l. In the hen metabolism study M656H032 was observed at 1.05 mg/kg in excreta. M656H032 was also observed in an in vitro metabolism study on liver and kidney and should be considered tentative mammalian metabolite. Both studies were previously reviewed under Annex I.

A Structural alerts for M656PH032

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times predicted M656PH032 [see molecule 7 of report DocID 2014/1088460] to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 7 of report DocID 2014/1088461] the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into a ring-open structure similar to transformation products of M656PH030 and M656PH031. For this alpha-beta unsaturated carbonyl was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins. Again the prediction was out of the total domain for this model. All other presumed transformation products (13 in total) gave no alert for chromosomal aberration. Formation of M656PH032 as a metabolite of M656PH031 is predicted by OASIS times [see metabolite 6.4 of DocID 2014/1088461].

In the DEREK analysis conducted structural alerts for M656PH032 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (Molecule 12) in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for chromosomal aberration in vitro with metabolic activation considered of low relevance for the in vivo situation.

B Genotoxicity studies of M656PH032

Report:	CA 5.8.1/17 Woitkowiak C., 2013a Reg.No. 395234 (metabolite of BAS 656 H, Dimethenamid) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2013/1113379
Guidelines:	OECD 471, (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656H032 (Reg.No. 395234, metabolite of Dimethenamid; Batch: RS-TGA-100396; Purity: 92.1%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent plate incorporation assays and a third preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation assays as well as in the preincubation test, M656H031 was tested up to 5600 µg/plate. In detail, concentrations of 33, 100, 333, 1000, 2800 and 5600 µg/plate were used. A bacteriotoxic effect was observed in the standard plate test depending on the strain and test conditions from about 2800 µg/plate onward and in the preincubation assay from about 333 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.
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 M656H031 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1113379)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg.No. 395234 (Metabolite of BAS 656 H, Dimethenamid)
Description:	Liquid; brown, clear
Lot/Batch #:	RS-TGA-100396
Purity:	92.1% (tolerance +/- 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2014 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay (1st and 2nd experiment):

Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2800 and 5600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above. In addition, a 2nd experiment with TA1537 was performed in the absence and presence of metabolic activation due to inconclusive bacteriotoxic values observed in the 1st approach.

Pre-incubation assay (3rd experiment): The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2800 and 5600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 05-Mar-2013 to 22-Mar-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his^- or trp^- background growth, slight decrease in the number of his^+ or trp^+ revertants, slight reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 2800 µg/plate onward. In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 333 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see **Table 5.8.1-42**).

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-42: Bacterial gene mutation assay with M656H032 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	15	51	39	11	9	11	9	93	85
M656H032										
33 µg/plate	26	18	60	42	17	7	8	5	88	77
100 µg/plate	30	19	56	43	8	8	10	6	81	87
333 µg/plate	30	17	57	45	12	8	8	4	93	80
1000 µg/plate	24	18	52	36	13	10	10	4	88	92
2800 µg/plate	22	15	41	44	9	10	5	5	87	88
5600 µg/plate	13	17	34	27	12	10	3	3	46	50
Pos. control§	1291	837	1464	923	411	1071	224	545	372	740
2nd experiment: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	-	-	-	-	-	-	8	7	-	-
M656H032										
33 µg/plate	-	-	-	-	-	-	9	5	-	-
100 µg/plate	-	-	-	-	-	-	8	6	-	-
333 µg/plate	-	-	-	-	-	-	8	8	-	-
1000 µg/plate	-	-	-	-	-	-	5	7	-	-
2800 µg/plate	-	-	-	-	-	-	8	3	-	-
5600 µg/plate	-	-	-	-	-	-	6	3	-	-
Pos. control§	-	-	-	-	-	-	514	627	-	-
3rd experiment: Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	24	16	39	31	10	9	11	8	65	68
M656H032										
33 µg/plate	24	14	36	30	10	9	11	7	66	66
100 µg/plate	20	15	37	29	9	9	10	7	64	69
333 µg/plate	24	16	38	22	11	10	8	6	60	73
1000 µg/plate	17	10	26	21	10	6	5	7	61	65
2800 µg/plate	17	15	35	24	8	5	7	3	67	68
5600 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	0B	0B
Pos. control§	983	1148	806	883	323	731	218	554	407	870

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B= reduced background growth

III. CONCLUSION

According to the results of the present study, the test substance M656H032 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Conclusion on genotoxicity of M656PH032
M656PH031 was not genotoxic in the Ames test conducted. The limited structural alert identified for chromosomal aberration in vitro was considered adequately covered by the genotoxicity testing conducted with M656PH030 and M656PH031. M656PH032 is a presumed metabolite of M656PH031. By weight of evidence M656PH032 is not considered to be genotoxic.

C Toxicological evaluation of metabolite M656PH032

M656PH032 is not mutagenic in the Ames test. M656PH032 is a presumed metabolite of M656PH031. The further toxicological evaluation was based on the grouping proposal presented and discussed in Doc N4, chapter 3.3 to 3.5 of this submission. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
As a member of the M31-group metabolite M656PH032 is considered to be of <u>no</u> toxicological relevance.

7. <u>Metabolite M656PH043 former assigned M43/M44</u>

M656PH043 is a metabolite of dimethenamid-P determined in groundwater. The estimated exposure via groundwater is 0.1 µg/L < M656PH043 ≤ 000.75 µg/L.

A Structural alerts for M656PH043

In the OECD-toolbox no alerts for DNA-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. For protein-binding according to the OASIS module a nucleophilic addition to carbon-hetero double-bonds was predicted isntead of nucleophilic substitution, while in the OECD module for both structures the prediction was to act via direct acylation to formation of actetates. No prediction on protein binding potency was possible for M656PH043, while dimethenamid-P was predicted to be moderately reactive. Thus these limited alerts are not considered of relevance.

OASIS-Times predicted M656PH043 [see molecule 12 of report DocID 2014/1088460 and DocID 2014/1088461] to be not mutagenic in the Ames test and not to induce chromosomal aberration in vitro with the limitation that the molecule was out of the prediction domain.

In the DEREK analysis conducted structural alerts for M656PH043 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P.

No alert for genotoxicity was identified in this model. The Vega prediction (Molecule 14) in both modules (CAESAR and SarPy) was not mutgenic, however the reliability of this prediction was low.

In conclusion there was no alert for genotoxicity.

B Genotoxicity studies of M656PH043

Report:	CA 5.8.1/18 Woitkowiak C., 2014a Reg.No. 5917262 (metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhy murium/Escherichia coli, reverse mutation assay 2013/1361332
Guidelines:	OECD 471 (1997), (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.13 No. L 142, (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.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656PH043 (Reg. No. 5917262, former assigned M43; metabolite of BAS 656-PH, Dimethenamid-P; Batch: L82-113; Purity: 94.6%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In both approaches M656PH043 was tested at concentrations of 33, 100, 333, 1000, 2650 and 5300 µg/plate. A bacteriotoxic effect was observed in the standard plate test depending on the strain and test conditions from about 2650 µg/plate onward and in the preincubation assay under all test conditions from about 2650 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.
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 M656PH043 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1361332)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg.No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6% (tolerance +/- 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 01 Sep 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation: S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:

Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay:

The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-Sep-2013 to 10-Oct-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance in the vehicle dimethyl sulfoxide (DMSO) was verified analytically (BASF Project No. 01Y0077/13Y011).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 2650 µg/plate onward. In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed under all test conditions from about 2650 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see **Table 5.8.1-43**).

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-43: Bacterial gene mutation assay with M656PH043 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	25	19	91	73	11	10	9	5	72	66
M656PH043										
33 µg/plate	26	20	88	70	12	8	9	6	72	65
100 µg/plate	28	18	84	72	11	10	8	5	69	65
333 µg/plate	26	17	84	70	9	9	8	5	71	64
1000 µg/plate	27	20	91	70	11	9	8	5	65	59
2650 µg/plate	17	18	64	74	12	10	7	3	50	59
5300 µg/plate	12	12	60	38	9	7	7	4	36	36
Pos. control§	1107	570	1432	1255	245	1417	259	567	510	857
Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	19	71	54	11	11	6	6	72	68
M656PH043										
33 µg/plate	28	19	70	55	12	11	5	6	67	71
100 µg/plate	27	20	69	57	11	10	6	6	66	68
333 µg/plate	27	20	73	55	12	10	5	8	73	68
1000 µg/plate	28	19	71	51	10	11	6	6	73	60
2650 µg/plate	11	6	54	0B	7	7	2	2	54	22
5300 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	0B	0B
Pos. control§	1182	444	1240	1031	243	1138	363	416	238	1030

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B= reduced background growth

III. CONCLUSION

According to the results of the present study, the test substance M656PH043 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/19
Wollny H.-E., 2013a
Reg.No. 5917262 (metabolite of BAS 656-PH, Dimethenamid-P): In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1246089

Guidelines: OECD 476 (1997), (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.17, EPA 870.5300

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH043 (Reg.No. 5917262, metabolite of Dimethenamid-P; Batch: L82-113, Purity: 94.6%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations between 93.8 and 3000 µg/mL were used in the main experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects were observed in the first culture of experiment II at 375.0 µg/mL and above without metabolic activation. No visible precipitation of the test item in the culture medium was observed. No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656PH043 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1246089)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6% (tolerance ± 1.0%) for details see Certificate of Analysis ASAP13_121 (Dose selection adjusted to purity considering the preliminary information concerning the purity at the start of the experiment (97.37%).)
Stability of test compound:	Stable in DMSO
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

S9 was produced from the livers of induced 8-12 weeks old male Wistar [Hsd Cpb: WU] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3) parts followed by centrifugation at 9000 g.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism: The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}$M), Aminopterin ($2.0x10^{-5}$M), Thymidine ($1.6x10^{-3}$M) and Glycin ($5.0x10^{-3}$M) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}$M) and Thymidine ($1.6x10^{-3}$M). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 192 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 23.44 to 3000 µg/mL

b) Mutation assay:

1st and 2nd experiment: 93.8, 187.5, 375.0, 750.0, 1500.0, 3000.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Jul-2013 to 16-Sep-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.

$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression:	In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection:	After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:	Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).
Calculations:	**Pre-test**

Pre-test

<u>total suspension growth (4 h treatment):</u>
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>total suspension growth (24 h treatment):</u>
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>relative suspension growth:</u>
total suspension growth × 100 / total suspension growth of corresponding control

Main test

<u>total suspension growth (4 h treatment):</u>
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>total suspension growth (24 h treatment):</u>
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control
relative total growth:
relative suspension growth × relative cloning efficiency / 100
cloning efficiency (viability):
ln(mean number of empty wells per plate / 96) / cells seeded per well
relative cloning efficiency:
cloning efficiency × 100 / cloning efficiency of corresponding control
cells survived:
cloning efficiency × cell number seeded in TFT medium
mutant colonies / 106 cells:
small mutant colonies + large mutant colonies
threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_121).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 23.44 µg/mL and 3000 µg/mL were chosen with regard to the molecular weight (287.3 g/mol) and the purity of the test item (according to the preliminary information concerning the purity of the test item (97.37%)). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.

Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in both main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Relevant cytotoxic effects indicated by a relative total growth of less than 50% of survival were observed in the first culture of experiment II at 375.0 µg/mL and above without metabolic activation. No visible precipitation of the test item in the culture medium was observed.

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached (see **Table 5.8.1-44**, **Table 5.8.1-45**).

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table 5.8.1-44: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	115	241	100.0	68	194
Pos. Control MMS	19.5	-	23.4	416	241	17.3	532	194
M656PH043								
	93.8	-	culture was not continued#			culture was not continued#		
	187.5	-	68.4	122	241	61.2	79	194
	375.0	-	63.4	146	241	87.8	89	194
	750.0	-	64.1	108	241	79.5	62	194
	1500.0	-	83.7	113	241	92.3	94	194
	3000.0	-	79.4	114	241	65.9	72	194
Solv. Control DMSO		+	100.0	132	258	100.0	71	197
Pos. Control CPA	3.0	+	37.3	550	258	56.9	449	197
Pos. Control CPA	4.5	+	36.1	478	258	23.4	281	197
M656PH043								
	93.8	+	culture was not continued#			culture was not continued#		
	187.5	+	87.1	135	258	129.2	58	197
	375.0	+	103.3	104	258	110.1	77	197
	750.0	+	113.7	105	258	143.0	57	197
	1500.0	+	89.4	139	258	156.6	59	197
	3000.0	+	117.1	78	258	111.9	51	197

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-45: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	78	204	100.0	56	182
Pos. Control MMS	13.0	-	11.5	348	204	22.5	338	182
M656PH043								
	93.8	-	culture was not continued#			culture was not continued#		
	187.5	-	164.4	57	204	139.4	56	182
	375.0	-	42.8	86	204	128.1	48	182
	750.0	-	45.6	92	204	124.9	59	182
	1500.0	-	42.1	97	204	112.2	42	182
	3000.0	-	27.1	47	204	162.1	53	182
Experiment II / 4 h treatment								
Solv. Control DMSO		+	100.0	60	186	100.0	50	176
Pos. Control CPA	3.0	+	49.8	197	186	54.7	215	176
Pos. Control CPA	4.5	+	24.4	354	186	29.8	324	176
M656PH043								
	93.8	+	culture was not continued#			culture was not continued#		
	187.5	+	114.1	55	186	91.7	64	176
	375.0	+	107.3	58	186	110.5	42	176
	750.0	+	91.4	34	186	87.0	93	176
	1500.0	+	86.3	57	186	93.7	63	176
	3000.0	+	88.2	72	186	107.4	55	176

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656PH043 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/20 Bohnenberger S., 2013a Reg.No. 5917262 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro micronucleus test in chinese hamster V79 cells 2013/1246088
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH043 (Reg. No. 5917262, former assigned M43; metabolite of BAS 656-PH, Dimethenamid-P; Batch: L82-113; Purity: 94.6%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations of 5.9 to 3000 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since cytotoxicity and precipitation were not observed and the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.
In Experiment II the exposure period was 24 hours without metabolic activation and 4 hours with metabolic activation. Concentrations of 5.9 to 3000 µg/mL were used for the experiments without and 93.8 to 3000 µg/mL for the experiments with metabolic activation. Concentrations of 750, 1500 and 3000 µg/ml were chosen for evaluation. Cytotoxicity as well as precipitation were not observed in any experiment.
No mutagenicity was observed in Experiment I in the absence and presence of S9 mix and in Experiment II in the presence of S9 mix. In Experiment II in the absence of S9 mix statistically significant increases were observed after treatment with 1500.0 and 3000.0 µg/mL (1.25 and 1.95 % micronucleated cells). Dose-dependency was observed and the highest value exceeded the range of the historical control data (0.05 – 1.50 % micronucleated cells). Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.
Based on the results of this study, M656PH043 is considered to induce micronuclei in vitro in V79 cells in the absence of metabolic activation.
(BASF DocID 2013/1246088)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in DMSO (solvent)
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive controls, -S9:	Mitomycin C (MMC, 0.1 µg/mL, dissolved in deionised water) Griseofulvin (8.0 µg/mL, dissolved in DMSO)
Positive control, +S9:	Cyclophosphamide (CPA, 10.0 µg/mL, dissolved in saline)
3. Activation:	S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice. The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥ 70 %).

5. Culture medium/conditions:

About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay:

Experiment I:

5.9 - 3000.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I (see **Table 5.8.1-46**).

b) Cytogenicity assay:

Experiment II:

5.9 – 3000.0 µg/mL without metabolic activation

93.8 - 3000.0 µg/mL with metabolic activation

Table 5.8.1-46: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with M656PH043

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 hrs	4 hrs	I	5.9	11.7	23.4	46.9	93.8	187.5	375.0	**750.0**	**1500.0**	**3000.0**
24 hrs	24 hrs	II	5.9	11.7	23.4	46.9	93.8	187.5	375.0	**750.0**	**1500.0**	**3000.0**
												With S9 mix
24 hrs	4 hrs	I	5.9	11.7	23.4	46.9	93.8	187.5	375.0	**750.0**	**1500.0**	**3000.0**
24 hrs	4 hrs	II					93.8	187.5	375.0	**750.0**	**1500.0**	**3000.0**

Evaluated experimental points are shown in bold characters

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-Jul-2013 - 27-Aug-2013

2. Preliminary cytotoxicity assay: With respect to the molecular weight and the purity (97.37 %, preliminary information at the start of the experiment) of the test item, 3000.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 5.9 and 3000.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose-related and reproducible increase in the number of cells containing micronuclei is observed
- if the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_121).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix.

C. CYTOGENICITY ASSAYS:

In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. In addition, no visible precipitation of the test item in the culture medium was observed.

No mutagenicity was observed in Experiment I in the absence and presence of S9 mix and in Experiment II in the presence of S9 mix. The rates of micronucleated cells after treatment with the test item (0.35 – 1.35 %) were close to the rates of the solvent control values (0.55 - 1.25 %) and within the range of the laboratory historical solvent control data. In Experiment II in the absence of S9 mix statistically significant increases were observed after treatment with 1500.0 and 3000.0 µg/mL (1.25 and 1.95 % micronucleated cells). Dose-dependency was observed and the highest value exceeded the range of the historical control data (0.05 – 1.50 % micronucleated cells) [see **Table 5.8.1-47**].

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-47: Summary of results of the micronucleus test with M656PH043

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.87	0.80
		Positive control[2]	2.60	**4.95**[S]
		750.0	2.85	0.70
		1500.0	2.80	1.35
		3000.0	2.74	0.90
Exposure period 24 hrs without S9 mix				
II	24 hrs	Solvent control[1]	2.92	0.55
		Positive control[3]	2.47	**14.45**[S]
		750.0	2.82	0.55
		1500.0	2.87	**1.25**[S]
		3000.0	2.65	**1.95**[S**]
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	2.07	1.25
		Positive control[4]	1.80	**8.85**[S]
		750.0	1.97	1.25
		1500.0	2.24	1.10
		3000.0	2.10	1.35
II	24 hrs	Solvent control[1]	1.82	1.05
		Positive control[4]	1.57	**12.95**[S]
		750.0	1.94	0.35
		1500.0	1.79	0.80
		3000.0	1.97	1.30

* The total number of micronucleated cells was determined in a sample of 2000 cells
** The total number of micronucleated cells was determined in a sample of 4000 cells
S Number of micronucleated cells statistically significantly higher than corresponding control values
1 DMSO 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 Griseofulvin 8.0 µg/mL
4 CPA 10.0 µg/mL

III. CONCLUSIONS

Based on the results of the study, M656PH043 is considered as mutagenic in this in vitro micronucleus test, when tested up to the highest required concentrations.

Report: CA 5.8.1/21
[redacted] 2013b
Reg.No. 5917262 (metabolite of BAS 656-PH Dimethenamid-P) - Micronucleus assay in bone marrow cells of the mouse
2014/1001781
Guidelines: OECD 474 (1997), (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.12, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.8.1/22
Grauert M.,Kamp H., 2014a
Reg. No. 5917262 (metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in 30 % dimethyl sulphoxide + 70 % polyethylenglycol (v+v)
2014/1098005
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.8.1/23
Becker M.,Landsiedel R., 2014a
Reg. No. 5917262 (metabolite of BAS 656-PH, Diemethenamid-P) - Plasma analysis for external studies
2014/1092436
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656PH043 (Reg. No. 5917262, former assigned M43, metabolite of Dimethenamid-P; Batch: L82-113; Purity: 94.6%) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. For this purpose, the test substance dissolved DMSO/PEG 400 (3/7) was administered once orally to groups of 7 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 male animals/control). The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

The oral administration of the test substance did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was below or very close to the vehicle control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Signs of systemic toxicity were not observed up to the highest tested dose of 2000 mg/kg bw.
The positive control chemical cyclophosphamide led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system. Based on the results of the study it is considered that M656PH043 did not induce micronuclei in the bone marrow cells of the mouse under the test conditions chosen.

(BASF DocID 2014/1001781)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5917262 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-113
Purity:	94.6% (tolerance ± 1.0%)
Stability of test compound:	Confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0077/13Y042). Homogeneity of the preparations was ensured by mixing.
Solvent used:	30% Dimethyl sulphoxide (DMSO), 70 % Polyethylene glycol (PEG)

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	DMSO / PEG 400 (3/7)
Positive control:	Cyclophosphamide (CPA) 40 mg/kg

3. Test animals:

Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 9 weeks
Weight at dosing:	Males mean value 34.6 g (SD ± 1.7 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon Type II (pre-test) / III (main study) cages, with wire mesh top

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 15-Oct-2013 to 13-Nov-2013

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).

3. Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once with either the vehicle or 500, 1000 or 2000 mg M656PH043 / kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation:	In general, 2000 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. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.

5. Evaluation criteria:

A test item was considered as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.
A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0077/13Y042).

B. PRELIMINARY RANGE FINDING TEST

None of the male or female mice died after oral administration of 1000 and 2000 mg/kg bw. At 1000 mg /kg bw clinical signs comprised ruffled fur and eyelid closure in both sexes. At 2000 mg/kg bw ruffled fur only was observed in one mal animal. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Signs of systemic toxicity were not observed up to 2000 mg/kg bw test substance. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle. Plasma-analytics however [see DocID 2014/1092436], demonstrated that M656PH043 was systemically available.

The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that M656PH043 did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item [see **Table 5.8.1-48**]. The mean values of micronuclei observed after treatment with the test substance were below or very near to the value of the vehicle control group. Moreover, microneucleus values obtained in all dose groups were within the historical negative control range.

The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

Table 5.8.1-48: Micronucleus test in mice administered M656PH043 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Vehicle	24	0.130	1-4	1280
M656PH043				
500 mg/kg bw	24	0.093	0-5	1243
1000 mg/kg bw	24	0.171	0-8	1225
2000 mg/kg bw	24	0.100	0-4	1241
Positive control				
Cyclophosphamide	24	1.950	24-56	1161
48 h sampling				
Sterile water	48	0.080	1-3	1191
M656PH043				
2000 mg/kg bw	48	0.064	1-2	1143

III. CONCLUSION

Based on the result of this study M656PH043 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Conclusion on genotoxicity of M656PH043

Overall, with regard to in vitro genotoxicity testing there was no indication for mutagenicity neither in the bacterial Ames-test nor in the mammalian Mouse-Lymphoma test. However, in the in vitro micronucleus test in in V79 cells conducted with Reg. No. 5917262 a potential chromosomal aberration effect was observed in the absence of metabolic activation. In contrast in the subsequently conducted in vivo micronucleus test in mice no treatment-related induction of micronuclei could be determined up to the limit dose of 2000 mg/kg a dose level that demonstrated clinical signs of toxicity in the pre-test. Plasma-analytics confirmed that M656PH043 was systemically available. Thus, the in vivo study conducted for the same endpoint did not demonstrate a treatment-related effect. By weight of evidence M656PH043 was not considered to be genotoxic.

C Toxicological evaluation of metabolite M656PH043

No relevant toxicological alert was identified for M656PH043. By weight of evidence M656PH043 was not considered to be genotoxic in the in vitro and in vivo genotoxicity studies conducted for evaluation of ground-water metabolites. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water and also below the 0.75µg/l threshold for toxicological screening of the Sanco guidance document Step 4 (Sanco/221/2000 – rev. 10 final 25-Feb-2003).

In conclusion M656PH043 is considered to be of no toxicological relevance.

8. Metabolite M656PH045 formerly assigned M45/M46

M656PH045 is a metabolite of dimethenamid-P determined in ground-water. The predicted exposure levels in ground-water are 0.75 µg/l < M656PH045 ≤ 4.5 µg/l.

A Structural alerts for M656PH045

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times [see molecule 22 of report DocID 2014/1088460] predicted M656PH045 to be not mutagenic in the Ames test with and without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 22 of report DocID 2014/1088461] the prediction was negative for the metabolite itself and the structural alerts identified for transformation products (alpha,beta polarized carbonyls) were not predicted to become active with the limitation that the molecules were out of the prediction domain.

In the DEREK analysis conducted structural alerts for M656PH045 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (Molecule 15) in both modules (CAESAR and SarPy) was non-mutagenic, however the reliability of this prediction was low.

In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

B Genotoxicity studies of M656PH045

Report:	CA 5.8.1/24 Woitcowiak C., 2013a Reg.No. 5917261 (metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhymurium/Eschericia coli, reverse mutation assay 2013/1361403
Guidelines:	OECD 471 (1997), (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656PH045 (Reg.No. 5917261, former assigned M45, metabolite of Dimethenamid-P; Batch: L82-128; Purity: 98.4%) using ultrapure water as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation experiment, M656PH045 was tested at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate. In the preincubation experiment, 10, 33, 100, 333, 1000 and 2500 µg/plate were tested. A bacteriotoxic effect was observed depending on the strain and test conditions from about 1000 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.
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 M656PH045 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1361403)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description: Solid, grey

Lot/Batch #: L82-128

Purity: 98.4% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Sep 2015 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Ultrapure water

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay (1st experiment):	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay (2nd experiment):	The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 18-Sep-2013 to 10-Oct-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions. The stability of the test substance in the vehicle ultrapure water was determined analytically (BASF Project No. 01Y0081/13Y021). Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_155).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

Bacteriotoxic effects (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) were observed in the standard plate test and in the preincubation assay depending on the strain and test conditions from about 1000 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see **Table 5.8.1-49**].

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-49: Bacterial gene mutation assay with M656PH045 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	21	16	61	58	11	11	8	6	61	58
M656PH045										
33 µg/plate	20	16	64	61	11	10	8	7	65	54
100 µg/plate	22	16	64	60	10	10	8	6	68	54
333 µg/plate	20	14	61	64	10	10	8	6	64	56
1000 µg/plate	11	14	55	64	8	12	5	6	68	54
2500 µg/plate	9	11	22	60	8	11	5	3	37	54
5000 µg/plate	9	12	20	54	5	09	0/B	4	30	24
Pos. control§	1297	328	1353	1305	375	1236	263	699	447	752
2nd experiment: Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	31	18	75	69	11	13	6	6	78	71
M656PH045										
10 µg/plate	26	18	86	67	10	12	7	5	74	70
33 µg/plate	28	22	81	70	11	13	6	6	73	66
100 µg/plate	28	21	81	75	11	12	7	6	77	70
333 µg/plate	27	18	80	67	10	10	6	6	73	66
1000 µg/plate	26	18	62	71	11	10	7	6	75	62
2500 µg/plate	0/B	4	39	37	7	7	0/B	3	16	16
Pos. control§	1182	444	1240	1031	243	1138	363	416	238	1030

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B = Reduced Background Growth

III. CONCLUSION

According to the results of the present study, the test substance M656PH045 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/25 Wollny H.-E., 2013b Reg.No. 5917261 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246091
Guidelines:	OECD 476 (1997), (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.17, EPA 870.5300
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH045 (Reg.No. 5917261, metabolite of Dimethenamid-P; Batch: L82-122, Purity: 99.7%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay six concentrations from 156.3 to 5000 µg/mL were used in the main experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
No relevant cytotoxic effects were observed in any of the experiments. No visible precipitation of the test item in the culture medium was observed. No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656PH045 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1246091)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description: Solid, beige

Lot/Batch #: L82-122

Purity: 99.7% (tolerance ± 1.0%)

Stability of test compound: Stable in deionised water (The stability of a comparable batch (L82-128) was verified analytically.)

Solvent used: deionised water

2. Control Materials:

Negative control: A negative control was not employed in this study.

Solvent control: deionised water

Positive control -S9: Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)

Positive control +S9: Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I and II.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism: The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}$M), Aminopterin ($2.0x10^{-5}$M), Thymidine ($1.6x10^{-3}$M) and Glycin ($5.0x10^{-3}$M) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}$M) and Thymidine ($1.6x10^{-3}$M). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 290 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 39.06 to 5000 µg/mL

b) Mutation assay:

1st and 2nd experiment: 156.3, 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 29-Jul-2013 to 30-Sep-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection: After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies: Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test

<u>total suspension growth (4 h treatment):</u>
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>total suspension growth (24 h treatment):</u>
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>relative suspension growth:</u>
total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control

relative total growth:
relative suspension growth × relative cloning efficiency / 100

cloning efficiency (viability):
ln(mean number of empty wells per plate / 96) / cells seeded per well

relative cloning efficiency:
cloning efficiency × 100 / cloning efficiency of corresponding control

cells survived:
cloning efficiency × cell number seeded in TFT medium

mutant colonies / 106 cells:
small mutant colonies + large mutant colonies

threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126

cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_154).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 39.06 µg/mL and 5000 µg/mL were chosen). No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.

Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in both main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 5000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No relevant cytotoxic effect indicated by a relative total growth of less than 50% of survival in both parallel cultures was observed in experiment I and II with and without metabolic activation. A single, moderately cytotoxic effect was noted in the second culture of experiment II at the maximum concentration of 5000 µg/mL in the presence of metabolic activation. However, this moderate reduction of the relative total growth was not judged as true cytotoxic effect as it was not reproduced in the parallel culture or in the first experiment with metabolic activation. No precipitation was observed by the unaided eye up to the maximum concentration.

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The induction factor of 126 above the corresponding solvent control was exceeded in the first experiment with metabolic activation at 2500 µg/mL in culture I. However, this isolated increase was judged as biologically irrelevant as it was neither dose dependent as indicated by the lacking statistical significance nor reproduced in the parallel culture under identical experimental conditions [see **Table 5.8.1-50**, **Table 5.8.1-51**].

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table 5.8.1-50: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with DMSO		-	100.0	60	186	100.0	71	197
Pos. Control with MMS	19.5	-	20.8	398	186	21.0	388	197
M656PH045								
	156.3	-	culture was not continued#			culture was not continued#		
	312.5	-	78.1	112	186	88.3	109	197
	625.0	-	95.4	92	186	92.6	121	197
	1250.0	-	96.5	80	186	106.3	65	197
	2500.0	-	75.7	98	186	62.2	136	197
	5000.0	-	83.8	104	186	68.5	128	197
Solv. Control with DMSO		+	100.0	95	221	100.0	95	221
Pos. Control with CPA	3.0	+	43.0	553	221	36.9	537	221
Pos. Control with CPA	4.5	+	39.4	642	221	21.9	788	221
M656PH045								
	156.3	+	culture was not continued#			culture was not continued#		
	312.5	+	116.4	120	221	68.0	119	221
	625.0	+	136.7	90	221	65.7	206	221
	1250.0	+	98.4	155	221	84.6	106	221
	2500.0	+	53.5	280	221	90.8	141	221
	5000.0	+	62.8	177	221	85.9	123	221

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-51: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control with DMSO		-	100.0	148	274	100.0	75	201
Pos. Control with MMS	13.0	-	19.6	402	274	25.7	271	201
M656PH045								
	156.3	-	culture was not continued#			culture was not continued#		
	312.5	-	78.8	137	274	78.0	56	201
	625.0	-	70.6	108	274	84.6	55	201
	1250.0	-	88.4	124	274	55.6	89	201
	2500.0	-	56.4	169	274	60.4	50	201
	5000.0	-	62.0	112	274	56.5	46	201
Experiment II / 4 h treatment								
Solv. Control with DMSO		+	100.0	135	261	100.0	106	232
Pos. Control with CPA	3.0	+	37.3	234	261	30.5	331	232
Pos. Control with CPA	4.5	+	30.7	352	261	25.4	403	232
M656PH045								
	156.3	+	culture was not continued#			culture was not continued#		
	312.5	+	77.9	162	261	56.1	97	232
	625.0	+	59.0	117	261	55.4	130	232
	1250.0	+	115.6	107	261	59.1	115	232
	2500.0	+	121.7	83	261	62.3	145	232
	5000.0	+	88.1	81	261	42.1	156	232

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656PH045 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/26 Bohnenberger S., 2013b Reg.No. 5917261 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro micronucleus test in Chinese hamster V79 cells 2013/1246090
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH045 (Reg.No. 5917261, former assigned M45, metabolite of Dimethenamid-P; Batch L82-122; purity 99.7%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations of 9.8 to 5000 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Cytotoxicity was not observed. Precipitation of the test item was observed microscopically at the end of treatment at 625.0 µg/mL and above in the absence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.
In Experiment II the exposure period was 24 hours without metabolic activation and 4 hours with metabolic activation. Concentrations of 9.8 to 5000 µg/mL were used for the experiments without and 156.3 to 5000 µg/mL for the experiments with metabolic activation. Cytotoxicity and precipitation were not observed in experimental part II.
No biologically relevant mutagenic effects were observed in both experiments under the respective metabolic conditions. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.
Based on the results of this study, M656PH045 is considered to not induce micronuclei in vitro in V79 cells under the experimental conditions chosen in this test.
(BASF DocID 2013/1246090)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Solid, beige
Lot/Batch #:	L82-122
Purity:	99.7 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in deionised water (solvent) (The stability of a comparable batch (L82-128) was verified analytically.)
Solvent used:	deionised water

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: deionised water

Positive controls, -S9: Mitomycin C
(MMC, 0.1 µg/mL, dissolved in deionised water)
Griseofulvin (9.0 µg/mL, dissolved in DMSO)

Positive control, +S9: Cyclophosphamide
(CPA, 10.0/15.0 µg/mL in Exp. I/II, dissolved in saline)

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥ 70 %).

5. Culture medium/conditions:

About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay:

Experiment I:

9.8 - 5000.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I (see **Table 5.8.1-52**).

b) Cytogenicity assay:

Experiment II:

9.8 – 5000.0 µg/mL without metabolic activation

156.3 - 5000.0 µg/mL with metabolic activation

Table 5.8.1-52: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5917261 (Metabolite of BAS 656-PH, Dimethenamid-P)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 hrs	4 hrs	I	9.8	19.5	39.1	78.1	**156.3**	**312.5**	**625.0^{P}**	1250.0^{P}	2500.0^{P}	5000.0^{P}
24 hrs	24 hrs	II*	9.8	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0^{P}**
24 hrs	24 hrs	II	9.8	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**
			With S9 mix									
24 hrs	4 hrs	I	9.8	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**
24 hrs	4 hrs	II*					156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**
24 hrs	4 hrs	II					156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**

Evaluated experimental points are shown in bold characters

*: Was repeated due to technical problems

^{P}: Precipitation occurred at the end of treatment

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-Jul-2013 - 02-Dec-2013

2. Preliminary cytotoxicity assay: 5000.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 9.8 and 5000.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours:	The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.
Preparations of cultures:	For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.
Analysis of micronuclei and cytotoxicity:	Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose-related and reproducible increase in the number of cells containing micronuclei is observed
- if the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_154).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the pre-test no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix. Precipitation of the test item was observed microscopically at the end of treatment at 625.0 µg/mL and above in the absence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

C. CYTOGENICITY ASSAYS:

In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. Visible precipitation of the test item in the culture medium was observed microscopically at the end of treatment in the absence of S9 mix at 625.0 µg/mL and above in Experiment I.

The rates of micronucleated cells after treatment with the test item in Experiment I in the presence of S9 mix and in Experiment II in the absence and presence of S9 mix (0.70 - 1.40 %) were close to the rates of the solvent control values (1.00 - 1.60 %) and within the range of the laboratory historical solvent control data. However, in Experiment I in the absence of S9 mix after treatment with 156.3 µg/mL one single increase in micronucleated cells (1.70 %) above the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) was observed. Since the value was not statistically significant, the finding was regarded as biologically irrelevant [see **Table 5.8.1-53**].

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-53: Summary of results of the micronucleus test with M656PH045

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.90	1.60
		Positive control[2]	2.60	**6.10**[S]
		156.3	2.96	1.70
		312.5	2.93	0.85
		625.0[P]	2.94	1.15
		5000.0[P]	2.92	n.e.
Exposure period 24 hrs without S9 mix				
II	24 hrs	Solvent control[1]	3.05	1.00
		Positive control[3]	2.65	**11.50**[S]
		1250.0	2.89	1.25
		2500.0	2.86	1.00
		5000.0	2.73	1.25
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	2.19	1.30
		Positive control[4]	1.74	**9.65**[S]
		1250.0	2.27	0.70
		2500.0	2.41	1.40
		5000.0	2.28	1.20
II	24 hrs	Solvent control[1]	2.25	1.35
		Positive control[5]	1.73	**12.55**[S]
		1250.0	2.28	1.10
		2500.0	2.16	1.60
		5000.0	2.13	1.20

* The number of micronucleated cells was determined in a sample of 2000 cells
S Number of micronucleated cells statistically significantly higher than corresponding control values
P Precipitation occurred microscopically at the end of treatment
1 Deionsed water 10.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 Griseofulvin 9.0 µg/mL
4 CPA 10.0 µg/mL
5 CPA 15.0 µg/mL
n.e. not evaluated

III. CONCLUSIONS

Based on the results of the study, M656PH045 is considered as non-mutagenic in this in vitro micronucleus test, when tested up to the highest required concentrations.

Conclusion on genotoxicity of M656PH045

Overall there is no evidence for genotoxicity of M656PH045 based on the in vitro genotoxicity studies conducted fulfilling the requirement of the DG Sanco guidance document for evaluation of ground-water metabolites.

C Short-term toxicity of M656PH045

Report:	CA 5.8.1/27 [redacted] 2014c Reg. No. 5917261 (metabolite of BAS 655-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018092
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of M656H045 (Reg.No. 5917261, metabolite of Dimethenamid-P; Batch: L82-128; Purity: 98.4%) to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm for at least 28 days did not cause any test substance-related adverse signs of toxicity.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1174 mg/kg bw/d) and in female (1298 mg/kg bw/d) Wistar rats.

(DocID 2014/1018092)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** **Reg.No. 5917261, metabolite of Dimethenamid-P**
   Description: Solid/grey
   Batch/purity #: L82-128, 98.4%
   Stability of test compound: Stable until 01 Sep 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. **Vehicle and/or positive control:** Rodent diet

3. **Test animals:**

Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 141.8 – 156.8 g, ♀ 119.9 -136.4 g
Source:	Charles River, Germany
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:	Group housing (5 animals per cage) in polysulfonate cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 05-Nov-2013 – 05-Mar-2014
(In life dates: 11-Nov-2013 (start of administration) to 10-Dec-2013 (necropsy))

2. Animal assignment and treatment:

M656H045 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. One diet preparation per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 34 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level single samples were analysed. No test-article was determined in control diets.

Table 5.8.1-54: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1200 ppm	8. Nov. 13	1220.1 ± 81.5	101.7	6.8
4000 ppm	8. Nov. 13	3675.600	91.9	
12000 ppm	8. Nov. 13	12014.2 ± 116.8	100.1	1.0

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 1.0 to 6.8% indicate the homogenous distribution of M656H045 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 91.9% to 101.7% of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H045 in the vehicle.

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 5.8.1-55: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-56: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table 5.8.1-57: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmos
15. feces discharge during examination (appearance/consistency)
16. urine discharge during examination
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y body weight [g] at 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from 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 hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands[£]	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides[¥]	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetized animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles[£]				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology, [£]seminal vesicles and coagulation weight determined together

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes including quantitative parameters. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.

Comparing the single intervals with the control groups, no significant deviations were measured.

C. BODY WEIGHT AND BODY WEIGHT GAIN

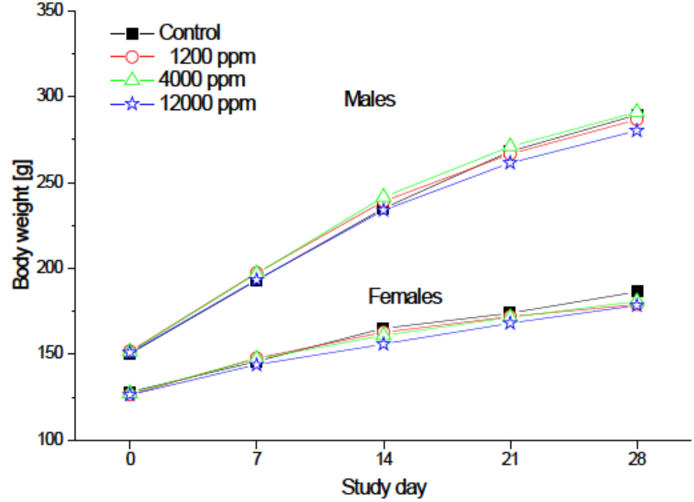
No statistically significant differences of absolute body weights or body weight gain were noted [see **Table 5.8.1-58** and **Figure 5.8.1-5**].

Table 5.8.1-58: Mean body weight of rats administered M656PH045 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
Body weight [g]								
- Day 0	150.3	151.8	151.4	150.9	128	126.4	127.3	126.5
- Day 28	289.3	286.7	291.0	280.1	186.4	179	180.8	178.4
Δ% (compared to control)#		-0.9	0.6	-3.2		4.0	-3	-4.3
Overall body weight gain [g]	139.0	135.0	139.6	129.2	58.4	52.6	53.5	51.9
Δ% (compared to control)#		-2.9	0.5	-7.1		9.9	-8.4	-11.2

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-5: Body weight development of rats administered M656PH045 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed. All recorded values were within the biological range typical for this strain of rats. Some values were not taken into account for groups summaries as these were declared as outliers. Increased food spilling was assumed for male animals the 4000 ppm group between study days 18 to 21 and 25 to 28 and for male animals of the 12000 ppm group between study days 25 to 28.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following **Table 5.8.1-59**.

Table 5.8.1-59: Calculated intake of M656PH045

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1200	102	100
2	4000	376	391
3	12000	1174	1298

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes among hematological parameters were observed. Prothrombin time could not be measured due to a technical failure of the instrument.

2. Clinical chemistry findings

No treatment-related changes among clinical chemistry parameters were observed.

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed.

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls.

When compared to the control group, all mean absolute and relative organ weights of treated animals did not show significant differences when compared to the control group.

2. Gross and histopathology

Isolated macroscopic findings were recorded in one male control group animal (focus in epdidymidis) and one low dose animal (reduced testes size). A spermatogonic granuloma was identified as histopathological correlate for the focus in the epididymidis. Tubular degeneration of the testis was diagnosed for the animal showing the testis size reduction and also for a male control group animal. These lesions were considered to be incidental or spontaneous in nature and without any relation to treatment.

All other histopathological findings were either single observations, were equally distributed between control and treated groups. Therefore, these findings were considered to be incidental.

III. CONCLUSIONS

The administration of M656H045 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance-related adverse signs of toxicity at concentrations of 1200, 4000 and 12000 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 12000 ppm in male (1174 mg/kg bw/d) and in female (1298 mg/kg bw/d) Wistar rats.

D Toxicological evaluation of M656PH045

No conclusive, relevant toxicological alerts were identified for M656PH045. There was no evidence for genotoxicity of M656PH045 in the in vitro genotoxicity studies conducted fulfilling the requirements for evaluation of ground-water metabolites. The available data on systemic toxicity – short-term toxicity study in rats - clearly demonstrated that the compound is of low toxicity and thus less toxic than the parent molecule dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
In conclusion M656PH045 is considered to be of <u>no</u> toxicological relevance.

9. Metabolite M656PH047 formerly assigned M47/M48

M656PH047 is a metabolite of dimethenamid-P determined in ground-water. The predicted human exposure levels via ground-water are 0.1 µg/l < M656PH047 ≤ 0.75 µg/l.

A Structural alerts for M656PH047

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times [see molecule 27 of report DocID 2014/1088460] predicted M656PH047 to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration in vitro [see molecule 27 of report DocID 2014/1088461] the prediction was negative for the metabolite itself and negative for all presumed transformation products thereof (7 in total). The identified alert for a thiol was not predicted to become active. The overall prediction was therefore negative for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain.

In the DEREK analysis conducted the structural alerts for M656PH047 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction [see Molecule 16 of DocID 2014/1088457] in both modules CAESAR and SarPy was inconclusive. While the CAESAR module predicted M656PH047 to be Ames mutagenic the prediction of the SarPy module was non-mutagenic. However the reliability of these predictions was low as no similar compounds with known experimental data were found in the database and some similar molecules found in the database disagree with the prediction.

In conclusion no conclusive, relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

B Genotoxicity studies of M656PH047

Report:	CA 5.8.1/28 Woitkowiak C., 2014b Reg.No. 5917260 (metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhymurium/Escherichia coli reverse mutation assay 2013/1397766
Guidelines:	OECD 471, (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656PH047 (Reg. No. 5917260, former assigned M47, metabolite of Dimethenamid-P; Batch: L82-137; Purity: 90.7%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In both approaches M656PH047 was tested at concentrations of 33, 100, 333, 1000, 2750 and 5500 µg/plate. A bacteriotoxic effect was observed in the preincubation assay depending on the strain and test conditions at 5500 µg/plate. Precipitation of the test substance was found in the preincubation experiment at 5500 µg/plate with and without S9 mix.
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 M656PH047 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1397766)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5917260 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description: Solid, beige

Lot/Batch #: L82-137

Purity: 90.7% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

The optical density of the fresh bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 10^9 cells per mL).

5. Test concentrations:

Plate incorporation assay:	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay:	The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-Oct-2013 to 14-Nov-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

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 dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripled (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (BASF Project No. 01Y0082/13Y015).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

No bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test up to the highest required concentration.
In the preincubation assay bacteriotoxicity (slight decrease in the number of his^+ or trp^+ revertants) was occasionally observed depending on the strain and test conditions at 5500 µg/plate.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see **Table 5.8.1-60**].

Test substance precipitation was found in the preincubation test at a concentration of 5500 µg/plate with and without S9 mix.

Table 5.8.1-60: Bacterial gene mutation assay with M656PH047 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	24.3	24.3	54.0	42.3	14.0	7.0	10.7	6.7	62.7	59.7
M656PH047										
33 µg/plate	28.0	19.0	52.3	40.7	11.3	7.7	8.7	6.7	58.0	57.3
100 µg/plate	26.3	18.3	52.3	40.3	10.3	9.7	7.3	6.7	62.0	57.3
333 µg/plate	26.0	15.7	43.0	39.3	9.0	10.0	6.0	4.0	62.7	60.3
1000 µg/plate	29.7	18.7	49.0	41.3	9.0	7.3	8.7	5.0	59.0	59.7
2750 µg/plate	19.7	21.3	44.0	40.7	10.3	10.3	8.7	5.7	65.3	50.7
5500 µg/plate	20.0	16.3	46.0	37.0	10.3	8.3	7.3	5.7	57.7	52.7
Pos. control§	2110.0	343.3	2803.7	4304.7	208.0	5337.0	248.0	1691.0	149.0	829.7
Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	25.7	24.3	44.0	38.7	9.0	7.0	7.7	6.7	69.7	67.3
M656PH047										
33 µg/plate	30.7	14.7	47.7	28.3	12.3	11.0	7.7	6.7	70.7	67.7
100 µg/plate	28.7	19.7	67.0	38.3	11.7	13.0	7.0	5.3	74.3	60.7
333 µg/plate	28.0	18.3	57.0	35.7	10.7	10.0	7.3	10.3	73.3	70.0
1000 µg/plate	31.7	20.3	66.7	45.7	13.7	9.7	9.3	7.7	69.7	58.0
2750 µg/plate	21.7	19.0	64.7	37.0	9.3	5.7	6.3	9.7	64.7	59.7
5500 µg/plate	15.3P	13.3P	58.7P	30.3P	9.3P	6.0P	4.3P	3.0P	59.0P	26.0P
Pos. control§	2420.7	375.0	3005.7	3191.3	170.3	3234.7	225.0	1169.7	148.3	351.3

§ = Compound and concentrations see Material and Methods (I.A.2.) above
P = Precipitation

III. CONCLUSION

According to the results of the present study, the test substance M656PH047 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/29 Wollny H.-E., 2014a Reg.No. 5917260 (metabolite of BAS 656-PH, Dimethenamid-P): In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2014/1018055
Guidelines:	OECD 476 (1997), (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.17, EPA 870.5300
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH047 (Reg. No. 5917260, former assigned M47, metabolite of Dimethenamid-P; Batch: L82-137; Purity: 90.7%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations between 120.9 and 3870 µg/mL in DMSO were used in the main experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects indicated by a relative total growth (RTG) below 50% in both parallel cultures solely occurred at the highest two concentrations of the second experiment without metabolic activation. RTG levels below 50% were also noted in the second culture of the second experiment with metabolic activation at 483.8 and 1935 µg/mL. However, no comparable effect occurred in the parallel culture under identical experimental conditions. No visible precipitation of the test item in the culture medium was observed. No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656PH047 does not induce forward mutations in mammalian cells in-vitro.
(BASF DocID 2014/1018055)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No. 5917260 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-137
Purity:	90.7% (tolerance ± 1.0%), dose calculation adjusted to purity
Stability of test compound:	Stable in DMSO over 4 hours
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 29.8 mg/mL (Lot. No.: 050913) in the pre-experiment and in experiment I and II.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism:

The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}M$), Aminopterin ($2.0x10^{-5}M$), Thymidine ($1.6x10^{-3}M$) and Glycin ($5.0x10^{-3}M$) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}M$) and Thymidine ($1.6x10^{-3}M$). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 192 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 15.1 to 1930 µg/mL

b) Mutation assay:

1st and 2nd experiment: 120.9, 241.9, 483.8, 967.5, 1935.0, 3870.0 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 05-Nov-2013 to 02-Dec-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression:	In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.
Selection:	After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative

total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies: Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test

total suspension growth (4 h treatment):

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

relative suspension growth:

total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:

total suspension growth × 100 / total suspension growth of corresponding control

relative total growth:

relative suspension growth × relative cloning efficiency / 100

cloning efficiency (viability):

ln(mean number of empty wells per plate / 96) / cells seeded per well

relative cloning efficiency:

cloning efficiency × 100 / cloning efficiency of corresponding control
cells survived:
cloning efficiency × cell number seeded in TFT medium
mutant colonies / 106 cells:
small mutant colonies + large mutant colonies
threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126
cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC, Q-NMR analysis (see BASF study report ASAP13_206).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 15.1 µg/mL and 1930 µg/mL were chosen. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment. A minor reduction of the relative suspension growth to approximately 50% occurred at the maximum concentration following 24 hours treatment without metabolic activation.

Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. The pH was adjusted to neutral using 2M sodium hydroxide solution. There was no relevant shift of the osmolarity at the maximum concentration.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3870 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Relevant and reproducible cytotoxic effects indicated by a relative total growth (RTG) below 50% in both parallel cultures solely occurred at the highest two concentrations of the second experiment without metabolic activation. RTG levels below 50% were also noted in the second culture of the second experiment with metabolic activation at 483.8 and 1935 µg/mL. However, no comparable effect occurred in the parallel culture under identical experimental conditions. No visible precipitation of the test item in the culture medium was observed.

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached (see **Table 5.8.1-74**, **Table 5.8.1-75**).

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls.

Table 5.8.1-61: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	59	185	100.0	110	236
Pos. Control MMS	19.5	-	33.5	313	185	34.2	319	236
M656PH047								
	120.9	-	culture was not continued#			culture was not continued#		
	241.9	-	91.5	91	185	139.0	74	236
	483.8	-	95.6	65	185	138.7	70	236
	967.5	-	86.6	76	185	102.7	210	236
	1935.0	-	72.9	121	185	83.0	120	236
	3870.0	-	91.4	92	185	97.6	86	236
Solv. Control DMSO		+	100.0	47	173	100.0	91	217
Pos. Control CPA	3.0	+	80.8	123	173	106.9	218	217
Pos. Control CPA	4.5	+	39.8	261	173	63.1	391	217
M656PH047								
	120.9	+	culture was not continued#			culture was not continued#		
	241.9	+	72.6	119	173	161.4	67	217
	483.8	+	56.4	138	173	184.1	115	217
	967.5	+	67.6	162	173	196.2	55	217
	1935.0	+	83.7	88	173	127.6	69	217
	3870.0	+	62.9	137	173	194.6	58	217

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-62: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control DMSO		-	100.0	65	191	100.0	53	179
Pos. Control MMS	13.0	-	11.1	411	191	8.2	441	179
M656PH047								
	241.9	-	culture was not continued#			culture was not continued#		
	483.8	-	87.3	67	191	113.8	49	179
	967.5	-	71.3	72	191	92.4	50	179
	1935.0	-	81.7	52	191	51.2	57	179
	2902.5	-	19.5	50	191	32.4	78	179
	3870.0	-	21.9	62	191	25.4	66	179
Experiment II / 4 h treatment								
Solv. Control DMSO		+	100.0	147	273	100.0	53	179
Pos. Control CPA	3.0	+	47.8	285	273	49.9	220	179
Pos. Control CPA	4.5	+	43.8	372	273	14.8	746	179
M656PH047								
	120.9	+	culture was not continued#			culture was not continued#		
	241.9	+	107.9	60	273	60.9	93	179
	483.8	+	96.4	79	273	33.5	174	179
	967.5	+	77.0	156	273	50.4	147	179
	1935.0	+	77.5	123	273	41.9	118	179
	3870.0	+	78.0	149	273	70.4	77	179

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656PH047 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report: CA 5.8.1/30
[redacted] 2014a
Reg.No. 5917260 (metabolite of BAS 656, Dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1018032
Guidelines: OECD 474 (1997), (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.12, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.8.1/31
Grauert M.,Kamp H., 2014b
Reg. No. 5917260 (metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in sterile water
2014/1098002
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.8.1/32
Becker M.,Landsiedel R., 2014b
Reg.No. 5917260 (metabolite of BAS 656-PH, Dimethenamid-P) - Plasma analysis for external studies
2014/1092434
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656PH047 (Reg. No. 5917260, former assigned M47, metabolite of Dimethenamid-P; Batch: L82-137; Purity: 90.7%) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. For this purpose, the test substance dissolved sterile water was administered once orally to groups of 7 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 male animals/control). The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

The oral administration of the test substance did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was even slightly below the value of the vehicle control groups in all dose groups. Moreover, micronucleus values obtained in all dose groups were very well within the historical negative control range. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. The only clinical symptom observed was ruffled fur in four of 14 mice treated with the high dose of test item observed 1 hour post application. The animals treated with the mid dose level and low dose level did not exhibit any clinical symptoms. The positive control chemical cyclophosphamide led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system. Based on the results of the study it is considered that M656PH047 did not induce micronuclei in the bone marrow cells of the mouse under the test conditions chosen.

(BASF DocID 2014/1018032)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5917260 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-137
Purity:	90.7%
Stability of test compound:	Confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0082/13Y060).
Solvent used:	sterile water

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	sterile water
Positive control:	Cyclophosphamide (CPA) 40 mg/kg

3. Test animals:

Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks
Weight at dosing:	Males mean value 35.8 g (SD ± 1.5 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon Type II (pre-test) / III (main study) cages, with wire mesh top

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 12-Nov-2013 to 09-Dec-2013

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).

3. Micronucleus test:

Treatment and sampling: Groups of male mice were treated once with either the vehicle or 500, 1000 or 2000 mg M656PH047 / kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose animals were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study.

Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide preparation: A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.

Slide evaluation: In general, 2000 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.

To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.

5. Evaluation criteria:

A test item was considered mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0082/13Y060).

B. PRELIMINARY RANGE FINDING TEST

None of the male or female mice died after oral administration of 1000 and 2000 mg/kg bw. At 2000 mg /kg bw clinical signs comprised ruffled fur and eyelid closure in both sexes. At 1000 mg/kg bw ruffled fur only was observed. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

The only clinical symptom in the main experiment was ruffled fur in four of 14 mice treated with the high dose of test item observed 1 hour post application. The animals treated with the mid dose level and low dose level did not exhibit any clinical symptoms. Analysis in blood plasma confirmed the systemic availability of M656PH047 [see DocID 2014/1092434].

The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that M656PH047 did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item [see **Table 5.8.1-97**]. The mean values of micronuclei observed after treatment with the test substance were even slightly below the value of the vehicle control groups in all dose groups. Moreover, micronucleus values obtained in all dose groups were very well within the historical negative control range.

The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

Table 5.8.1-63: Micronucleus test in mice administered M656PH047 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
		24 h sampling		
Vehicle	24	0.120	1-4	1287
M656PH047				
500 mg/kg bw	24	0.107	0-4	1241
1000 mg/kg bw	24	0.114	2-3	1240
2000 mg/kg bw	24	0.086	0-5	1234
Positive control				
Cyclophosphamide	24	3.030	57-67	1109
		48 h sampling		
Vehicle	48	0.140	2-5	1153
M656PH047				
2000 mg/kg bw	48	0.057	0-4	1208

III. CONCLUSION

Based on the result of this study M656PH047 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Conclusion on genotoxicity of M656PH047

Overall, with regard to in vitro genotoxicity testing there was no indication for mutagenicity neither in the bacterial Ames-test nor in the mammalian Mouse-Lymphoma test. There was no treatment related induction of micronuclei in an in vivo micronucleus test in mice up to the limit dose of 2000 mg/kg a dose level that demonstrated clinical signs of toxicity. Plasma-analytics confirmed that M656PH047 was systemically available. Thus, M656PH047 is considered to be not genotoxic.

C Short-term toxicity of M656PH047

Report:	CA 5.8.1/33 [redacted], 2014d Reg.No. 5917260 (metabolite of BAS 656-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018064
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of M656H047 (Reg.No. 5917260, metabolite of Dimethenamid-P; Batch: L82-137; Purity: 90.70%) to Wistar rats at dietary dose levels of 0, 1320, 4400 and 13200 ppm; dose levels adjusted to purity of the test compound in order to obtain intended dose levels of 1200, 4000 and 12000 ppm for at least 28 days did not cause any test substance-related adverse signs of toxicity. As a treatment-related effect higher serum total bile acid levels in males as well as increased urinary urobilinogen levels in rats of both sexes in the same test group indicated a higher rate of excretion of bilirubin and bile acids with the bile, followed by a higher intestinal resorption of bile acids and urobilinogen, the latter excreted by the kidneys. This effect might have been a consequence of an increased secondary metabolism induced by the compound. It was regarded as an adaptive and not an adverse effect.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 13200 ppm corresponding to 1161 mg/kg bw/day in male and 967 mg/kg bw/day in female Wistar rats when corrected for purity.

(DocID 2014/1018092)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** **Reg.No. 5917260, metabolite of Dimethenamid-P**
   Description: Solid/brown
   Batch/purity #: L82-137, 90.7%
   Stability of test compound: Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. **Vehicle and/or positive control:** Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 155.9 – 169.8 g, ♀ 118.8 – 145.1 g
Source:	Charles River, Germany
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:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 09-Dec-2013 – 05-Mar-2014
(In life dates: 17-Dec-2013 (start of administration) to 15-Jan-2014 (necropsy))

2. Animal assignment and treatment:

M656H047 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1320 (low dose), 4400 (intermediate dose) and 13200 ppm (top dose) for at least 28 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. Eight diet preparation per dose were performed for this study (preparation twice a week).

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for 4 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1320 ppm) and top dose level (13200 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level single samples were analysed. No test-article was determined in control diets.

Table 5.8.1-64: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD	% of nominal concentration	Relative standard deviation [%]
1320 ppm	17. Dez. 13	1313.3 ± 57.8	99.5	4.4
4400 ppm	17. Dez. 13	4346.9	98.8	
13200 ppm	17. Dez. 13	13039.8 ± 332.4	98.8	2.5

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 2.5 to 4.4% indicate the homogenous distribution of M656H047 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 98.8 to 99.5% of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H047 in the vehicle.

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 5.8.1-65: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-66: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table 5.8.1-67: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmos
15. feces discharge during examination (appearance/consistency)
16. urine discharge during examination
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y body weight [g] at 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane anesthetized animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands[£]	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides[¥]	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetized animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles[£]				
[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology, [£]seminal vesicles and coagulation weight determined together											

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. Most deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental. The statistically significantly reduced forelimb grip strength in males of the low dose did not show a dose response relationship and thus was not considered to be treatment related.
With regard to the overall motor activity as well as single intervals no significant deviations were observed between treated animals and the control group.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No test substance-related differences with regard to the mean body weights and body weight change values were noted for male and female animals of all dose groups [see **Table 5.8.1-68** and **Figure 5.8.1-6**]. The significantly lower body weight change value in female animals of the 13200 ppm group in the interval from day 0 up to day 14 was assessed as being spontaneous in nature and not related to treatment, because no significant change occurred for the other intervals or the absolute mean body weight.

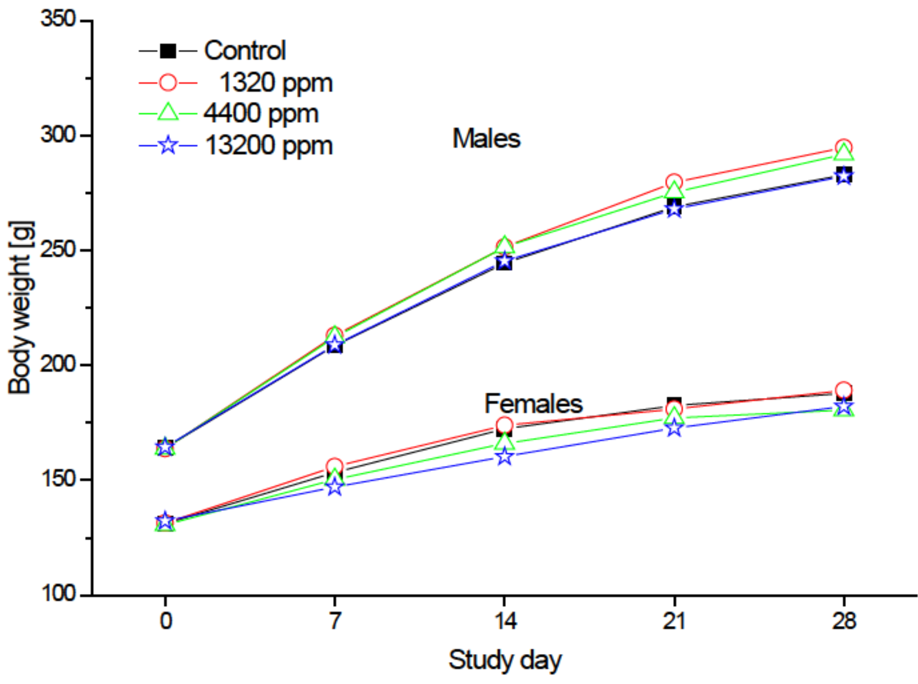
Table 5.8.1-68: Mean body weight of rats administered M656H047 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1320**	**4400**	**13200**	**0**	**1320**	**4400**	**13200**
Body weight [g]								
- Day 0	164.1	163.7	163.8	164.4	131.1	131.4	130.5	132.3
- Day 28	282.9	294.7	291.8	282.3	187.8	189.1	180.5	182.2
Δ% (compared to control) #		4.2	3.1	-0.2		0.7	-3.9	-3
Body weight change [g]								
- day 0 - 7	44.5	49.3	48.5	44.5	22.2	24.6	19.9	14.8
- day 0 - 14	80.3	87.7	87.6	81.0	41.2	42.6	35.5	**27.9 ***
- day 0 - 21	104.8	115.9	111.5	103.6	51.5	49.5	46.7	40.5
- day 0 - 28	118.8	131.1	128.0	117.9	56.6	57.7	50.1	49.8
Δ% (compared to control)#		10.3	7.7	-0.7		1.9	-11.6	-12

* $p \leq 0.05$ (Dunnett-test, two sided)

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-6: Body weight development of rats administered M656H047 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related, adverse findings were observed. An trend for an increase of food consumption during administration period was observed in male animals of the high dose group [**Table 5.8.1-69**]. These changes were assessed as being spontaneous in nature and not related to treatment. All other recorded values were within the biological range typical for this strain of rats.

Table 5.8.1-69: Mean food consumption of rats administered M656H047 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1320**	**4400**	**13200**	**0**	**1320**	**4400**	**13200**
Food consumption [g]								
- Day 3-7	18.5	18.7	18.7	20.0	12.7	13.7	12.0	12.7
- Day 10-14	20.5	20.4	20.9	24.2	14.5	14.8	13.5	13.2
- Day 17-21	21.4	20.9	20.9	25.9	14.0	14.4	14.1	14.2
- Day 24-28	17.8	16.5	16.9	27.2	12.3	13.4	11.3	13.0
Total	78.2	76.5	77.4	97.3	53.5	56.3	50.9	53.1

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following **Table 5.8.1-70**.

Table 5.8.1-70: Calculated intake of M656H047

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1320	99	107
2	4400	336	333
3	13200	1280	1066
Intake corrected for purity of M656PH047 of 90.7%			
1	1200	90	97
2	4000	304	302
3	12000	1161	967

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related adverse findings among hematological parameters were observed.

In male animals of the 13200 ppm group, neutrophilic counts were significantly increased as compared to controls [see **Table 5.8.1-71:** Selected hematology findings in rats administered M656H047 for at least 28 days (group means)**Table 5.8.1-71**]. This was the only altered differential cell count fraction and even total white blood cell counts were not changed. The neutrophil cell counts in males of this test group were at the upper border of the historical control range. No alteration in hematology occurred in females. Therefore, the higher absolute neutrophil cell counts in males the 13200 ppm were regarded as incidental and not treatment-related.

Table 5.8.1-71: Selected hematology findings in rats administered M656H047 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
[mg/kg bw/day		**90**	**304**	**1161**		**97**	**302**	**967**
Neutrophilic leucocytes[giga/l]	0.63	0.56	0.75	**1.09***	0.45	0.65	0.63	0.42
[%]	11.7	13.1	15.1	15.7	11.8	14.8	17.6	13.7
	Historical control: 0.53 – 1.09 giga/l							

*$p \leq 0.05$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Clinical chemistry findings

No treatment-related changes among clinical chemistry parameters were observed.

At the end of the study in males of mid and high dose group total bile acid levels were increased (not statistically significant in test group 3). At least in males of 4400 ppm this was the only altered clinical chemistry parameter in these individuals. Therefore, the higher total bile acid levels in males of 4400 ppm were regarded as treatment-related, but not adverse [ECETOC 2002a; DocID 2002/1027057]. Regarding the 12000 ppm dose group higher serum total bile acid levels in males [see **Table 5.8.1-72**] as well as increased urinary urobilinogen levels in rats of both sexes in the same test group [see **Table 5.8.1-73**] indicated a higher rate of excretion of bilirubin and bile acids with the bile, followed by a higher intestinal resorption of bile acids and urobilinogen, the latter excreted by the kidneys. This effect might have been a consequence of an increased secondary metabolism induced by the compound. It was regarded as an adaptive and not an adverse effect.

Table 5.8.1-72: Selected clinical chemistry findings in rats administered M656H047 for at least 28 days (group means)

Sex	**Males**				**Females**			
Dose [ppm]	**0**	**1200**	**4000**	**12000**	**0**	**1200**	**4000**	**12000**
[mg/kg bw/day		**90**	**304**	**1161**		**97**	**302**	**967**
Total bile acids [µmol/l]	16.6	25.3	**34.6****	**44.7**	14.5	31.2	17.6	29.4

*p ≤ 0.05 (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Urinalysis

In males and females of the 13200 ppm urinary urobilinogen levels were increased and additionally in females of this test group specific gravity of the urine was higher compared to controls. The higher urine specific gravity in females per se without any other finding in clinical pathology was regarded as treatment-related, but not adverse. As discussed above the increased urobilinogen levels were attributed to an increased excretion of bilirubin and bile acids with subsequent intestinal resorption of bile acids and urobilinogen the latter being renal excreted. No other treatment-related changes among urinalysis parameters were observed.

Table 5.8.1-73: Selected urinary parameter findings in rats administered M656H047 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1320	4400	13200	0	1320	4400	13200
[mg/kg bw/day		99	336	1280		107	333	1066
Urobilinoge[K]	1	1	2	**2***	1	1	1	**3****
Specific gravidity[W] [g/l]	1.068	1.062	1.071	1.066	1.057	1.051	1.058	**1.093***

*p ≤ 0.05 **p≤ 0.01

K = Kruskal-Wallis + Wilcoxon test (two sided)

W = Wilcoxon test (one sided)

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls. When compared to the control group, the mean absolute organ weights of treated animals showed no significant deviations.

2. Gross and histopathology

A single macroscopic finding (pelvic dilation of the kidney) recorded in males of test group 3 (12000 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.

There was no histopathological finding considered treatment related. All recorded histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental.

III. CONCLUSIONS

The administration of M656H047 via the diet to male and female Wistar rats for 4 weeks did not cause any test substance-related adverse signs of toxicity at concentrations of 1320, 4400 and 13200 ppm. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 13200 ppm corresponding to 1161 mg/kg bw/day in male and 967 mg/kg bw/day in female Wistar rats when corrected for purity.

E Toxicological evaluation of M656PH047

No conclusive, relevant toxicological alerts were identified for M656PH047. By weight of evidence M656PH047 was considered not to be genotoxic in the in vitro and in vivo genotoxicity studies conducted for evaluation of ground-water metabolites. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
In conclusion M656PH047 is considered to be of <u>no</u> toxicological relevance.

10. Metabolite M656PH049 former assigned M49

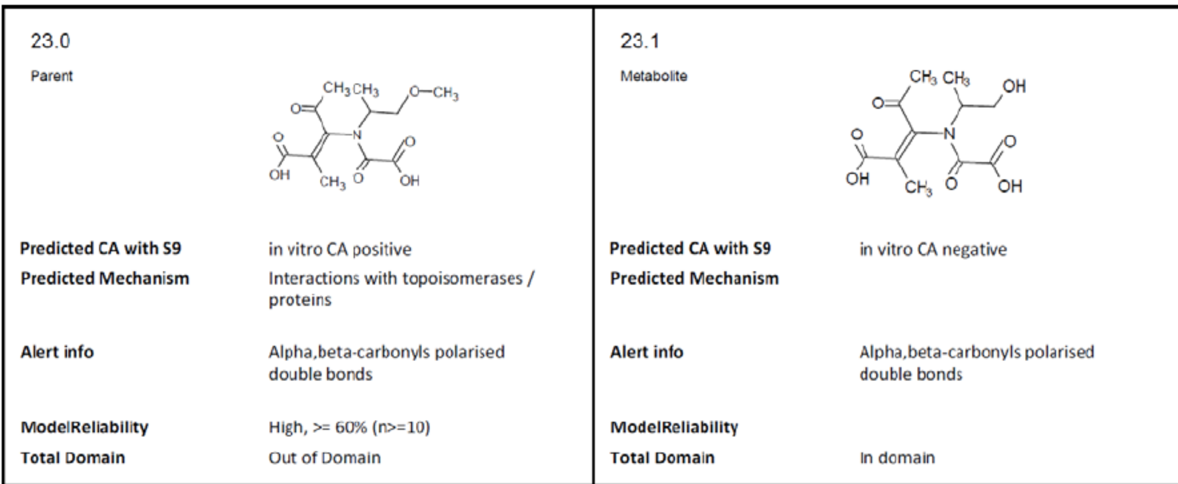
M656PH049 is a ground-water metabolite. The predicted exposure in ground-water is 0.1 µg/l < M656PH049 ≤ 0.75 µg/l.

A Structural alerts for M656PH049

In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule Dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential nucleophilic addition to the carbo-hetero double bond (keto-group) was identified in the OECD and the OASIS module. In contrast no DNA alert was identified for M656PH049 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox only provides the alerts based on the functional groups identified only and does not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.

OASIS-Times [see molecule 23 of report DocID 2014/1088460] however predicted M656PH049 to be not mutagenic with regard to Ames test alerts without or with metabolic activation with the limitation that the molecule was out of the prediction domain. There was however an alert for in vitro chromosomal aberration [see molecule 23 of report DocID 2014/1088461] based on the alpha, beta-carbonyls polarized double bonds. Again the prediction reliability was low as the structure was out of the prediction domain. Moreover the demethylated degradate (contained in the predicted metabolic pathway, molecule 23.1) was considered negative with the difference that this structure was covered by the prediction domain [see **Figure 5.8.1-7**].

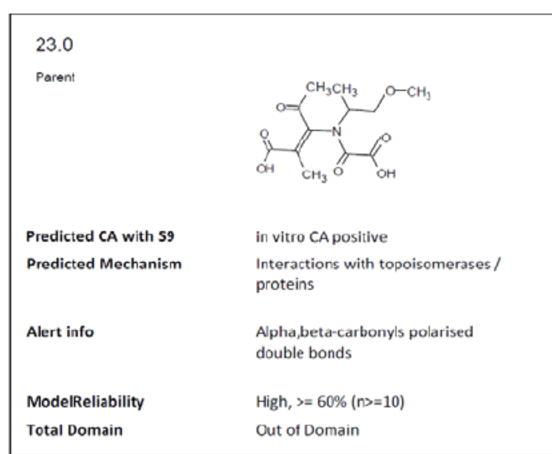
Figure 5.8.1-7: M656PH049 and its degradate OASIS times prediction for chromosomal aberration in vitro



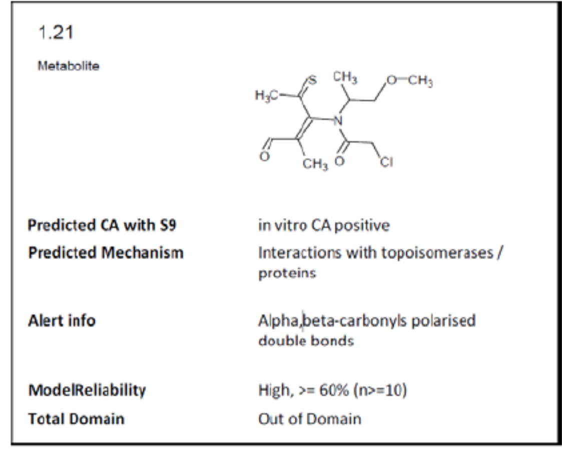
23.0 Parent		23.1 Metabolite	
Predicted CA with S9	in vitro CA positive	**Predicted CA with S9**	in vitro CA negative
Predicted Mechanism	Interactions with topoisomerases / proteins	**Predicted Mechanism**	
Alert info	Alpha,beta-carbonyls polarised double bonds	**Alert info**	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)	**ModelReliability**	
Total Domain	Out of Domain	**Total Domain**	In domain

In addition the structural alert of concern was considered to be intrinsically covered by the toxicological testing of dimethenamid-P, and metabolites M656PH023 and M656PH054, as similar degradation product were presumed in their metabolic pathways.

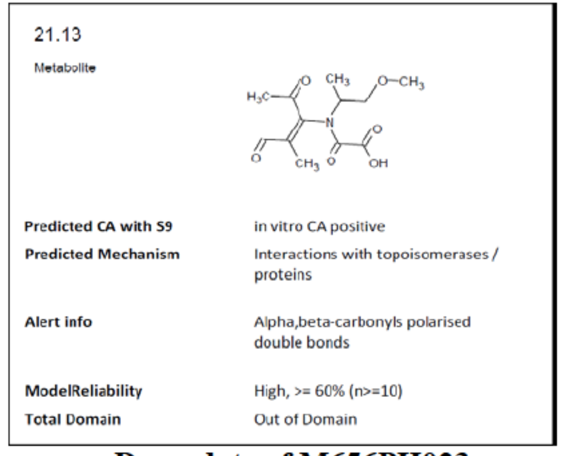
Figure 5.8.1-8: Coverage of M656PH049 by presumed degradates of tested compounds



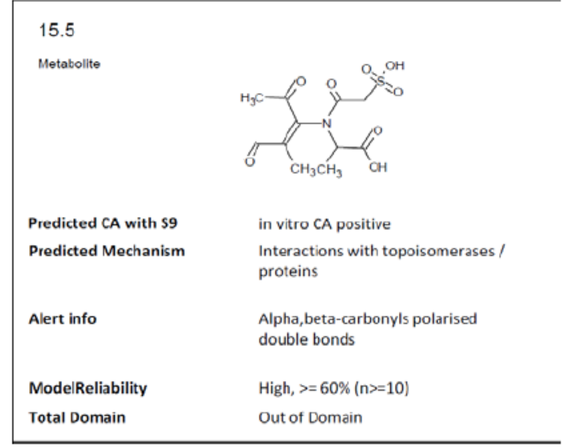
M656PH049



Degradate of dimethenamid-P



Degradate of M656PH023



Degradate of M656PH054

In the DEREK analysis conducted structural alerts for M656PH049 [DocID 2014/1088454 here called M49a] were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. Moreover, there was also the alert for alpha-beta unsaturated ketone substructure indicating chromosome damage in vitro. As this alert is specific for an in vitro effect it was not considered relevant by the evaluator in adding an additional risk.

The Vega prediction [see Molecule 17 in 2014/1088457] in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. Only moderately similar compounds with known experimental values have been found in the database and some similar molecules found in the database have experimental data that disagree with the prediction. Note: although the structure presented in the input mask of Vega showed the correct ring-open structure, in the output mask it was transferred to a ring-closed structure. Thus there remains some inclarity whether the calculations made are applicable to M656PH049.

The structural alert identified in several of the structure activity relationship models applied was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl and is mostly characterized for small, low molecular weight molecules (e.g. cyclohexen-1-one and methyl vinyl ketone). This alert is known to become active in in vitro systems but in particular for greater complex molecules like M656PH049 the evidence to become effective in vivo is lacking.

In conclusion a structural alert for chromosomal aberration in vitro was identified in several of the QSAR models employed that is considered of low relevance for mammalian systems in vivo. Moreover, the structural alert identified was considered to be covered by the toxicological testing conducted with dimethenamid-P, M656PH023 and M656PH054.

B Toxicological evaluation of M656PH049

A structural alert for in vitro chromosomal aberration was identified that was considered of low relevance for mammals in vivo. The structural alert identified was considered to be covered by the toxicological testing conducted with dimethenamid-P, M656PH023 and M656PH054. Any efforts to synthestize M656PH049 were not successful [see section 2 of Doc N4 of this submission]. Consequently the toxicological evaluation is made based on the grouping proposal presented and discussed in Doc N4, chapter 3.3 to 3.5 of this dossier. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
As a member of the M23-group metabolite M656PH049 is considered to be of <u>no</u> toxicological relevance.

11. <u>Metabolite M656PH050 formerly assigned M50</u>

M656PH050 is a ground-water and soybean metabolite, but was not determined in edible commodities, The predicted exposure levels in ground-water are 0.1 µg/l < M656PH050 ≤ 0.75 µg/l.

A Structural alerts for M656PH050

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times [see molecule 24 of report DocID 2014/1088460] predicted M656PH050 to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration in vitro [see molecule 24 of report DocID 2014/1088461] the prediction was negative for the metabolite itself and negative for all presumed transformation products thereof (12 in total). The identified alerts for alfa,beta unsaturated aldehydes, alfa,beta polarized carbonyls were not predicted to become active. The overall prediction was therefore negative for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain.

In the DEREK analysis conducted structural alerts for M656PH050 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction [Molecule 18 of DocID 2014/1088457] in both modules (CAESAR and SarPy) was not mutagenic, however the reliability of this prediction was low. No similar compounds with known experimental values have been found in the database, some similar molecules found in the database have experimental data that disagree with the prediction and the accuracy of prediction for similar molecules in the training set is not optimal.

In conclusion no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

B Toxicological evaluation of M656PH050

The toxicological evaluation is made based on the grouping proposal made. Please refer to Doc N4, chapter 3.3 to 3.5 of this dossier. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water and even below the threshold of 0.75 µg/l for toxicological screening.
As a member of the M23-group metabolite M656PH049 is considered to be of <u>no</u> toxicological relevance.

12. Metabolite M656PH051 former assigned M51

M656PH051 is a metabolite observed in soybean and rotational crop metabolism but was not observed in any edible commodity. It is also a ground-water metabolite with predicted exposure in ground water in the range of 0.1 µg/l < M656PH051 ≤ 0.75 µg/l.

1. Structural alerts for M656PH051

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times predicted M656PH051 [see molecule 8 of report DocID 2014/1088460] to be not mutagenic in the Ames test without or with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 8 of report DocID 2014/1088461] the prediction was negative for the metabolite itself, however there was a prediction of metabolic transformation into ring-open structures similar to transformation products of M656PH030 and M656PH031. For this alpha-beta unsaturated carbonyls was an alert given for induction of chromosomal aberration by interaction with topomerases / proteins. Again the prediction was out of the total domain for this model.

In the DEREK analysis conducted structural alerts for M656PH051 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction [Molecule 2 of the Amendment report DocID 2014/1088458] was inconclusive. The CAESAR module predicted M656PH051 to be mutagenic. The reliability was low as no similar compounds with known experimental data were in the database and as similar molecules found in the database had experimental values that disagree with the prediction. Instead the SarPy module predicted M656PH051 to be not mutagenic. Again the reliability of this prediction was low as no similar compounds with known experimental value were found in the database and as the accuracy of prediction for similar molecules found in the database is not optimal. In conclusion no conclusive relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

2. Toxicological evaluation of M656PH051

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for chromosomal aberration in vitro with metabolic activation considered of low relevance for the in vivo situation. The toxicological evaluation is made based on the grouping proposal presented in Doc N4, chapter 3.3 to 3.5 of this dossier.
As a member of the M31-group metabolite M656PH051 is considered to be of no toxicological relevance.

13. Metabolite M656PH052 former assigned M52

M656PH052 is a ground-water metabolite. The predicted exposure in groud-water is 0.1 µg/l < M656PH052 ≤ 0.75 µg/l.

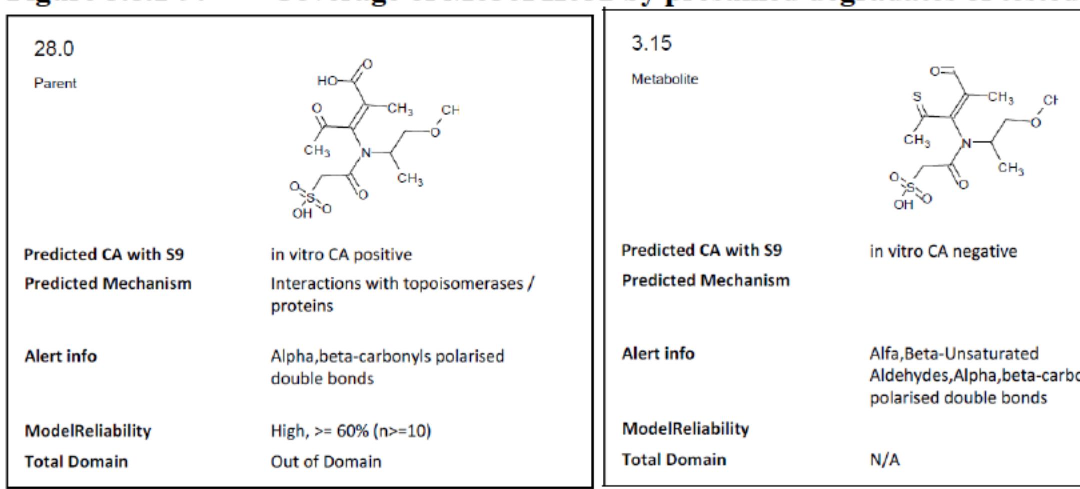
A Structural alerts for M656PH052

In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule Dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential nucleophilic addition to the carbo-hetero double bond (keto-group) was identified in the OECD and the OASIS module. In contrast no DNA alert was identified for M656PH052 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox only provides the alerts based on the functional groups identified only and does not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.

OASIS-Times [see molecule 28 of report DocID 2014/1088460] predicted M656PH052 to be not mutagenic in the Ames test without and with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 28 of report DocID 2014/1088461] the prediction was positive for the metabolite itself and for the demethylated transformation product thereof based on the structural alert for alpha,beta carbonyls with polarized double bonds. The overall prediction was therefore positive for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain. As indicated by the TIMES prediction on S9-induced metabolization formation of such ring opening structures is a plausible metabolic pathway in mammals and thus several similar ring-open thiols and alpha-beta unsaturated carbonyl degradates have been predicted for e.g. dimethenamid-P, **M656PH023, M656PH027, M656PH047, M656PH030, M656PH031, M656PH032, M656PH043, M656PH054** [please refer to DocID 2014/1088461].

The identity of considered closest related transformation products is shown in **Figure 5.8.1-10** below.

Figure 5.8.1-9: Coverage of M656PH052 by presumed degradates of tested compounds



28.0 Parent	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

M656PH052

3.15 Metabolite	
Predicted CA with S9	in vitro CA negative
Predicted Mechanism	
Alert info	Alfa,Beta-Unsaturated Aldehydes,Alpha,beta-carbonyls polarised double bonds
ModelReliability	
Total Domain	N/A

Metabolite of M656PH027

15.5 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Metabolite of M656PH054

6.16 Metabolite	
Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interactions with topoisomerases / proteins
Alert info	Alpha,beta-carbonyls polarised double bonds
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

Presumed metabolite of M656PH031

Thus it is reasonable to assume that the toxicological testing conducted with **M656PH027**, **M656PH054** and **M656PH031** [see section CA 5.8.1 3 and CA 5.8.1 5 above and CA 5.8.1 4 below] covers intrinsically potentially structural alerts of **M656PH052**.

In the DEREK analysis conducted [see DocID 2014/1088454] structural alerts for M656PH052 were the alpha,beta-unsaturated ketone alert for chromosome damage in vitro. This alert is considered specifically for an in vitro effect, not observed in vivo, hence this metabolite is not considered to add to any risk of in vivo effects of the parent molecule.

The Vega prediction [see molecule 3 of report Addendum DocID 2014/1088458] was inconclusive. In the CAESAR module the prediction was mutagenic. The reliability of this prediction was low as no similar compounds with known experimental data have been found in the database and similar compound within the database have experimental values that disagree with the prediction. In particular the molecules identified in the databse that gave evidence for mutagenicity contained other structural alerts not identified in M656PH052 that were considered potentially responsible for the mutagenic activity.

Figure 5.8.1-10: Comparison of structural alerts of M656PH052 with similar structures identified by Vega CAESAR as mutagenic

The SarPy module prediction was non-mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. Some similar molecules with experimental data disagree with the prediction and the accuracy of the prediction for similar molecules is not optimal.

B Toxicological evaluation of M656PH052

A structural alert for in vitro chromosomal aberration was identified that was considered of low relevance for mammals in vivo. The structural alert identified was considered to be covered by the toxicological testing conducted with M656PH027, M656H031 and M656PH054. Any efforts to synthestize M656PH052 were not successful [see section 2 of Doc N4 of this submission]. Consequently the toxicological evaluation is made based on the grouping proposal presented and discussed in Doc N4, chapter 3.3 to 3.5 of this dossier. The determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
As a member of the M27-group metabolite M656PH052 is considered to be of <u>no</u> toxicological relevance.

3. <u>Metabolite M656PH053 former assigned M53/M57</u>

M656PH053 is a ground-water metabolite. The predicted exposure levels in ground-water are 0.75 µg/l < M656PH053 ≤ 4.5 µg/l.

A Structural alerts for M656PH053

In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule Dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential direct acylation was identified in the OECD and the OASIS module. In contrast no alert was identified for M656PH053 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox profiles provide the alerts based on the functional groups identified only and do not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.

OASIS-Times predicted M656PH053 [see molecule 29 of report DocID 2014/1088460] to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 29 of report DocID 2014/1088461] the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for alpha,beta carbonyls with polarized double bonds was not considered to become active as all presumed transformation products were predicted to be negative for chromosomal aberration in vitro due to the entire considered structural properties of the molecule (12 in total). The overall prediction was therefore negative for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain.

The Vega prediction [see molecule 1 of report Addendum II DocID 2014/1088458] was negative. The CAESAR and SarPy module predictions were both non-mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. The accuracy of the prediction for similar molecules is not optimal and a prominent number of atom centered fragments of the molecule have not been found or are rare in the database.

B Toxicological evaluation of M656PH053

No conclusive toxicological alert is identified and the toxicological evaluation is made based on the grouping proposal presented and discussed in Doc N4, chapter 3.3 to 3.5 of this dossier.
As a member of the M27-group metabolite M656PH053 is considered to be of no toxicological relevance.

4. Metabolite M656PH054 former assigned M54/M58

M656PH054 is a ground-water metabolite. Estimated exposure levels via groundwater are 0.75 µg/l < M656PH054 ≤ 4.5 µg/l.

A Structural alerts for M656PH054

In the OECD-toolbox no alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not also identified for the parent molecule Dimethenamid-P. Thus these limited alerts are not considered of relevance.

OASIS-Times predicted M656PH054 [see molecule 15 of report DocID 2014/1088460] to be not Ames mutagenic with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 15 of report DocID 2014/1088461] the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for a ring-opened alpha,beta carbonyl with polarized double (one out of 5 presumed transformation products). The overall prediction was therefore positive for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain. For such a complex unsaturated system as determined for the transformation product of M656PH054, the relevance of the alert given is however of doubt for the in vivo situation.

In the DEREK analysis conducted the structural alerts for M656PH054 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (Molecule 19) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was reasonable as no similar compound with experimental data were in the training set.

Overall there was no alert for Ames mutagenicity in the QSAR models applied. There was however a limited alert for chromosomal aberration in vitro in the OASIS times module for a presumed transformation product. The structural alert was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems in vitro. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive center for nucleophilic addition and is mostly characterized for small, low molecular weight molecules. This alert is known to become active in in vitro systems but in particular for greater complex molecules like M656PH054 the evidence to become effective in vivo is lacking.

B Genotoxicity studies of M656PH054

Report:	CA 5.8.1/34 Woitkowiak C., 2014c Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhimurium / Escherichia Coli reverse mutation assay 2013/1363556
Guidelines:	OECD 471 (1997), (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656PH054 (Reg. No. 5920718, former assigned M54, metabolite of Dimethenamid-P; Batch: L82-121; Purity: 85.1%) using ethanol as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation test, M656PH054 was tested at concentrations of 33, 100, 333, 1000, 3000 and 6000 µg/plate. In the preincubation experiment, concentrations of 10, 33, 100, 333, 1000 and 3000 µg/plate were tested. A bacteriotoxic effect was observed in the standard plate test depending on the test conditions at 6000 µg/plate and in the preincubation assay depending on the strain and test conditions from about 1000 µg/plate onward. Test substance precipitation was found in the preincubation assay with S9 mix from about 1000 µg/plate onward.
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 M656PH054 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1363556)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5920718 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description: Solid, beige

Lot/Batch #: L82-121

Purity: 85.1% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Aug 2014 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Ethanol

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate. The optical density of the fresh thawed bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 10^9 cells per mL).

5. Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3000 and 6000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 3000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-Oct-2013 to 24-Oct-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

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 dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.
The stability of the test substance in the vehicle ethanol was verified analytically (BASF Project No.: 01Y0083/13Y010).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test depending on the test conditions at 6000 µg/plate. In the preincubation assay, bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 1000 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see **Table 5.8.1-74**).

Test substance precipitation was found in the preincubation assay with S9 mix from about 1000 µg/plate onward.

Table 5.8.1-74: Bacterial gene mutation assay with M656PH054- Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.3	21.3	65.0	50.0	11.7	11.0	7.7	7.0	73.7	53.3
M656PH054										
33 µg/plate	27.3	19.0	63.0	44.7	8.3	14.3	9.3	7.3	59.7	48.7
100 µg/plate	27.7	17.7	54.3	48.3	9.3	11.3	9.7	9.7	53.0	61.3
333 µg/plate	29.3	16.3	67.0	59.3	9.3	11.3	12.3	8.0	60.0	60.7
1000 µg/plate	25.7	22.7	60.0	52.7	12.0	6.0	9.7	7.0	54.0	56.3
3000 µg/plate	22.7	21.3	44.3^{B}	49.3	11.0	8.7	8.0	4.7	58.3	54.0
6000 µg/plate	0.0^{B}	15.0^{B}	0.0^{B}	53.3^{B}	7.3^{B}	7.3^{B}	5.0^{B}	3.3^{B}	50.0^{B}	32.0^{B}
Pos. control§	2240.0	483.3	2275.0	4092.0	214.7	5197.3	244.0	2277.7	282.0	867.0
Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	32.7	23.0	67.3	37.3	11.0	13.0	11.0	8.0	72.0	64.3
M656PH054										
10 µg/plate	27.3	18.7	64.0	36.0	14.0	10.0	10.3	7.0	75.3	64.7
33 µg/plate	29.3	21.0	58.0	49.3	8.0	8.0	9.0	6.7	79.3	63.0
100 µg/plate	30.0	18.3	65.7	49.3	12.7	11.7	7.7	8.0	70.0	71.3
333 µg/plate	32.7	19.7	65.0	49.7	10.3	10.0	13.3	9.7	67.7	57.7
1000 µg/plate	33.0^{BP}	21.0^{B}	58.7^{BP}	43.3^{B}	9.7^{BP}	6.3^{B}	6.7^{BP}	4.3^{B}	79.3^{BP}	63.7^{B}
3000 µg/plate	24.0^{BP}	6.3^{B}	60.3^{BP}	38.3^{B}	6.3^{BP}	4.3^{B}	5.0^{BP}	1.7^{B}	47.3^{BP}	32.3^{B}
Pos. control§	2087.3	387.7	2382.3	2246.3	215.3	1812.0	199.3	954.7	157.3	327.3

§ = Compound and concentrations see Material and Methods (I.A.2.) above
P= Precipitation

III. CONCLUSION

According to the results of the present study, the test substance M656PH054 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/35 Wollny H.-E., 2013a Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1246093
Guidelines:	OECD 476 (1997), (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.17, EPA 870.5300, EPA 712-C-98-221
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 5.8.1/36 Wollny H.-E., 2013d Amendment No. 1 - Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1404743
Guidelines:	OECD 476 (1997), (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.17, EPA 870.5300, EPA 712-C-98-221
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH054 (Reg.No. 5920718, metabolite of Dimethenamid-P; Batch: L82-121, Purity: 85.1%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Three independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay six concentrations from 100.4 to 3214 µg/mL were used in the 1st and 2nd experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. An additional experiment no. III was performed after data on the purity of the test item were available to test an additional concentration of 3800 µg/mL (approx. 10 mM) in the absence of metabolic activation for 4 and 24 h, respectively, and for 4 h in the presence of metabolic activation. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.

Cytotoxic effects indicated by a relative total growth of less than 50% of survival were observed in the second culture of the 1st experiment at 803.5 µg/mL and above with metabolic activation. In the second experiment cytotoxicity was noted in both cultures at 803.5 µg/mL and above without metabolic activation, in the second culture with metabolic activation at 1607.0 µg/mL and above and in the additional third experiment in both cultures after 24 hours treatment performed without metabolic activation. No biologically relevant and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656PH054 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1246093, 2013/1404743)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 5920718 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-121
Purity:	85.1% (tolerance ± 1.0%)
Stability of test compound:	Stable in ethanol
Solvent used:	Ethanol

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	Ethanol
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I and III); 13.0 µg/mL (experiment III)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation: Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I and II, and 29.8 mg/mL (Lot. No.: 050913) in experiment III.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism:

The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}$M), Aminopterin ($2.0x10^{-5}$M), Thymidine ($1.6x10^{-3}$M) and Glycin ($5.0x10^{-3}$M) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}$M) and Thymidine ($1.6x10^{-3}$M). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 290 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 25.1 to 3214 µg/mL

b) Mutation assay:

1st, 2nd and 3rd experiment: 100.4, 200.9, 401.8, 803.5, 1607.0, 3214.0 µg/mL without and with metabolic activation (1st and 2nd experiment)

3800.0 µg/mL without and with metabolic activation (3rd experiment; this experiment was performed after data on the purity of the test item were available to test an additional concentration of 3800 µg/mL. This additional concentration was identical to a molar concentration of approximately 10 mM taking into account the purity of the test item (85.1%) as provided by the sponsor)

B. TEST PERFORMANCE:

1. Dates of experimental work: 02-Sep-2013 to 04-Nov-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.
$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection: After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies: Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations:

Pre-test

total suspension growth (4 h treatment):
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control

relative total growth:
relative suspension growth × relative cloning efficiency / 100

cloning efficiency (viability):
-ln(mean number of empty wells per plate / 96) / cells seeded per well

relative cloning efficiency:
cloning efficiency × 100 / cloning efficiency of corresponding control

cells survived:
cloning efficiency × cell number seeded in TFT medium

mutant colonies / 106 cells:
small mutant colonies + large mutant colonies

threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126

cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_137).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 25.1 µg/mL and 3214 µg/mL were chosen. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.

Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. In the pre-experiment and in the main experiments the pH was adjusted at the two highest concentrations using 2N NaOH. There was no relevant shift of the osmolarity at the maximum concentration.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 3214 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Relevant cytotoxic effects indicated by a relative total growth of less than 50% of survival were observed in the second culture of the first experiment at 803.5 µg/mL and above with metabolic activation. In the second experiment cytotoxic effects were noted in both cultures at 803.5 µg/mL and above without metabolic activation, and in the second culture with metabolic activation at 1607.0 µg/mL and above. In the additional third experiment relevant cytoxic effects of less than 50% were noted in both cultures after 24 hours treatment performed without metabolic activation.

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. Isolated increases exceeding the threshold of 126 above the corresponding solvent control were noted at 803.5 and 1607.0 µg/mL of the second culture of the first experiment with metabolic activation. These increases however, were not reproduced in the parallel culture at identical experimental conditions or in the second experiment with metabolic activation [see **Table 5.8.1-75** and **Table 5.8.1-76**). A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was solely detected in the second culture of the second experiment without metabolic activation. This trend however, was judged as irrelevant since it actually was reciprocal, going down versus increasing concentrations.

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies with at least one of the concentrations of the controls.

Table 5.8.1-75: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with ethanol		-	100.0	137	263	100.0	98	224
Pos. Control with MMS	19.5	-	29.2	360	263	29.7	373	224
M656PH054								
	100.4	-	culture was not continued#			culture was not continued#		
	200.9	-	103.9	105	263	99.4	134	224
	401.8	-	103.3	119	263	57.2	153	224
	803.5	-	114.0	152	263	123.4	96	224
	1607.0	-	93.1	139	263	116.7	98	224
	3214.0	-	96.1	126	263	165.0	46	224
Solv. Control with ethanol		+	100.0	96	222	100.0	90	216
Pos. Control with CPA	3.0	+	54.4	326	222	45.8	312	216
Pos. Control with CPA	4.5	+	22.0	831	222	51.3	532	216
M656PH054								
	100.4	+	culture was not continued#			culture was not continued#		
	200.9	+	103.0	81	222	101.6	101	216
	401.8	+	128.3	54	222	80.2	151	216
	803.5	+	118.7	62	222	49.0	238	216
	1607.0	+	124.4	63	222	44.2	276	216
	3214.0	+	155.9	54	222	49.3	185	216

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-76: Gene mutation in mammalian cells - 2nd and 3rd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control with ethanol		-	100.0	64	190	100.0	98	224
Pos. Control with MMS	13.0	-	10.0	682	190	12.5	1098	224
M656PH054								
	100.4	-	culture was not continued#			culture was not continued#		
	200.9	-	94.7	62	190	102.9	113	224
	401.8	-	164.2	56	190	97.3	125	224
	803.5	-	27.3	46	190	20.1	107	224
	1607.0	-	27.5	64	190	31.0	85	224
	3214.0	-	21.0	83	190	27.2	71	224
Experiment II / 4 h treatment								
Solv. Control with ethanol		+	100.0	94	220	100.0	101	227
Pos. Control with CPA	3.0	+	41.4	277	220	25.9	339	227
Pos. Control with CPA	4.5	+	46.8	338	220	18.0	458	227
M656PH054								
	100.4	+	culture was not continued#			culture was not continued#		
	200.9	+	127.1	88	220	93.6	63	227
	401.8	+	123.6	55	220	74.5	108	227
	803.5	+	182.7	81	220	72.3	135	227
	1607.0	+	125.3	71	220	49.0	106	227
	3214.0	+	112.0	104	220	41.9	137	227
Experiment III / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with ethanol		-	100.0	197	323	100.0	146	272
Pos. Control with MMS	19.5	-	18.6	749	323	17.4	604	272
M656PH054	3800.0	-	87.1	195	323	45.2	201	272
Solv. Control with ethanol		+	100.0	99	225	100.0	72	198
Pos. Control with CPA	3.0	+	53.9	266	225	60.4	204	198
Pos. Control with CPA	4.5	+	34.1	449	225	38.6	374	198
M656PH054	3800.0	+	69.5	152	225	56.5	122	198
Experiment III / 24 h treatment								
Solv. Control with ethanol		-	100.0	104	230	100.0	93	219
Pos. Control with MMS	13.0	-	10.3	392	230	13.9	356	219
M656PH054	3800.0	-	25.5	106	230	27.1	149	219

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656PH054 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/37 Bohnenberger S., 2013c Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - In vitro micronucleus test in Chinese hamster V79 cells 2013/1246092
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656PH054 (Reg. No. 5920718, metabolite of Dimethenamid-P; Batch L82-121; purity 85.1%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in one experiment. Concentrations of 6.3 to 3214 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since cytotoxicity was not observed, the cultures fulfilled the requirements for cytogenetic evaluation and mutagenicity was observed, this preliminary test was designated main experiment. Concentrations of 803.5, 1607 and 3214 µg/ml were chosen for evaluation in the experimental part without metabolic activation and 50.2, 100.4, 200.9, 401.8, 803.5 and 3214.0 in the part with metabolic activation. Precipitation of the test item in the culture medium was observed microscopically at 1607.0 µg/mL and above in the presence of S9 mix at the end of treatment.
In the absence of S9 mix statistically significant increases in micronucleated cells were observed but considered as being biologically irrelevant, since the values were clearly in the range of the laboratory historical control data. However, in the presence of S9 mix statistically significant increases in micronucleated cells clearly exceeding the range of the laboratory historical control data were observed after treatment with 200.9, 401.8 and 803.5 µg/mL. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.
Based on the results of this study, M656PH054 is considered to induce micronuclei in vitro in V79 cells.

(BASF DocID 2013/1246092)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 5920718 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description: Solid, beige

Lot/Batch #: L82-121

Purity: 85.1 % (tolerance ± 1.0 %)

Stability of test compound: Stable in ethanol

Solvent used: ethanol

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: ethanol

Positive controls, -S9: Mitomycin C (MMC, 0.1 µg/mL, dissolved in deionised water)

Positive control, +S9: Cyclophosphamide (CPA, 10.0 µg/mL, dissolved in saline)

3. Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥ 70 %).

5. Culture medium/conditions:

About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

Preliminary toxicity assay/
Cytogenicity assay:

6.3, 12.6, 25.1, 50.2, 100.4, 200.9, 401.8, 803.5, 1607.0, 3214.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated main experiment (see **Table 5.8.1-77**).

Table 5.8.1-77: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5920718 (Metabolite of BAS 656-PH, Dimethenamid-P)

Preparation interval	Exposure period	Concentration in µg/mL									
		Without S9 mix									
24 hrs	4 hrs	6.3	12.6	25.1	50.2	100.4	200.9	401.8	**803.5**	**1607.0**	**3214.0**
		With S9 mix									
24 hrs	4 hrs	6.3	12.6	25.1	**50.2**	**100.4**	**200.9**	**401.8**	**803.5**	1607.0^{P}	**3214.0**^{P}

Evaluated experimental points are shown in bold characters

^{P}: Precipitation occurred at the end of treatment

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Jul-2013 - 06-Aug-2013

2. Preliminary cytotoxicity assay: With respect to the molecular weight of the test item, 3214.0 µg/mL of the test substance was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 6.3 and 3214.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:
A test item was classified as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose-related and reproducible increase in the number of cells containing micronuclei is observed
- if the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP13_137).

B. CYTOGENICITY ASSAYS:

No cytotoxicity was observed up to the highest applied concentration in the presence and absence of metabolic activation. Visible precipitation of the test item in the culture medium was observed microscopically at 1607.0 µg/mL and above in the presence of S9 mix at the end of treatment.

In the absence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 803.5 and 3214.0 µg/mL (1.05 and 0.85 %). These values were clearly in the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) and therefore considered as being biologically irrelevant. In the presence of S9 mix statistically significant increases in micronucleated cells clearly exceeding the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) were observed after treatment with 200.9, 401.8 and 803.5 µg/mL (2.75, 8.55 and 7.15 %) [see **Table 5.8.1-78**]. Since the test item was considered to be mutagenic after the first experiment, only one experiment was performed.

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-78: Summary of results of the micronucleus test with M656PH054

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.82	0.25
		Positive control[2]	2.33	**7.85**[S]
		803.5	2.97	**1.05**[S]
		1607.0	2.94	0.60
		3214.0	2.90	**0.85**[S]
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	2.03	1.20
		Positive control[3]	1.63	**10.35**[S]
		50.2	2.06	1.30
		100.4	2.06	1.80
		200.9	2.00	**2.75**[S]
		401.8	1.83	**8.55**[S]
		803.5	1.69	**7.15**[S]
		3214.0[P]	2.12	n.e.

* The total number of micronucleated cells was determined in a sample of 2000 cells
S Number of micronucleated cells statistically significantly higher than corresponding control values
P Precipitation occurred microscopically at the end of treatment
n.e. not evaluated
1 Ethanol 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 CPA 10.0 µg/mL

III. CONCLUSIONS

Based on the results of the study, M656PH054 is considered as mutagenic with metabolic activation in this in vitro micronucleus test.

Report: CA 5.8.1/38
[redacted] 2014b
Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1005221
Guidelines: OECD 474 (1997), (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.12, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.8.1/39
Grauert E.,Kamp H., 2014a
Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in sterile water
2014/1098011
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.8.1/40
Becker M.,Landsiedel R., 2014e
Analytical report - Reg. No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - Plasma analysis for external studies
2014/1092437
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656PH054 (Reg. No. 5920718, Metabolite of BAS 656-PH, Dimethenamid-P; batch: L82-136, purity: 86.5%) was tested its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. Based on the result of two pre-tests, the test substance dissolved in sterile water was administered once orally to groups of 7 male mice at dose levels of 500, 1000, and 2000 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 animals/group). The animals were sacrificed 24 or 48 hours (additional high dose and vehicle group) after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.
Oral administration of the test substance did not lead to any biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used. The rate of micronuclei was near to the value of the negative control and within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Signs of systemic toxicity were observed at the highest tested dose of 2000 mg/kg bw and comprised reduction of spontaneous activity, eyelid closure, ruffled fur,

hunchback, tiptoe walk, sunken flanks, moribund condition, discharge from the eyes and swollen abdomen. At necropsy 48 h after application, the stomach of all animals treated with the high dose of the test item was filled with fluid and enlarged. In the mid dose of 1000 mg/kg bw reduction of spontaneous activity and ruffled fur was observed. The positive control led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.
According to the results of the study, the test substance Reg. No. 5920718 did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and is therefore considered non-mutagenic.

(BASF DocID 2014/1005221)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige to brownish
Lot/Batch #:	L82-136
Purity:	86.5%
Stability of test compound:	Stable in solvent (Confirmed indirectly by dose formulation analytics (see BASF study 04Y0083/13Y040)
Solvent used:	Sterile water

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	Sterile water
Positive control:	Cyclophosphamide (CPA) 40 mg/kg bw

3. Test animals:

Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 11 weeks
Weight at dosing:	35.7 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively

Acclimation period:	At least 5 days
Diet:	Pelleted standard diet, ad libitum
Water:	Tap water, ad libitum
Housing:	The animals were housed in groups in Makrolon Type II (pre-test) / III (main study), with wire mesh top.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound concentration:

Range finding test:	1000 and 2000 mg/kg bw (administered once orally)
Micronucleus assay:	500, 1000 and 2000 mg/kg bw The test substance was administered once orally using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 22-Oct-2013 to 21-Nov-2013

2. Preliminary cytotoxicity assay:

Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 1000 mg/kg bw (1st pre-experiment) and 2000 mg/kg bw (2nd experiment).

3. Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once orally with either the vehicle, positive control substance or 500, 1000 and 2000 mg M656PH054 / kg bw. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period (48 hours). The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four/48 hours after the administration the mice were killed and the femora were removed. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with fetal calf serum and

	subsequently centrifuged at 390xg for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet re-suspended.
Slide preparation:	A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:	In general, 2000 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 and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

5. Evaluation criteria:

A test item was considered as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods to determine the significance of effects were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results. A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle (water) was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y0083/13Y040).

B. PRELIMINARY RANGE FINDING TEST

None of the male or female mice died after single oral dosing of 1000 and 2000 mg/kg bw. However, clinical signs including reduction of spontaneous activity, eyelid closure, ruffled fur, abdominal posture, sunken flanks and salivation in the 2000 mg/kg bw dose group. No clinical signs were observed in the 1000 mg/kg bw dose group. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Clinical signs observed in the animals treated with the high dose of 2000 mg/kg bw comprised reduction of spontaneous activity, eyelid closure, ruffled fur, hunchback, tiptoe walk, sunken flanks, moribund condition, discharge from the eyes and swollen abdomen. At necropsy 48 h after application, the stomach of all animals treated with the high dose of the test item was filled with fluid and enlarged. In the mid dose of 1000 mg/kg bw reduction of spontaneous activity and ruffled fur was observed. No clinical signs were observed in any of the animals treated with low of the test substance, the positive control or the vehicle. Concurrent analysis for M656PH054 in Plasma [see DocID 2014/1092437] confirmed that M656PH054 was systemically available.

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control in most mice. Two mice dosed with 2000 mg/kg showed lower PCE numbers 48 hours after treatment.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were near to the value of the vehicle control group and within the historical vehicle control data range [see **Table 5.8.1-1**].

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.97%), thereby demonstrating the sensitivity of the test system.

Table 5.8.1-79: Micronucleus test in mice orally administered M656PH054

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Sterile water	24	0.090	1-3	1184
M656PH054				
500 mg/kg bw	24	0.107	0-3	1178
1000 mg/kg bw	24	0.129	0-7	1167
2000 mg/kg bw	24	0.143	1-6	1062
Positive control				
Cyclophosphamide	24	2.970	33-81	1089
48 h sampling				
Sterile water	48	0.100	1-5	1225
M656PH054				
2000 mg/kg bw	48	0.114	0-5	929

III. CONCLUSION

Based on the results of this study, M656PH054 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under in vivo conditions.

Conclusion on genotoxicity of M656PH054

Overall, with regard to in vitro genotoxicity testing there was no indication for mutagenicity neither in the bacterial Ames-test nor in the mammalian Mouse-Lymphoma test. However, in the in vitro micronucleus test in in V79 cells conducted with M656PH054 a potential chromosomal aberration effect was observed with metabolic activation. In contrast in the subsequently conducted in vivo micronucleus test in mice no treatment-related induction of micronuclei could be determined up to the limit dose of 2000 mg/kg a dose level with clear clinical signs of toxicity. Plasma-analytics confirmed that M656PH054 was systemically available. Thus, the in vivo study conducted for the same endpoint did not demonstrate a treatment-related effect. By weight of evidence M656PH054 was not considered to be genotoxic.

C Short-term toxicity of M656PH054

Report:	CA 5.8.1/41 [redacted] 2014e Reg.No. 5920718 (metabolite of BAS 656-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2014/1018065
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of M656H054 (Reg.No. 5920718, metabolite of Dimethenamid-P; Batch: L82-136; Purity 86.5%) to Wistar rats at dietary dose levels of 0, 1390, 4630 and 13900 ppm based on purity of M656PH054 to obtain dose levels of 1200, 4000, 12000 ppm. Due to significant reduced food consumption in males as well as impaired body weight development in males and females, the dose level was reduced to 9250 ppm from study day 7 onwards. No other treatment related adverse effects were observed throughout the study.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) for M656PH054 was 4630 ppm corresponding to 346 mg/kg bw/day in males and 472 mg/kg bw/day in females when corrected for purity of 86.5%.

(DocID 2014/1018065)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** **Reg.No. 5920718, metabolite of Dimethenamid-P**
   Description: solid / beige to brownish
   Batch/purity #: L82-136 / 86.5%
   Stability of test compound: Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. **Vehicle and/or positive control:** Rodent diet

3. **Test animals:**

Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 167.1 ± 7 g, ♀ 127.1 ± 4.6 g
Source:	Charles River, Germany
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:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 09-Dec-2013 – 05-Mar-2014
(In life dates: 19-Dec-2013 (start of administration) to 17-Jan-2014 (necropsy))

2. Animal assignment and treatment:

M656H054 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1390 (low dose), 4630 (intermediate dose) and 13900 ppm (top dose). As body weight loss was determined in all males of the high dose group between study day 0 and study day 7, the diet concentration was reduced to 9250 ppm from study day 7 onwards. For all female dose groups as well as for the other male dose groups the concentrations remained throughout the 28-day study period. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. Three diet preparations per dose were performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 10 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1390 ppm) and top dose level (13900 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level and for the reduced high dose for males single samples were analysed. No test-article was determined in control diets.

Table 5.8.1-80: Analysis of diet preparations for homogeneity and test-item content

Dose level	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1390 ppm	18. Dec. 13	1256.9 ± 51#	90.4	4.1
4630 ppm	18. Dec. 13	4957.7	107.1	n.a.
9250 ppm	26. Dec. 13	8965.6	96.9	n.a.
13900 ppm	18. Dec. 13	12836.7 ± 101#	92.4	0.8

n.a.: not applicable;
based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 0.8 to 4.1% indicate the homogenous distribution of M656H054 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 90.4 to 107.1% of the nominal concentrations. These results demonstrated the correctness of the concentrations of M656H054 in the vehicle.

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 5.8.1-81: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-82: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table 5.8.1-83: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmos
15. feces discharge during examination (appearance/consistency)
16. urine discharge during examination
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

BW_x and BW_y body weight [g] at 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane anesthetized animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	✓	kidneys[£]	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands[£]	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	#	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides[¥]	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetized animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles[£]				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; [£]additional stained with CAB for hyaline droplets, *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology, [£]seminal vesicles and coagulation weight determined together

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes including quantitative parameters. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.

With regard to the overall motor activity as well as single intervals no significant deviations were observed between treated animals and the control group.

C. BODY WEIGHT AND BODY WEIGHT GAIN

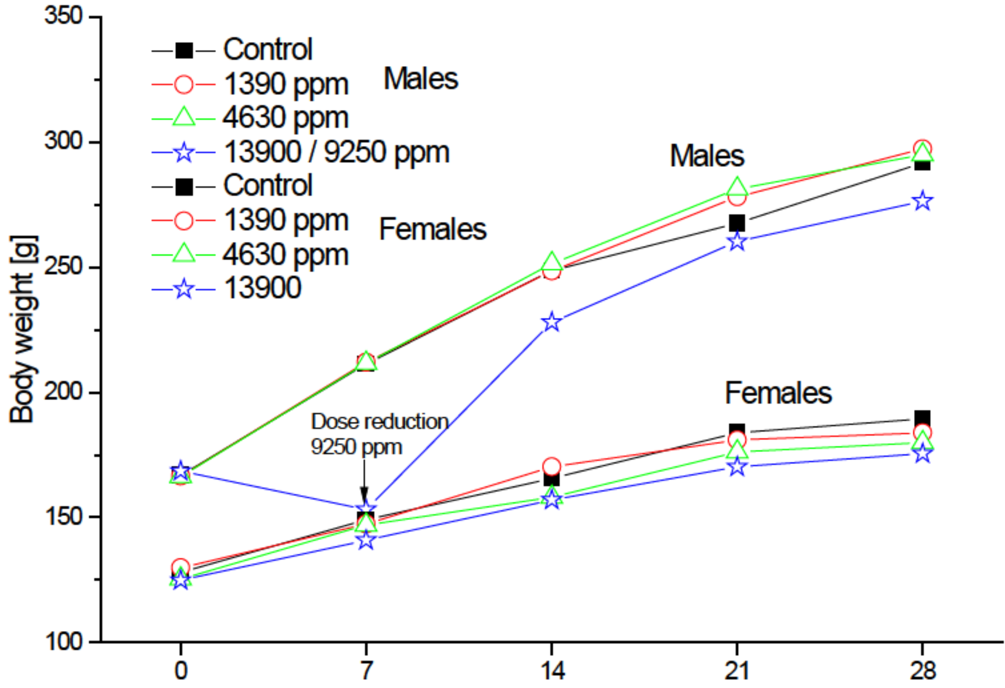
In the first week of treatment males of the high dose group showed significant body weight loss in combination with a severe reduced food consumption [see **Table 5.8.1-84**, **Table 5.8.1-85** below and **Figure 5.8.1-11**]. Although not significantly altered, a tendency to impaired body weight development was also observed in female animals after 7 days of treatment. Thus it was decided to reduce the diet concentration of that dose group from day 7 onwards from 13900 towards 9250 ppm. After the dose reduction the male animals showed an increased body weight gain from day 7 to 14, but body weight was still significantly reduced on day 14. The final body weight (94.8% of control) and the overall body weight gain (86.4% of control) still showed a trend of being lower than control. In the female high dose group (13900/9250 ppm) a slight, not significantly reduced body weight development was noticed throughout the study, leading to a final significantly reduced body weight of 92.7% of control and a not statistically significant reduction in body weight gain of 82.6% of control, respectively [see **Table 5.8.1-84**].

Table 5.8.1-84: Mean body weight of rats administered M656H054 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1390**	**4630**	**13900 / 9250[1]**	**0**	**1390**	**4630**	**13900 / 9250[1]**
Body weight [g]								
- Day 0	166.8	166.5	166.4	168.5	128.1	129.9	125.4	124.9
- Day 7	211.4	212.1	211.7	**153.2****	149.1	147.3	147	140.9
- Day 14	248.7	248.6	251.5	**228.1***	165.6	170.2	158.1	157
- Day 21	267.6	278.2	281.4	260.3	183.8	181	176.2	170.2
- Day 28	291.8	297.4	295.0	276.5	189.4	183.9	179.9	**175.5***
Δ% (compared to control)[#]		1.9	1.1	-5.2		2.9	-5	-7.3
Body weight gain [g]								
d 0 -> 7	44.6	45.5	45.3	**-15.4****	21	17.4	21.6	16
d 0 -> 14	81.9	82.1	85.1	**59.5****	37.5	40.3	32.8	32.2
d 0 -> 21	100.8	111.7	115.0	91.8	55.7	51.1	50.9	45.4
d 0 -> 28	125.0	130.9	128.5	108.0	61.3	54.0	54.5	50.6
Δ% (compared to control)[#]		4.7	2.9	-13.6		11.9	-11.1	-17.4

[1] Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm from study day 7 onwards.
* $p \leq 0.05$; $p \leq 0.01$; Dunnett test (two-sided)
[#] Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-11: Body weight development of rats administered M656H054 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

In the high dose males a severe decrease in food consumption was observed for males between days 4-7. After the reduction of the diet concentration on study day 7 the food consumption was comparable to control group values [**Table 5.8.1-85**]. All other recorded values were within the biological range typical for this strain of rats. Increased food spilling was observed for male animals of the 4630 ppm between study days 11 to 14 and 18 to 21, thus the group mean values were declared as outliers and not given.

Table 5.8.1-85: Mean food consumption of rats administered M656H054 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1390**	**4630**	**13900 / 9250[1]**	**0**	**1390**	**4630**	**13900 / 9250[1]**
Food consumption [g]								
- Day 4-7	19.4	20.9	20.5	**7.1**	14.1	14.3	14.7	13.4
- Day 10-14	21.1	24.1	21.5	22.5	15.0	15.9	-	14.1
- Day 17-21	19.8	32.0	21.8	21.1	15.6	21.1	-	15.3
- Day 24-28	23.9	29.2	25.2	24.3	16.9	16.8	24.4	16.8
Total	84.2	106.2	89.0	75.0	61.6	68.1	39.1	59.6

[1] Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm from study day 7 onwards.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the study period calculated on the values for week 2 to4 is shown in the following **Table 5.8.1-86**.

Table 5.8.1-86: Calculated intake of M656H054

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)	
		Males	Females
1	1390	142	140
2	4630	400	546
3	9250 (from day 7 onwards)	826	850
	13900 (until day 7)	645	1319
Intake corrected for purity of M656PH054 of 86.5%			
1	1200	123	120
2	4000	346	472
3	8000 (from day 7 onwards)	714	736
	12000 (until day 7)	558	1141

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes among hematological parameters were observed.

2. Clinical chemistry findings

No treatment-related changes among clinical chemistry parameters were observed. In males of the low 1390 ppm dose group triglyceride levels were higher compared to controls, but the increase was not dose-dependent. Therefore, this alteration was regarded as incidental and not treatment-related.

Table 5.8.1-87: Selected clinical chemistry findings in rats administered M656H054 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000	0	1200	4000	12000 / 8000
[mg/kg bw/day		123	346	558 / 714		120	472	1141 / 736
Triglycerides [mmol/l]	0.90	**1.28***	0.76	0.85	0.56	0.51	0.49	0.54

[1] Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm corresponding to 8000 ppm when corrected for purity of M656PH054 from study day 7 onwards.
*p ≤ 0.05 (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed.

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats displayed no statistically significant differences to the controls [see **Table 5.8.1-88**].

When compared to the control group, the only significant deviation was the decrease in absolute testes weights of the high dose group (13900 / 9250 ppm). The decreased absolute weight of testes in male animals of test group 3 (13900 ppm) could be explained by the decreased terminal body weight in these animals. Additionally, there was no histopathological finding correlating to the decreased weight. Therefore, the decreased absolute weight of testes was not regarded as a treatment-related effect. All other mean absolute organ weights of either male or female animals treated with M656PH054 showed no significant deviations.
In relation to terminal body weight no significant deviations from control were determined.

Table 5.8.1-88: Selected mean absolute and relative organ weights of rats administered M656H054 for at least 28 days

Sex	Males				Females			
Dose [ppm]	**0**	**1200**	**4000**	**12000 / 8000**	**0**	**1200**	**4000**	**12000 / 8000**
[mg/kg bw/day		**123**	**346**	**558 / 714**		**120**	**472**	**1141 / 736**
Terminal bodyweight	265.96	269.5	267.3	253.34	172.34	168.9	165.38	161.36
[% of control]	100	101	101	95	100	98	96	94
Testes weight, absolute [g]	3.53	3.568	3.296	**3.176****				
[% of control]	100	101	93	**90**				
Testes weight, relative [%]	1.33	1.326	1.238	1.253				
[% of control]	100	100	93	94				

[1] Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm corresponding to 8000 ppm when corrected for purity of M656PH054 from study day 7 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross and histopathology

A single macroscopic finding (pelvic dilation of the kidney) recorded in one male of the low dose group (1390 ppm) belongs to the spectrum of background lesion and was considered to be incidental in nature and not related to treatment.

All histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

The kidneys of male animals all dose groups (1390, 4630 as well as 13900 and 9250 ppm) showed a marginally increased severity of eosinophilic droplets in proximal tubular epithelial cells [see **Table 5.8.1-89**]. Since there was no clear dose-response relationship and since the grading and severity of eosinophilic droplets in treated animals were within the range of historical control data (see PART III, Supplement), the finding was regarded not to be treatment-related.

Table 5.8.1-89: Selected histopathological findings of rats administered M656H054 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000	0	1200	4000	12000 / 8000
[mg/kg bw/day		123	346	558 / 714		120	472	1141 / 736
Animals in group	5	5	5	5	5	5	5	5
Kidneys, eosinophilic droplets	5	5	5	5	0	0	0	0
[%]	100	100	100	100				
minimal	5	2	4	2				
moderate	0	3	1	3				
	Historical control: 0 – 100%; grading minimal up to moderate							

[1] Due to significant weight loss from day 0 to day 7 the diet concentration of the high dose group was reduced to 9250 ppm corresponding to 8000 ppm when corrected for purity of M656PH054 from study day 7 onwards.

III. CONCLUSIONS

The administration of M656H054 via the diet to male and female Wistar rats for 4 weeks test substance-related adverse signs of toxicity taking int account reduced food consumption in male animals as well as impaired body weight development in male and female animals at concentrations of 13900 ppm in males leading to a concentration reduction to 9250 ppm from day 7 of treatment onwards. No adverse signs of toxicity were noticed at the low dose (1390 ppm) or mid dose (4360 ppm).
Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) in Wistar rats was 4630 ppm corresponding to 346 mg/kg bw/day in males and 472 mg/kg bw/day in females when corrected for purity of 86.5%.

D Toxicological evaluation of M656PH054

There was a limited alert for chromosomal aberration in vitro with metabolic activation identified for M656PH054 that was confirmed in the in vitro micronucleus test. The concurrent in vivo micronucleus test was clearly negative and the systemic availability of M656PH054 in this test system was demonstrated. The bacterial and mammalian gene mutation tests in vitro were both negative. Thus, by weight of evidence M656PH054 is considered to be not genotoxic in the in vitro and in vivo genotoxicity studies conducted fulfilling the requirements for evaluation of ground-water metabolite evaluation. The available data on systemic toxicity – short-term toxicity study in rats - clearly demonstrated that the compound is of low toxicity and thus less toxic than the parent molecule dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.
In conclusion M656PH054 is considered to be of no toxicological relevance.

5. Metabolite M656H055 former assigned M55

M656H055 is a ground-water metabolite. Estimated exposure levels via groundwater are 0.1 µg/l < M656H055 ≤ 0.75 µg/l.

A Structural alerts for M656H055

In the OECD-toolbox no alerts for DNA-binding were identified. The only alert for genotoxicity (H-acceptor path) was also identified for the parent molecule Dimethenamid-P. There was a deviating alert for protein-binding in the OASIS-module by amide formation via ester-aminolysis, instead of nucleophilic substitution at the sp3 carbon. The relevance of this alert is however questionable.

OASIS-Times [see molecule 16 of report DocID 2014/1088460] predicted M656H055 to be not mutagenic in the Ames test with and without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 16 of report DocID 2014/1088461] the prediction was negative for the metabolite itself but positive for structural alerts contained in presumed transformation products thereof. This prediction based on the one side on the the structural alert for alpha,beta carbonyl with polarized double bond. The presumed transformation products were partly in the predictivity domain. Moreover there was an alert for cleavage into primary aromatic amine structures whose predicted chromosomal aberration was again in the predicitictivity domain. The overall prediction was therefore positive for in vitro chromosomal aberration and considered reasonable.

In the DEREK analysis conducted the structural alerts for M656H055 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.
The Vega prediction (Molecule 16) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was reasonable as no similar compound with experimental data were in the training set.

In conclusion, there was an alert for chromosomal aberration in vitro.

B Genotoxicity studies of M656H055

Report:	CA 5.8.1/42 Woitkowiak C., 2012a Reg.No. 5749263 (metabolite of BAS 656 H, Dimethenamid) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220415
Guidelines:	OECD 471, (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656H055 (Reg.No. 5749263, metabolite of Dimethenamid; Batch: L80-154; Purity: 69.8%) using ultrapure water as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In both experiments, M656H055 was tested at concentrations of 33, 100, 333, 1000, 3600 and 7200 µg/plate. A bacteriotoxic effect was not observed in any experiment. Precipitation of the test substance did not occur up to the highest tested concentration.
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 M656H055 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2012/1220415)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5749263 (Metabolite of BAS 656 H, Dimethenamid)

Description: Solid, beige

Lot/Batch #: L80-154

Purity: 69.8% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2013 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Ultrapure water

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay (1st experiment): Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3600 and 7200 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay (2nd experiment): The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 3600 and 7200 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 16-May-2012 to 25-May-2012

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

Purity of the test item was verified by Q-NMR analysis (see BASF study report ASAP11_177).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

No bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed in the standard plate test and in the preincubation assay up to the highest required concentration.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see **Table 5.8.1-90**].

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-90: Bacterial gene mutation assay with M656H055 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	31	19	88	72	15	10	8	6	82	73
M656H055										
33 µg/plate	27	20	90	73	13	12	9	7	89	79
100 µg/plate	28	15	93	73	11	10	10	6	91	72
333 µg/plate	22	17	88	68	16	13	8	7	88	82
1000 µg/plate	31	20	91	73	16	9	8	6	88	83
3600 µg/plate	29	18	82	71	14	12	5	6	81	80
7200 µg/plate	29	17	87	72	15	11	10	5	95	88
Pos. control§	762	632	817	608	308	768	180	423	248	1142
2nd experiment: Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (water)	28	21	90	79	13	11	8	6	44	36
M656H055										
33 µg/plate	26	20	91	79	12	13	9	7	43	41
100 µg/plate	26	21	92	81	12	10	8	6	46	41
333 µg/plate	26	19	82	87	12	11	7	7	42	37
1000 µg/plate	33	20	100	86	12	11	8	7	45	40
2800 µg/plate	27	18	87	82	13	11	6	6	41	37
5600 µg/plate	23	18	98	81	10	12	7	6	41	40
Pos. control§	715	627	874	731	139	720	141	448	265	749

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance M656H055 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/43
Kapp M.-D.,Landsiedel R., 2013a
Reg.No. 5749263 (metabolite of BAS 656 H, Dimethenamid) - In vitro gene mutation test in CHO cells (HPRT locus assay)
2013/1282610

Guidelines: OECD 476 (1997), (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.17, EPA 870.5300

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

M656H055 (Reg. No. 5749263, metabolite of Dimethenamid; Batch: L80-154, Purity: 69.8%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 3600 µg/mL were used in the original and the confirmatory experiment. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Ethylmethanesulfonate (EMS) and 7,12-dimethylbenz[a]anthracene (DMBA) served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments after the incubation period treatment media were replaced by culture medium and the cells were incubated for 6-8 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.
Cytotoxic effects indicated by reduced cloning efficiencies of below 20 % of the respective vehicle control were not observed in any of the experiments irrespective of treatment interval and the presence of metabolic activation. In addition, no precipitation was observed.
Neither in the original nor in the confirmatory studies was a relevant increase in the mutant frequency observed. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test M656H055 does not induce forward mutations in mammalian cells in-vitro.
(BASF DocID 2013/1282610)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No. 5749263 (Metabolite of BAS 656 H, Dimethenamid)

Description:	Solid, beige
Lot/Batch #:	L80-154
Purity:	69.8% (tolerance +/- 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2013 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Solvent used:	Culture medium

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	Culture medium
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	7,12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organism: Chinese hamster CHO cells with a high proliferation rate (doubling time of about 12-16 h), high plating efficiency (about 90%) and a karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL and 1% (v/v) stable glutamine (200 mM)

All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericin B (250 µg/mL)

During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 14.1 to 3600 µg/mL

b) Mutation assay:

1st experiment: 450, 900, 1800 and 3600 µg/mL with and without metabolic activation

2nd experiment: 450, 900, 1800 and 3600 µg/mL without metabolic activation

625, 1250, 2500 and 3600 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Apr-2013 to 06-Aug-2013

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation) after an attachment period of 20-24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask were seeded into 175 cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 - 24 hours with 5% CO_2 at 37°C and > 90% humidity for cell attachment. 2 flasks were used for each test group.

	After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive/vehicle control or test substance. In case of metabolic activation the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL vehicle control or test substance preparation and 4 mL S9-mix. Analogously, for the positive control group 16 mL of medium was supplemented with 0.2 mL vehicle and 4 mL S9-mix. For the exposure period of more than 4 hours Hams´s F12 medium with 10% FCS was used.
	Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity.
Expression:	After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).
Selection:	For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10 % FCS. After a 20-24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the treatment medium was replaced by Ham's F12

medium with 10% FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted.

Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE_2) was determined in parallel to the selection of mutants after the expression period under the same conditions as described for cloning efficiency 1.

Calculations:

Mutant frequency:

Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{\text{total number of colonies in the test group}}{\text{total number of seeded cells in the test group}} \times 100$$

relative, in comparison to control:

$$CE_{relative} = \frac{\text{CE of the dose group}}{\text{CE of the vehicle control}} \times 100$$

4. Statistics:
Due to the negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.

- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor.

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment in the presence and absence of metabolic activation a significant test substance induced cytotoxicity leading to a reduction of relative cloning efficiency below 20% was not observed. The relative cloning efficiency ($CE_{1\ relative}$) after treatment with the highest concentration (3600 µg/mL) ranged between 72.6 to 111.7% depending on the treatment interval and metabolic activation.

Precipitation of the test substance was not observed up to the highest tested concentration with and without metabolic activation. In the pretest the parameters pH value and osmolarity were not influenced by the addition of the test substance preparation to the culture medium at the concentrations measured.

Based on these data the highest concentration tested in the mutagenicity experiments was 3600 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

A significant cytotoxic effect was not observed in both experiments up to the highest tested concentration irrespective of treatment interval and presence of metabolic activation. The obtained relative cloning efficiency did not drop below 85.9% under any of the tested conditions.

A relevant increase in the number of mutant colonies was not observed in both experiments with and without metabolic activation [see **Table 5.8.1-91**].
The mutant frequencies obtained at any tested concentration with or without metabolic activation were close to the range of the concurrent vehicle control and within the range of the historical negative control data.

The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 3600 µg/mL.

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.

Table 5.8.1-91: Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Genotoxicity ** MF corr. [per 10^6 cells]	Cytotoxicity ***	
						CE_1 [%]	CE_2 [%]
1	4	Negative control	-	-	9.16	100.0	100.0
		M656H055					
		450.0	-	-	5.26	96.0	105.8
		900.0	-	-	5.44	108.2	115.1
		1800.0	-	-	1.88	105.7	117.8
		3600.0	-	-	2.23	102.3	112.1
		Positive control[1]	-	-	202.16	116.4	85.6
2	24	Negative control	-	-	4.61	100.0	100.0
		M656H055					
		450.0	-	-	0.38	101.8	110.4
		900.0	-	-	3.33	98.0	116.3
		1800.0	-	-	0.71	95.0	109.2
		3600.0	-	-	2.64	85.9	102.4
		Positive control[1]	-	-	227.43	80.4	95.3
1	4	Negative control	+	-	5.50	100.0	100.0
		M656H055					
		450.0	+	-	3.57	112.5	102.8
		900.0	+	-	1.99	97.9	108.5
		1800.0	+	-	3.99	100.2	112.7
		3600.0	+	-	7.40	103.6	116.0
		Positive control[2]	+	-	438.70	90.9	87.8
2	4	Negative control	+	-	1.11	100.0	100.0
		M656H055					
		625.0	+	-	3.39	93.4	95.8
		1250.0	+	-	3.21	88.6	98.4
		2500.0	+	-	0.36	92.9	96.9
		3600.0	+	-	2.05	94.9	94.3
		Positive control[2]	+	-	416.09	70.0	79.9

* Precipitation in culture medium at the end of exposure period
** Mutant frequency MF_{corr}: mutant colonies per 10^6 cells corrected with the CE_2 value
*** Cloning efficiency related to the respective vehicle control
1 EMS 300 µg/mL
2 DMBA 1.25 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test M656H055 does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report:	CA 5.8.1/44 2013a Reg. No. 5749263 (metabolite of BAS 656 H, Dimethenamid) - Micronucleus assay in bone marrow cells of the mouse intraperitoneally administration 2012/1205857
Guidelines:	OECD 474 (1997), EPA 870.5395, (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.12
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M656H055 (Reg.No. 5749263, metabolite of Dimethenamid; batch: L80-154, purity: 69.8%) was tested its ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. Based on the result of two pre-tests, the test substance dissolved in sterile water was administered once intraperitoneally to groups of 7 male mice at dose levels of 525, 1000, and 2000 mg/kg body weight in a volume of 20 mL/kg body weight. The intraperitoneal administration route was selected to promote systemic exposure, as no clinical signs were observed in a first pre-experiment after oral administration at the limit dose level. The vehicle served as negative and cyclophosphamide as positive control (5 animals/group). The animals were sacrificed 24 or 48 hours (additional high dose and vehicle group) after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.
Intraperitoneal administration of the test substance did not lead to any biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used. The rate of micronuclei were below or near to the value of the negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Signs of systemic toxicity were observed at the highest tested dose of 2000 mg/kg bw and comprised reduced spontaneous activity, eyelid closure, ruffled fur and hunchback. The positive control led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.
According to the results of the study, the test substance M656H055 did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and is therefore considered non-mutagenic.

(BASF DocID 2012/1205857)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg. No. 5749263 (metabolite of BAS 656 H, Dimethenamid)
Description:	Solid, beige
Lot/Batch #:	L80-154
Purity:	69.8%
Stability of test compound:	stable in solvent (Confirmed indirectly by dose formulation analytics (see BASF study 04Y0266/10Y062)
Solvent used:	sterile water

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	sterile water
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw

3. Test animals:

Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	10 - 11 weeks
Weight at dosing:	37.1 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex/dose
Micronucleus assay:	7 males/dose/test group, 5 males per vehicle and control group, respectively
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V.; Horst; The Netherlands), ad libitum
Water:	Tap water, ad libitum
Housing:	The animals were housed in groups in Makrolon Type II/III, with wire mesh top.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 85%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound concentration:

Range finding test:	2068 (per os) and 2000 (i.p.) mg/kg bw
Micronucleus assay:	525, 1000 and 2000 mg/kg bw The test substance was administered once intraperitoneally using an application volume of 20 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 23-Jul-2012 to 05-Sep-2012

2. Preliminary cytotoxicity assay:

Male and female NMRI mice were administered the test substance once by oral gavage at a dose of 2068 mg/kg bw (1st pre-experiment) and by intraperitoneal injection with 2000 mg/kg bw (2nd experiment).

3. Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once with either the vehicle, positive control substance or 525, 1000 and 1500 mg Reg.No. 5749263 / kg bw by intraperitoneal injection. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period (48 hours). The application volume was 20 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The application volume for the positive control groups was 10 mL/kg b.w. The animals were surveyed for evident clinical signs of toxicity throughout the study.

	Twenty-four/48 hours after the administration the mice were killed and the two femora were prepared free of all soft tissue. After cutting the epiphyses, the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390xg for 10 minutes. Afterwards, the supernatant was discarded and the cell pellet re-suspended.
Slide preparation:	A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted. At least one slide was made from each bone marrow sample. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide evaluation:	In general, 2000 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 and to investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same samples and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

5. Evaluation criteria:

A test item was classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods to determine the significance of effects were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results. A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle (water) was verified in a separate study under the responsibility of the sponsor and the results are reported in a separate report (BASF study code 04Y0266/10Y062).

B. PRELIMINARY RANGE FINDING TEST

None of the male or female mice died after single oral dosing of 2068 mg/kg bw or single i.p. injection of 2000 mg/kg bw. However, clinical signs including reduction of spontaneous activity, eyelid closure, tumbling, straub phenomena, hunchback, ruffled fur, stiff-legged walking, dark eyes, sunken flanks and pharyngeal reflex were observed within 6 hours after administration. On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No substantial sex specific differences were observed with regard to clinical signs. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Clinical signs were restricted to the animals treated with high dose of 2000 mg/kg bw and comprised reduction of spontaneous activity, eyelid closure, ruffled fur and hunchback. No clinical signs were observed in any of the animals treated with low and mid dose of the test substance, the positive control or the vehicle.

After treatment with the test item at 24h and 48h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that the test substance did not induce cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after test substance treatment were below or near to the value of the vehicle control group and all values in all dose groups were very well within the historical vehicle control data range [see **Table 5.8.1-92**].

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (2.91%), thereby demonstrating the sensitivity of the test system.

Table 5.8.1-92: Micronucleus test in mice administered M656H055 by i.p. injection

Treatment	Sampling time	PCEs with micronuclei (5%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Sterile water	24	0.090	0-4	1384
M656H055				
525 mg/kg bw	24	0.079	0-4	1270
1000 mg/kg bw	24	0.100	0-4	1242
2000 mg/kg bw	24	0.050	0-2	1252
Positive control				
Cyclophosphamide	24	2.910	36-93	1200
48 h sampling				
Sterile water	48	0.080	0-6	1244
M656H055				
2000 mg/kg bw	48	0.057	0-4	1255

III. CONCLUSION

Based on the results of this study, M656H055 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under in vivo conditions.

Conclusion on genotoxicity of M656H055

There was no evidence in the in vitro and in vivo genotoxicity studies conducted fulfilling the requirement for genotoxicity testing of ground-water metabolites.

C Toxicological evaluation of metabolite M656H055

For M656H055 structural alerts for presumed degradates were identified for chromosomal aberration in vitro. M656H055 was however, not genotoxic in the in vitro and in vivo genotoxicity studies conducted fulfilling the requirements for evaluation of ground-water metabolites. The predicted exposure levels in ground-water are clearly below the threshold of 0.75 µg/l water that would require further toxicological testing.

In conclusion M656H055 is considered to be of <u>no</u> toxicological relevance.

6. <u>Metabolite M656PH059 former assigned M59/M60/M61</u>

M656PH059 is a ground-water metabolite. Estimated exposure levels via groundwater are 0.75 µg/l < M656PH059 ≤ 4.5 µg/l.

A Structural alerts for M656PH059

In the OECD-toolbox some deviating alerts for DNA-binding, protein-binding, genotoxicity or carcinogenicity were determined that were not identified for the parent molecule Dimethenamid-P. There was a structural alert for having an alpha-beta unsaturated carbonyl group identified by the ISS modules. This alert was considered for point-mutations (Ames) module, for inducing chromosomal aberrations (micronuclei) and as a consequence being a genotoxic carcinogen. With regard to protein binding a potential direct acylation was identified in the OECD and the OASIS module. In contrast no alert was identified for M656PH059 for Ames, Micronucleus and Chromosomal aberration in the OASIS module. Thus the identified alerts for genotoxicity are somewhat contradictory. As discussed in the general section on the QSAR modules applied the OECD toolbox profiles provide the alerts based on the functional groups identified only and do not take into consideration the influence on reactivity by neighboured functional groups and/or sterical hindrance.

OASIS-Times predicted M656PH059 [see molecule 30 of report DocID 2014/1088460] to be not mutagenic in the Ames test with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 30 of report DocID 2014/1088461] the prediction was negative for the metabolite itself but positive for a structural alert contained in presumed transformation products thereof. This prediction based on the structural alert for alpha,beta carbonyls with polarized double bonds was not considered to become active as all presumed transformation products were predicted to be negative for chromosomal aberration in vitro due to the entire considered structural properties of the molecule (12 in total). The overall prediction was therefore negative for in vitro chromosomal aberration, with the limitation that the structures were out of the prediction domain.

The Vega prediction [see molecule 21 of report DocID 2014/1088457] was inconclusive. In the CAESAR module the prediction was mutagenic. The reliability of this prediction was low as no similar compounds with known experimental data have been found in the database and similar compound within the database have experimental values that disagree with the prediction. Moreover, a prominent number of atom centered fragments of the compound have not been found or are rare in compounds found in the database. Vice versa compounds of the database that gave evidence for mutagenicity contained significantly other atom centered fragments not identified in M656PH059 that were considered potentially responsible for the mutagenic activity [see **Figure 5.8.1-12**].

Figure 5.8.1-12: Comparison of structural alerts of M656PH059 with similar structures identified by Vega CAESAR as mutagenic

The SarPy module prediction was not mutagenic, however the reliability of this prediction was also low. No similar molecules with known experimental data were in the database. Some similar molecules with experimental data disagree with the prediction. Moreover, a prominent number of atom centered fragments of the compound have not been found or are rare in compounds found in the database. Overall the structural similarity is considered insufficient.

There were contradictory predictions for Ames mutagenicity. While OECD toolbox ISS module considered M656PH059 Ames positive for the alert of an alpha, beta unsaturated carbonyl, this alert was not considered in the OASIS module and there was also no alert for Ames mutagenicity in the OASIS TIMES prediction neither for the molecule per se nor for presumed transformation products. The Vega CAESAR prediction for being mutagenic is not considered reliable as other structural alerts are considered responsible for the mutagenicity of the identified similar but not close enough related compounds. Thus there was no conclusive Ames alert identified for M656PH059. Overall there was some inconsistency in the prediction of an alert for chromosomal aberration in vitro predicted for either the molecule per se or presumed transformation product. The structural alert was that of unsaturated carbonyl compounds. They are known to present toxicological concerns in biological systems in vitro. This toxic potential originates from the alpha-beta unsaturated system adjacent to the carbonyl building a reactive center for nucleophilic addition and is mostly characterized for small, low molecular weight molecules. This alert is known to become active in in vitro systems but in particular for greater complex molecules like M656PH059 the evidence to become effective in vivo is lacking.

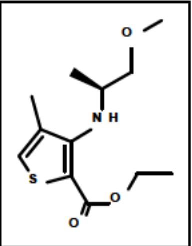
B Toxicological evaluation of M656PH059

No conclusive toxicological alert is identified and the toxicological evaluation is made based on the grouping proposal presented and discussed in Doc N4, chapter 3.3 to 3.5 of this dossier.
As a member of the M27-group metabolite M656PH059 is considered to be of <u>no</u> toxicological relevance.

7. <u>M656PH062 former assigned M62</u>

M656PH062 is a ground-water metabolite. The predicted exposure levels in ground-water are 0.75 µg/l < M656PH062 ≤ 4.5 µg/l.

It was not possible to obtain M656PH062 as stable test item for toxicological testing as the compound was rapidly decarboxylated at the thiophene ring. Thus, decision was taken to test the ethylesterderivate of this metabolite as a surrogate taking into account that ester-bonds are easily cleaved in metabolic capable test-systems. The structure for the surrogate test item is presented below.



A Structural alerts for M656PH062

In the OECD-toolbox the alert for DNA-binding of the OECD module differed from what was identified for the parent compound dimethenamid-P. For dimethenamid-P the alert was for iminium ion formation of the tertiary amine. Instead for M656PH062 it was the alert of nitrenium ion formation of the secondary heterocyclic amine. There was however no alert for DNA binding in the OASIS v. 1.2 module for both compounds. The only alert for genotoxicity (H-acceptor path) was also identified for the parent molecule Dimethenamid-P.

OASIS-Times [structure 19 of report DocID 2014/1088460] predicted M656PH062 to be not mutagenic in the Ames neither without nor with metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see molecule 19 of report DocID 2014/1088461] the prediction was negative for the metabolite itself and the structural alerts identified for transformation products (thiols) were not predicted to become active with the limitation that the molecules were out of the prediction domain.

In the DEREK analysis conducted the structural alerts for M656PH062 were the thiophene alert for hepatotoxicity and nephrotoxicity which were also identified for the parent compound Dimethenamid-P. No alert for genotoxicity was identified in this model.

The Vega prediction (Molecule 22) in both modules CAESAR and SarPy was not mutagenic. The reliability of these predictions was low as no similar compound with experimental data were in the training set and as similar molecules found in the database have experimental values that disagree with the prediction.
In conclusion, no relevant toxicological alert was identified in any of the structure activity evaluation tools employed.

B Genotoxicity studies of M656PH062

Report:	CA 5.8.1/45 Woitkowiak C., 2013b Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, Dimethenamid-P) - Salmonella typhymurium / Escherichia coli reverse mutation assay 2013/1373303
Guidelines:	OECD 471, (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.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to M656PH062 (Reg. No. 5936274, former assigned M62, metabolite of Dimethenamid-P; Batch: L82-129; Purity: 90.1%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and in a preincubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation test, M656PH062 was tested at concentrations of 33, 100, 333, 1000, 2650 and 5300 µg/plate. In the preincubation test, M656PH062 was tested at concentrations of 10, 33, 100, 333, 1000 and 2650 µg/plate. A bacteriotoxic effect was occasionally observed depending on the strain and test conditions from about 333 µg/plate onward. Precipitation of the test substance was found in the standard plate test from about 100 µg/plate onward with and without S9 mix.
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 M656PH062 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1373303)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5936274 (Derivate of metabolite of BAS 656-PH, Dimethenamid-P)

Description: Liquid, yellow/clear

Lot/Batch #: L82-129

Purity: 90.1% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions over the test period was guaranteed until 01 Nov 2015 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

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: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from the livers of induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last 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 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
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

The optical density of the fresh bacteria cultures was determined. Fresh cultures of bacteria were grown up to late exponential or early stationary phase of growth (approximately 10^9 cells per mL).

5. Test concentrations:

Plate incorporation assay: Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2650 and 5300 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: The test substance / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2650 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-Oct-2013 to 25-Oct-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

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 dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripled (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance in the vehicle DMSO was verified analytically (BASF Project No. 01Y0291/13Y029).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his^- and trp^- background growth, decrease in the number of his^+ revertants) was occasionally observed in the standard plate test depending on the strain and test conditions from about 2650 µg/plate onward.
In the preincubation assay bacteriotoxicity (reduced his^- and trp^- background growth, decrease in the number of his^+ revertants) was occasionally observed depending on the strain and test conditions from about 333 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see **Table 5.8.1-93**).

In the standard plate test, test substance precipitation was found from about 100 µg/plate onward with and without S9 mix.

Table 5.8.1-93: Bacterial gene mutation assay with M656PH062 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.0	17.7	56.0	52.0	12.0	15.7	11.3	10.0	58.0	53.3
M656PH062										
33 µg/plate	26.7	16.3	66.3	50.3	12.0	15.7	9.3	10.3	51.0	52.0 P
100 µg/plate	23.7 P	17.3 P	67.0 P	52.7 P	11.7 P	14.3 P	16.0 P	7.0 P	55.3 P	61.0 P
333 µg/plate	22.0 P	15.7 P	54.0 P	65.3 P	11.7 P	13.0 P	8.0 P	8.7 P	59.3 P	52.0 P
1000 µg/plate	21.3 P	14.3 P	58.3 P	52.3 P	8.7 P	8.7 P	10.7 P	8.7 P	56.3 P	50.7 P
2650 µg/plate	17.0 P	11.7 P	46.0 P	44.0 P	10.0 P	10.7 P	5.0 P	5.7 P	61.3 P	50.0 P
5300 µg/plate	22.7P/B	15.3P/B	41.0P/B	48.0P/B	7.0P/B	12.0P/B	3.0P/B	0.7P/B	51.7P/B	52.3P/B
Pos. control§	2240.0	483.3	2275.0	4092.0	214.7	5197.3	244.0	2277.7	282.0	867.0
Preincubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27.0	15.7	51.7	35.7	12.7	9.3	8.0	9.3	43.3	48.0
M656PH062										
10 µg/plate	26.7	15.0	43.7	33.0	14.7	9.7	7.0	7.0	59.7	42.7
33 µg/plate	23.3	16.7	50.0	39.0	8.3	9.7	5.7	7.0	51.3	59.0
100 µg/plate	23.0	13.7	46.0	43.0	9.3	8.3	6.3	6.7	53.3	57.3
333 µg/plate	25.3	14.0	30.7	38.0	9.3	7.3	6.7	5.7	51.3	56.0
1000 µg/plate	25.0	11.0	42.7	33.7	9.3	11.0	6.3	4.0	63.0	37.7
2650 µg/plate	18.7	12.0 B	31.7	25.3 B	11.0	6.0 B	7.7	2.7 B	53.3	37.7 B
Pos. control§	1900.0	379.3	684.7	316.7	194.7	1242.7	191.3	1670.7	120.7	406.0

§ = Compound and concentrations see Material and Methods (I.A.2.) above
P = Precipitation

III. CONCLUSION

According to the results of the present study, the test substance M656PH062 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/46
Wollny H.-E., 2013c
Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, Dimethenamid-P) - In vitro cell mutation assay at the Thymidine Kinase Locus (TK+/-) in mouse lymphoma L5178Y cells
2013/1307624

Guidelines: OECD 476 (1997), (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.17, EPA 870.5300, EPA 712-C-98-221

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The ethylester derivate of M656PH062 (Reg. No. 5936274, ethylester derivate of metabolite of Dimethenamid-P; Batch: L82-129, Purity: 90.1%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation. In the 1st main experiment concentrations between 10.6 and 255 µg/mL were used without and 2.7 to 63.8 µg/mL with metabolic activation. In the 2nd experiment concentrations between 2.6 and 63.0 µg/mL were tested without and 5.3 to 63.0 µg/mL with metabolic activation. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.
Cytotoxic effects were observed in both cultures of the first experiment at 170 µg/mL without metabolic activation and at 42.5 µg/mL and above with metabolic activation. In the second experiment without metabolic activation cytotoxic effects were noted at 63.0 µg/mL without metabolic activation and at 31.5 µg/mL and above with metabolic activation. No visible precipitation of the test item in the culture medium was observed. No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. The positive control substances, however, induced a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test the ethylesterderivate of M656PH062 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1307624)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg. No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)
Description:	Liquid; yellow, clear
Lot/Batch #:	L82-129
Purity:	90.1% (for details see Certificate of Analysis ASAP13_203; at the start of the experiment preliminary information on the purity of the test item indicated a purity ≥ 94.859 area-%)
Stability of test compound:	Stable in Ethanol
Solvent used:	Ethanol

2. Control Materials:	
Negative control:	A negative control was not employed in this study.
Solvent control:	Ethanol
Positive control -S9:	Methyl methanesulfonate (MMS) 19.5 µg/mL (experiment I); 13.0 µg/mL (experiment II)
Positive control +S9:	Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313) in the pre-experiment and in experiment I, and 29.8 mg/mL (Lot. No.: 050913) in experiment II.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 10 % v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism:

The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}$M), Aminopterin ($2.0x10^{-5}$M), Thymidine ($1.6x10^{-3}$M) and Glycin ($5.0x10^{-3}$M) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}$M) and Thymidine ($1.6x10^{-3}$M). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 192 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 21.2 to 2713.0 µg/mL

b) Mutation assay:

1st experiment:	10.6, 21.3, 42.5, 85.0, 170.0, 255.0 µg/mL without metabolic activation
	2.7, 5.3, 10.6, 21.3, 42.5, 63.8 µg/mL with metabolic activation
2nd experiment:	2.6, 5.3, 10.5, 21.0, 42.0, 63.0 µg/mL without metabolic activation
	5.3, 10.5, 21.0, 31.5, 42.0, 63.0 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-Sep-2013 to 04-Nov-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation.

$1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium with 3% horse serum (15% horse serum during 24 h exposure) were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection: After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.

Size distribution of the colonies: Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).

Calculations: **Pre-test**

<u>total suspension growth (4 h treatment):</u>

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

<u>total suspension growth (24 h treatment):</u>

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

relative suspension growth:

total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):

(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):

(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:

total suspension growth × 100 / total suspension growth of corresponding control

relative total growth:

relative suspension growth × relative cloning efficiency / 100

cloning efficiency (viability):

ln(mean number of empty wells per plate / 96) / cells seeded per well

relative cloning efficiency:

cloning efficiency × 100 / cloning efficiency of corresponding control

cells survived:

cloning efficiency × cell number seeded in TFT medium

mutant colonies / 10^6 cells:

small mutant colonies + large mutant colonies

threshold:

number of mutant colonies per 10^6 cells of each solvent control plus 126

cloning efficiency (viability):

cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent solvent control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.
A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_203).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 21.2 µg/mL and 2713.0 µg/mL were chosen with regard to the molecular weight (257.4 g/mol) corresponding to a molar concentration of about 10 mM and considering the preliminary information concerning the purity of the test item (94.859 area-%) at the start of the experiment. No relevant toxic effect occurred up to the maximum concentration tested with and without metabolic activation following 4 and 24 hours of treatment.
Both, pH value and osmolarity were determined at the maximum concentration of the test item and in the solvent control without metabolic activation. There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test item. Precipitation was observed by the unaided eye at 678.3 µg/mL and above with and without metabolic activation following 4 and 24 hours treatment.

The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 255 µg/mL without and 68.3 µg/mL with metabolic activation.

C. MUTAGENICITY ASSAYS

Relevant cytotoxic effects indicated by a relative total growth of less than 50% of survival were observed in both cultures of the first experiment at 170 µg/mL without metabolic activation and at 42.5 µg/mL and above with metabolic activation. In the second experiment without metabolic activation cytotoxic effects as described above were noted at 63.0 µg/mL without metabolic activation and at 31.5 µg/mL and above with metabolic activation. Precipitation of the test substance was not observed.

No substantial and reproducible dose dependent increase of the mutation frequency was observed with and without metabolic activation. Isolated increases exceeding the threshold of 126 above the corresponding solvent control were noted. As these increases were no reproducible and occurred and cytotoxic concentrations, they were considered as biologically not relevant [see **Table 5.8.1-94** and **Table 5.8.1-95**].

The positive controls MMS and CPA were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies.

Table 5.8.1-94: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with ethanol		-	100.0	107	233	100.0	127	253
Pos. Control with MMS	19.5	-	13.0	504	233	14.1	475	253
Ethylesterderivate of M656PH062								
	10.6	-	106.9	71	233	135.1	136	253
	21.3	-	93.0	126	233	122.8	130	253
	42.5	-	169.5	80	233	83.5	113	253
	85.0	-	116.4	77	233	75.2	105	253
	1700.0	-	42.8	88	233	37.8	88	253
	255.0	-	culture was not continued#			culture was not continued#		
Solv. Control with ethanol		+	100.0	52	178	100.0	69	195
Pos. Control with CPA	3.0	+	17.8	241	178	23.3	369	195
Pos. Control with CPA	4.5	+	14.4	599	178	15.1	649	195
Ethylesterderivate of M656PH062								
	2.7	+	culture was not continued##			culture was not continued##		
	5.3	+	84.7	56	178	142.0	74	195
	10.6	+	80.5	65	178	106.6	70	195
	21.3	+	60.5	85	178	68.1	151	195
	42.5	+	10.6	172	178	46.0	201	195
	63.8	+	2.1	207	178	4.0	172	195

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued due to exceedingly severe cytotoxic effects
culture was not continued as a minimum of only four concentrations is required by the guidelines

Table 5.8.1-95: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control with ethanol		-	100.0	58	184	100.0	63	189
Pos. Control with MMS	13.0	-	9.9	348	184	10.4	330	189
Ethylesterderivate of M656PH062								
	2.6	-	culture was not continued##			culture was not continued##		
	5.3	-	96.0	50	184	124.4	56	189
	10.5	-	74.6	79	184	110.7	67	189
	21.0	-	80.4	71	184	110.7	62	189
	42.0	-	81.9	68	184	76.9	68	189
	63.0	-	29.3	49	184	46.7	46	189
Experiment II / 4 h treatment								
Solv. Control with ethanol		+	100.0	52	178	100.0	66	192
Pos. Control with CPA	3.0	+	95.7	184	178	47.7	137	192
Pos. Control with CPA	4.5	+	24.9	341	178	25.2	332	192
Ethylesterderivate of M656PH062								
	5.3	+	culture was not continued##			culture was not continued##		
	10.5	+	117.3	83	178	151.9	33	192
	21.0	+	54.0	69	178	126.1	32	192
	31.5	+	43.1	109	178	46.2	48	192
	42.0	+	20.5	94	178	47.6	54	192
	63.0	+	9.7	187	178	11.7	75	192

threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test the ethylesterderivate of M656PH062 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/47 Bohnenberger S., 2014a Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, Dimethenamid-P): In vitro micronucleus test in chinese hamster V79 cells 2013/1307623
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The ethylester derivate of M656PH062 (Reg. No. 5936274, ethylester derivate of metabolite of Dimethenamid-P, Batch L82-129; purity 90.1%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation. Concentrations of 5.3 to 2713 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since the cultures fulfilled the requirements for cytogenetic evaluation and the test item was considered to be mutagenic, this preliminary test was designated Main Experiment and concentrations of 5.3, 10.6 and 21.2 µg/mL were chosen for evaluation. Cytotoxicity as well as precipitation were not observed at concentrations evaluated for cytogenicity.
In the absence of S9 mix statistically significant increases in micronucleated cells, clearly exceeding the range of the laboratory historical control data were observed after treatment with 10.6 and 21.2 µg/mL. In the presence of S9 mix one single statistically significant increase in micronucleated cells, clearly exceeding the range of the laboratory historical control data was observed after treatment with 21.2 µg/mL. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.
Based on the results of this study, ethylesterderivate of M656PH062 is considered to induce micronuclei in vitro in V79 cells when tested up to the highest evaluable concentration in the absence and presence of metabolic activation.

(BASF DocID 2013/1307623)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Liquid; yellow, clear
Lot/Batch #:	L82-129
Purity:	90.1 %
Stability of test compound:	Stable in Ethanol (solvent)
Solvent used:	Ethanol

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	Ethanol
Positive controls, -S9:	Mitomycin C (MMC, 0.1 µg/mL, dissolved in deionised water)
Positive control, +S9:	Cyclophosphamide (CPA, 15.0 µg/mL, dissolved in saline)

3. Activation: Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 is prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 is routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test. The protein concentration of the S9 preparation was 38.4 mg/mL (Lot. No.: 220313).

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of approx. 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells were used in the experiments. This is a continuous cell line with a population doubling time of 13 hours and a reasonable plating efficiency of untreated cells (in general ≥ 70 %).

5. Culture medium/conditions:

About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity and cytogenicity assay:

5.3 - 2713.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation and the test item was considered to be mutagenic, this preliminary test was designated Main Experiment (see **Table 5.8.1-96**).

Table 5.8.1-96: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg. No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 hrs	4 hrs	I	**5.3**	**10.6**	**21.2**	42.4	84.8	169.6 PS	339.1 PS	678.3 PS	1356.5 PS	2713.0 PS
			With S9 mix									
24 hrs	4 hrs	I	**5.3**	**10.6**	**21.2**	42.4	84.8	169.6	339.1 PS	678.3 PS	1356.5 PS	2713.0 PS

Evaluated experimental points are shown in bold characters

PS Phase separation was observed at the end of treatment

B. TEST PERFORMANCE:

1. Dates of experimental work: 18-Sep-2013 - 25-Sep-2013

2. Preliminary cytotoxicity assay: With regard to the molecular weight and the preliminary information on purity (94.859%) of the test item, 2713.0 µg/mL of Reg. No. 5936274 (Derivate of Metabolite of BAS 656-PH, Dimethenamid-P) (approx. 10 mM) were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 5.3 and 2713.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:
A test item was considered as mutagenic if:

- the number of micronucleated cells exceeds both the value of the concurrent negative control and the range of the historical negative control data
- a significant, dose-related and reproducible increase in the number of cells containing micronuclei is observed

A test item can be considered as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_203).

B. PRELIMINARY CYTOTOXICITY ASSAY:

Since the cultures fulfilled the requirements for cytogenetic evaluation and the test item was considered to be mutagenic, the preliminary test was designated Main Experiment (see below).

C. CYTOGENICITY ASSAYS:

In the absence and presence of S9 mix, concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage.

In the absence of S9 mix statistically significant increases in micronucleated cells, clearly exceeding the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells) were observed after treatment with 10.6 and 21.2 µg/mL (3.05 and 6.30 %). In the presence of S9 mix one single statistically significant increase in micronucleated cells, clearly exceeding the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) was observed after treatment with 21.2 µg/mL (8.55 %) [see **Table 5.8.1-97**].

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-97: Summary of results of the micronucleus test with ethylester-derivate of M656PH062

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.91	1.10
		Positive control[2]	2.74	**3.70**[S]
		5.3	2.87	1.10
		10.6	2.68	**3.05**[S]
		21.2	2.05	**6.30**[S]
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	2.24	1.45
		Positive control[3]	1.72	**9.55**[S]
		5.3	2.16	1.40
		10.6	2.08	1.35
		21.2	1.94	**8.55**[S]

* The number of micronucleated cells was determined in a sample of 2000 cells
S Number of micronucleated cells statistically significantly higher than corresponding control values
1 Ethanol 0.5 % (v/v)
2 Mitomycin C 0.1 µg/mL
3 CPA 15.0 µg/mL

III. CONCLUSIONS

Based on the results of the study, ethylester-derivate of M656PH062 is considered as mutagenic in this in vitro micronucleus test, when tested up to the highest required concentrations.

Report: CA 5.8.1/48
2014c
Reg.No. 5936274 (derivate of metabolite of BAS 656-PH, Dimethenamid-P): Micronucleus assay in bone marrow cells of the mouse
2014/1028628

Guidelines: OECD 474 (1997), (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.12, EPA 870.5395

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report: CA 5.8.1/49
Becker M.,Landsiedel R., 2014c
Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, Dimethenamid-P) - Plasma analysis for external studies
2014/1092433

Guidelines: <none>

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.8.1/50
Grauert E.,Landsiedel R., 2014a
Analytical report - Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, Dimethenamid-P) - Concentration control analyses in corn oil
2014/1101991

Guidelines: <none>

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The ethylester derivate of M656PH062 (Reg. No. 5936274, ethylester derivate of metabolite of Dimethenamid-P, Batch L82-129; purity 90.1%) was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. For this purpose, the test substance dissolved corn oil was administered once orally to groups of 7 male mice at dose levels of 250, 500 and 1000 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 male animals/control). The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

The oral administration of the test substance did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The mean values of micronuclei observed after treatment with the test substance were even below to the value of the vehicle control group. Moreover, micronucleus values obtained in all dose groups were within the historical negative control range. Clinical signs comprised ruffled fur (at ≥ 250 mg/kg bw), reduction of spontaneous activity (at ≥ 500 mg/kg bw) and eyelid closure as well as abdominal position (at 1000 mg/kg bw). The positive control chemical cyclophosphamide led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system. Based on the results of the study it is considered that M656PH062 did not induce micronuclei in the bone marrow cells of the mouse under the test conditions chosen. (BASF DocID 2014/1028628)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5936274 (Metabolite of BAS 656-PH, Dimethenamid-P)

Description:	Solid, beige
Lot/Batch #:	L82-129
Purity:	90.1% (tolerance ± 1.0%)
Stability of test compound:	Confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0291/13Y051).
Solvent used:	corn oil

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	corn oil
Positive control:	Cyclophosphamide (CPA) 40 mg/kg

3. Test animals:

Species:	Mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 11 weeks
Weight at dosing:	Males mean value 35.9 g (SD ± 1.7 g)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon Type II (pre-test) / III (main study) cages, with wire mesh top

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	1000 and 2000 mg/kg (administered once orally)
Micronucleus assay:	250, 500 and 1000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 30-Oct-2013 to 26-Nov-2013

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 2000 mg/kg bw (2nd pre-test).

3. Micronucleus test:

Treatment and sampling: Groups of male mice were treated once with either vehicle or 250, 500 or 1000 mg M656PH062/ kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study.

Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide preparation: A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.

Slide evaluation: In general, 2000 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.

To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.

5. Evaluation criteria:

A test item was considered as mutagenic if it induces either a dose-related increase or a clear increase in the number of micro-nucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics (see separate report, BASF study code 04Y0291/13Y051).

B. PRELIMINARY RANGE FINDING TEST

One male animal of the 2000 mg/kg bw dose group died. No further mortalities were observed. At 1000 mg /kg bw clinical signs comprised reduction of spontaneous activity, abdominal position, hunchback, sunken flanks, ruffled fur and eyelid closure in both sexes. At 2000 mg/kg bw reduction of spontaneous activity, abdominal position, hunchback, sunken flanks, ruffled fur, eyelid closure, tumbling, apathy and tiptoe walk in both sexes was observed in animals of both sexes. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Clinical symptoms in the main experiment included ruffled fur, abdominal position, reduced spontaneous activity and eyelid closure in the animals treated with the high dose of the test item. The animals treated with the mid dose level exhibited reduced spontaneous activity and ruffled fur. The animals treated with the low dose exhibited ruffled fur only. Most signs occurred transiently after test item administration, only ruffled fur was observed in some mice up to the end of the observation period. Plasmaanalytics confirmed that ethylester derivate of M656PH062 is systemically available [see DocID 2014/1092433].

The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that the test substance did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item [see **Table 5.8.1-98**]. The mean values of micronuclei observed after treatment with the test substance were even below to the value of the vehicle control group. Moreover, micronucleus values obtained in all dose groups were within the historical negative control range.

The positive control cyclophosphamide showed a statistically significant increase of induced micronucleus frequency thereby ensuring the validity of the test system.

Table 5.8.1-98: Micronucleus test in mice administered M656PH062 by oral gavage

Treatment	Sampling time	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
24 h sampling				
Vehicle	24	0.110	0-7	1154
M656PH062				
250 mg/kg bw	24	0.086	0-4	1149
500 mg/kg bw	24	0.050	0-2	1186
1000 mg/kg bw	24	0.100	0-4	1195
Positive control				
Cyclophosphamide	24	2.430	36-61	1144
48 h sampling				
Sterile water	48	0.110	0-4	1222
M656PH062				
1000 mg/kg bw	48	0.071	0-2	1075

III. CONCLUSION

Based on the result of this study M656PH062 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Conclusion on genotoxicity of M656PH062

Studies were conducted with the ethylester derivate of M656PH062 as M656PH062 could not be stably syntethized. Overall, with regard to in vitro genotoxicity testing there was no indication for mutagenicity neither in the bacterial Ames-test nor in the mammalian Mouse-Lymphoma test from the studies conducted with ethylester derivate of M656PH062. However, in the in vitro micronucleus test in in V79 cells conducted with ethylester derivate of M656PH062 a potential chromosomal aberration effect was observed without and with metabolic activation. In contrast in the subsequently conducted in vivo micronucleus test in mice no treatment-related induction of micronuclei could be determined up to the limit dose of 2000 mg/kg a dose level with clear clinical signs of toxicity. Plasma-analytics confirmed that ethylester derivate M656PH062 was systemically available. Thus, the in vivo study conducted for the same endpoint did not demonstrate a treatment-related effect. By weight of evidence M656PH062 was not considered to be genotoxic.

C Short-term toxicity of M656PH062

Report: CA 5.8.1/51
[redacted], 2014f
Reg.No. 5936274 (derivative of metabolite of BAS 656-PH, Dimethenamid-P) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet
2014/1018066

Guidelines: OECD 407, (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.7 No. L 142, EPA 870.3050, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Ethylester-derivate of M656PH062 (Reg.No. 5926274, derivate of metabolite of Dimethenamid-P; Batch: L82-129; Purity 90.1%) was initially administered to Wistar rats at dietary dose levels of 0, 1200, 4000 and 12000 ppm. Due to severely impaired body weight development in males the high dose level of 12000 ppm was reduced to 8000 ppm form day 18 onwards.
Decreased food consumption in high dose males and females was noticed between study days 11 to 14. After reduction of dose to 8000 ppm decreased food consumption was noticed in females between study days 25 to 28. Mean body weight and body weight change were significantly lower in male animals treated with 12000 ppm from study day 7 onwards that were still significantly lower after reduction of dose to 8000 ppm. A tendency to impaired body weight development was also observed in females treated with 12000 ppm from study day 7 onwards until reduction of dose level.
Altered clinical chemistry parameters indicative for liver enzyme induction, increases in absolute and relative liver weight together with a centrilobular hepatocellular hypertrophy were seen in the high dose male and female dose group treated with 12000 / 8000 ppm. In addition follicular cell hypertrophy/hyperplasia in the thyroid gland assessed to be secondary to liver enzyme induction was noticed.
No treatment-related adverse effects were observed at the mid dose of 4000 pppm and the low dose of 1200 ppm
Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 4000 ppm that is 323 mg/kg bw/day in male and 385 mg/kg bw/day in female Wistar rats.

(DocID 2014/1018066)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** **Reg.No. 5936274, derivate of metabolite of Dimethenamid-P**

Description:	liquid / yellow, clear
Batch/purity #:	L82-129 / 90.1%
Stability of test compound:	Stable until 01 Nov 2015. The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. **Vehicle and/or positive control:** Rodent diet

3. **Test animals:**

Species:	Rat
Strain:	Wistar Crl:WI (Han)
Male and female	
Age:	42 ± 1 day at start of administration
Weight at dosing:	♂: 160.1 ± 6.3 g, ♀ 130.7 ± 7.7 g
Source:	Charles River, Germany
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:	Group housing (5 animals per cage) in polysulfonate H cages (Techniplast, Hohenpeißenberg, Germany), floor area about 2065 cm² with dust-free wooden bedding, Wooden gnawing blocks (NGM E-022) supplied by Abedd Lab. And Vet. Science GmbH, Vienna, Austria and play tunnel large supplied by PLEXX B.V., Elst Netherlands for environmental enrichment Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 air changes 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: 05-Nov-2013 – 26-Feb-2014
(In life dates: 14-Nov-2013 (start of administration) to 13-Dec-2013 (necropsy))

2. Animal assignment and treatment:

The ethylester derivate of M656H062 was administered to groups of 5 male and 5 female rats at dietary concentrations of 0, 1200 (low dose), 4000 (intermediate dose) and 12000 ppm (top dose). As body weight development was significantly impaired in all males of the high dose group and was also impaired in females between study day 0 and study day 14, the diet concentration was reduced to 8000 ppm from study day 18 onwards in males as well as in females. For the other dose groups the concentrations remained throughout the 28-day study period. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

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. One diet preparations per dose was performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 32 days.

Homogeneity analyses of the diet preparations were performed at the beginning of the administration. According to the SOP, three specimen were sampled from the top, middle and bottom to storage containers for the low (1200 ppm) and top dose level (12000 ppm) and subsequently analyzed. The samples were also used for determination of the test-article concentration. For the mid dose level (4000 ppm) and for the reduced high dose level (8000 ppm) single samples were analysed. No test-article was determined in control diets.

Table 5.8.1-99: Analysis of diet preparations for homogeneity and test-item content

Dose level	Sampling	Concentration Mean ± SD [ppm]	% of nominal concentration	Relative standard deviation [%]
1200 ppm	12. Nov. 13	1143.7 ± 13[#]	95.3	1.1
4000 ppm	12. Nov. 13	3876.4	96.9	n.a.
8000 ppm	1. Dez. 13	7473.7	93.4	n.a.
12000 ppm	12. Nov. 13	11660.4 ± 147[#]	97.2	1.2

n.a.: not applicable;
[#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity samples in the range of 1.1 to 1.2% indicate the homogenous distribution of the ethylester derivate of M656H062 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 93.4 to 97.2% of the nominal concentrations. These results demonstrated the correctness of the concentrations of ethylester derivate of M656H062 in the vehicle.

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 5.8.1-100: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, 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
feces, rearing, grip strength forelimbs, grip strength hind limbs, foot-splay test, motor activity	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 equal medians

Table 5.8.1-101: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except for urine color and turbidity	For parameters with bidirectional changes: 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 equal medians For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Table 5.8.1-102: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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. For this 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. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmos
15. feces discharge during examination (appearance/consistency)
16. urine discharge during examination
17. pupil size

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once weekly as representative value over 3 days and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ \text{to}\ x}} \times 100$$

BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), $FC_{y\ \text{to}\ x}$ 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.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:
Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. No quantitative determination of water consumption was conducted

5. Ophthalmoscopy:
Not performed in this study.

6. Functional observation battery (FOB):
A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), 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 not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

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

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

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 hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity examinations were performed in a darkened room using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 infrared beams per cage. For the measurements animals were placed in new clean polycarbonate cages with absorbent material. Motor activity measurements started at 14:00 h. Because of the staggered measurement procedure, the starting time varied according to the time needed to place the animals in the cages. The numbers of beam interrupts were counted over 12 intervals of 5 minutes each. Measurement started individually for each animal when the 1st beam was interrupted and lasted exactly 1 hour. No food or water was offered during the measurements.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane anesthetized animals from the retro-orbital plexus. For hormone determinations blood was taken at necropsy after decapitation, these samples were stored frozen but not analysed. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test) (HQT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count(PLT)
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activatged partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (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	✓	Bile acids (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

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	✓	✓	#	kidneys	✓			skin
✓		#	aorta	✓			lachrymal glands, extraorbital	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	#	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	coagulating glands[£]	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	colon	✓			mammary gland (♂ and ♀)	✓	✓	✓	thymus
✓		#	duodenum	✓		#	muscle, skeletal	✓	✓	#	thyroid/parathyroid
✓	✓	#	epididymides[¥]	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	urinary bladder
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓	✓	#	uterus with cervix
✓			femur (with joint)	✓		#	pancreas	✓		#	vagina
			gall bladder	✓			pharynx				
✓		✓	gross lesions	✓		#	pituitary				
✓			Harderian glands	✓	✓	#	prostate		✓		body (anesthetized animals)
✓	✓	#	heart	✓		#	rectum				
✓		#	ileum	✓			salivary glands*				
✓		#	jejunum (w. Payer's plaque)	✓	✓	#	seminal vesicles[£]				
[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [‡]1 histopathology at level III, [¥]left epididymidis collected for histopathology, [£]seminal vesicles and coagulation weight determined together											

The organs or tissues were fixed in 4% formaldehyde, except for the eyes with optic nerve and the testes, which were fixed in modified Davidson's solution. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast.

After the completion of the histopathological assessment by the study pathologist an internal peer review was performed by a second senior pathologist (Dr. Karin Küttler, Ludwigshafen, Germany) on liver and thyroid glands of all animals. Results presented in the study report reflect the consensus opinion of both the study pathologist and the reviewing pathologist.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:	
•	Increased/decreased grade of cortico-medullar ratio (related only to area)
•	Increase of starry sky cells
•	Changes of cellular density in the cortex
•	Changes of cellular density in the medulla
Spleen:	
•	Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
•	Altered cellular composition of follicles
•	Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):	
•	Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
•	Altered cellular composition of paracortex
•	Altered number of germinal centers
•	Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):	
•	Changes of the cellularity of follicles (including mantle zone and germinal centers)
•	Changes of the cellularity of interfollicular area
Bone marrow:	
•	Changes of the cellularity
•	Changes of the myeloid/erythropoid ratio

Special attention was given for the synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Comparing the single intervals with the control groups, no significant deviations were measured for motor activity neither in males nor in females.

C. BODY WEIGHT AND BODY WEIGHT GAIN

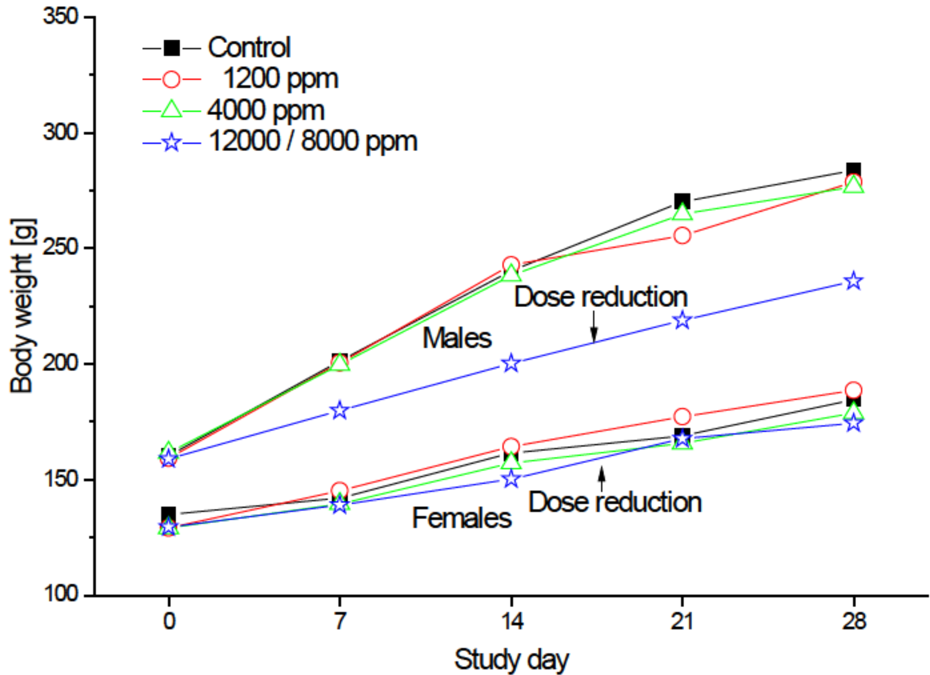
In the first and second week of treatment males of the high dose group showed significant body weight gain reduction in combination with a significantly reduced food consumption in the second week [see **Table 5.8.1-103**, **Table 5.8.1-104** below and **Figure 5.8.1-13**]. Although not significantly altered, a tendency to impaired body weight development was also observed in female animals at that time, but the effect on food consumption was even more pronounced. As a consequence the concentration in the diet was reduced to 8000 ppm from study day 18 onwards in both sexes. In males the dose reduction prevented the further increase in effects on body weight development but the animals did not recover throughout the rest of the study period. The female animals however, seemed to recover as demonstrated by the comparable body weight of controls and high dose on day 21, however in the last week of treatment there was again the tendency to decreased body weight development in relation to the decreased food consumption observed. Overall the high dose males showed a 16.8% reduction in body weight and a 37.7% reduction in body weight gain at the end of the 28-day study period, in females the reduction was 5.5% and 9.4% for body weight and body weight gain, respectively [see **Table 5.8.1-103**]. The significantly decreased body weight change value in male animals and the significantly increased body weight change value in female animals of the 1200 ppm on study day 21 were assessed as being incidental and not related to treatment as no dose-response relationship occurred.

Table 5.8.1-103: Mean body weight of rats administered ethylester derivate of M656H062 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1200**	**4000**	**12000 / 8000[1]**	**0**	**1200**	**4000**	**12000 / 8000[1]**
Body weight [g]								
- Day 0	160.3	159.3	161.7	159	134.9	129.1	129.1	129.5
- Day 7	201.3	200.5	199.8	**179.9****	142.1	145.2	139.6	139
- Day 14	240.3	242.8	238.3	**200.2****	161.5	164.3	157.1	150.2
- Day 21	270.2	255.5	264.8	**218.9****	168.9	177.3	165.7	167.7
- Day 28	283.7	278.7	276.7	**235.9****	184.7	188.6	178.8	174.5
Δ% (compared to control)[#]		-1.8	-2.5	-16.8		2.1	-3.2	-5.5
Body weight gain [g]								
d 0 -> 7	41	41.2	38.1	**20.9****	7.2	16.1	10.5	9.5
d 0 -> 14	80	83.4	76.6	**41.2****	26.6	35.2	28	20.8
d 0 -> 21	109.9	**96.2***	103.1	**60****	34	**48.2***	36.6	38.2
d 0 -> 28	123.4	119.4	115	**76.9****	49.8	59.5	49.7	45.1
Δ% (compared to control)[#]		-3.2	-6.8	-37.7		19.5	-0.2	-9.4

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards..
* $p \leq 0.05$; $p \leq 0.01$; Dunnett test (two-sided)
[#] Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.8.1-13: Body weight development of rats administered ethylester derivate of M656H062 for at least 28 days



D. FOOD CONSUMPTION AND COMPOUND INTAKE

In the high dose males and females food consumption was clearly decreased between study days 11 to 14. After the reduction of the diet concentration on study day 18 the food consumption in males was in the comparable range to control group values with a trend to compensatory higher values in the week after the dose reduction [**Table 5.8.1-104**]. However, in females where the compensatory increase in food consumption after decrease of the dose level was even more pronounced during week 3 (d18 to 21) lower food consumption was again noticed thereafter in the fourth measurement (day 25 to 28). Overall the effects on food consumption were considered related to treatment.

Table 5.8.1-104: Mean food consumption of rats administered the ethylester derivate of M656H062 for at least 28 days

	Males				Females			
Dose level [ppm]	**0**	**1200**	**4000**	**12000 / 8000[1]**	**0**	**1200**	**4000**	**12000 / 8000[1]**
Food consumption [g]								
- Day 4-7	18.0	19.4	19.5	22.0	13.7	14.7	12.9	19.7
- Day 11-14	20.7	22.1	19.9	**11.1**	15.3	23.5	16.7	**6.2**
- Day 18-21	20.9	17.9	18.1	23.2	15.8	17.0	15.3	21.1
- Day 25-28	24.0	24.0	20.3	20.0	17.1	17.9	16.8	**11.5**
Total	83.6	83.4	77.8	76.3	61.9	73.1	61.7	58.5

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/day) over the study period calculated on the values for week 1 to 4 for the 1200 and 4000 ppm dose group and calculated for the 12000 / 8000 ppm dose group on the weeks 1 to 2 for 12000 ppm and on weeks 3 to 4 for 8000 ppm respectively is shown in the following **Table 5.8.1-105**.

Table 5.8.1-105: Calculated intake of ethylester derivate of M656H062

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/day)	
		Males	Females
1	1200	103	131
2	4000	323	385
3	12000	1065	1096
	8000	763	767

E. WATER CONSUMPTION

No test substance-related, adverse changes with regard to water consumption were observed.

F. BLOOD ANALYSIS

1. Hematological findings

At the end of the study in rats of both sexes of the high 12000 and 8000 ppm group prothrombin time (HQT: Hepatoquick's test) was significantly shortened [see **Table 5.8.1-106**]. However, in both gender prothrombin time mean was within the historical control ranges whereas those of the controls were below this range. Thus this alteration was considered incidental and not related to treatment.

The isolated finding of significantly increased reticulocytes in males of the high dose group was considered incidental and not treatment related as it was within the historical control range and no other parameters of the red-blood cell system were affected [see **Table 5.8.1-106**].

Table 5.8.1-106: Selected hematology findings in rats administered ethylester derivate of M656H062 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000[1]	0	1200	4000	12000 / 8000[1]
[mg/kg bw/day		103	323	1065 / 763		131	385	1096 /767
Reticulocytes [%]	1.8	2.0	1.3	**2.5***	2.1	1.8	2.2	1.7
	Historical control: 1.1 – 3.2							
Prothrombin time [sec]	42	39.4	38.7	**35.4****	37.2	37	34.6	**34.4***
	Historical control: 33.3 – 39.6				Historical control: 30.3-36.7			

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
*$p \leq 0.05$; **$p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Clinical chemistry findings

At the end of the study in males of the 12000 / 8000 ppm high dose group, γ-glutamyl transferase activities and cholesterol levels were increased and urea and total bile acid levels were decreased. In female animas of the high dose group triglyceride levels were increased. These were considered treatment-related adverse findings. γ-glutamyl transferase activities and higher cholesterol levels indicated an altered liver cell metabolism most probably due to liver enzyme induction. This was confirmed by decreased ALT activities [see **Table 5.8.1-107**] in these individuals [Hall et al., 2012a, DocID 2012/1365642].

TBA was already lower in males of the mid dose group (4000 ppm), but in this test group it was the only altered parameter and, therefore, this change was regarded as treatment-related but not adverse [ECETOC 2002a, DocID 2002/1027057].

In male animals of low and high dose group alanine aminotransferase (ALT) activities were decreased. The alteration was not dose-dependent and even in the high dose group the activity decrease was not toxicologically relevant as the values were not below 50% [see UK PSD 2007a, DocID 2007/1070125]. In male animals of high dose group chloride levels were marginally above the historical control range [see **Table 5.8.1-107**]. However, this was the only altered electrolyte level in these individuals. Therefore, this change was regarded as incidental and not treatment-related. Therefore these mentioned parameter changes were regarded as incidental and not treatment-related.

In females of the mid and high dose group (4000 as well as 12000 / 8000 ppm) cholesterol, total protein and globulin levels were higher and glucose levels were lower compared to controls. However, none of the mentioned parameters were changed dose-dependently. All means were within historical control ranges except for cholesterol in the mid dose group which was marginally above this range [see **Table 5.8.1-107** below]. Inorganic phosphate levels were higher in females of the 4000 ppm dose group, but the alteration was not dose-dependent. These changes in females were therefore regarded incidental and not treatment related.

Table 5.8.1-107: Selected chlinical chemistry findings in rats administered ethylester derivate of M656H062 for at least 28 days (group means)

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000[1]	0	1200	4000	12000 / 8000[1]
[mg/kg bw/day		103	323	1065 / 763		131	385	1096 /767
Bile acids, total [µmol/l]	29.6	16.8	**11.1****	**6.6****	14.8	12.3	11	4,6
Cholesterol [mmol/l]	1.89	2.24	2.05	**2.64***	1.38	1.34	**2.11****	**1.95***
					Historical control: 0.95 – 1.96			
Triglycerides [mmol/l]	0.86	1.19	0.99	1.15	0.50	0.40	0.75	**1.24***
Protein, total [g/l]	60.64	61.46	63.58	57.98	59.9	59.75	**63.39***	**62.38***
					Historical control: 58.40 – 66.83			
Globulins [g/l]	22.26	23.05	23.93	21.54	20.46	20.56	**23.35****	**22.37****
					Historical control: 21.07 – 27.67			
Urea [mmol/l]	5.83	5.67	5.62	**4.43***	5.42	6.40	5.40	5.69
Glucose [mmol/l]	5.80	6.67	5.89	5.61	5.76	5.13	**4.63****	**5.00***
					Historical control: 4.07 – 6.72			
ALT [µkat/l]	0.79	**0.63***	0.67	**0.51****	0.53	0.57	0.53	0.45
γ-GT [nkat/l]	0	0	0	**40****	0	1	3	4
Cl [mmol/l]	100.7	99.9	100.5	**104.1***	102.4	102.6	103.2	102.6
	Historical control: 99.2 – 104.0							

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
*p ≤ 0.05 ; **p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

3. Urinalysis

In rats of both sexes of the 4000 ppm as well as 12000 / 8000 ppm group as well as in males of the 1200 ppm group urobilinogen levels in the urine were increased [see **Table 5.8.1-108**]. This finding was not accompanied by increased bilirubin levels in serum or urine. Red blood cell parameters (red blood cell counts, hemoglobin and hematocrit levels) were also in the normal range. Total bile acid levels were lower at least in males of the 4000 ppm group as well as 12000 / 8000 ppm group indicating a reduced synthesis or more probably an increased excretion of conjugated bile acids via bile but a reduced intestinal reabsorption. Higher urobilinogen levels in the urine demanded a higher rate of biliary excretion of conjugated bilirubin followed by an increased urobilinogen formation and intestinal absorption. Higher urobilinogen levels in the urine per se without any other findings in clinical pathology or in anatomical pathology of the kidneys and/or liver cannot be regarded as adverse, but as an adaptive effect due to the increased conjugation rate in the liver.
In females of the 1200 and 4000 ppm group pH values of the urine were lower compared to controls, but the decrease was not dose-dependent and therefore it was regarded as incidental and not treatment-related.

Table 5.8.1-108: Selected urinalysis findings in rats administered ethylester derivate of M656H062 for at least 28 days (group means)

Sex	**Males**				**Females**			
Dose [ppm]	**0**	**1200**	**4000**	**12000 / 8000[1]**	**0**	**1200**	**4000**	**12000 / 8000[1]**
[mg/kg bw/day		**103**	**323**	**1065 / 763**		**131**	**385**	**1096 /767**
pH [µmol/l]	6.5	**5.8***	**5.4***	5.7	5.9	5.7	5.7	5.6
Urobilinogen [mmol/l]	1	**3****	**3****	**3****	1	2	**2****	**2***

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
*$p \leq 0.05$; **$p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

G. NECROPSY

1. Organ weight

Terminal body weights of treated rats were significantly (-17%) reduced in high dose male rats [see **Table 5.8.1-109**].

The only statistically significant weight effect considered treatment-related was the dose-dependent increase in absolute and relative liver weights in both male and female animals. The effects were statistically significant in males in all dose-groups, while effects were seen in the md and high dose group in females only. The absolute liver weights of treated males were within the historical control range. Relative liver weights in test group 2 and 3 were above the historical control range while the relative weight of test group 1 males was within that range. The weight increase was therefore assessed as treatment-related in test groups 2 and 3 only. Because of concurrent findings in clinical pathology [see **Table 5.8.1-107** above] indicating an altered liver cell metabolism, the liver weight increases in rats of both sexes of the 12000 / 8000 ppm group [see **Table 5.8.1-109** below] and the moderate centrilobular liver cell hypertrophy in females of this group [see **Table 5.8.1-110** below] were regarded as adverse. The slight liver weight increases with only minimal histological findings and without any clinical pathological changes in rats of the 4000 ppm group however, were evaluated as adaptive effect.

Secondary to the decrease in body weight and, therefore, only indirectly related to treatment, there was a decrease in absolute kidneys and prostate weights and also an increase in relative brain weight in high dose group [see **Table 5.8.1-110**]. The statistically significant increases in absolute and relative brain and spleen weights in low dose males did not show a dose-response relationship and therefore were considered to be incidental.

Table 5.8.1-109: Selected mean absolute and relative organ weights of rats administered the ethylester derivate of M656H062 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000[1]	0	1200	4000	12000 / 8000[1]
[mg/kg bw/day		103	323	1065 / 763		131	385	1096 /767
Terminal bodyweight	257.48	263.28	251.3	**214.38****	165.82	170.68	163.56	159.46
[% of control]	100	102	98	83	100	103	99	96
Brain weight, absolute [g]	1.878	**2.01***	1.968	1.808	1.792	1.8	1.816	1.81
[% of control]	100	**113**	107	91	100	1000	101	101
Brain weight, relative [%]	0.73	**.0764***	0.784	**0.844****	1.082	1.057	1.114	1.136
[% of control]	100	**105**	107	**116**	100	98	103	105
Kidney weight, absolute [g]	1.822	1.924	1.876	**1.602***	1.338	1.346	1.348	1.32
[% of control]	100	106	103	**88**	100	101	101	99
Kidney weight, relative [%]	0.707	0.731	0.748	0.747	0.808	0.79	0.824	0.827
[% of control]	100	103	106	106	100	98	102	102
Liver weight, absolute [g]	6.552	**7.304****	**7.794****	**7.882****	4.72	4.646	**5.224***	**5.3***
[% of control]	100	**111**	**119**	**120**	100	98	**111**	**112**
	Historical control: 6.402 – 10.131							
Liver weight, relative [%]	2.548	**2.776***	**3.102****	**3.682****	2.849	2.728	**3.196***	**3.324****
[% of control]	100	**109**	**122**	**145**	100	96	**112**	**117**
	Historical control: 2.245% to 3.091%							
Spleen weight, absolute [g]	0.43	**0.526****	0.454	0.398	0.332	0.336	0.338	0.304
[% of control]	100	**122**	106	93	100	101	102	92
Spleen weight, relative [%]	0.167	**0.2***	0.18	0.185	0.2	0.197	0.207	0.191
[% of control]	100	**119**	108	111	100	99	104	95
Prostate weight, absolute [g]	0.53	0.48	0.478	**0.36***	-	-	-	-
[% of control]	100	91	90	**68**	-	-	-	-
Prostate weight, relative [%]	0.206	0.182	0.19	0.167	-	-	-	-
[% of control]	100	89	93	81	-	-	-	-

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross and histopathology

The isolated macroscopic findings (focus in adrenal gland of one mid dose male animal and in one high dose female animal, focus in epididymidis of one mid dose male animal, kidney cyst in one low dose male animal and pelvic dilation of the kidney in one low dose male and one female control group animal) belong to the spectrum of background lesions and were considered to be incidental in nature and not related to treatment. Histopathological correlates were determined for the adrenal foci in the mid dose male and the high dose female in form of accessory cortical tissue and for the epididymal focus in the 4000 ppm group identified as a spermatogenic granuloma.

A histopathological correlate of centrilobular hypertrophy was found for the liver weight increases in females of the mid and high dose group only and without any adverse histopathological findings (e.g. necrosis, fatty change, degeneration). Given the severity of the findings the effects in the high dose group were considered as adverse while the effects in the mid dose were considered adaptive.

The follicular hypertrophy/hyperplasia determined in males and females of the mid and high dose group [see **Table 5.8.1-110**] was considered to be treatment related. In the 12000 / 8000 ppm group, this finding was graded slight in all males and 4 of 5 females, while it was graded minimal in 2 of 5 males and 1 of 5 females of the 4000 ppm group. This finding was also assessed as treatment-related and secondary to enzyme induction in the liver. Due to the low incidence and grading the effect in the 4000 ppm group was considered adaptive.

Table 5.8.1-110: Selected histopathological findings of rats administered ethylester derivate of M656H062 for at least 28 days

Sex	Males				Females			
Dose [ppm]	0	1200	4000	12000 / 8000[1]	0	1200	4000	12000 / 8000[1]
[mg/kg bw/day]		103	323	1065 / 763		131	385	1096 /767
Number of animals evaluated	5	5	5	5	5	5	5	5
Liver								
- hypertrophy centrilobular	0	0	0	0	0	0	4	4
• minimal	-	-	-	-	-	-	4	
• severe	-	-	-	-	-	-		4
Thyroid								
- hypertrophy/hyperplasia, follicular	0	0	2	5	0	0	1	4
• minimal	-	-	2		-	-	1	
• moderate	-	-		5	-	-		4

[1] As body weight development was significantly impaired in all males of the high dose group the diet concentration was reduced in males and females to 8000 ppm from study day 18 onwards.
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

All other histopathological findings were either single observation, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental.

III. CONCLUSIONS

The administration of ethylester derivate of M656H062 via the diet to male and female Wistar rats for 4 weeks test substance-related adverse signs of toxicity at concentrations of 12000 ppm in males leading to a concentration reduction in males and females to 8000 ppm from day 18 of treatment onwards. Treatment related adverse effects were noticed after administered 12000 / 8000 ppm as indicated by impaired body weight development and food consumption, altered clinical chemistry parameters, increased absolute and relative liver weights e and histopathological findings in livers and thyroids of male and/or female rats indicative for liver enzyme induction. The findings in the 4000 ppm group – increased absolute and relative liver weight – without any associated alteration in clinical chemistry or and only minimal histopathological changes was considered to be treatment related but not adverse. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 4000 ppm in male (323 mg/kg bw/day) and in female (385 mg/kg bw/day) Wistar rats.

D Toxicological evaluation of M656PH062

No relevant toxicological alerts were identified for M656PH062. By weight of evidence M656PH062 was considered to be not genotoxic base on the in vitro and in vivo genotoxicity studies conducted with the ethylelester derivate of M656PH062 fulfilling the requirements for evaluation of ground-water metabolites. The available data on systemic toxicity – short-term toxicity study in rats - demonstrated that the compound is of lower toxicity than the parent molecule dimethenamid-P. Furthermore the determined exposure levels in ground-water are clearly below the threshold of toxicological concern for non-genotoxic compounds in Cramer class 3 i.e. 4.5 µg/l water.

In conclusion M656PH062 is considered to be of no toxicological relevance.

CA 5.8.2 Supplementary studies on the active substance

Studies evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: A plasma-protein binding study conducted with racemic dimethenamid done in rat and human blood cells and an enzyme induction study with racemic dimethenamid in rats are available. These studies have been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.8.2-1: Summary of supplementary studies conducted with dimethenamid-P and racemic dimethenamid

Study	Main effects	Reference
Investigation of the Potential of a Covalent Binding of [^{14}C]-dimethenamid (SAN 582 H) or its Derivatives to Rat and Human Hemoglobin	No increase in methemoglobin in rats. Strong binding of dimethenamid to rat hemoglobin, primarily to globin protein, but no incorporation of dimethenamid to human hemoglobin.	1992/12484
Investigation of Liver Enzyme Induction by dimethenamid (SAN 582 H) in Rats	Metabolism of dimethenamid involves oxidation steps mainly by cytochrome P450 dependent enzymes and glutathione conjugation and glucuronidation.	1994/11897

The pharmacokinetic studies indicated that dimethenamid may bind to blood components in rats. This was based on 3% of the radiolabeled material administered remaining in the blood fraction. Therefore, the nature of the interaction between dimethenamid and rat blood was investigated. The results of the study showed that racemic dimethenamid did not produce methemoglobin in rat blood following a four day treatment. Dimethenamid was shown to bind to rat hemoglobin, primarily to the globin portion, but no binding was demonstrated using human blood.
The difference in hemoglobin binding between humans and rats is explained by the difference in three dimensional structures between the 2 species. It is known from the literature that the cysteine residue β-125 in rat hemoglobin is accessible for chemical substitution, but in human hemoglobin, the sequence does not contain a cysteine residue in position 125. In summary, it can be concluded that the interaction between dimethenamid and hemoglobin is a species-specific reaction. This binding is irrelevant for humans.

In a further in vivo study with rats, the qualitative and quantitative effects of racemic dimethenamid on liver enzymes, blood and urine parameters were investigated. Oral administration of racemic dimethenamid to rats for 4 days induced several liver enzyme systems. It was demonstrated that the metabolism of racemic dimethenamid involves oxidation steps mainly by cytochrome P450 dependent enzymes, and glutathione conjugation and glucuronidation. Upon removal from treatment, there is a recovery from the liver changes.

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph are provided below.

Based on the available data the following assessment was drawn in the Annex I listing of Dimethenamid-P:

Binding of dimethenamid to blood components and effects on methemoglobin	- no effect on methemoglobin in rat blood - binding of dimethenamid to rat hemoglobin primarly to globin, but practically no binding to human hemoglobin..
Effects of Dimethenamid on liver enzymes	Induction of P-450 dependent liver enzymes in rats

In addition to these studies already evaluated an immunotoxicity study has been conducted to fulfill data requirements of the US-EPA.

The study conducted with dimethenamid-P in female mice in the presence of systemic toxicity, did not reveal any signs of immunotoxicity (by T-cell dependent antigen response) when administered via the diet over a period of 4-weeks

Thus, the conclusion for relevant endpoints for the current re-registration was amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Supplementary studies on the active substance	**Binding of dimethenamid to hemoglobin, production of methemoglobin*:** - no effect on methemoglobin in rat blood - binding of dimethenamid to rat hemoglobin primarly to globin, but practically no binding to human hemoglobin
	Liver enzyme induction of dimethenamid-P*: Induction of P-450 dependent liver enzymes in rats; 4-d, rat: NOAEL = 25 mg/kg bw/d *
	Immunotoxicity of Dimethenamid-P No evidence for immunotoxicity **No classification required**

* based on studies performed with racemic dimethenamid

A summary of the supplementary studies on dimethenamid evaluated for Annex I inclusion of Dimethenamid-P is given below.

Hemoglobin binding potential (1992/12484)

Two experiments were conducted. In the first experiment the amount of methemoglobin was determined. As part of a liver enzyme study (described below, 1994/11987), Wistar rats were administered unlabeled racemic dimethenamid via gavage in corn oil for 4 days at dosages of 0, 25, 100, 200 and 400 mg/kg bw. At the end of the treatment period, blood samples were collected. Methemoglobin levels were determined and reported as part of this study. In the second experiment the hemoglobin binding was observed. Blood samples were collected from a human volunteer and Wistar rats. The packed red blood cell component was obtained and hemolyzed blood components were incubated with 1 µL (0.2 µCi) ^{14}C-dimethenamid for 15 minutes at 37°C in a shaking water bath. The incubation mixture was then separated into globin and heme fractions and the radioactivity counted.

Blood samples from Wistar rats treated for four consecutive days with various concentrations of dimethenamid did not indicate an increase in methemoglobin.
Dimethenamid bound strongly to rat hemoglobin. However, no incorporation of dimethenamid into human hemoglobin was detected. Further investigation demonstrated that in rats the binding to hemoglobin was almost exclusively to the globin portion and very little radioactivity was found in the heme portion. The difference in hemoglobin binding between humans and rats is explained by the difference in three dimensional structures between the two species. It is known from the literature the cysteine residues β-125 in rat hemoglobin is accessible for chemical substitution, but in human hemoglobin the sequence does not contain a cysteine residue in position 125.

Conclusion
Racemic dimethenamid did not produce methemoglobin in rat blood following a 4 day treatment. Dimethenamid was shown to bind to rat hemoglobin, primarily to the globin portion, but no binding was demonstrated using human blood. It can be concluded that the interaction between dimethenamid and hemoglobin is a species specific reaction. This binding is irrelevant for humans.

Liver enzyme induction (1994/11987)

To determine liver enzyme levels, racemic dimethenamid was administered to groups of 6 male Sprague-Dawley rats via gavage in corn oil at dose levels of 0, 25, 100, 200 and 400 mg/kg bw for 4 consecutive days. A second group of 6 male rats were treated with dimethenamid at a dose level of 400 mg/kg bw/day for 4 days and then allowed a 4 day recovery period. At the end of the treatment period urine was collected for standard analysis and the animals were sacrificed and blood samples were drawn. All animals were subjected to a gross pathological examination and the liver, brain and kidneys were weighed.

No substance related mortality and no clinical signs were observed. Absolute and relative liver weights were increased at doses from 100 to 400 mg/kg bw/day. The only blood liver enzyme change was increased alanine aminotransferase at the high dose. At the high dose, urine volume was decreased and urine protein, creatinine and urea were decreased. The liver enzyme analysis demonstrated significant changes with treatment with racemic dimethenamid. Total cytochrome P450 and specifically PROD and EROD were increased with treatment. UDP-glucuronyl transferase was also increased at the two highest dose levels. Racemic dimethenamid also induces an increase in glutathione s-transferase and NADPH reductase levels at all treatment levels. However, these changes were slight at low dose. In addition, the inductions of these enzymes represent a physiological adaptation in the liver to remove the chemical and are not an adverse effect. In the absence of liver weight change or other liver enzyme changes at the low dose, the slight changes on these two enzyme systems is not considered an adverse effect. Glutathion was decreased at the high dose. This indicates that the glutathione conjugation pathway was saturated at the high dose level. All parameters investigated returned to control or near control levels following the four day recovery period.

Conclusion
Oral administration of racemic dimethenamid to rats for 4 days induces several liver enzyme systems. It was demonstrated that the metabolism of dimethenamid involves oxidation steps mainly by cytochrome P450 dependent enzymes and glutathione conjugation and glucuronidation. Upon cessation of treatment, the observed liver effects were almost fully reversible within a 4 day recovery period. The NOAEL was considered to be 25 mg/kg bw/day.

Report:	CA 5.8.2/1 [redacted] 2013f BAS 656-PH - Immunotoxicity study in female C57BL/6J Rj mice - Administration via the diet for 4 weeks 2013/1028329
Guidelines:	EPA 870.7800
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of Dimethenamid-P (Batch: COD-001509; Purity: 95.9%) in female C57BL/6J Rj mice was analyzed using dietary dose levels of 0, 500, 1500 and 4000 ppm (corresponding to mean intake levels of 120, 385 and 1167 mg/kg/d, respectively) for 28 days.
Treatment with Dimethenamid-P did result in systemic toxicity as assessed by body weight development and a treatment related increase of liver weights. 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). None of the parameters mentioned above was affected by treatment with Dimethenamid-P up to the highest dose level tested (limit dose).

Concurrent treatment with positive control substance, cyclophosphamide (10 mg/kg bw/d by oral gavage) 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 Dimethenamid-P does not bear an immunomodulatory/immunotoxic potential under the conditions of this study. The NOAEL for immunotoxicity was 4000 ppm corresponding to 1167 mg/kg bw/day. The NOAEL for systemic toxicity was 500 ppm corresponding to 120 mg/kg bw/day in female C57BL mice.

(DocID 2013/1028329)

I. MATERIAL AND METHODS

1. Test Material:	Dimethenamid-P
Description:	liquid/ brown, clear
Lot/Batch #:	COD-001509, Dimethenamid-P (BAS 656-PH):
Purity:	95.9%
Stability of test compound:	The test substance was stable over the study period (Expiry date Oct. 01, 2013).
2. Vehicle control:	Rodent diet
3. Positive control:	Cyclophosphamide monohydrate (CPA)
Description:	Solid / white
Lot/Batch #:	SLBC0666V
Purity:	102.3% (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date March 2015).
Vehicle for CPA:	Drinking water
4. Test animals:	
Species:	Mouse
Strain:	C57BL/6J Rj
Sex:	Female
Age:	41 ± 1 days at delivery; approx. 49 ± 1 days at start of administration
Weight at dosing:	18.2 ± 0.6 g
Source:	Centre d´Elevage R. Janvier, Route des Chênes Secs - B.P. 5, 53940 Le Genest St Isle, France
Acclimation period:	8 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum

Housing:	Pairwise in polycarbonate cages type M III with wire cover (Becker & Co., Castrop-Rauxel, Germany) with dust free wooden bedding. Mouse tunnel (red, transparent) and Nestlets NES 3600 (PLEXX b.v.; Elst Netherlands) were added for enrichment.
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: 10/02/2012 - 03/06/2013
(In life dates: 10-Oct-2012 (start of administration) to 08-Nov_2012 (necropsy))

2. Animal assignment and treatment:
Dimethenamid-P was administered to groups of 8 female mice at dietary concentrations of 0, 500, 1500 and 4000 ppm for 28 days. Additionally 8 female mice were treated orally (gavage) with 10 mg Cyclophosphamide monohydrate (CPA; positive control substance) per kilogram per day. 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

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. Diet preparations were performed once before the start of administration.

Analyses performed using a comparable batch prior to the start of the administration period revealed that the test-substance was stable in the diet for up to 46 days.

Homogeneity and concentration analyses of the diet preparations were performed at the beginning of the administration period for all concentrations. No test-article was determined in control diets.

Table 5.8.2-2: Results of homogeneity and concentration control analysis of Dimethenamid-P in rodent diet

Nominal Dose level [ppm]	Sampling	Concentration Mean ± SD# [ppm]	Relative standard deviation [%]	Mean of nominal concentration [%]
500	Oct. 09, 2012	479.5 ± 19.9	4.2	95.9
1500	"	1389.8 ± 19.3	1.4	92.7
4000	"	3966.7 ± 101.7	2.6	99.2

Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity of the Dimethenamid-P samples were in the range of 1.4 to 4.2%, which indicate the homogenous distribution of Dimethenamid-P in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 92.7 to 99.2% 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.

Table 5.8.2-3: Results of concentration control analysis of CPA in drinking water

Nominal Concentration [mg/mL]	Sampling / Analysis	Analytical concentration [mg/mL]	Mean of nominal concentration [%]
1	Oct. 08, 2012 / Feb. 19, 2013	0.928	92.8

The actual CPA concentrations was 92.8% of the nominal concentration confirming the correctness of the concentration.

The stability analysis conducted revealed the stability of the CPA solution for 32 days when stored frozen and for 7 days when stored ambient. Indirectly the concentration control analysis revealed a stability of CPA solution for 134 days when stored frozen [see **Table 5.8.2-3** above].

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 5.8.2-4: Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change and food efficiency	For test substance and the vehicle control groups: A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the dose group with the control group was performed using the t-test (two-sided) for the hypothesis of equal means

Table 5.8.2-5: Statistics of clinical pathology

Parameter	Statistical test
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 Wilcoxon-test (two-sided) for the equal medians

Table 5.8.2-6: Statistics of pathology

Parameter	Statistical test
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 pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

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.

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	

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this 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.

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated based upon individual values for body weight and mean weekly food consumption per cage-group of animals:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

with BW_x and BW_y body weight [g] at 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 dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Individual water consumption was observed by daily visual inspection of the water bottles for any overt changes in the volume. No water consumption values were recorded:

5. Analysis of the primary immune response:

Blood was drawn in the morning from non-fasted, isoflurane anesthetized animals after decapitation (in case the blood volume from retro-orbital sampling was insufficient). The blood sampling procedure and the subsequent analysis of the blood samples were carried out in a randomized sequence.

The assays of serum parameters were performed under internal laboratory quality control conditions with reference controls to assure reliable test results.

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 (cat. No. 4200-1, Life Diagnostics Inc, West Chester, USA). Each sample was diluted 1:101. SRBC-IgM concentrations outside the standard curve range were measured in a second test run with an appropriate dilution. Generally, two in-house anti-SRBC positive serum 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. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

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	
✓	✓		kidneys	✓	✓		spleen		✓		body (anesthetized animals)
✓	✓		liver	✓	✓		thymus				

No histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weight of animals of the high dose (4000 ppm) was significantly lower by -7.2% on study day 14 [see **Table 5.8.2-7** and **Figure 5.8.2-1**]. Consequently, the body weight change value was significantly lower by -97.1% on study day 14. These findings occurred in the absence of a similar pattern in mean food consumption, and therefore, were considered to be treatment-related, direct adverse systemic effects of Dimethenamid-P.

In contrast, the increased body weight change in the mid dose (1500 ppm) on study days 21 and 28 was considered to be incidental due to lacking dose-response relationship. Impaired body weight development was observed in CPA treated mice. Absolute mean body weight and body weight gain were reduced by 9% and 103.1%, respectively.

Figure 5.8.2-1: Body weight development of mice administered Dimethenamid-P for 28 days

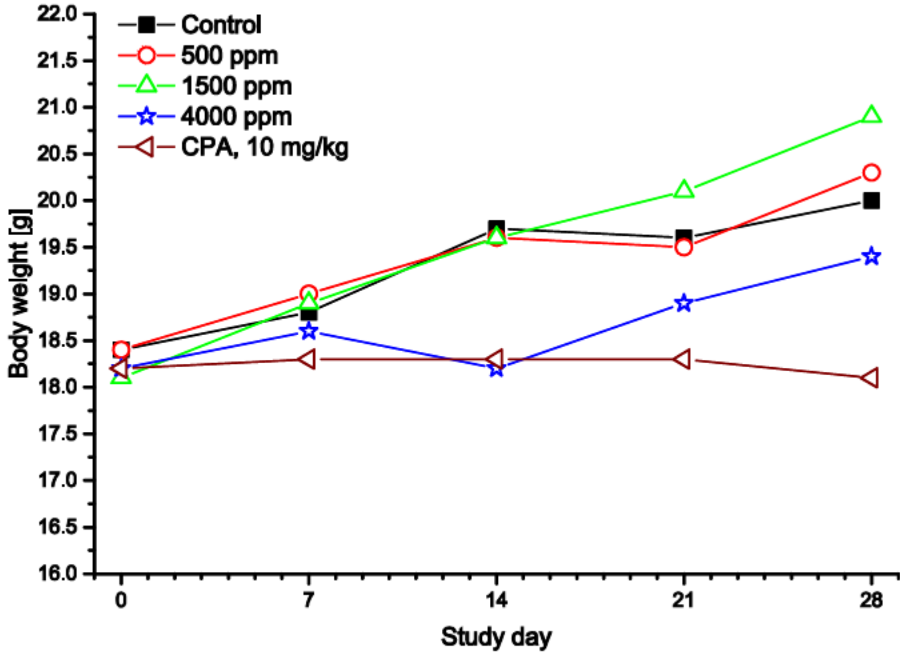


Table 5.8.2-7: Mean body weight of mice administered BAS 656 PH or Cyclophosphamide (CPA) for 28 days

Treatment	Dimethenamid-P				CPA
Dose level	0 ppm	500 ppm	1500 ppm	4000 ppm	10 mg/kg
Body weight [g]					
- Day 0	18.4	18.4	18.1	18.2	18.2
- Day 7	18.8	19	18.9	18.6	18.3
- Day 14	19.7	19.6	19.6	**18.2 ****	**18.3****
- Day 21	19.6	19.5	20.1	18.9	18.3*
- Day 28	20	20.3	20.9	19.4	**18.1****
Day 28 Δ% (compared to control)#		1.6	4.6	-2.6	**-9.0**
Overall body weight gain Day 0 to day 28 [g]	1.6	1.8	2.8 **	1.2	**0****
Δ% (compared to control)#		14.1	75.8	-23.4	**-103**

Values may not calculate exactly due to rounding of figues
* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnet-test, two sided)

C. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

Food consumption was significantly increased from study day 14 to 21 for mice of test group 3 (4000 ppm) [see **Table 5.8.2-8**]. However, food spilling was observed prior to the determination of food consumption on study day 21 in one cage of the test group. Thus, the finding was assessed as being not toxicologically relevant.

Table 5.8.2-8: Mean cumulative food consumption of mice administered Dimethenamid-P or Cyclophosphamide (CPA) for 28 days

Treatment	Dimethenamid-P				CPA
Dose level	0 ppm	500 ppm	1500 ppm	4000 ppm	10 mg/kg
Food consumption [g/animal*day]					
- Day 0 to 7	3.9	3.8	4.1	4.9	3.7
- Day 7 to 14	4.2	5.2	5.1	4.8	4.7
- Day 14 to 21	4.5	4.8	5.6	**6.5****	5.4
- Day 21 to 28	5.5	4.8	5.3	5.5	**4***
Cumulative food consumption [g/animal]					
- Day 0 to 28[§]	126.7	130.2	140.7	151.9	124.6
Δ% (compared to control)[#]		3%	11%	20%	-2%

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnet test, two sided)
[§] Values were calculated based on mean daily food consumption
[#] Values may not calculate exactly due to rounding of figures

The food consumption of the CPA treated animals was statistically reduced on days 21 and 28. This value was still within the normal range typical for the strain of mice. Taking into account the observed effects on body weight for CPA a treatment relation (see **Table 5.8.2-7:** above) can however not be excluded.

The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown in the following table:

Table 5.8.2-9: Calculated intake of Dimethenamid-P

Test group	Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/d)
		Females
1	500	120
2	1500	385
3	4000	1167

No treatment-related effects on water consumption were noted.

D. IMMUNOLOGICAL ANALYSES

1. Analysis of the primary T-cell dependent immune response

The SRBC specific IgM titers of the plasma samples from the animals treated with Dimethenamid-P were not relevantly altered as compared to the vehicle control group, whereas the SRBC titers were lower in mice of the positive control group (CPA, 10 mg/kg bw/d) [see **Table 5.8.2-10**].

Table 5.8.2-10: Analysis of the specific primary (IgM) immune response to SRBC in mice treated with Dimethenamid-P or Cyclophosphamide for 28 days

Treatment	Dimethenamid-P				CPA
Dose [ppm]	0	500	1500	4000	
[mg/kg bw/day]		120	385	1167	10
Specific IgM Titer (U/mL)					
- Mean ± SD	3,604 ± 2,568	3,788 ± 3697	2176 ± 1315	2389 ± 1755	**277**** ± 113
- Median	2,826	2576	1597	1988	242
- min. value	551	1938	1149	47	177
- max value	8305	12195	4353	5867	528

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test. two sided)

Even though the means seem to indicate trend towards a decrease immune response, the immune response was not considered to be affected by treatment. This is due to the following reasons:

1) There was no dose-response relationship.
2) The determined values show a high variability of the immune response within animals of the same group, as evident by the fact that the statistical evaluation did not show a statistically significant difference.
3) Except for one value of the low dose group (that was above) and one value in the high dose group (that was below), all individual immune responses in Dimethenamid-P treated groups were within the range of individual responses in control animals [see **Table 5.8.2-10.**].

G. NECROPSY

1. Terminal body and organ weight

Mean terminal body weights of the Dimethenamid-P groups were not affected by treatment [see **Table 5.8.2-11**].

Absolute liver weights were significantly increased in the mid and high dose group administered 1500 and 4000 ppm respectively by 19 and 37%. Correspondingly the relative liver weights were increased by 7 and 41% respectively. These weight increases were considered to be treatment related.

Absolute and relative thymus weights were significantly increased by 29% and 33% in the high dose group [see **Table 5.8.2-11**]. These weight changes were however considered to be incidental as they were clearly within the historical control range for these parameters [see **Table 5.8.2-11** below].
Relative kidney weights were slightly increased (+15, +7, +11%) when compared to control. The significantly increased kidney weights were considered to be incidental due to the lacking dose-response relationship.

In the positive control group however, the terminal body weights were significantly decreased. Moreover,

Treatment-related and statistically significant decrease of absolute and relative terminal body and absolute spleen weight were observed in the animals with the positive control substance CPA [see **Table 5.8.2-11**].

Table 5.8.2-11: Mean absolute and relative organ weights of female mice treated with Dimethenamid-P or Cyclophosphamide for at least 28 days

Sex		**Dimethenamid-P**				**CPA**
Dose [ppm]		**0**	**500**	**1500**	**4000**	
[mg/kg bw/day			**120**	**385**	**1167**	**10**
Terminal bodyweight	[g]	17.175	17.2	17.375	16.638	**15.863****
	[% of control]	100	100	101	97	**92**
Kidney, absolute	[g]	0.243	0.279	0.26	0.27	0.229
	[% of control]	100	115	107	111	94
Kidney, relative	[%]	1.405	1.627	**1.507***	**1.616****	1.448
	[% of control]	100	116	**107**	**115**	103
Liver, absolute	[g]	0.758	0.816	**0.901****	**1.039****	**0.684****
	[% of control]	100	108	119	138	90
Liver, relative	[%]	4.411	**4.735***	**5.191****	**6.231****	4.315
	[% of control]	100	**107**	**118**	**141**	98
Spleen, absolute	[g]	0.064	0.063	0.066	0.063	**0.053***
	[% of control]	100	98	104	98	**82**
Spleen, relative	[%]	0.364	0.361	0.378	0.367	0.331
	[% of control	100	99	104	101	91
Thymus, absolute	[mg]	41.125	45.125	48.125	**53.0***	44.5
	[% of control]	100	110	117	**129**	**108**
	[mg]	Historical control: 38.200 – 66,600 (mean 44,993)				
Thymus, relative	[%]	0.239	0.263	0.277	**0.318****	0.286
	[% of control]	100	110	116	**133**	119
	[%]	Historical control: 0.222 – 0.353 (mean 0.265)				

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross pathology

No gross pathological lesions were observed in this study.

III. CONCLUSIONS

In the presence of systemic toxicity, BAS 656-PH did not reveal any signs of immunotoxicity (by T-cell dependent antigen response) when administered via the diet over a period of 4 weeks to female mice. Thus, the NOAEL for immunotoxicity was set to 4000 ppm (1167 mg/kg bw/d), the highest dose tested (limit dose). The NOAEL for systemic toxicity was 500 ppm corresponding to 120 mg/kg bw/day in female C57BL mice.

The oral administration of the positive control substance CPA (10 mg/kg bw/d) led to findings indicative of immunotoxicity by T-cell dependent antigen response. This was represented by significantly lower SRBC IgM antibody titers as well as reduced spleen weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in female C57BL/6J Rj mice.

CA 5.8.3 Endocrine disrupting properties

A separate evaluation of potential endocrine disruption was not a data-requirement at the time of Annex I inclusion of Dimethenamid-P. However, this endpoint is considered intrinsically covered by the respective pivotal toxicity studies on racemic dimethenamid and dimethenamid-P.

The data package of Dimethenamid-P does not indicate a potential of Dimethenamid-P to affect the estrogen or androgen system.

There is no indication for adverse alterations in the thyroidal hormonal system from the available database for Dimethenamid-P. The noticed parathyroidal hyperplasia in the long-term rat study is not related to any indication of adverse effects due to parathyroidal hormonal imbalance i.e. altered ossification.

Racemic dimethenamid is part of the ToxCast program. Several assay react to varying doses of racemic dimethenamid, however, no conclusive picture has emerged. E.g dimethenamid is positive for androgen receptor antagonism but negative for other assays on the androgen receptor system. Also dimethenamid does not activate any assays indicating a linkage to the estrogen receptor system. There is some evidence that link dimethenamid to the activation of the CAR/PXR-system, which would be in line with the known pattern of liver enzyme induction and observed liver effects in the short- and long-term toxicity studies.

In addition to the pivotal toxicity studies the following literature was taken into consideration.

Table 5.8.3-1: Summary of literature studies indicating endocrine disruption with dimethenamid-P

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects	Reference BASF DocID
Literature: Reporter gene assays (not reported)	Multiple transcriptional targets			Activation of some transactivation systems, contradictory information for androgen receptor signaling no indication for estrogen receptor signalling, some indications of activation of the CAR/PXR axis.	AIR3-Dossier 5.8.3/1 Doc ID 2011/1295091
Literature: Reporter gene assays (not reported)	Multiple transcriptional targets			Limited indication for androgen receptor pathway, no indication for estrogen receptor pathway, no indication for thyroid axis	AIR3-Dossier 5.8.3/2 Doc ID 2010/1231552
Literature: Reporter gene assays (not reported)	Multiple transcriptional targets			Unclear linkage	AIR3-Dossier 5.8.3/3 Doc ID 2013/1371960

BASF response to in vitro studies – The presented in vitro studies below provide some evidence of interaction between dimethenamid-P and PXR pathway and to a lesser degree androgen receptor and PPAR in highly artificial cell systems These are in general lacking any form of metabolic competence which is especially critical in a setting where the substance is rapidly and nearly completely metabolized as is the case for dimethenamid-P.

In a recent evaluation of the predictivity of the high-throughput in vitro screening battery used in ToxCast, Thomas et al (Toxicological Sciences; 128(2), 398-417 (2012)) tested 84 statistical classification models (JMP Genomics software 5.0) with chemical descriptors (QSAR analysis) or in vitro assays as variables. After multiple iterations and cross validations, they identified balanced accuracy scores to be < 0.55 (or 55% predictivity) for 56 of the 60 endpoints. There was little to no predictive advantages of the cell based assays in comparison to QSAR tools, which are generally deemed to have low predictivity for complex endpoints (<70% accuracy, evaluation of multiple endpoints by EPA and EFSA).

This indicates that in vitro studies only provide an initial step in the evaluation of the toxicological properties of a compound.

A full set of higher tier studies is available for racemic dimethenamid and dimethenamid-P. This includes carcinogenicity studies in mouse and rats with no effect in oestrogen or androgen related organs either as neoplastic or non-neoplastic lesions. Furthermore a 1 year study in dogs and teratogenicity studies in rats and rabbits and are available.

In a two generation study in rats no indication of estrogenic or androgenic activity were observable. This includes no effect on time to pregnancy, pregnancy rate, gestation length, or any pup effects related to an estrogenic or androgenic activity were notable.
No spermatology investigation was performed. However, male fertility was indirectly assessed in the breedings in each generation which indicated no evidence of an effect on male fertility.

In conclusion BASF considers it highly unlikely that dimethenamid-P has estrogenic, androgenic, or anti-androgenic properties up to the maximum dose testable in mammalian systems.

There might be an enzyme induction related higher excretion of thyroid hormones considered, related to dimethenamid treatment in the rat. This potential increased excretion is considered an indirect effect not related to endocrine activity. Moreover, although there is evidence for enzyme induction, there is no indication that thyroid might be a target organ.

BASF considers it highly unlikely that dimethenamid-P does affect the thyroidal system up to the maximum dose testable in mammalian systems.

In conclusion, there is no evidence that dimethenamid-P has a human relevant endocrine related effect. Thus, the conclusion for relevant endpoints for the current re-registration was amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Endocrine disrupting properties	No endocrine effects on the oestrogen, androgen or thyroid hormone system.

In addition to the pivotal toxicity studies the following literature was taken into consideration.

Peer-reviewed Literature

Report:	CA 5.8.3/1 Shah I. et al., 2011a Using nuclear receptor activity to stratify hepatocarcinogens 2011/1295091
Guidelines:	<none>
GLP:	no

Executive Summary of the Literature

As part of the ToxCast program dimethenamid-P was tested for activity 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) in vitro. Dimethenamid-P was allocated to group D (negative for liver tumor progression). Dimethenamid-P was positive in activation assays for ATG_PPARg_Trans, , ATG_PXRE_CIS, CLZD_CYP2B6, CLZD_CYP3A4, NCGC_AR_Antagonist, NCGC_PXR_Agonist_human.
It is interesting to note, that dimethenamid-P was not associated with the activation of other assays targeting the same molecular endpoints. For example dimethenamid-P was negative in the other assays targeting the androgen receptor system (e.g. ATG_AR_Trans, NCGC_AR_Agonist, NVS_NR_hAR) the estrogen receptor system, or other assays linking a chemical to activation of CAR, PPAR or PXR.

In total it becomes apparent that the provided data on dimethenamid-P provides contradictory information not supported by the pivotal animal studies conducted and at best allows a linkage between dimethenamid-P and the CAR/PXR system, which would be in line with the in vivo observations of enzyme induction and an adaptive liver response.

Classification of study: Supplementary information

Report:	CA 5.8.3/2 Reif D.M. et al., 2010a Endocrine profiling and prioritization of environmental chemicals using ToxCast data 2010/1231552
Guidelines:	<none>
GLP:	no

Executive Summary of the Literature

This publication illustrates a profiling tool developed on the ToxCast database to prioritize chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this priorization tool was applied also to dimethenamid being part of the ToxCast program. The priorization tool focused on estrogen, androgen and thyroid pathways and thus incorporated those screening assays of the ToxCast program considered relevant for putative endocrine profiles. In addition it incorporated external molecular pathway databases i.e. Kyoto

Encyclopedia of Genes and Genomes (KEGG), Ingenuity software and the Online Mendelian Inheritance in Men repository. The tests in which dimethenamid showed an activity are the same as described in the publication of Shah et al 2011 [see CA 5.8.3/1 above]. The so-called ToxPi profile for dimethenamid shows no alert for thyroid or estrogen receptor pathways but a limited alert for androgen receptor pathways as well as for other nuclear receptors (not specified). Also a limited activity for the KEGG pathways is shown. The publication does not provide an absolute ranking but in visual comparison to activity alerts for other compound the ranking of activities for dimethenamid is low. No linkage between dimethenamid-P and other endpoints is provided.

Classification of study: Supplementary information

Report:	CA 5.8.3/3 Sipes N.S. et al., 2013a Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays 2013/1371960
Guidelines:	<none>
GLP:	no

Executive Summary of the Literature

Summary report on the ToxCast program. The publication makes some general statements on the progress of the ToxCast program. The data provided for dimethenamid- is the same as in Shah et al (2011). No linkage between dimethenamid-P and other endpoints is provided.

Classification of study: Supplementary information

CA 5.9 Medical Data

Information evaluated in the draft monograph of rapporteur member state Germany of Sep. 12, 2000: A survey was conducted in 1992 that did not report any cases of skin irritation, skin sensitization or other adverse health effects in personnel handling racemic dimethenamid and dimethenamid products. For the convenience of the reviewer, the surveillance is summarized below as extracted from the monograph. Furthermore no direct observation of e.g. clinical cases or poisoning incidents, no observations on exposure of the general population and no epidemiology studies were reported. Specific signs of poisoning or clinical tests were not known. This information has been evaluated by European authorities and Germany as Rapporteur member state (European Commission Peer Review Program).

Thus, the following conclusion was given in the list of endpoints for Annex I listing of Dimethenamid-P:

Medical data (Annex IIA, point 5.9)

	New product, no adverse health effects during research and experimental use of dimethenamid-P and its formulations.

Information on medical data obtained since then has been collected and evaluated and a literature search has been conducted to extend the evaluation basis. Thus, the conclusion for relevant endpoints for the current re-registration was adopted as follows:

Medical data (SANCO/11802 data point 5.9)

....	No adverse health effects during research, production and use of dimethenamid-p and its formulations.

A search in the literature databases listed below - restricted to "pps=human" and "ct d human" - has been performed on March 11th, 2014 via DIMDI-host for the following terms:

- Dimethenamid*
- CAS 90717-03-6 and 163515-14-8

Medline 66 (NLM)
Medline alert (NLM)
Embase 74 (Elsevier)
Embase alert (Elsevier)
Cochrane Library-Central
Biosys (Thomson Reuters)
gms (German Medical Science)
IPA International Pharmaceutical Abstracts (Thomson Reuters)

Deutsches Aerzteblatt (Aerzteverlag)

Cross check via Internet available databases:
CHEMID (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
Toxnet (http://toxnet.nlm.nih.gov/)

These searches revealed no relevant documents.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

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 dimethenamid. Thus, the medical monitoring program 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 dimethenamid-P exposure have not been observed.

Racemic Dimethenamid, medical survey

A survey was conducted in 1992 to determine if there were any adverse health effects in personnel handling dimethenamid and dimethenamid products that was evaluated for Annex I inclusion of dimethenamid-P. Interviews and information requests were made with 50 people handling dimethenamid and dimethenamid formulated products over a 7-year period. Personal was engaged in research on racemic dimethenamid and its product, in pesticidal production and filling and in field trials including mixing/loading and application of dimethenamid products. In conclusion there were no reported cases of skin irritation, skin sensitization or other adverse health effects.

CA 5.9.2 Data collected on humans

Some cases of irritation of the eyes have been registered in the BASF-internal clinical incident log exposed to dimethenamid in combination with other products. It is not clear whether dimethenamid was the cause for these irritations.

CA 5.9.3 Direct observations

Some cases of slight irritation of the skin, eyes or respiratory tract (including rhinitis and cough), head ache, nausea and dizziness have been reported to BASF in persons exposed to dimethenamid in combination with other active ingredients. These reports could not be verified, and it is not clear whether dimethenamid was the cause for these irritations. In one case sensitisation was discussed, which could not be verified.

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.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Specific signs of toxicity or specific clinical test methods are not known to BASF SE.

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.

Dimethenamid-P

DOCUMENT M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Compiled by:

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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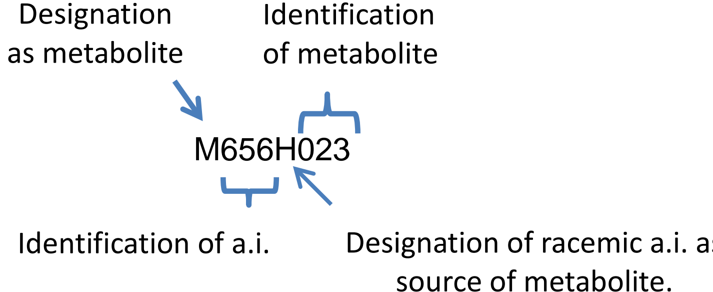
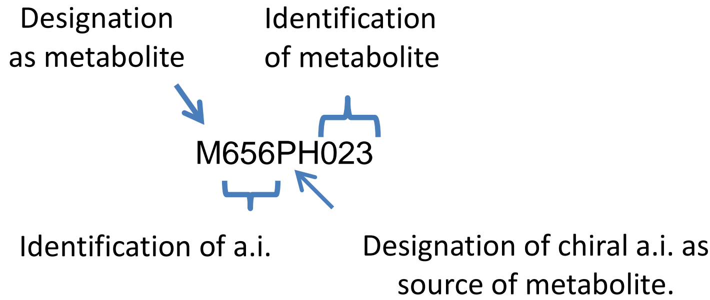
CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

General explanation on metabolite nomenclature in relation to stereoisomery.

Dimethenamid-P is the S-enantiomer of the racemic dimethenamid. For the active substance a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic pathway synthesis. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example M23:



Metabolites where the source of identification and the synthesis route could clearly be attributed to the chiral compound dimethenamid-P were assigned as follows:



The metabolic pathways in soil, water, mammals, and plants are equivalent for the racemic dimethenamid and dimethenamid-P (S-enantiomer). The metabolites derived from either racemic or enantio-enriched source are considered toxicologically equivalent and were taken into account for the assessment below.

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. For those studies which are considered still relevant for this section to give a complete picture of the metabolism and residue behavior of dimethenamid-P in plants and livestock, short summaries have been included for the reviewer's convenience.

Since over time different systems for metabolite designation evolved, the following concordance list has been included summarizing all metabolites observed in plants and livestock studies together with their various designations.

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
BAS 656 H (275.8)	**360720**	**-**	**87674-68-8**		
BAS 656 PH	**363851**	**-**	**163515-14-8**		
M656PH002	**Not assigned**	**M2**	**Not assigned**	Goat	
M656H003	**360717**	**M3**	**Not assigned**	Hen Goat	
M656H007	**360718**	**M7**	**Not assigned**	Goat Hen	
M656H008	**Not assigned**	**M8**	**Not assigned**	Hen	

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656H011	**403120**	**M11**	**Not assigned**	Hen Maize Sugarbeet	
M656PH011	**5886786**	**M11**	**Not assigned**	Soybean Maize	
M656PH014	**Not assigned**	**M14**	**Not assigned**	Goat[1] Soybean	
M656H017	**Not assigned**	**M17**	**Not assigned**	Goat Hen	
M656H022	**Not assigned**	**M22**	**Not assigned**	Goat Hen	
M656H023 (271.3)	**360715**	**M23**	**Not assigned**	Sugar beet Maize Soybean	
M656PH023	**5886780**	**M23**	**Not assigned**	Soybean	

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656H024	**Not assigned**	**M24**	**Not assigned**	Goat	
M656H025	**Not assigned**	**M25**	**Not assigned**	Goat Hen	
M656H026	**360716**	**M26**	**Not assigned**	Maize	
M656PH026 (361.5)	**5886781**	**M26**	**Not assigned**	Goat[1] Maize Soybean	
M656H027	**Not assigned**	**M27**	**Not assigned**	Hen Goat Sugar beet	
M656PH027 (343.4)	**5912598**	**M27**	**Not assigned**	Soybean	
M656H028	**Not assigned**	**M28**	**Not assigned**	Sugar beet	

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656H029	**Not assigned**	**M29**	**Not assigned**	Sugar beet	
M656H030	**Not assigned**	**M30**	**Not assigned**	Hen Maize Sugar beet	
M656PH030 (377.5)	**5296352**	**M30**	**Not assigned**	Goat[1] Maize Soybean	
M656H031	**360712**	**M31**	**Not assigned**	Goat Maize	
M656PH031	**5886777**	**M31**	**Not assigned**	Maize Soybean	
M656H032	**395234**	**M32**	**Not assigned**	Hen Maize	
M656H039	**368402**	**M39**	**Not assigned**	Hen	

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656H040	**Not assigned**	**M40**	**Not assigned**	Maize	
M656PH040	**Not assigned**	**M40**	**Not assigned**	Maize Soybean	
M656PH050	**Not assigned**	**M50**	**Not assigned**	Soybean	
M656H051	**Not assigned**	**M51**	**Not assigned**	Soybean Rotational Crop	
M656H067	**Not assigned**	**PL-3688**	**Not assigned**	Hen	
M656H073	**Not assigned**	**PL-1688**	**Not assigned**	Hen	
M656H075	**Not assigned**	**PL-2088**	**Not assigned**	Hen	
M656H079	**Not assigned**	**Not assigned**	**Not assigned**	Hen	

Table 6-1: Notations of parent and metabolites of dimethenamid-P

Metabolite designation				Compound found in	Structure/Name
BASF code/ Synonym (Mol. weight)	**Reg. No**	**Metabolite code**	**CAS-No**		
M656PH081	**Not assigned**	**M81**	**Not assigned**	Soybean Rotational Crop	
M656PH096	**Not assigned**	**Not assigned**	**Not assigned**	Goat[1]	
M656PH098	**Not assigned**	**Not assigned**	**Not assigned**	Goat[1]	

1 Metabolite was detected in the goat metabolism study conducted with the metabolite M656PH030

CA 6.1 Storage stability of residues

Storage stability in frozen crop matrices was evaluated during the initial EU Review of the active substance dimethenamid-P by the RMS Germany. It was considered applicable to extrapolate data on the stability of dimethenamid-P residues from the data on dimethenamid, the racemic mixture of S-dimethenamid (dimethenamid-P) and R-dimethenamid. Dimethenamid has been shown to be stable in commodities with high water content for at least 21 months when stored frozen at approximately -20°C. A study proving the stability dimethenamid residues up to 16 months in high oil content commodities was evaluated by JMPR.

In the present Annex I renewal dossier, storage stability for parent dimethenamid-P as well as its metabolites M23, M26, M27 and M30 data are provided. Data for strawberry fruits and maize whole plant representing high water content commodities, maize seed representing high starch content commodities, dried beans representing high protein content commodities as well as dried matrices are presented in report CA 6.1/1. This report contains also data on oilseed rape seeds which are not considered valid, possibly due to extraction issues. Additional data for oilseed rape seeds, soybean seed and sunflower seeds representing high oil content commodities are presented in report CA 6.1/2.

Storage stability could be demonstrated for parent dimethenamid-P as well as its metabolites M23, M26, M27 and M30 for all matrices. None of the studies summarized below were not part of the last peer review for dimethenamid-P and thus were evaluated before.

Since no additional animal feeding studies are submitted, a stability study of residues in products of animal origin is not considered necessary. All (labeled) residues obtained in animal metabolism studies were frozen within 24 hours and 6 months.

Report:	CA 6.1/1 Lehmann A., 2014a Investigation of the storage stability of BAS 656 H (Dimethenamid-P) and its metabolites M23, M26, M27 and M30 in plant matrices 2013/1335905
Guidelines:	EEC 7032/VI/97 rev. 5, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414, EPA 860.1380, OECD 506
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30
	Description:	
	Lot/Batch #:	BEAU201204, L74-120 (BAS 656 PH); L81-76, L59-90 (M23); L84-38-1, L82-95 (M26); 1213-32, 01311-28 (M27); L74-138 (M30)
	Purity:	96.4%, 96.5%; 98.8%, 98.4%; 91.5% (87.8%) 74.1%; 97.4%, 97.1%; 87.4%, 83.7%
	CAS#:	163515-14-8 (BAS 656 PH)
	Development code:	
	Spiking levels:	0.1 mg/kg
2.	**Test Commodity:**	
	Crop:	Strawberry (fruit), maize (whole plant, seed), dried bean (seed), oilseed rape (seed)
	Sample size:	Not reported

B. STUDY DESIGN AND METHODS

1. Test procedure

A freezer storage stability study was conducted dimethenamid-P (BAS 656 PH) and its metabolites M23, M26, M27 and M30 in plant matrices. Samples were fortified with the compounds at 0.1 mg/kg. The fortified samples were stored frozen at about -20°C in the dark and analyzed after 0, 31-52, 103-105, 179-181, 365-369, 544-550 and 745-748 days

2. Description of analytical procedures

For the analysis, either BASF method No L0179/01 or L0179/02 was used which determine the analytes by means of HPLC-MS/MS.

Dimethenamid-P and its metabolites M23, M26, M27 and M30 are extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant is diluted for determination by HPLC-MS/MS.

The limit of quantitation (LOQ) of the method is 0.01 mg/kg per analyte.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for procedural recoveries. In order to account for possible variations over the time investigated, mean procedural recovery results are given in addition.

After storage time of about 24 months dimethenamid-P proved to be stable in strawberry, maize (whole plant and seed) and dried beans for at least 745 days. In oilseed rape seed, dimethenamid-P was calculated to be stable for 36 days. M23 proved to be stable in strawberry, maize (whole plant and seed) and dried beans for at least 745 days. In oilseed rape seed, M23 was calculated to be stable for 2 days. M26 was calculated to be stable for 632 days in dried beans, for 432 days in maize seed, for 344 days in strawberry, for 8 days in maize whole plant and for 1 day in oilseed rape seed. M27 proved to be stable in strawberry, maize whole plant and dried beans for at least 745 days. In maize seed and oilseed rape seed, M27 was calculated to be stable for 50 and 2 days, respectively. M30 proved to be stable in strawberry, maize whole plant and dried beans for at least 745 days. In maize seed and oilseed rape seed, M30 was calculated to be stable for 517 and 2 days, respectively.

Table 6.1-1: Storage stability of dimethenamid-P in plant matrices

Day	**Mean recovery (%)**									
	A: mean in stored samples, % of nominal					B: mean procedural, in freshly spiked sample				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Strawberry fruit		**Maize whole plant**		**Maize seed**		**Dried bean seed**		**Oilseed rape seed**	
0	96 (125)	77	89 (99)	90	94 (97)	97	87 (119)	73	106 (102)	104
31-52	89 (109)	82	91 (93)	98	95 (86)	111	103 (99)	104	73 (70)	105
103-105	104 (105)	99	95 (95)	100	87 (82)	106	98 (94)	104	62 (62)	100
179-181	98 (99)	99	91 (91)	100	89 (94)	95	92 (89)	103	54 (53)	102
365-369	97 (97)	100	94 (89)	106	100 (89)	112	96 (88)	109	66 (62)	106
544-550	96 (99)	97	97 (93)	104	80 (81)	99	96 (91)	105	68 (66)	103
745-748	82 (83)	99	90 (93)	97	77 (76)	101	86 (83)	104	45 (42)	107

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-2: Storage stability of M23 in plant matrices

Day	Mean recovery (%)									
	A: mean in stored samples, % of nominal					B: mean procedural, in freshly spiked sample				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Strawberry fruit		**Maize whole plant**		**Maize seed**		**Dried bean seed**		**Oilseed rape seed**	
0	89 (100)	89	89 (99)	90	84 (98)	86	73 (100)	73	104 (98)	106
31-52	81 (92)	88	81 (96)	84	69 (73)	94	84 (85)	99	55 (50)	110
103-105	94 (98)	96	91 (95)	96	65 (70)	93	79 (81)	98	44 (44)	99
179-181	94 (94)	100	95 (92)	103	67 (72)	93	78 (79)	99	45 (45)	100
365-369	91 (97)	94	96 (94)	102	78 (75)	104	85 (82)	104	42 (38)	112
544-550	91 (95)	96	97 (101)	96	73 (77)	95	73 (72)	101	45 (42)	108
745-748	81 (84)	96	86 (90)	96	64 (70)	91	72 (64)	112	35 (32)	109

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-3: Storage stability of M26 in plant matrices

Day	Mean recovery (%)									
	A: mean in stored samples, % of nominal					B: mean procedural, in freshly spiked sample				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Strawberry fruit		**Maize whole plant**		**Maize seed**		**Dried bean seed**		**Oilseed rape seed**	
0	88 (102)	86	87 (104)	84	62 (102)	61	66 (103)	64	117 (110)	106
31-52	75 (100)	75	55 (76)	72	70 (80)	87	57 (77)	74	54 (53)	102
103-105	104 (102)	102	61 (62)	99	69 (68)	101	79 (79)	100	46 (42)	109
179-181	77 (76)	101	48 (48)	99	69 (73)	95	84 (87)	97	46 (46)	101
365-369	87 (92)	95	41 (41)	101	76 (71)	107	62 (60)	104	47 (45)	105
544-550	56 (60)	93	37 (37)	99	74 (76)	98	86 (83)	104	37 (36)	103
745-748	34 (36)	94	25 (26)	95	65 (68)	96	67 (67)	100	35 (32)	109

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-4: Storage stability of M27 in plant matrices

Day	Mean recovery (%)									
	A: mean in stored samples, % of nominal					B: mean procedural, in freshly spiked sample				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Strawberry fruit		**Maize whole plant**		**Maize seed**		**Dried bean seed**		**Oilseed rape seed**	
0	88 (100)	88	85 (104)	82	90 (111)	81	84 (95)	88	92 (89)	103
31-52	80 (89)	90	69 (87)	79	54 (63)	86	83 (86)	96	49 (45)	109
103-105	86 (91)	95	86 (88)	98	54 (61)	88	76 (78)	98	41 (40)	102
179-181	91 (92)	99	93 (92)	101	61 (71)	86	75 (78)	96	41 (41)	99
365-369	85 (90)	94	87 (87)	100	66 (72)	92	79 (77)	102	40 (36)	111
544-550	88 (93)	95	85 (87)	98	61 (66)	92	79 (77)	103	42 (39)	108
745-748	74 (76)	97	75 (77)	98	58 (67)	87	67 (68)	98	29 (27)	108

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-5: Storage stability of M30 in plant matrices

Day	Mean recovery (%)									
	A: mean in stored samples, % of nominal					B: mean procedural, in freshly spiked sample				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Strawberry fruit		**Maize whole plant**		**Maize seed**		**Dried bean seed**		**Oilseed rape seed**	
0	91 (100)	91	78 (93)	84	69 (92)	75	71 (80)	89	85 (83)	103
31-52	84 (94)	89	67 (83)	81	56 (70)	80	60 (71)	85	37 (36)	104
103-105	90 (96)	94	84 (88)	95	57 (63)	90	74 (81)	91	40 (41)	97
179-181	85 (87)	98	80 (78)	102	59 (63)	93	70 (77)	91	38 (39)	97
365-369	77 (84)	92	83 (88)	94	73 (70)	104	73 (73)	100	39 (36)	107
544-550	88 (94)	94	86 (87)	99	65 (68)	95	78 (78)	100	34 (34)	100
745-748	62 (66)	94	73 (81)	90	60 (63)	95	56 (57)	98	29 (28)	105

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

III. CONCLUSION

Overall dimethenamid-P and M23 are stable in all tested matrices when stored at about -20°C for up to 24 months, except for oilseed rape seeds, where dimethenamid-P was calculated to be stable for up to 36 days and M23 for up to2 days. M26 was calculated to be stable for 632 days in dried beans, for 432 days in maize seed, for 344 days in strawberry, for 8 days in maize whole plant and for 1 day in oilseed rape seed. M27 and M30 were calculated to be stable in strawberry, maize whole plant and dried beans for at least 24 months. In maize seed M27 and M30 were calculated to be stable for 50 and 517 days respectively and in in oilseed rape seeds both analytes were stable for 2 days each

The results in oilseed rape seed are not considered valid, possibly due to extraction issues, and an additional storage stability study with sunflower seed, soybean seed and oilseed rape seed was conducted to investigate the stability of dimethenamid-P, M23, M27, M26 and M30 further in high oil matrices. The interim report of this study is presented below.

Report: CA 6.1/2
Oppinger M., 2014a
Investigation of the storage stability of BAS 656 H (Dimethenamid-P) and its metabolites M23, M26, M27 and M30 in oily matrices
2013/1335906
Guidelines: EEC 7032/VI/97 rev. 5, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex III (Part A Section 8), EPA 860.1380, OECD 506
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30
Description:
Lot/Batch #: L74-120 (BAS 656 PH); L59-90 (M23); L82-95 (M26); L82-97 (M27); L74-138 (M30)
Purity: 96.5%; 98.4%; 74.1%; 92.3%; 83.7%
CAS#: 163515-14-8 (BAS 656 PH)
Development code:
Spiking levels: 0.1 mg/kg

2. **Test Commodity:**
Crop: Oilseed rape (seed), soybean (seed), sunflower (seed)
Sample size: 13 samples each matrix and sampling date (per analyte 2 fortified and 3 unfortified samples)

B. STUDY DESIGN AND METHODS

1. Test procedure

A freezer storage stability study was conducted dimethenamid-P (BAS 656 PH) and its metabolites M23, M26, M27 and M30 in oily plant matrices. Samples were fortified with the compounds at 0.1 mg/kg. The fortified samples were stored frozen at about -20°C in the dark and analyzed after 0, 3, 7, 14, 29, 59 and 94 days.

2. Description of analytical procedures

For the analysis, either BASF method No L0179/01 or L0179/02 was used which determine the analytes by means of HPLC-MS/MS.

Dimethenamid-P and its metabolites M23, M26, M27 and M30 are extracted with methanol. A portion of the extract is centrifuged and an aliquot of the supernatant is diluted for determination by HPLC-MS/MS.

The limit of quantitation (LOQ) of the method is 0.01 mg/kg per analyte.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated the mean procedural recoveries results are given in addition. After a storage time of about 3 months the mean recovery of the nominal in all stored samples are above 70% and show stability for dimethenamid-P and its metabolites M23, M26, M27 and M30 in oilseed rape, soybean and sunflower seeds.

Dimethenamid-P and its metabolites M23, M26 and M27 proved to be stable in oilseed rape seeds, soybean seeds and sunflower seeds for at least 94 days. Only for M30, mean recoveries below 70% were found in some samples after 3-59 days of storage. Correcting these values for procedural recoveries resulted in values >70% in most cases. Calculations based on degradation percentage leads to the conclusion that dimethenamid-P and its metabolites M23, M26, M27 and M30 going to be stable about at least 290 days.

Table 6.1-6: Storage stability of dimethenamid-P in oily plant matrices

Day	Mean Recovery (%)					
	A: mean in stored samples, % of nominal			B: mean procedural, in freshly spiked sample		
	A	B	**A**	B	**A**	B
	Oilseed rape seed		**Soybean seeds**		**Sunflower seeds**	
0	102 (100)	102	98 (107)	92	101 (102)	99
3	95 (88)	108	100 (96)	105	101 (98)	104
7	93 (90)	103	97 (95)	103	102 (97)	105
14	98 (94)	105	111 (100)	111	112 (100)	112
29	112 (103)	108	114 (106)	107	114 (105)	108
59	98 (88)	111	107 104)	108	113 (103)	109
94	97 (89)	109	106 (95)	112	110 (104)	106

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-7: Storage stability of M23 in oily plant matrices

Day	Mean Recovery (%)					
	A: mean in stored samples, % of nominal			B: mean procedural, in freshly spiked sample		
	A	B	**A**	B	**A**	B
	Oilseed rape seed		**Soybean seeds**		**Sunflower seeds**	
0	103 (96)	107	85 (103)	83	87 (106)	82
3	82 (73)	113	75 (72)	105	81 (77)	105
7	80 (81)	98	72 (72)	100	81 (80)	101
14	92 (81)	113	89 (84)	106	88 (85)	102
29	106 (100)	106	105 (96)	109	107 (103)	104
59	97 (87)	112	95 (86)	110	102 (94)	108
94	99 (92)	108	101 (96)	105	109 (109)	101

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-8: Storage stability of M26 in oily plant matrices

Day	Mean Recovery (%)					
	A: mean in stored samples, % of nominal			B: mean procedural, in freshly spiked sample		
	A	B	**A**	B	**A**	B
	Oilseed rape seed		**Soybean seeds**		**Sunflower seeds**	
0	100 (98)	102	79 (95)	83	77 (94)	82
3	76 (60)	127	78 (59)	131	83 (71)	117
7	87 (73)	119	77 (63)	123	93 (79)	118
14	104 (76)	136	96 (74)	130	107 (87)	123
29	84 (79)	106	93 (89)	105	101 (94)	108
59	130 (78)	166	133 (72)	187	157 (89)	177
94	77 (54)	145	85 (63)	135	103 (78)	132

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-9: Storage stability of M27 in oily plant matrices

Day	Mean Recovery (%)					
	A: mean in stored samples, % of nominal			B: mean procedural, in freshly spiked sample		
	A	B	**A**	B	**A**	B
	Oilseed rape seed		**Soybean seeds**		**Sunflower seeds**	
0	102 (99)	103	89 (110)	81	77 (99)	78
3	73 (62)	118	77 (74)	105	77 (72)	107
7	71 (74)	96	75 (72)	104	72 (78)	101
14	84 (72)	117	87 (82)	107	77 (77)	100
29	105 (94)	112	115 (107)	107	104 (102)	102
59	93 (86)	108	92 (85)	108	96 (95)	101
94	82 (69)	118	107 (91)	118	104 (98)	106

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

Table 6.1-10: Storage stability of M30 in oily plant matrices

Day	Mean Recovery (%)					
	A: mean in stored samples, % of nominal			B: mean procedural, in freshly spiked sample		
	A	B	**A**	B	**A**	B
	Oilseed rape seed		**Soybean seeds**		**Sunflower seeds**	
0	99 (101)	98	86 (110)	78	76 (100)	76
3	74 (75)	98	73 (78)	93	68 (69)	100
7	62 (70)	88	65 (72)	90	63 (68)	92
14	75 (77)	97	83 (85)	98	66 (75)	88
29	111 (107)	104	100 (95)	105	109 (105)	104
59	56 (63)	89	67 (75)	90	79 (98)	81
94	88 (63)	138	98 (77)	128	88 (81)	109

() Values in parentheses give mean recoveries corrected for mean procedural recoveries

III. CONCLUSION

Dimethenamid-P and its metabolites M23, M26, M27 and M30 have proven to be stable in oily plant matrices for at least 94 days. Stabilities of at least about 290 days have been calculated for all analytes.

This estimation going to be verified by results of this study up to 180 days. An extension up to 730 days by an amendment is planned.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

During the initial EU review of the active substance dimethenamid-P the metabolism was investigated for foliar application of the racemic mixture on root and tuber vegetables (sugar beet) and for soil application on cereals (maize). After Annex I inclusion, one additional metabolism studies with soil application of the racemic mixture on soybeans (pulses and oilseeds) was evaluated from France 2013. For Annex I renewal two new metabolism studies with foliar respective soil application of pure dimethenamid-P on soybeans (pulses and oilseeds) and maize (cereals) were conducted and summaries are below. The characteristics of these studies are summarized in Table 6.2.1-1.

Table 6.2.1-1: Summary of metabolism studies in plants

Group	Crop	Label position	Application and sampling details					Author, Year	DocID
			Method, F or G [a]	Rate (kg a.s./ha)	No	Sampling (DAT)	Remarks[d]		
Metabolism Studies- Peer Reviewed									
Root and tuber vegetables	Sugar beet	[3-^{14}C-tienyl]-labelled R,S-dimethenamid [b]	Foliar treatment, G [c]	0.45 (total: 135) 0.90+1.80+1.35+1.35 (total:5.4)	3 4	126 105	Germany 2000	Lam, 1998	1998/5173
Pulses and oilseeds	Soy-beans	[3-^{14}C-tienyl]-labelled R,S-dimethenamid [b]	Soil treatment, F	1.34	1	49, 100, 118	France, 2013	Atallah 1991	1991/11879
Cereals	Maize	[3-^{14}C-tienyl]-labelled R,S-dimethenamid [b]	Soil treatment, F	1.68 4.4	1 1	19, 50, 116, 130	Germany, 2000	Moore, Wendt 1995	1995/10129
Metabolism Studies – Not Peer Reviewed									
Cereals	Maize	[3-^{14}C-tienyl]-labelled R,S-dimethenamid [b] + [3-^{14}C-tienyl]-labelled dimethenamid-P	Foliar treatment, G	1.3 0.72	1 1	30 80-81 119-120	-	Bross, 2006	2006/1024513
Pulses and oilseeds	Soy-beans	[thienyl-2(5)-^{14}C]-labelled dimethenamid-P	Soil treatment, G	1.008	1	119	-	Schweda, 2012	2012/1144379

(a): Outdoor/field application (F) or glasshouse/protected/indoor application (G)
(b): Racemic mixture
(c): The plants were kept in a greenhouse for the first 41 days and then were moved to an outdoor shed until harvest. The first application was conducted after the cotyledons were completely unfolded (BBCH 10)
(d): Evaluator and Year, Remark given in EFSA Reasoned Opinion 2013

Dimethenamid is rapidly metabolized in plants and metabolism occurs through similar pathways in the three crops studied. The proposed metabolic pathway in plants involves conjugation of dimethenamid with glutathione and hydrolysis to the cysteine conjugate, both being considered transient intermediates undergoing rapid oxidation, deamination and/or decarboxylation to form many relatively polar metabolites. In maize and sugar beet matrices, all these metabolites are present at levels of less than 0.05 mg/kg or less than 10% of the TRR. Nevertheless, in soya bean forage and hay, more significant levels of M23 and M27 were recovered. These metabolites were identified in the rat metabolism and are not expected to be of higher concern than the parent compound. Moreover, bound radiocarbon increased with time, indicating incorporation of residues into the plant matrix. No parent compound was detected in any of the plant tissues at any sampling interval."

While the studies noted above are sufficient in the investigation of the metabolism of dimethenamid-P, there are some deficiencies in the maize and soybean study. The most notable are the application rate with racemic dimethenamid and the methods of identification of metabolite. In both the maize and soybean study TLC was used with minimal confirmation using GC or HPLC to confirm the metabolites. Since the sugar beet study was dosed at a high enough rate of the racemic mix to cover the use of dimethenamid-P and used HPLC for confirmation of metabolites, it is sufficient for covering the metabolism of dimethenamid-P. Therefore, new studies were conducted to investigate the metabolism of dimethenamid-P in maize and soybean. The 2006 maize study was performed using both racemic dimethenamid and dimethenamid-P. There was no difference observed in metabolism. In general, the results from the first two studies were confirmed in the new studies. Any differences observed were quantitative and not qualitative. However, these two studies were carefully designed to cover the use of dimethenamid-P and used state of the art techniques in metabolite quantification and identification. Therefore, results from the 2006 maize and 2012 soybean can be used to replace the results from the 1995 maize and 1991 soybean studies.

Report: CA 6.2.1/1
Bross M.,Glaessgen W.E., 2007a
Metabolism of 14C-Dimethenamid (14C-BAS 656 H) in corn
2006/1024513

Guidelines: EPA 860.1000, EPA 860.1300, EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants (draft of 22 July 1997), PMRA 98-02 (June 1998)

GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: Dimethenamid-P, BAS 656 PH; racemic dimethenamid, BAS 656 H
   Lot/Batch #: 824-1030 (thienyl-3-^{14}C): 96.8% (radiochemical purity), specific activity of a.s. 8.48 MBq/mg; 824-1029 (thienyl-3-^{14}C): 96.1% (radiochemical purity), specific activity 8.27 MBq/mg
   Purity: 824-1030: 100% (chemical purity)
   824-1029: 99.7% (chemical purity)
   CAS#: 163515-14-8 (dimethenamid-P)
   87674-68-8 (racemic dimethenamid)
   Development code:
   Spiking levels: Not reported

2. **Test Commodity:**
   Crop: Maize
   Type: Cereals
   Variety: Benicia
   Botanical name: *Zea mays*
   Crop parts(s) or processed commodity: Forage, forage/husks, grain/cobs, straw, husks, cobs and grain
   Sample size: Forage (ca. 1.2-1.6 kg), forage/husks (1.4-1.8 kg), grain/cobs (600-800g) straw (1.5-2.3 kg), husks (170-300 g), cobs (450-650 g) and grain (900-1300 g)

B. STUDY DESIGN AND METHODS

1. Test procedure

The study was conducted at the Agricultural Research Center of BASF in Limburgerhof, Germany. The cultivation of the crop took place in plastic containers located in a glass house, climatic chambers (phytotron) and in a glass roofed vegetation hall.

Maize was sowed into 10 plastic containers with soil. Four containers were used for dimethenamid-P and six containers were used for racemic dimethenamid. The maintenance of the crop was performed in accordance with normal agricultural practice. In experiment 1, the crop was treated once with racemic ^{14}C-dimethenamid at a rate of 1300 g a.s./ha. In experiment 2, the crop was treated once with ^{14}C-dimethenamid-P at a rate of 720 g a.s./ha. The application took place at growth stage BBCH 13-15.

For each experiment, a separate application formulation was prepared and applied with an automatic spray track. Unlabeled ^{12}C-dimethenamid-P was mixed with the radiolabeled test item (1:1) ^{14}C-dimethenamid-P and blank EC formulation BAS 656 08 H. Likewise, Unlabeled racemic ^{12}C-dimethenamid was mixed with the radiolabeled test item ^{14}C-dimethenamid (1/1.3, ^{14}C/^{12}C) and blank EC formulation BAS 656 02 H. This resulted in a nominal target rate of 720 g a.s./ha. The application formulations were analyzed by radio-HPLC for identity and purity check.

For both experiments, forage samples were taken 30 days after application (30 DAT). Samples of forage/husks and grain/cobs were taken at growth stage BBCH 83-85, 81 DAT for dimethenamid-P/ 80 DAT for racemic dimethenamid). At harvest (BBCH 89, 120 DAT for dimethenamid-P/ 119 DAT for racemic dimethenamid), mature plants were separated into straw, husks, cobs and grain. All samples were stored in a freezer at -18°C or below. Extracts were stored in a refrigerator or, for longer periods, in a freezer.

In order to demonstrate the storage stability of the residues in plants, the extraction and HPLC investigations were carried out at the beginning of the study. At the end of the metabolism study, a sample of maize forage (dimethenamid-P) was re-extracted. In addition, stored methanol extracts of maize forage (dimethenamid-P) and straw (racemic dimethenamid) were re-analyzed by HPLC.

Table 6.2.1-2: Design of the plant-uptake part – maize

Position of label	Racemic dimethenamid		Dimethenamid-P	
Intended use rate [g a.s./ha]	1300		720	
Number of applications	1		1	
Interval between applications [days]	-		-	
Sampling material	forage, husks, cobs, grain, straw		forage, husks, cobs, grain, straw	
PHI [days after last application]	forage	30	forage	30
	Forage/husks	81	Forage/husks	80
	Grain/cobs	81	Grain/cobs	80
	Straw	120	Straw	119
	Husks	120	Husks	119
	Cobs	120	Cobs	119
	Grain	120	Grain	119

2. Description of analytical procedures

The identification of the metabolites is based on the LC-MS/MS analysis of purified fractions from partition phases of the methanol extracts of forage and forage/husk (both racemic dimethenamid). Peak identification in the other samples was done by comparison of the authentic reference items. Quantitation is based on HPLC with radio-detection.
Frozen subsamples of the different maize matrices were mixed with liquid nitrogen and homogenized using a mill. Subsamples were extracted with methanol, and another set of subsamples was extracted with water.

Radioactivity was measured in aliquots using common combustion and LSC techniques. To characterize and purify the metabolites extracted with methanol, liquid/liquid partitioning was carried out and aliquots of the liquid phases were analyzed by LSC measurement.
For HPLC analysis, gradient elution on reversed-phase columns was applied. For metabolite profiles and quantitation of metabolites, one HPLC system was used, while further HPLC systems were used for comparison of the metabolite profiles and for confirmation of metabolite identification, for storage stability investigations, for fractionation of some phases obtained after liquid/liquid partition of the methanol extracts of maize forage / husks (racemic dimethenamid), for purification of some collected metabolite fractions, and for analysis of the diluted application solutions. Details of the system parameters are described in the study report.

II. RESULTS AND DISCUSSION

The uptake and the metabolism of dimethenamid (BAS 656 H) in maize was investigated after post-emergence spray application at a rate of 1 x 1300 g a.s./ha racemic ^{14}C-dimethenamid, or 1 x 720 g a.s./ha ^{14}C-dimethenamid-P, respectively.

After extraction procedures and various solubilization treatments of the residual radioactive residues (RRR) after solvent extraction, HPLC analyses were carried out for extracts and supernatants with a sufficient level of radioactivity. Identification of the metabolites is mainly based on LC-MS/MS investigations performed with purified fractions from forage (30 DAT) and forage/husks (80 DAT; both racemic dimethenamid). The metabolites in the other samples were assigned by comparison of the retention times with those purified fractions or reference items and of the elution profiles with those of the methanol extracts and the fractions after liquid/liquid partition, from which the fractions had been isolated. If identification was not possible, the peaks were characterized by their retention times. The grain samples taken 119/120 DAT represent the most important raw agricultural commodity (RAC) since grain is used for human food.

Total radioactive residues (TRRs)

The total radioactive residues (TRR) were determined by direct combustion analysis of small aliquots of homogenized sample material as well as by summarizing the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. The results for the samples under investigation are summarized in Table 6.2.1-3. No major differences were observed when comparing these calculated values with the results of replicate combustion analyses. Minor variations were due to inhomogeneities of the sample material and the limited amounts used for combustion. The TRR values calculated as the sum of ERR and RRR were used as 100% TRR for all further calculations.

Table 6.2.1-3: Total radioactive residues (TRRs) in maize samples following seed treatment of BAS 656 PH and BAS 656 H

TRRs in treated maize			
Matrix	**DAT***	**TRR determined by direct combustion [mg/kg]**	**TRR calculated [mg/kg]****
Dimethenamid-P			
Forage	30	0.748	0.718
Forage (re-extraction)	30	0.806	0.692
Forage/husks	81	0.189	0.179
Grain/cobs	81	0.015	0.015
Straw	120	0.538	0.483
Husks	120	0.044	0.042
Cobs	120	0.018	0.018
Grain	120	0.026	0.026
Racemic dimethenamid			
Forage	30	2.125	2.495
Forage/husks	80	0.279	0.278
Grain/cobs	80	0.020	0.019
Straw	119	0.822	0.694
Husks	119	0.075	0.069
Cobs	119	0.029	0.028
Grain	119	0.039	0.039

* DAT = Days after treatment
** TRR was calculated as the sum of ERR and RRR

Extractability

The extractability of radioactive residues from maize forage after post emergence treatment was high. As given in Table 6.2.1-4, 89.2% of the TRR were extracted for dimethenamid-P and 85.7% of the TRR for racemic dimethenamid.

Table 6.2.1-4: Extraction efficiency for residues of BAS 656 PH and BAS 656 H in maize samples

Matrix	DAA*	TRR calc.**	Distribution of radioactive residues							
			Combined methanol extract		Combined aqueous extract		ERR***		RRR****	
		[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Dimethenamid-P										
Forage	30	0.718	0.588	81.8	0.053	7.4	0.641	89.2	0.077	10.8
Forage/husks	81	0.179	0.126	70.3	0.011	6.3	0.137	76.6	0.042	23.4
Grain/cobs	81	0.015	0.009	60.5	0.001	4.0	0.010	64.4	0.005	35.6
Straw	120	0.483	0.292	60.4	0.068	14.1	0.360	74.5	0.123	25.5
Husks	120	0.042	0.024	57.0	0.002	4.5	0.026	61.5	0.016	38.5
Cobs	120	0.018	0.010	54.5.	0.001	5.2	0.011	59.6	0.007	40.4
Grain	120	0.026	0.010	36.4	0.003	9.7	0.012	46.1	0.014	53.9
Racemic dimethenamid										
Forage	30	2.495	1.946	78.0	0.192	7.7	2.138	85.7	0.357	14.3
Forage/husks	80	0.278	0.198	71.1	0.018	6.6	0.216	77.6	0.062	22.4
Grain/cobs	80	0.019	0.013	65.0	0.001	3.9	0.013	68.9	0.006	31.1
Straw	119	0.694	0.443	63.9	0.084	12.1	0.527	75.9	0.167	24.1
Husks	119	0.069	0.048	68.9	0.004	5.5	0.052	74.4	0.018	25.6
Cobs	119	0.028	0.016	58.2	0.002	5.8	0.018	63.9	0.010	36.1
Grain	119	0.039	0.017	42.7	0.004	9.7	0.020	52.4	0.019	47.6

* DAA = Days after application
** TRR was calculated as the sum of ERR + RRR
*** ERR = Extractable Radioactive Residue
**** RRR = Residual Radioactive Residue (after solvent extraction)

Partition characteristics

In order to classify the metabolites into organo-soluble and water-soluble ones and to support metabolite identification and assignment, the combined methanol extracts of forage, forage/husks, grain/cobs, straw, husks, cobs and grain were reduced to the aqueous phase and then partitioned with isohexane, dichloromethane and ethyl acetate. The phases obtained were concentrated and examined by means of HPLC and identification of metabolites was achieved by LC-MS/MS analyses of purified fractions from the dichloromethane phase of the methanol extract of maize forage and from three phases obtained after liquid/liquid partition of the methanol extract of forage/husks. Results are presented in Table 6.2.1-5.

Table 6.2.1-5: Extraction efficiency for residues of BAS 656 H and BAS 656 PH in maize samples

Matrix	DAA*	Combined methanol extract		Organosoluble						Sum organo-soluble		Water soluble		Re-covery %**
				Isohexane		Dichlor-methane		Ethyl acetate						
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	
Dimethenamid-P														
Forage	30	0.588	81.8	0.024	3.4	0.125	17.4	0.068	9.5	0.217	30.3	0.394	54.9	104.1
Forage/ husks	81	0.126	70.3	0.004	2.3	0.015	8.2	0.012	6.8	0.031	17.3	0.094	52.8	99.7
Grain/ cobs	81	0.009	60.5	0.001	3.7	0.002	16.2	0.001	6.3	0.004	26.2	0.004	26.7	87.5
Straw	120	0.292	60.4	0.009	1.9	0.051	10.6	0.034	7.0	0.094	19.5	0.207	42.9	103.2
Husks	120	0.024	57.0	0.001	3.2	0.004	9.3	0.003	6.7	0.008	19.2	0.015	36.1	97.0
Cobs	120	0.010	54.5	0.001	3.0	0.001	6.1	0.001	5.7	0.003	14.8	0.007	38.2	97.4
Grain	120	0.010	36.4	0.001	2.7	0.002	7.7	0.001	5.2	0.004	15.6	0.004	15.1	84.5
Racemic dimethenamid														
Forage	30	1.946	78.0	0.016	0.6	0.303	12.1	0.307	12.3	0.626	25.0	1.269	50.9	97.3
Forage/ husks	80	0.198	71.1	0.007	2.5	0.027	9.7	0.022	7.8	0.056	20.0	0.141	50.7	99.5
Grain/ cobs	80	0.013	65.0	0.001	3.9	0.004	20.0	0.001	7.7	0.006	31.6	0.006	30.1	94.8
Straw	119	0.443	63.9	0.015	2.1	0.073	10.5	0.050	7.3	0.138	19.9	0.306	44.2	100.3
Husks	119	0.048	68.9	0.002	3.4	0.008	11.5	0.006	8.1	0.016	23	0.030	43.0	95.7
Cobs	119	0.016	58.2	0.001	3.1	0.002	7.6	0.002	6.2	0.005	16.9	0.011	37.6	93.7
Grain	119	0.017	42.7	0.001	2.3	0.004	9.8	0.002	6.1	0.007	18.2	0.006	16.6	81.5

* DAA = Days after application

** Recovery calculated as isohexane + dichlormethane + ethyl acetate + water soluble [mg/kg] x 100 / combined methanol extract [mg/kg]

Identification and characterization of the extractable radioactive residues (ERRs)

The composition of the ERRs in maize samples is specified in Table 6.2.1-6.

Table 6.2.1-6: Summary of identified components in maize samples after treatment with ^{14}C-dimethenamid (720 g a.s./ha) and racemic ^{14}C-dimethenamid (1300 g a.s./ha)

Metabolite code (Reg. No of reference substance)	Metabolite identity	Matrix	Dimethenmid-P [mg/kg] (% TRR)	Racemic Dimethenamid [mg/kg] (% TRR)
M40 (one to several isomers)		Forage	0.017 (2.3)	0.098 (3.9%)
		Forage/ husks	0.021 (11.7)	0.028 (10.0)
		Grain/ cobs	<0.0005 (2.7)	<0.0005 (2.3)
		Straw	0.023 (4.7)	0.028 (4.1)
		Husks	0.001 (2.3)	0.003 (4.2)
		Cobs	<0.0005 (1.1)	<0.0005 (1.5)
		Grain	<0.0005 (1.1)	<0.0005 (1.0)
M30		Forage	0.128 (17.9)	0.461 (18.5)
		Forage/ husks	0.008 (4.6)	0.016 (5.6)
		Grain/cobs	<0.0005 (0.6)	<0.0005 (1.1)
		Straw	0.031 (6.4)	0.073 (10.5)
		Husks	0.001 (2.2)	0.001 (1.1)
		Cobs	<0.0005 (0.8)	<0.0005 (0.7)
		Grain	n.d. (n.d.)	<0.0005 (0.2)
M31 (or isomer) (Reg. No.: 360712)		Forage	0.031 (4.4)	0.052 (2.1)
		Forage/ husks	0.005 (2.6)	0.007 (2.6)
		Grain/cobs	<0.0005 (0.6)	<0.0005 (1.7)
		Straw	0.012 (2.5)	0.018 (2.7)
		Husks	<0.0005 (0.4)	0.001 (2.0)
		Cobs	<0.0005 (0.4)	<0.0005 (1.0)
		Grain	<0.0005 (0.2)	<0.0005 (0.2)

Table 6.2.1-6: Summary of identified components in maize samples after treatment with ^{14}C-dimethenamid (720 g a.s./ha) and racemic ^{14}C-dimethenamid (1300 g a.s./ha)

Metabolite code (Reg. No of reference substance)	Metabolite identity	Matrix	Dimethenmid-P [mg/kg] (% TRR)	Racemic Dimethenamid [mg/kg] (% TRR)
M26 (Reg. No.: 360716)		Forage	0.077 (10.8)	0.175 (7.0)
		Forage/ husks	0.002 (1.3)	0.003 (1.0)
		Grain/cobs	<0.0005 (0.6)	<0.0005 (1.9)
		Straw	0.006 (1.2)	0.012 (1.7)
		Husks	n.q. n.q.	n.q. (n.q.)
		Cobs	<0.0005 (0.4)	n.d. (n.d.)
		Grain	n.d. (n.d.)	n.d. (n.d.)
M23 (Reg. No.: 360715) and / or M39 (PL1588) and / or $C_{11}H_{13}NO_3S$ (isomer 2, charac-terized) and / or M37 (or isomer)	M23	Forage	n.d. (n.d.)	One to four of these components were represented by the corresponding HPLC peak and counted as "characterised".
	M39 (PL1588)	Forage/ husks	n.d. (n.d.)	0.005 (1.9)
	M37 (or isomer)	Grain/cobs	n.d. (n.d.)	One to four of these components were represented by the corresponding HPLC peak and counted as "characterised"
		Straw	n.d. (n.d.)	n.d. (n.d.)
		Husks	n.d. (n.d.)	n.d. (n.d.)
		Cobs	n.d. (n.d.)	n.d. (n.d.)
		Grain	n.d. (n.d.)	n.d. (n.d.)

n.d. Not detected

n.q. Not quantified; the metabolite M26 was detected in the concentrated methanol extract as a shoulder eluting after the peak at approximately 60.225 minutes and no separate quantitation was achieved; The complete peak was therefore counted as "characterized"

Storage stability
Storage stability investigations were performed at the beginning and at the end of the study. A subsample of maize forage (dimethenamid-P) was initially extracted approximately six months after sampling. No noticeable change of the extract was detected. Extracts were stable over a period of more than 15 months.

Proposed metabolic pathway
Dimethenamid is intensively metabolized in maize: the parent molecule BAS 656 H was not detected in extracts (only traces were detectable after liquid/liquid partition), but a large number of different HPLC peaks was observed. The metabolic pathway is comparable for both isomeric test items. The main degradation route proceeds via glutathione conjugation, enzymatic cleavage of the tripeptide and subsequent metabolic reactions on the resulting cysteine conjugate (loss of the amino group of the cysteine moiety and S-oxidation). Various sulfur-containing secondary metabolism products (modified cysteine conjugates) M30, M31 (or isomer), M26 and M37 (or isomer) represented the major part of the extractable radioactive residues. A second metabolic route is hydrolytic/oxidative displacement of the chlorine atom, followed by glycosylation (to form one to several isomers of the metabolite M40) or further transformation (e. g. oxidation, leading to the oxalamide M23, or cleavage of the acetamide to form the amine metabolite M39).

The proposed metabolic pathway of BAS 656 H in maize is shown in Figure 6.2.1-1. The results of this study are in accordance with previous investigations in maize, with metabolism studies in sugar beet and soybean, and also with data published in literature for related active substances.

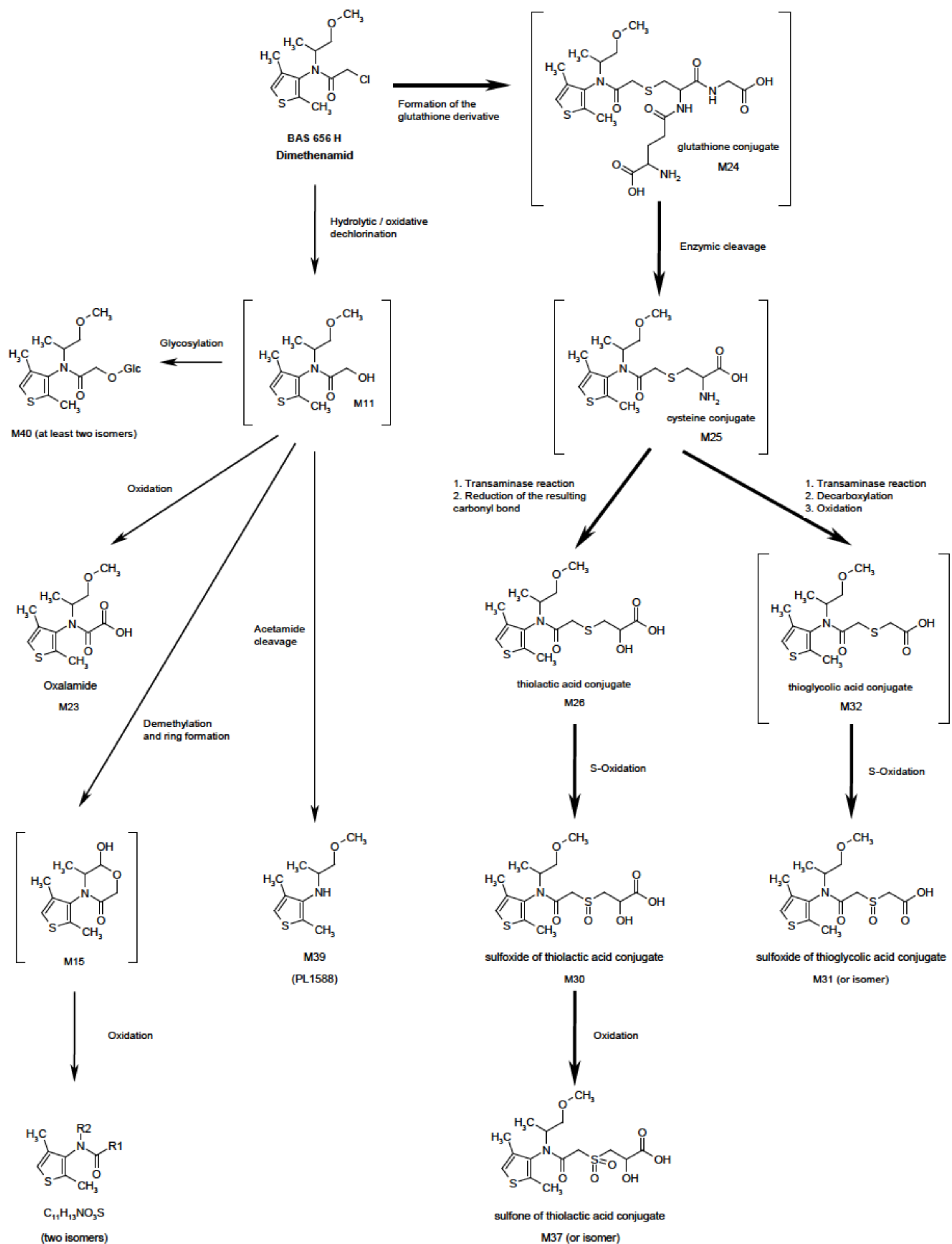
III. CONCLUSION

The maize metabolism study was conducted with ^{14}C-BAS 656 PH and ^{14}C-BAS 656 H after post emergence spray application at a rate of 720 g a.s./ha (dimethenamid-P) or 1300 g a.s./ha (racemic dimethenamid), respectively. The highest levels of total radioactive residues (TRR) were found in maize forage (sampled 30 days after treatment), straw (harvested 120/119 DAT) and forage/husks (sampled 81/80 DAT). In maize grain/cobs (sampled 81/80 DAT) and in husks, cobs and grain (120/119 DAT), the total radioactive residues were much lower. The extractability of radioactive residues with methanol and water were acceptable with similar results for dimethenamid-P and racemic dimethenamid.

Dimethenamid is intensively metabolized in maize: the parent molecule BAS 656 H was not detected in the extracts, but a large number of different HPLC peaks were observed. The metabolic pathway is comparable for both isomeric test items. The main degradation route proceeds via glutathione conjugation, enzymatic cleavage of the tripeptide, and subsequent metabolic reactions on the resulting cysteine conjugate. The various sulfur-containing secondary metabolism products (modified cysteine conjugates) M30, M31 (or isomer), M26 and M37 (or isomer) represented the major part of the extractable radioactive residues. A second metabolic route is hydrolytic/oxidative displacement of the chlorine atom, followed by glycosylation (to form one to several isomers of the metabolite M40) or further transformation (e. g. oxidation, leading to the oxalamide M23, or cleavage of the acetamide to form the amine metabolite M39).

The results of this study are in accordance with previous investigations in maize, with metabolism studies in sugar beet and soybean, and also with data published in literature for related active substances.

Figure 6.2.1-1: Metabolic pathway of ^{14}C-BAS 656 H in maize after post emergence treatment



Report: CA 6.2.1/2
Schweda Z. et al., 2012a
Metabolism of 14C-BAS 656-P H (14C-labeled Dimethenamid-P) in soybean
2012/1144379

Guidelines: EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF No 59 NohSan No 4200, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 6.2.1/3
Schweda Z., 2014a
Amendment No. 1 - Metabolism of 14C-BAS 656-P H (14C-labeled Dimethenamid-P) in soybean
2014/1036947

Guidelines: EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF No 59 NohSan No 4200, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: Dimethenamid-P, BAS 656 PH
   Lot/Batch #: 824-5070 (thienyl-2(5)-^{14}C): 98.9% (radiochemical purity), specific activity of a.s. 8.78 MBq/mg; L74-174 (unlabeled)
   Purity: 824-5070: 87.4% (chemical purity)
   L74-174: 99.4% (chemical purity)
   CAS#: 163515-14-8 (dimethenamid-P)
   Development code:
   Spiking levels: Not reported

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Pioneer 9091
Botanical name:	*Glycine max.* L.
Crop parts(s) or processed commodity:	Leaf, bean, hull and rest of plant
Sample size:	Leaf (622 g), bean (ca. 1 kg), hull (420 g) and rest of plant (430 g)

B. STUDY DESIGN AND METHODS

1. Test procedure

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany. The plant uptake part of the study was conducted in a phytotron, vegetation hall, or greenhouse.

Soybean was sowed into eight containers filled with soil. According to the study protocol, a single application was carried out on the soil (directly after sowing) with ^{14}C-dimethenamid-P at a nominal rate of 1008 g a.s./ha. The plants were harvested at 119 DAT.

For the preparation of the application formulation, ^{14}C-dimethenamid-P (dissolved in acetonitrile) and unlabeled dimethenamid-P were mixed to obtain a ratio of approximately 40:60. Thereafter, the test item was dissolved in a mixture of the blank formulation BAS 656 AB H and water. The purity of the application solution was confirmed using HPLC and the isotopic pattern was determined by HPLC-MS analysis. At growth stage BBCH 89, the leaves were picked up from the plants. The beans were picked from the stipes and separated from the hulls. The stipes were cut and minced. The samples were weighed and frozen.

2. Description of analytical procedures

Homogenized solid plant samples were weighed and combusted by means of an automatic sample oxidizer. The limit of quantitation in mg/kg was calculated from the twofold background radioactivity level (dpm/g matrix) divided by the corresponding specific radioactivity. For the quantitation of radioactivity in liquid samples a liquid scintillation counter was used.

Homogenization/solvent extraction: All samples (leaf, bean, hull and rest of plant) were homogenized. All samples were extracted three times with methanol and two times with water. After each extraction step solid material was separated from the extract by centrifugation and filtration. The filtered supernatants (methanol extracts and water extracts) were pooled and adjusted to a defined volume. The residue after solvent extraction was dried in a lyophilization device, homogenized and radio assayed.

Solubilization of the RRR: The residue after solvent extraction was extracted two times with 1% ammonia. The extraction procedure was similar to the solvent extraction. The residue after ammonium solubilization was resuspended in a sodium acetate buffer (pH 5), Macerozyme R-10 and Cellulase Onozuka R-10 were added in a ratio of 1:1 and the mixture was incubated on a shaker at 37°C for 48 h. After centrifugation, the resulting residue was resuspended in a phosphate buffer (pH 5.95), α-amylase, β-amylase and amyloglucosidase were added and the mixture was incubated on a shaker at 37°C over the weekend. After centrifugation, the resulting residue was dried in a lyophilisation device. The dried residue was resuspended in 10% NaOH and was heated for 3 h under reflux. Thereafter, the sample was filtered and the residue was washed with 10% NaOH at 80°C and then with water at 25°C. The extract and the washing solutions were pooled. The residue after NaOH solubilization was dried in an oven at 50°C. The NaOH solubilizate was mixed with concentrated HCI until pH 1 was reached. The mixture was stored overnight in a refrigerator and lignin was separated by centrifugation.

II. RESULTS AND DISCUSSION

Total Radioactive Residue (TRR)

In the present study, the TRR was calculated by summarizing the extractable radioactive residue (ERR) and the residual radioactive residue (RRR) after solvent extraction. The measured TRR of all four matrices showed no major differences to the calculated TRR values.

Table 6.2.1-7: Total radioactive residues (TRRs) in soybean samples following the application of BAS 656 PH

TRRs in treated soybean			
Matrix	DAT*	TRR determined by direct combustion [mg/kg]	TRR calculated [mg/kg]**
Leaf	119	2.816	2.595
Bean	119	0.648	0.693
Hull	119	0.719	0.821
Rest of plant	119	0.666	0.629

* DAT = Days after treatment
** TRR was calculated as the sum of ERR and RRR

Extractability
The extractability of the soybean matrices with methanol and water is summarized in Table 6.2.1-8.

Table 6.2.1-8: Extraction efficiency for residues of BAS 656 PH in soybean samples

Matrix	DAA*	TRR calc.**	Distribution of radioactive residues							
			Combined methanol extract		Combined water extract		ERR***		RRR****	
		[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Leaf	119	2.595	1.215	46.8	0.607	23.4	1.822	70.2	0.773	29.8
Bean	119	0.693	0.110	15.8	0.216	31.2	0.326	47.1	0.367	52.9
Hull	119	0.821	0.107	13.0	0.103	12.6	0.210	25.6	0.611	74.4
Rest of plant	119	0.629	0.188	29.9	0.051	8.0	0.239	38.0	0.390	62.0

* DAA = Days after application
** TRR was calculated as the sum of ERR + RRR
*** ERR = Extractable radioactive residue
**** RRR = Residual radioactive residue (after solvent extraction)

Identification, characterization and quantification of radioactive residues in leaf
Analysis of the leaf methanol extract with HPLC resulted in a pattern of 54 peaks, of which 15 were identified. The double peak at 56.8 and 57.4 min was identified as metabolite M27 and was the most abundant component (0.276 mg/kg or 10.7% TRR). The peak group eluting at about 6-8 min was assigned as polar fraction (0.051 mg/kg or 2.0% TRR). All other identified components (M81, M50, M40, M14 isomer A/ M14 isomer B / M30 / others, M31, M23 / M51, M26/ M11) were present at significantly lower amounts and accounted from 0.7% TRR (metabolite M50) up to 3.6% TRR (M81). HPLC analysis of the leaf water extract led to a pattern of 66 peaks, of which six were identified. The peak at 6.1 min was assigned as polar fraction and was the most abundant component (0.066 mg/kg or 2.5% TRR). Other identified components were M14 isomer A / M14 isomer B / M30 / others, M27, M23 / M51 and M26 / M11, which ranged from 0.3% TRR (M26 / M11) up to 2.0% TRR (M14 isomer A / M14 isomer B / M30 / others). In the solvent extracts 0.806 mg/kg or 31.1% TRR were identified and 1.016 mg/kg or 39.2% TRR were characterized by HPLC (each peak below or equal to 1.7% TRR). Taken together a total of 1.822 mg/kg or 70.2% TRR was identified and characterized in the ERR of soybean leaf.

Analysis of the ammonia solubilizate resulted in a pattern of 49 peaks, of which nine were identified. The peak group eluting at about 6-12 min was assigned as polar fraction and accounted 0.057 mg/kg and 2.2% TRR. Other identified metabolites were M14 isomer A / M14 isomer B / M30 / others, M23 / M51 and M26 / M11, which were significantly less abundant and accounted for up to 0.7% TRR (M14 isomer A / M14 isomer B / M30 / others). Analysis of the macerozyme and amylase solubilizate with another HPLC method predominantly led to early eluting peaks (about 5-12 min), which were assigned as polar fraction. The polar fraction accounted for 0.111 mg/kg or 4.3% TRR (macerozyme solubilizate) and 0.057 mg/kg or 2.2% TRR (amylase solubilizate). The residue after amylase solubilization was further characterized by NaOH solubilization and lignin precipitation. In the solubilizates 0.254 mg/kg or 9.8% TRR were identified and 0.149 mg/kg or 5.7% TRR were characterized by HPLC (each peak below or equal to 0.5% TRR). Taken together, a total of 0.618 mg/kg or 23.8% TRR were identified and characterized in the RRR of soybean leaf.

A summary of identified and characterized residues is compiled in Table 6.2.1-9. In soybean leaf, amounts of 2.440 mg/kg or 94.0% TRR were identified and characterized.

Identification, characterization and quantification of radioactive residues in bean
Analysis of the protease solubilizate of the methanol extract with HPLC resulted predominantly in early eluting peaks (about 7-12 min), which were assigned as polar fraction (0.046 mg/kg or 6.7% TRR). Analysis of the corresponding supernatant (supernatant of methanol extract) with HPLC led to a pattern of 109 peaks, of which each was below or equal to 0.4% TRR. The cyclohexane phase of the methanol extract and the supernatant of the water extract were also analyzed with HPLC. Similarly to the methanol extract, analysis of the protease solubilizate of the water extract with HPLC resulted predominantly in early eluting peaks (6-13 min), which were assigned as polar fraction (0.154 mg/kg or 22.3% TRR). A significant part of the radioactive residues in the methanol and water extract was precipitated with acetone and solubilized by protease. Therefore, the corresponding radioactivity, which was mainly assigned as polar fraction, was most probably incorporated into proteins. In the solvent extracts 0.201 mg/kg or 29.0% TRR were identified and 0.048 mg/kg or 7.0% TRR were characterized by HPLC (each peak below or equal to 1.2% TRR). Taken together, a total of 0.293 mg/kg or 42.4% TRR was identified and characterized in the ERR of soybean.

HPLC analysis of the solubilizates of the RRR (supernatant of ammonia solubilizate, macerozyme solubilizate and amylase solubilizate) with HPLC resulted predominantly in early eluting peaks (about 5-12 min), which were assigned as polar fraction. The polar fraction of the supernatant of the ammonia solubilizate accounted for 0.021 mg/kg or 3.0% TRR, the polar fraction of the macerozyme solubilizate for 0.112 mg/kg or 16.2% TRR and the polar fraction of the amylase solubilizate for 0.021 mg/kg or 3.1% TRR. The residue after amylase solubilization was further characterized by NaOH solubilization and lignin precipitation. In the solubilizates 0.154 mg/kg or 22.3% TRR were identified and 0.007 mg/kg or 1.1% TRR were characterized by HPLC (each peak below or equal to 0.7% TRR). The residues in the pellet of the ammonia solubilizate (0.109 mg/kg or 15.8% TRR) were most likely proteins, since they were precipitated by acetone, similar to the methanol and water extract. Taken together, 0.343 mg/kg or 49.5% TRR were identified and characterized in the RRR of soybean.

A summary of identified and characterized residues is compiled in Table 6.2.1-9. In soybean, amounts of 0.637 mg/kg or 91.9% TRR were identified and characterized.

Identifcation, characterization and quantification of radioactive residues in hull
HPLC analysis of the hull methanol extract resulted in a pattern of 64 peaks, of which eleven were identified. The peak group which eluted about 6-10 min was assigned as polar fraction (0.022 mg/kg or 2.6% TRR). The remaining identified components (M14 isomer A / M14 isomer B / M30 / others, M31, M23 / M51 and M27) were less abundant and accounted from 0.7% TRR (M14 isomer A /M14 isomer B / M30 / others) up to 1.1% TRR (M23 / M51 and M27). Analysis of the water extract with HPLC led to a pattern of 81 peaks, of which six were identified. The peak group at about 6-9 min was assigned as polar fraction and represented the main component (0.036 mg/kg or 4.4% TRR). The two other identified peaks (peak group) M14 isomer A / M14 isomer B / M30 / others and M23 / M51 were significantly less abundant and accounted for 0.5% TRR and 0.8% TRR, respectively. In the solvent extracts 0.099 mg/kg or 12.0% TRR were identified and 0.112 mg/kg or 13.6% TRR were characterized by HPLC (each peak below or equal to 0.4% TRR). Taken together, a total of 0.210 mg/kg or 25.6% TRR was identified and characterized in the ERR of soybean hull.

HPLC analysis of the solubilizates of the RRR (ammonia solubilizate, macerozyme solubilizate and amylase solubilizate) resulted predominantly in early eluting peaks (5-12 min), which were assigned as polar fraction. The polar fraction of the ammonia solubilizate accounted for 0.085 mg/kg or 10.4% TRR, the polar fraction of the macerozyme solubilizate for 0.230 mg/kg or 28.0% TRR and the polar fraction of the amylase solubilizate for 0.051 mg/kg or 6.3% TRR. The residue after amylase solubilization was further characterized by NaOH solubilization and lignin precipitation. In the solubilizates 0.367 mg/kg or 44.6% TRR were identified. Taken together, 0.486 mg/kg or 59.1% TRR were identified and characterized in the RRR of soybean hull.

A summary of identified and characterized residues is compiled in Table 6.2.1-9. In soybean hull, amounts of 0.696 mg/kg or 84.7% TRR were identified and characterized.

Identification, characterization and quantification of radioactive residues in rest of plant
HPLC analysis of the rest of plant methanol extract resulted in a pattern of 62 peaks, of which nine were identified. The peak group which eluted about 7-8 min was assigned as polar fraction (0.017 mg/kg or 2.7% TRR). The remaining identified components (M81, M14 isomer A / M14 isomer B / M30 / others, M31, M27, M23 / M51) were less abundant and accounted from 0.7% TRR (M23 / M51) up to 2.6% TRR (M27). Analysis of the water extract with HPLC led to a pattern of 72 peaks, of which one peak was identified. The peak at 5.9 min was assigned as polar fraction and represented the main component (0.012 mg/kg or 2.0% TRR). In the solvent extracts 0.078 mg/kg or 12.5% TRR were identified and 0.160 mg/kg or 25.5% TRR were characterized by HPLC (each peak below or equal to 1.2% TRR). Taken together, a total of 0.239 mg/kg or 38.0% TRR was identified and characterized in the ERR of soybean rest of plant.

HPLC analysis of the solubilizates of the RRR (ammonia solubilizate, macerozyme solubilizate and amylase solubilizate) resulted predominantly in early eluting peaks (6-13 min), which were assigned as polar fraction. The polar fraction of the ammonia solubilizate accounted for 0.046 mg/kg or 7.3% TRR, the polar fraction of the macerozyme solubilizate for 0.089 mg/kg or 14.2% TRR and the polar fraction of the amylase solubilizate for 0.020 mg/kg or 3.2% TRR. The residue after amylase solubilization was further characterized by NaOH solubilization and lignin precipitation. In the solubilizates 0.156 mg/kg or 24.7% TRR were identified. Taken together, 0.244 mg/kg or 38.8% TRR were identified and characterized in the RRR of soybean rest of plant.

A summary of identified and characterized residues is compiled in Table 6.2.1-9. In soybean rest of plant, amounts of 0.482 mg/kg or 76.7% TRR were identified and characterized.

Table 6.2.1-9: Summary of identified components in soybean matrices

Metabolite	Leaf		Bean		Hull		Rest of plant	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Polar fraction	0.342	13.2	0.355	51.2	0.425	51.7	0.185	29.4
M81	0.093	3.6	n.r.		n r.		0.011	1.7
M50	0.019	0.7	n.r.		n r.		n r.	
M40	0.034	1.3	n.r.		n r.		n r.	
M14 isomer A/ M14 isomer B/ M30/ others	0.135	5.2	n.r.		0.010	1.2	0.007	1.2
M31	0.025	1.0	n.r.		0.006	0.8	0.010	1.6
M27	0.321	12.4	n.r.		0.009	1.1	0.016	2.6
M23 /M51	0.053	2.1	n.r.		0.015	1.9	0.005	0.7
M26 / M11	0.038	1.5	n.r.		n r.		n r.	
Total identified	1.060	40.9	0.355	51.2	0.465	56.6	0.234	37.2
Total characterized	1.380	53.2	0.282	40.7	0.231	28.1	0.248	39.5
Total identified and characterized	2.440	94.0	0.637	91.9	0.696	84.7	0.482	76.7
Final residue	0.135	5.2	<0.001	<0.1	0.121	14.7	0.151	24.0
Grand total	2.576	99.3	0.637	91.9	0.816	99.4	0.633	100.8

n.r. Not reported

Metabolic pathway

The proposed metabolic pathway of dimethenamid-P in soybean is shown in Figure 6.2.1-2. A summary of the detected metabolites is given in Table 6.2.1-9. The initial step of most metabolites of dimethenamid-P is a presumed substitution of the chlorine atom by glutathione, which leads to the intermediate M24, which was proposed in the metabolic pathway in maize (DocID 2006/1024513). Cleavage of the amino acids glutamic acid and glycine from the glutathione side-chain of M24, followed by deaminiation and reduction, leads to the 3-mercapto-lactic acid derivative M26. Oxidation of the thioether group of M26 (S-oxidation) yields the sulfoxide M30. Decarboxylation of M30 followed by oxidation leads to metabolite M31. Oxidation of the sulfoxide group of M31 results in the sulfone M51. Further decarboxylation and oxidation (C- and S-oxidation) steps on M51 yield the sulfonic acid derivative M27. Alternatively, after the first decarboxylation step, the ether group is cleaved, which results in the isomers M14 isomer A and B. Glycosylation of M14 isomer A or M14 isomer B yields metabolite M81. Alternatively to the glutathione conjugation and thioether cleavage, hydrolytic dechlorination of dimethenamid-P yields the hydroxylated derivative M11. Oxidation of the hydroxyl group to a carboxyl residue leads to metabolite M23. Cleavage of the ether group of M23 results in metabolite M50. Metabolite M40 is the result of glycosylation of M11. The radioactivity of dimethenamid-P was also partially incorporated into sugar molecules. Since incorporation of dimethenamid-P moieties into sugar molecules requires the transformation of the degradation products into suitable compounds for the biosynthesis of carbohydrates, it is likely that C1 and / or C2 entities of dimethenamid-P enter anabolic biosynthetic pathways. Considerable portions of non-extractable residues were solubilized with macerozyme / cellulose and amylases / amyloglucosidase, and thereafter, identified by HPLC as polar fraction. Release

of ^{14}C-labeled polar components upon treatment of the residues after solvent extraction with polysaccharide-cleaving enzymes most probably reflects an incorporation of radioactivity into polysaccharides. Likewise, detection of polar components after protease treatment of the solvents extracts of bean reflects an incorporation of radioactivity into proteins.

Storage Stability
The quantitative analyses of the solvent extracts (methanol and water) of all matrices (leaf, bean, hull and rest of plant) with HPLC were generally carried out within a maximum of 91 days after sampling, besides analysis of the methanol extract of soybean rest of plant (294 days). The confirmatory analyses of the solvent extracts of all matrices with HPLC were generally carried out within a maximum of 151 days after sampling, besides analysis of the methanol extract of soybean leaf (273 days). However, initial analyses of the methanol extracts of rest of plant and leaf were carried out 84 days and 139 days after sampling, respectively. Thereby, the metabolite patterns of the methanol extract of rest of plant and leaf were similar to the above mentioned later analyses. Additionally, storage stability investigations (initial analysis, re-analysis of stored extract and re-extraction of stored sample and analysis) were performed exemplarily for the methanol extract of soybean leaf and rest of plant with HPLC. Thereby, the extracts were stored for at least 216 days. Re-extraction was performed after 270 days of storage of the samples. For both matrices the metabolite patterns were similar for initial analysis, re-analysis and re-extraction and no major changes were observed with HPLC. Therefore, the residues in the methanol extracts of leaf and rest of plant were at least stable for a period of 194 days and the residues in the samples were at least stable for a period of 210 days. Taken together, the storage stability was confirmed for all matrices and extracts over the period of investigation.

III. CONCLUSION

The present study describes the metabolism of dimethenamid-P (BAS 656 PH) in soybean after soil application of 1008 g a.s./ha (directly after sowing). Soybean plants were harvested 119 days after treatment/sowing and were separated into leaf, bean, hull and rest of plant.

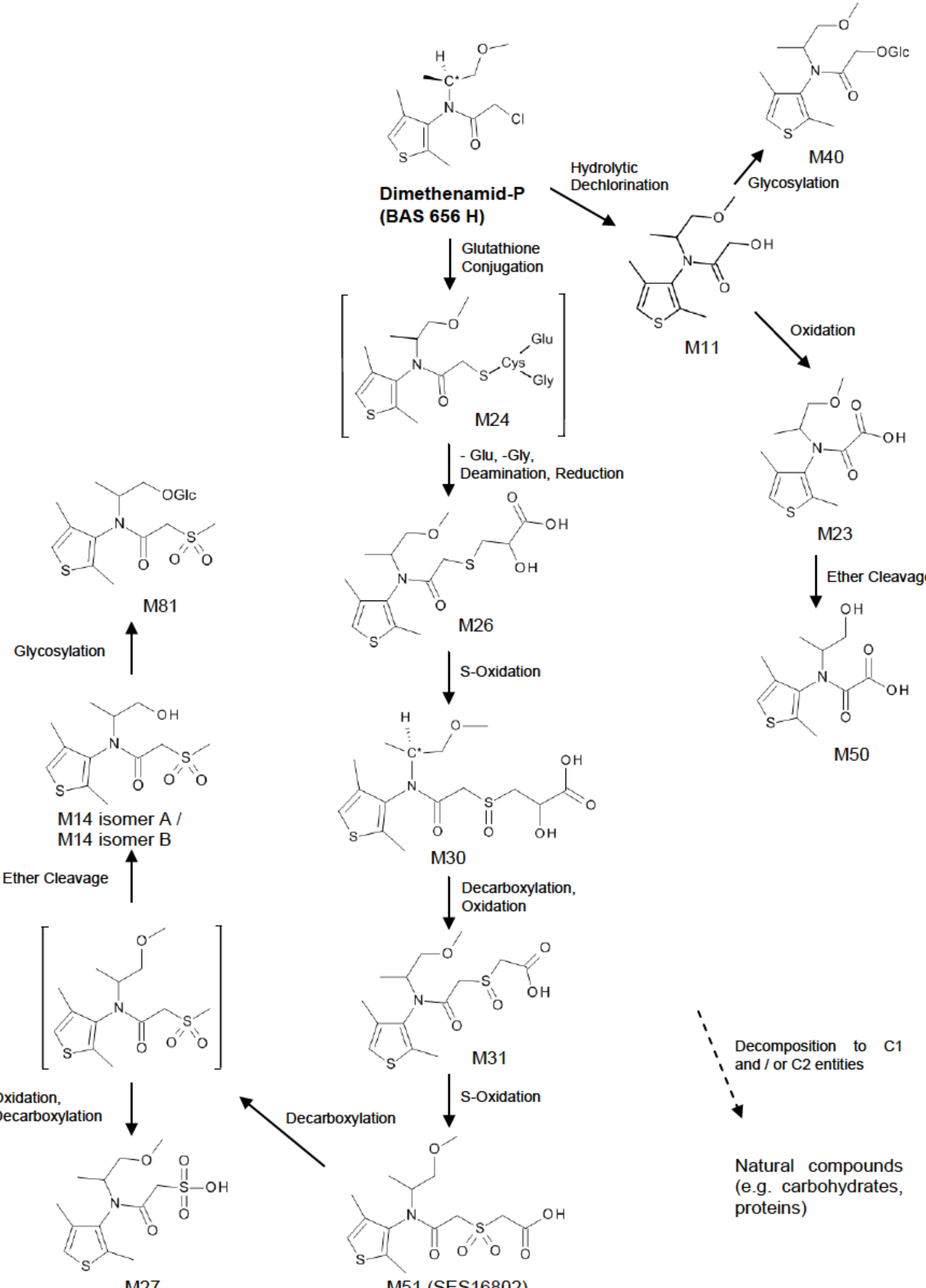
The highest total radioactive residue (TRR) was found in soybean leaf accounting for 2.595 mg/kg. TRR in the other matrices (bean, hull and rest of plant) was approximately 3-4 times lower. For bean the TRR accounted for 0.693 mg/kg, for hull 0.821 mg/kg and for rest of plant 0.629 mg/kg. The extractability of leaf with methanol and water was high and accounted for 70.2% of the TRR. The extractability for the remaining matrices was lower and accounted for 47.1% (bean), 25.6% (hull) and 38.0% (rest of plant) of the TRR. The residue after solvent extraction of all matrices was further solubilized, whereby 23.9% (leaf) to 59.0% (hull) of the TRR were additionally released.

HPLC-MS investigations of isolated fractions from soybean leaf methanol extract led to the identification of metabolites M11, M40, M23, M50, M26, M30, M31, M51, M14 isomer A, M14 isomer B, M81 and M27. Metabolites M11, M40, M23 and M50 are dechlorinated derivatives of the parent compound. The remaining metabolites result from substitution of the chlorine atom with glutathione and subsequent degradation of the glutathione moiety. The isolated MS fractions were used for co-chromatography experiments for peak assignment. Additionally, sugars were identified by co-chromatography experiments with a polar fraction from soybean leaf methanol extract, whereby two saccharide-specific HPLC methods were applied.

In all matrices the polar fraction was the most abundant (leaf, hull and rest of plant) or the only identified (bean) component and accounted from 13.2% (leaf) up to 51.7% (hull) of the TRR. The polar fraction was partially composed of sugars. Generally, the major part of the radioactivity of the polar fraction was incorporated into polysaccharides and / or proteins, because polar components were released upon treatment with polysaccharide cleaving enzymes (RRR of all matrices) and / or protease (ERR of bean). In soybean leaf metabolite M27, a sulfonic acid derivative of the parent compound, was the second most abundant component and accounted for 12.4% TRR. Other identified metabolites in soybean leaf, hull and rest of plant were significantly less abundant and were present at concentrations from 0.7% TRR (M23 / M51 in rest of plant) up to 5.2% TRR (M14 isomer A / M14 isomer B / M30 / others in leaf).

Initial HPLC analyses of the solvent extracts of all matrices were carried out within a maximum of 151 days after sampling. Additional storage stability experiments (re-analysis of stored extracts and re-extraction of stored samples) with leaf and rest of plant confirmed the stability of the metabolic pattern over the time interval used for this study.

Figure 6.2.1-2: Proposed metabolic pathway of dimethenamid-P in soybean



Overall summary plant metabolism

The metabolite profile was essentially identical for sugar beet, soybean and maize treated with dimethenamid and this pathway is common to several chloroacetamide herbicides. Essentially all metabolic processes occur after glutathione conjugation and are related to the glutathione moiety of the conjugate. Metabolic modification at other sites on the thiophene ring is not likely to occur.

The proposed metabolic pathway of dimethenamid-P in soybean, maize and sugar beet is similar and was confirmed as involving conjugation of dimethenamid with glutathione and hydrolysis to the cysteine conjugate. Both intermediates were considered as transient that undergo further rapid oxidation, deamination and/or decarboxylation. This results in many relatively polar metabolites. Incorporation of residues into the plant matrix increasing over time was found by bound radiocarbon.

The new metabolism studies in maize and soybean generally confirmed the results of the previous evaluated metabolism studies. Comparison with racemic dimethenamid and pure dimethenamid-P resulted in no significant differences in the metabolic pathway. It should be noted there are quantifiable differences in M23 and M27 between the older (1991) soybean and (1995) maize study compared to the new studies with dimethenamid-P in (2012) soybean and (2006) maize. In the case of maize (dimethenamid-P), M23 and M27 were not detectable in the 2006 study and were lower in the soybean study. This is due to better techniques in identification and quantification of metabolites. The limited presence of M23 and M27 was also confirmed in residues studies across multiple crop groups (see MCA, Section 6, Chapter 6.3). Since DMTA-P is metabolized so rapidly and extensively, it can be difficult to identify relevant metabolites across all crops tested. The only metabolite that was measurable in all commodities is M30 and therefore should be considered the most relevant. No parent compound was detected in any of the plant tissues at any sampling interval. The prevalence of Parent and M30 were also confirmed with residue studies (See MCA, Section 6, Chapter 6.3).

Dimethenamid is rapidly metabolized in plants and metabolism occurs through similar pathways in the three crops studied. Therefore metabolism of dimethenamid-P is sufficiently investigated.

Table 6.2.1-10: Overall summary of dimethenamid-P and metabolites found in metabolism studies

Crop	Soybean (1991/11879)			Maize (1995/10129)			Sugar beet (1998/5173)		Maize (2006/1024513)							Soybean (2012/1144379)			
Metabolite	Seed	Forage	Hay	Forage	Silage	Fodder	Root	Tops	Forage (30 DAT)	Forage/ Husks (81 DAT)	Grain/ Cobs (81 DAT)	Straw (120 DAT)	Husks (120 DAT)	Cobs (120 DAT)	Grain (120 DAT)	Leaf	Bean	Hull	Rest of Plant
mg/kg (%TRR)																			
Parent	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Polar Fraction^	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0 342 (13.2)	0.355 (51.2)	0.425 (51.7)	0.185 (29.4)
M23	0.03 (6.55)	0.47 (16.76)	0.14 (5.28)	0.0110 (3.58)	0.0023-0.0144 (0.57-3.57)	0.0072 (1.43)**	0.009 (1.09)	nd	nd	nd	nd	nd	nd	nd	nd	-	nd	-	-
M23/M51	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.053 (2.1)	nd	0.015 (1.9)	0.005 (0.7)
M26	nd	nd	nd	0.0070 (2.28)	0.0048-0.0169 (1.19-4.19)	nd	nd	nd	0.077 (10.8)	0.002 (1.3)	<0.0005 (0.6)	0.006 (1.2)	nq	<0.0005 (0.4)	nd	nd	nd	nd	nd
M26/M11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.038 (1.5)	nd	nd	nd
M27	0.03 (7.54)	0.20 (7.03)	0.28 (10.60)	0.186 (6.06)	0.0297 (7.38)	0.0126 (2.5)	0.0047 (6.01)	0.0185 (6.5)	nd	nd	nd	nd	nd	nd	nd	0.321 (12.4)	nd	0.009 (1.1)	0.016 (2.6)
M28	nd	nd	nd	nd	nd	nd	0.0018 (2.25)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
M29	nd	nd	nd	nd	nd	nd	0.0044 (5.66)	0.0029 (1.01)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
M30	-	-	-	0.0051 (1.66)	0.0117 (2.90)	0.0034 (0.67)	nd	0.0267 (9.39)	0.128 (17.9)	0.008 (4.6)	<0.0005 (0.6)	0.031 (6.4)	0.001 (2.2)	<0.0005 (0.8)	nd	-	-	-	-
M30/M31	0.04 (11.7)	0.17 (6.0)	0.20 (7.8)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M30/M14/ others	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.135 (5.2)	nd	0.010 (1.2)	0.007 (1.2)
M31	-	-	-	0.0049 (1.60)	0.0149 (3.70)	0.0102 (2.02)	nd	nd	0.031 (4.4)	0.005 (2.6)	<0.0005 (0.6)	0.012 (2.5)	<0.0005 (0.4)	<0.0005 (0.4)	<0.0005 (0.2)	0.025 (1.0)	nd	0.006 (0.8)	0.010 (1.6)
M40*1	nd	nd	nd	nd	nd	nd	nd	nd	0.017 (2.3)	0.021 (11.7)	<0.005 (2.7)	0.023 (4.7)	0.001 (2.3)	<0.0005 (1.1)	<0.0005 (1.1)	0.034 (1.3)	nd	nd	nd
M50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.019 (0.7)	nd	nd	nd
M51	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	-
M32/M11/ Unknowns	nd	nd	nd	0.0114 (3.71)	0.0024-0.0145 (0.60-3.60)	0.0283 (5.62)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
M81	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.093 (3.6)	nd	nd	0.011 (1.7)

nd= not detected, nq= not quantifiable M26 was detected as a shoulder peak and could not be fully separated
* one to several isomers
^ Polar Fraction consists of 2 or more sugars

CA 6.2.2 Poultry

During the initial EU Review of the active substance dimethenamid-P the metabolism was investigated in laying hen. Therefore, only a brief summary is included below for completeness. The characteristics of these studies are summarized in Table 6.2.2-1.

Table 6.2.2-1: Summary of available metabolism studies in laying hen

Group	Species	Label position	No of animal	Application details		Sample details		Author, Year	DocID
				Dose (mg/kg bw per d)	Duration (days)	Commodity	Time		
Laying poultry	hens	[3-^{14}C-tienyl]-labelled R,S-dimethenamid	3	10	4	Eggs	Daily	[redacted] 1990	1990/11110 1992/12430
						Excreta	Daily		
						Tissues	After sacrifice		

Following the administration of ^{14}C-dimethenamid to laying hens, the radioactivity was rapidly excreted: Based on the ^{14}C-radioactivity from excreta alone, it accounted for more than 77% of the total applied dose at sacrifice.

The residue concentrations in egg white were 0.19 (24 h), 0.20 (72 h) and 0.30 mg/kg (79 h). The egg yolk residue concentrations at 24, 72 and 79 h were 0.01, 0.24 and 0.62 mg/kg, respectively. Residue concentrations for fat, muscle (breast), muscle (thigh) and liver were 0.29, 0.45, 0.58 and 8.33 mg/kg, respectively. The low residue concentration in fat indicated that there was no risk of bioaccumulation (BAF = 0.002). Total organo-solubles in egg, different tissues and excreta ranged from approximately 30 to 75%TRR. The amount of radioactivity released into organic solvents varied from approximately 13 to 66%TRR.

Dimethenamid was rapidly and extensively metabolized and excreted. The metabolic pathway was via glutathione conjugation, reductive dechlorination followed by the formations of cysteine and mercapturate conjugations, and dimerization of a mercaptan intermediate as can be seen in excreta. The other pathways included O-demethylation and reductive dechlorination. In liver, the metabolites ≥0.05 mg/kg were M3 (reductive dechlorination) and M8 (O-demethylation and cyclization). The analysis of fat indicated parent dimethenamid was above 0.05 mg/kg. A large number of metabolites were detected which were not exceeding 10% of the TRR.

The residues in hens will be ≤0.01 mg/kg, which is below the LOQ of 0.01 mg/kg. Therefore, quantifiable residues are highly unlikely to occur in poultry eggs, organs and tissues originating from normal agricultural practice. No residues above LOQ are expected when animals are fed with feed items produced under GAP.

CA 6.2.3 Lactating ruminants

During the initial EU Review of the active substance dimethenamid-P the metabolism was investigated in lactating ruminant. Therefore only a brief summary of these studies are included below. The characteristics of this study are summarized in Table 6.2.3-1.
In preparation for active ingredient re-registration, an additional goat metabolism study was performed using M30. It is included in full below.

Table 6.2.3-1: Summary of available metabolism studies in lactating goats

Group	Species	Label position	No of animal	Application details		Sample details		Author, Year	DocID
				Dose (mg/kg bw per d)	Duration (days)	Commodity	Time		
Lactating ruminants	Goat	[3-^{14}C-tienyl]-labelled R,S-dimethenamid	1	8.9	4	Milk	Twice daily	1990	1990/11112 1992/12431 1990/11113 1992/12432 1992/12499
						Urine and feces	Daily		
						Tissues	After sacrifice		

Following the administration of ^{14}C-dimethenamid to lactating goats, the radioactivity was rapidly excreted. More than 59% and 28% of the dose was excreted in the urine and feces, respectively. The residue concentrations in milk were 0.17 to 0.51 mg/kg equivalent in the first 24 h, 0.69 to 0.90 mg/kg in 48 h, and 0.62 to 0.98 in 72 h. Thus, the residue level reached a plateau within 3 days. Residue concentrations in kidney, fat, muscle and liver were 9.92, 0.97, 0.97 and 16.62 mg/kg, respectively. The low residue concentration in fat indicated there was no risk of bioaccumulation (BAF = 0.004). Total organosolubles in urine, feces, milk and tissues ranged from approximately 30 to 82%TRR. Additional residues were partitioned into organic solvents, which varied from approximately 1 to 62%TRR.

Dimethenamid was rapidly and extensively metabolized in the goat study. The major metabolic pathway was through glutathione conjugation, followed by the formations of cysteine, mercapturate, sulfoxide of thioglycolic acid conjugations, and dimerization of a mercaptan intermediate. The other pathways included O-demethylation and reductive dechlorination. In liver, the metabolites ≥0.05 mg/kg were the M22 (dimer), M17 (mercapturate conjugate), M24 (glutathione conjugate) and M25 (cysteine conjugate). In kidney, metabolites M7 (O-demethylated parent), M17, M24 and M25 were above 0.05 mg/kg (M7 amounted for 24.1% TRR). The analysis of milk indicated M17, M24 and M25 were at 0.05 mg/kg or above. For muscle, the metabolites above 0.05 mg/kg were: M17, M24 and M25. Metabolites in fat above 0.05 mg/kg were M7 and M17. In urine and feces, metabolites M3 (reductive dechlorinated), M7, M17, M24, M25, and M31 (sulfoxide of thioglycolic acid) were identified.

This study was conducted using racemic dimethenamid. Therefore, the metabolism with the dimethenamid-P was included since the racemic mixture contained 50% of the P-isomer (dimethenamid-P). The residue in beef cattle will be ≤0.01 mg/kg, which is below the LOQ of 0.01 mg/kg. Therefore, quantifiable residues are highly unlikely to occur in ruminant milk, organs and tissues originating from normal agricultural practice. No residues above LOQ are expected when animals are fed with feed items produced under GAP.

While this study is considered adequate for informational purposes, metabolism and residue results indicate animals will never be exposed to dimethenamid-P but are consistently exposed to M30. Therefore, a metabolism study was conducted in 2013-2014 to investigate the metabolism of M656PH030 in lactating ruminants.

Report:	CA 6.2.3/1 [redacted] 2014 M656PH030 – Metabolism of 14C-M656PH030 in the lactating goat 2013/7002636
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock,OECD Test Guideline 503 - Metabolism in livestock,PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada),EEC 91/414 (1607/IV/97 Rev. 1),EEC 91/414 (7030/VI/95)
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	M656PH030
Lot/Batch #:	1089-1003 (thienyl-5-^{14}C); 1088-1005 (thienyl-5-^{13}C), L74-138 (unlabeled)
Purity:	^{14}C: 98% (radiochemical); specific activity 5.17 MBq/mg ^{13}C: 92.6% (chemical purity) Unlabeled: 83.7% (chemical purity)
CAS#:	Not specified
Development code:	
Spiking levels:	Not reported

2. **Test animals:** Goat

Species:	*Capra hircus*
Gender:	Lactating female
Age:	2-4 years
Weight at dosing:	40.9-43.6 kg
Number of animals:	1 treated animal + 1 companion animal
Acclimation period:	10 days
Diet:	Sprout Sweet Pellet 16% (Mills Fleet Farm, 1101 W. Upham Street, Marshfield, WI 54449) and hay ad libitum. Fresh feed (grain and hay) was offered twice daily. Feed consumption for the test animal was recorded from receipt until termination of the animal.
Water:	*Ad libitum*
Housing:	Throughout the study period, test animal was kept in individual stainless-steel metabolism cage. Cages were maintained in a closed building.

Environmental conditions

Temperature:	12.6-29.1°C
Humidity:	31-99%
Air changes:	Ventilation to maintain fresh air by an exhaust fan
Photoperiod:	12 h light/12 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:

Amount of dose:	12 mg/kg in the diet equivalent to 23.9 mg/capsule/day.
Food consumption:	About 2 kg/day
Vehicle:	Gelatin capsule as vector
Timing:	Once daily
Duration:	10 days

2. Sample collection

Milk collection:	Twice daily
Blood collection:	The blood was collected at the following time points: 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after the first dose. The 24-h collection occurred immediately before the administration of the second dose.
Urine and Feces collection:	Once daily
Interval from last dose to sacrifice:	2 h
Tissues collected and analyzed:	Liver, bile, muscle (flank and loin), kidney and fat (omental, renal and subcutaneous), GI-tract

3. Description of analytical procedures

The dosing solution, ^{14}C-M656PH030 was prepared by isotopic dilution with ^{13}C-M656PH030 and ^{12}C-M656PH030 in a 1:1:1 ratio to facilitate metabolite identification by mass spectrometry. The total amount of M656PH030 was 256.1 mg.

An aliquot of the dosing solution containing 23.9 mg of test material corresponding to a nominal dose of 12 mg/kg diet was added to each gelatin capsule half containing cellulose. The solvent was allowed to evaporate overnight and capped with the remaining capsule half. A total of ten dosing capsules were prepared. The capsules were stored under freezer conditions. Two stability capsules were prepared similarly and were used to verify concentration and stability of the test substance in the capsules during the dosing period.

Dosing and determination of TRR in blood, urine, feces, milk and tissues:

One lactating goat was administered ten consecutive daily oral doses of ^{14}C-M656PH030 (nominal dose of 12 mg/kg feed/day). The average actual dose was 13.79 mg/kg of feed/day and 0.5663 mg/kg body weight/day. The average actual dose was 115% of the nominal dose.

Day 1 blood was collected at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after the first dosing for combustion and LSC counting to determine the T_{max}. T_{max} was determined to be 2 h. As specified in the protocol, the goat was sacrificed 2 h (T_{max}) following the last (10th) dose.

During the course of the study, urine, feces and cage wash were collected daily. Milk was collected twice daily. Tissues (liver, kidney, loin muscle, flank muscle, omental fat, renal fat, subcutaneous fat and GI tract and contents), blood, and bile were collected at termination. Aliquots of all samples were assayed by combustion (except for milk), and LSC for ^{14}C content.

Extraction and metabolite patterns of feces, edible tissues and milk

Feces: An aliquot of homogenized Day 8 feces was weighed and extracted three times with methanol and twice with water. Each extract was centrifuged to separate solids, decanted volumes recorded and aliquots of each were analyzed by LSC. The methanol extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate which was analyzed by HPLC.

Liver: An aliquot of homogenized liver tissue was weighed and extracted once with ACN, twice with ACN-water, once with ACN, once with methanol and once with water. Each extract was centrifuged and aliquots of each were analyzed by LSC. The residual radioactive residues (RRR) were allowed to dry in the hood, and then aliquots were assayed by combustion analysis. The acetonitrile extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate which was analyzed by HPLC.

Kidney: An aliquot of homogenized kidney tissue was weighed and extracted once with ACN and twice with ACN-water. Each extract was centrifuged and aliquots of each were analyzed by LSC. The ACN extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate which was analyzed by HPLC. The post-extracted solid (PES) was subjected to protease digestion followed by extraction with acetonitrile. The acetonitrile extract was concentrated and then analysed by HPLC. The PES2 was further subjected to HCl hydrolysis and then extracted with ethyl acetate. The ethyl acetate extract was concentrated and reconstituted in HPLC mobile phase prior to HPLC analysis.

Loin muscle: An aliquot of homogenized loin muscle tissue was weighed and extracted once with ACN, three times with ACN-water and once with acetone. Each extract was centrifuged and aliquots of each were analyzed by LSC. The ACN extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate which was analyzed by HPLC.

Milk: An aliquot of Day 8 PM and Day 8 milk was weighed and extracted once with ACN and twice with ACN-water. Each extract was centrifuged and aliquots of each were analyzed by LSC. The extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate which was analyzed by HPLC.

Renal fat: An aliquot of homogenized renal fat tissue was weighed and extracted once with ACN, once with ACN-water and once with acetone and once with methanol. Each extract was centrifuged and aliquots of each were analyzed by LSC. The extracts were pooled and concentrated to the aqueous phase to give an aqueous concentrate. The aqueous concentrate was analyzed by HPLC with fractions collected every 30 seconds for LSC.

Metabolite patterns and isolation and characterization of metabolites in **urine:** Day 1-9 urine was analyzed by HPLC equipped with a liquid cell to determine the metabolite pattern. Day 2 urine and Day 8 urine were injected onto an HPLC equipped with a solid cell to isolate major and minor urinary metabolites for LC-MS identification. Day 1-9 urine samples were further analyzed by a second HPLC method to determine the isomeric metabolite pattern. The low residues found in edible tissues precluded metabolite isolation, purification, and characterization/identification of metabolites from tissues. Since the radioprofile in urine was qualitatively similar to the observed radioprofile in tissues, identification of metabolites was accomplished from pooled urine sample.

The isolated HPLC fractions were directly analyzed by LC-MS without further treatment. All isolated HPLC fractions were first analyzed by Q1 scanning in search for the distinct isotope pattern. If a metabolite was observed, MS2 scanning of all three isotopes (^{12}C, ^{13}C and ^{14}C) of the M656PH030 ion to reveal the daughter ions was performed.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Feces, milk, liver, kidney, loin muscle and renal fat were extracted to determine the nature of the radioactive residues. Urine and bile samples were amenable to HPLC analysis without any further purification. The extractability is summarized in Table 6.2.3-2.

Table 6.2.3-2: Extractability of residues of [^{14}C]-M656PH030 (Reg. No. 5296352) in goat matrices

Matrix	TRR		ERR[1]		RRR[2]		Recovery[3]
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Liver	0.219	100.0	0.096	43.7	0.149	68.0	111.7
Kidney	0.243	100.0	0.240	99.1	0.031	12.9	112.0
Loin muscle	0.016	100.0	0.011	64.6	0.006	35.2	99.7
Renal fat	0.014	100.0	0.008	54.3	0.004	28.8	83.1
Day 8 PM milk	0.027	100.0	0.021	79.5	0.003	11.0	90.5
Day 8 milk	0.018	100.0	0.016	87.6	0.001	5.8	93.4

1 ERR = Extractable radioactive residue
2 RRR = Residual radioactive residues
3 Sum of all extracts and the residue

The overall recovery of radioactivity is provided in Table 6.2.3-3. Approximately 100.6% of the administered dose was recovered, the majority of which was present in the urine (51.7%), feces (36.8%) and GI tract contents (11.7%) indicating that urinary and fecal excretion is the major elimination pathway. A small amount of radioactivity was recovered in bile (0.03%) and cage wash (0.3%). Radioactivity associated with edible portions (milk and tissues) accounted for 0.2% of the administered dose.

Table 6.2.3-3: Recovery of radioactivity after administration of ^{14}C-M656PH030 to lactating goats and total radioactive residues (TRRs) in milk, excreta and tissues

	^{14}C-M656PH030 Equivalents	
	TRR [mg/kg]	**% of the dose**
Urine	n r.	51.7
Cage wash	n r.	0.3
Feces	n r.	36.8
Muscle - loin	0.016	n.r.
Muscle - flank	0.017	n.r.
Liver	0.219	0.1
Kidney	0.243	<0.1
Fat - omental	0.011	n.r.
Fat - renal	0.014	n.r.
Fat - subcutaneous	0.017	n.r.
GI tract and intestinal contents	n r.	11.7
Termination bile	1.221	<0.1
Milk	n r.	0.1
Total	n r.	**100.6**

n.r. Not reported

The concentration of radioactivity in **blood** was measured at regular intervals throughout the 24 h period following the first dose administration. Results are provided in Table 6.2.3-4. The level of radioactivity in the blood reached to a maximum of 0.124 mg equiv/kg at 2 h post first dose.

Table 6.2.3-4: Residues after administration of ^{14}C-M656PH030 to lactating goats and total radioactive residues (TRRs) in blood from Day 1

	^{14}C-M656PH030 equivalents
Day 1 Blood	**TRR [mg/kg]**
0.5 h	0.016
1 h	0.046
2 h	0.124
3 h	0.111
4 h	0.094
6 h	0.083
8 h	0.055
10 h	0.047
12 h	0.040
24 h	0.013

TRR data for **milk** are provided in Table 6.2.3-5. Residues in the milk reached to a plateau maximum of 0.018 mg/kg within 3 days. Recovery of radioactivity in milk accounted for 0.1% of the administered dose, indicating that transfer of residues to milk was low. The ratio of radioactive residues in the cream and skimmed milk fractions was determined in a representative 24 h milk sample from the plateau region. The ratio of residues in the cream to skimmed milk was 1.1 : 1, with residues equivalent to 0.009 mg/kg and 0.008 mg/kg in the cream and skimmed milk, respectively. For this reason, whole milk was analyzed for unchanged M656PH030M and metabolites. The milk sample was extracted with acetonitrile and acetonitrile (1:1, v:v), resulting in 87.6% TRR (0.016 mg/kg) in the extract. The remaining 5.8% TRR was present in the solid debris which was determined by combustion analysis. The extracts were combined and concentrated for HPLC analysis.

Table 6.2.3-5: Residues after administration of ^{14}C-M656PH030 to lactating goats and total radioactive residues (TRRs) in milk

Day	**^{14}C-M656PH030 equivalents**
	TRR [mg/kg]
1	0.012
2	0.016
3	0.018
4	0.015
5	0.016
6	0.015
7	0.016
8	0.018
9	0.017
10	NA

Urine samples were directly measured and Day 8 **feces** sample was extracted with methanol and water, resulting in 58.9% TRR in the extract. The remaining 51.5% TRR was present in the solid debris which was analyzed by combustion analysis. The extracts were combined and concentrated for HPLC analysis (see Table 6.2.3-6).

Table 6.2.3-6: Residues after administration of ^{14}C-M656PH030 to lactating goats and total radioactive residues (TRRs) in urine, feces and cage wash

Time [h]	**Feces**		**Urine**	
	TRR [mg/kg]	**% of dose**	**TRR [mg/kg]**	**% of dose**
24	4.903	2.1	6.565	5.1
48	7.497	3.9	6.692	5.7
72	8.013	4.1	5.797	6.0
96	7.205	4.5	6.236	5.9
120	6.663	4.3	7.096	5.8
144	5.877	4.3	6.350	5.9
168	6.274	4.0	6.377	5.6
192	7.260	4.8	8.043	5.8
216	6.091	4.2	5.525	5.9
218	5.209	0.5	n r.	n.r.

n.r. Not reported

The homogenized **liver** sample was extracted with acetonitrile and acetonitrile/water (1:1, v/v), resulting in extraction of 43.7% TRR (0.096 mg/kg). The solid debris was analyzed by combustion analysis and contained 68.0% TRR (0.149 mg/kg). The extracts were combined and concentrated for HPLC analysis. The nature of unextracted radioactivity in the liver debris was investigated by treatment with digestive enzyme (protease) and further extraction of the digested material with acetonitrile. The process resulted in a further 10.6% TRR (0.023 mg/kg) being liberated. This protease hydrolyzate was concentrated for HPLC analysis.

The liver debris after initial extraction and protease treatment/extraction was investigated by acid hydrolysis. The process resulted in a further 36.0% TRR (0.079 mg/kg) being liberated.

The **kidney** sample was extracted with acetonitrile and acetonitrile/water (1:1, v:v), resulting in 98.9% TRR (0.240 mg/kg) in the extract. The remaining 12.9% TRR was present in the solid debris which was determined by combustion analysis. The extracts were combined and concentrated for HPLC analysis.

The homogenized **loin muscle** sample was extracted with acetonitrile, acetonitrile/water (1:1, v/v), and acetone, resulting in extraction of 64.6% TRR (0.011 mg/kg). The solid debris was analyzed by combustion analysis and contained 35.2% TRR (0.006 mg/kg). The extracts containing significant radioactivity were combined and concentrated for HPLC analysis.

The homogenized **renal fat** sample was extracted with acetonitrile, acetonitrile/water (1:1, v/v), and acetone, resulting in extraction of 54.3% TRR (0.008 mg/kg). The solid debris was analyzed by combustion analysis and contained 28.8% TRR (0.004 mg/kg). The extracts containing significant radioactivity were combined and concentrated for HPLC analysis.

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

Analysis of Day 2 **urine** using HPLC showed a pattern of 10 peaks, the most predominant component was identified as M656PH026. Three more metabolites were identified as M656PH098, M656PH096 and M656PH030 The relatively low concentration of other minor metabolites precluded further identification and they collectively accounted for 0.693 mg/kg (10.4% TRR). Analysis of the same urine sample using another HPLC method revealed the numbers of isomers (rotamers\stereoisomers) observed for M656PH098 (three peaks in a 1:2:1 ratio, consistent with four isomers) and in M656PH096 (two peaks, consistent with two isomers). Co-chromatography of Day 2 urine and a diluted [^{14}C]-M656PH030 dosing solution further confirmed the identification of M656PH030 in urine (see Table 6.2.3-7 for numerical results).

Analysis of Day 8 **urine** using HPLC showed a pattern of 8 peaks, similar to Day 2 urine, except M656PH096 was no longer detected and two additional metabolites M656PH002 and M6565PH014 were also identified. The other minor components were not identified due to relatively low concentration and they collectively accounted for 0.615 mg/kg (8.5% TRR). Analysis of the same urine sample using another HPLC method revealed the numbers of isomers (rotamers\stereoisomers) observed for, in M656PH002 (three peaks in a 1:2:1 ratio, consistent with four isomers) and for M656PH0014 (two peaks were observed, consistent with two isomers) (see Table 6.2.3-7 for numerical results).

Analysis of **bile** using HPLC showed a pattern of 3 peaks and analysis of **feces** extract using HPLC led to a pattern of 10 peaks. The metabolites observed were similar to those found in urine (see Table 6.2.3-7).

Table 6.2.3-7: Summary of identified components in various goat excreta

Metabolite	Urine (Day 2)		Urine (Day 8)		Feces (Day 8)		Bile	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
M656PH030	0.410	6.1	0.101	1.3	0.948	13.1	0.484	39.7
M656PH098	1.345	20.1	0.602	7.5	n r.		0.418	34.2
M656PH002	n.r.		0.770	9.6	0.163	2.2	n.r.	
M656PH096	0.673	10.1	n.r.		n r.		n.r.	
M656PH014	n.r.		0.683	8.5	0.235	3.2	n.r.	
M656PH026	3.570	53.4	5.205	64.7	2.436	33.5	n.r.	

n.r. Not reported

Analysis of **milk** extract using HPLC led to a pattern of 5 peaks which were identified with similar metabolites to urine. Analysis of **liver** extract using HPLC led to a pattern of 11 peaks. Analysis of **kidney** extract using HPLC led to a pattern of 10 peaks. Four unknown components in **kidney** accounted for 0.039 mg/kg (16.2% TRR) were detected. Analysis of **loin muscle** extract using HPLC led to a pattern of 8 peaks. Three unknown components in **loin muscle** accounting for 0.002 mg/kg (14.7% TRR) were detected. Analysis of **renal fat** extract using HPLC led to a pattern of 3 peaks. The radioactive residues identified in urine, feces, milk and edible tissues are summarized in Table 6.2.3-8 below:

Table 6.2.3-8: Summary of identified components in various goat tissues and milk

Metabolite	Milk (Day 8)		Liver		Kidney		Loin muscle		Renal fat	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
M656PH030	0.001	7.6	0.027	12.4	0.048	19.8	0.002	14.1	0.003	23.7
M656PH098	0.002	13.4	0.011	5.3	0.032	13.4	0.002	12.8	0.002	14.1
M656PH002	n.r.		0.010	4.5	0.014	5.9	n r.		n.r.	
M656PH096	n.r.		0.001	0.6	0.035	14.4	0.001	3.5	n.r.	
M656PH014	0.001	5.8	0.013	6.1	0.011	4.6	0.001	7.3	n.r.	
M656PH026	0.002	13.2	0.011	5.0	0.060	24.8	0.002	12.2	0.002	16.5
Total identified	0.007	40.1	0.074	33.8	0.201	82.9	0.008	49.9	0.008	54.3
Total characterized	0.008	47.5	0.045	20.5	0.039	16.2	0.002	14.7	n.r.	
Total identified and characterized	0.016	87.6	0.119	54.3	0.240	99.1	0.011	64.6	0.008	54.3
RRR	0.001	5.8	0.095	43.4	0.031	12.9	0.006	35.2	0.004	28.8
Grand total	0.017	93.4	0.214	97.7	0.272	112.0	0.016	99.7	0.012	83.1

n.r. Not reported

Proposed metabolic pathway
The proposed metabolic pathway of [thienyl-5-^{14}C]-M656PH030 in lactating goat is provided in Figure 6.2.3-1. [Thienyl-5-^{14}C]-M656PH030 was extensively metabolized in the lactating goat. The unchanged M656PH030 was found in portions below 24% TRR in matrices except in bile where M656PH030 comprised 40% TRR. The main component in urine and feces was M656PH026, formed by reduction of M656PH030, indicating that urinary and fecal excretion is the major elimination pathway accounting for 89% of the administered dose. In extracts of milk, liver, kidney, loin muscle, and renal fat the main components were M656PH026 and M656PH030. The metabolite M656PH002 was formed by demethylation of the ether group and substitution of the 2-hydroxypropanoic acid with a methyl group, followed by oxidation of the sulphur group to sulfoxide and subsequent conjugation with glucuronic acid leading to M656PH098. This metabolite was present in significant levels in milk, kidney, loin muscle, and renal fat. Further oxidation of M656PH002 to the corresponding sulfone led to M656PH014 followed by glucuronidation yielding M656PH096. M656PH002, M656PH014 and M656PH096 which were present in some tissue extracts, generally at lower levels.

Thus the following metabolic transformations occurred

- mainly *via* reduction of the sulfoxide moiety yielding the metabolite M656PH026
- demethylation of the ether group and cleavage of the 2-hydroxypropanoic acid in M656PH026 followed by S-methylation and S-oxidation led to M656PH002 and M656PH014
- glucuronidation of M656PH002 and M656PH014 yielded M656PH098 and M656PH096.

Storage Stability
Initial analyses of the tissue, milk and excreta extracts were carried out within 6 months of sacrifice and as such no formal sample storage investigations were required for this study.

III. CONCLUSION

One lactating goat was administered ten consecutive daily oral doses of ^{14}C-M656PH030 (nominal dose of 12 mg/kg feed/day). The average actual dose was 13.79 mg/kg of feed/day and 0.5663 mg/kg body weight/day. The average actual dose was 115% of the nominal dose

The total radioactive residues in milk, muscle and fat were very low and accounted for a maximum of 0.018 mg/kg. The residues in the other edible matrices accounted for 0.219 mg/kg (liver) and 0.243 mg/kg (kidney).

Radioactivity in blood reached a maximum of 0.124 mg equiv/kg at 2 h post first dose. Residues in milk had reached steady state within 3 days and the sacrifice time was set as 2 h following the tenth dose.

Approximately 100.6% of the total dose was recovered, the majority of which was present in the urine (51.7%), feces (36.8%) and GI tract contents (11.7%). A small amount of radioactivity was recovered in bile (<0.1%) and cage wash (0.3%). Radioactivity associated with edible portions (milk and tissues) accounted for 0.2% of the administered dose.

Radioactive residues in milk accounted for a plateau concentration of approximately 0.016 mg equiv/kg. The ratio of residues associated with skimmed milk and cream was determined in a representative 24 h composite sample from the plateau and found to be 1:1.1.

The identification of metabolites was based on co-chromatography and confirmation by LC-MS/MS of isolated HPLC fractions from pooled urine samples. A summary of the metabolites identified in all feces, milk and tissue extracts is provided in Table 6.2.3-7 and Table 6.2.3-8.

The proposed metabolic pathway of [thienyl-5-^{14}C]-M656PH030 in the lactating goat is provided in Figure 6.2.3-1. [Thienyl-5-^{14}C]-M656PH030 was extensively metabolized in the lactating goat. The unchanged M656PH030 was found in portions below 24% TRR in matrices except in bile where M656PH030 comprised 40% TRR. The main component in urine and feces was M656PH026, indicating that urinary and fecal excretion is the major elimination pathway accounting for 89% of the administered dose. In extracts of milk, liver, kidney, loin muscle and renal fat the main components were M656PH026 and M656PH030. The metabolite M656PH002 was formed by demethylation of the ether group and substitution of the 2-hydroxypropanoic acid with a methyl group, followed by oxidation of the sulphur group to sulfoxide and subsequent conjugation with glucuronic acid leading to M656PH098. Further oxidation of M656PH02 to the corresponding sulfone led to M656PH014 followed by glucuronidation yielding M656PH096. M656PH002, M656PH014, and M656PH096 were present in some tissue extracts, generally at lower levels.

Thus metabolic transformations occurred

- mainly *via* reduction of the sulfoxide moiety yielded the metabolite M656PH026
- demethylation of the ether group and cleavage of the 2-hydroxypropanoic acid in M656PH026 followed by S-methylation and S-oxidation led to M656PH002 and M656PH014
- glucuronidation of M656PH002 and M656PH014 yielded M656PH098 and M656PH096.

Figure 6.2.3-1: Proposed metabolic pathway of ^{14}C-M656PH030 in lactating goat

M656PH030
$C_{15}H_{23}NO_6S_2$
Exact Mass: 377.10

Reduction

M656PH026
$C_{15}H_{23}NO_5S_2$
Exact Mass: 361.10

Oxidative dealkylation

M656PH002
$C_{12}H_{19}NO_3S_2$
Exact Mass: 289.08

Glucuronidation

M656PH098
$C_{18}H_{27}NO_9S_2$
Exact Mass: 465.11

Oxidation

M656PH014
$C_{12}H_{19}NO_4S_2$
Exact Mass: 305.08

Glucuronidation

M656PH096
$C_{18}H_{27}NO_{10}S_2$
Exact Mass: 481.11

Overall summary animal metabolism

After administration of ^{14}C-dimethenamid to **laying hens**, the radioactivity was rapidly excreted (77% of the total applied dose at sacrifice). Residue concentrations in egg white and egg yolk were below 0.62 mg/kg and for fat, muscle (breast), muscle (thigh) and liver were 0.29, 0.45, 0.58 and 8.33 mg/kg, respectively. Dimethenamid was rapidly and extensively metabolized and excreted. The metabolic pathway was via glutathione conjugation, reductive dechlorination followed by the formations of cysteine and mercapturate conjugations, and dimerization of a mercaptan intermediate as can be seen in excreta. The other pathways included O-demethylation and reductive dechlorination. Metabolism in hen is similar to metabolism in rats.

The radioactivity was also rapidly excreted after the administration of ^{14}C-dimethenamid to **lactating goats**. More than 59% and 28% of the dose was excreted in the urine and feces, respectively. The residue concentrations in milk were below 0.98 mg/kg and kidney, fat, muscle and liver were 9.92, 0.97, 0.97 and 16.62 mg/kg, respectively. Dimethenamid was rapidly and extensively metabolised in the goat study. The major metabolic pathway was through glutathione conjugation, followed by the formations of cysteine, mercapturate, sulfoxide of thioglycolic acid conjugations, and dimerization of a mercaptan intermediate. The other pathways included O-demethylation and reductive dechlorination. In liver, M22 (dimer), M17 (mercapturate conjugate), M24 (glutathione conjugate) and M25 (cysteine conjugate) were found. In kidney, M7 (O-demethylated parent), M17, M24 and M25 were found. The analysis of milk indicated M17, M24 and M25. For muscle, the main metabolites were: M17, M24 and M25. Metabolites in fat were M7 and M17. In urine and feces, metabolites M3 (reductive dechlorinated), M7, M17, M24, M25, and M31 (sulfoxide of thioglycolic acid) were identified.

The above mentioned studies were conducted using racemic dimethenamid. Therefore, the metabolism with the dimethenamid-P was included since the racemic mixture contained 50% of the P-isomer (dimethenamid-P). No evidence was shown that dimethenamid has properties for accumulation.

While livestock animal will never be exposed to dimethenamid-P but are consistently exposed to M30, a metabolism study was conducted in 2013-2014 to investigate the metabolism of M656PH030 in lactating ruminants. The results of this metabolism study indicate that M30 is mainly transformed to M26 and to smaller amounts to M98 and M96. However, the main two metabolites in tissues were M30 and M26.

CA 6.2.4 Pigs

No metabolism study in pigs was performed, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly. This is congruent with EFSA's 2013 statement that:

Metabolism in lactating ruminants and poultry was investigated and findings can be extrapolated to pigs as well.

CA 6.2.5 Fish

According to Commission 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. In principal, seeds of soybean and sunflower as well as their meal and oilseed rape meal may be considered to serve as feed item for fish. However, residues of dimethenamid-P in these crop parts are usually not detectable.

The exact conditions under which such a study should be performed are further described in the Working Document of the EU Commission SANCO/11187/2013, rev. 3 on the nature of pesticide residues in fish. The document specifies that the accumulation of compounds with low lipophilicity via the diet is known to be negligible and that fish metabolism studies are therefore only required for active substances with a log P_{OW} equal or greater than 3 (refer to p. 6/ paragraph 4 of the document).

The log P_{ow} of dimethenamid-P was determined to be 1.89, thus no accumulation in fish tissues is expected. A bioconcentration study for racemic dimethenamid-P in fish, including identification of the nature of residue was submitted in the EU Dossier BAS 656 PH, AII, M 8.2.3. According to SANCO/11802/2010 Rev. 7, the results from bioconcentration studies may be used as metabolism data (See MCA, Section 8, Chapter 8.2.2.3). Bioconcentration factors for fish based on total radioactivity were low in edible tissue (20) and moderate in non-edible tissue and whole fish (100 and 57, respectively).

Thus, since the log P_{OW} of dimethenamid-P is below the above mentioned trigger value and since it was demonstrated that dimethenamid-P residues do not accumulate, no fish metabolism study is deemed necessary.

CA 6.3 Magnitude of residues trials in plants

Most residue studies were performed using the representative formulation BAS 656 12 H and BAS 830 01 H.

CA 6.3.1 Sunflower

Table 6.3.1-1: Critical GAP for the use of BAS 656 PH in/on sunflower

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Sunflower	1 x 0.864 kg BAS 656 H/ha	100-400 L/ha	N.r.	Spray application	BBCH 00-09

PHI Pre-harvest interval
N r. Not reported

This GAP is currently registered for the product BAS 656 12 H (EC formulation, application rate of 0.8-1.2 L product/ha at BBCH 00-09).

Table 6.3.1-2: GAP information of residue trials conducted in/on sunflower in Northern and Southern Europe

Region	Country (trials)	Formulation	Application[0]				DALA[1]	DocID	EU submitted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Northern EU	Germany (2) France (1) The Netherlands (2) United Kingdom (1) Belgium (1) 2013	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	141-154	2013/ 1335422	No
Northern EU	Germany (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.864 (1.008)*	0.432 (0.504)*	1	147	2013/ 1335405	No
Northern EU	Germany (1) France (2) The Netherlands (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	115-152	2012/ 1272620	No
Southern EU	France (2) Italy (2) Greece (2) Spain (2) 2013	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	80-132	2013/ 1335422	No
Southern EU	France (1) Greece (1) Italy (1) Spain (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	90-150	2012/ 1272620	No

0 Actual application rates varied by 10% at most
1 Days after last application
* Exaggerated application on the same trial

Report: CA 6.3.1/1
Meyer M., 2014c
Residue behaviour of Dimethenamid-P (BAS 656 H) in sunflower after treatment with BAS 656 12 H under field conditions in Germany, Northern France, The Netherlands, United Kingdom, Belgium, Southern France, Italy, Greece and Spain, 2013
2013/1335422

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, 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. 9 (March 2011)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 656 12 H (EC)
Lot/Batch #: 0004701751, 720 g/L BAS 656 PH nominal
Purity: Not relevant
CAS#: 163515-14-8
Development code: Not applicable
Spiking levels: 0.01-1.0 mg/kg

2. Test Commodity:

Crop: Sunflower
Type: Oilseeds
Variety: Alisson, Maestro, PE64HE01, ES Paulina, Olencia, ES-ETHIC, Orasole, Sikklos, LG5520, P64HE01
Botanical name: *Helianthus annuus* L.
Crop parts(s) or processed commodity: Seeds
Sample size: 1 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2013 a total of 15 field trials in sunflower was conducted in Northern and Southern Europe (Germany, France, The Netherlands, United Kingdom, Belgium, Spain, Greece and Italy), in order to determine the magnitude of the residues of dimethenamid-P after treatment with BAS 656 12 H. The trials consisted of two plots, one untreated control plot and one plot treated once with 0.864 kg dimethenamid-P/ha. The application took place at BBCH 09 with a water volume of 200 L/ha. Seed specimens were sampled 80-154 days after the application at BBCH 89. All specimens were stored deep frozen until analysis, including transportation, for a maximum period of 16 days.

Table 6.3.1-3: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg/ha)	Water volume (L/ha)	Target date/ timing
2013	15	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	200	BBCH 09

2. Description of analytical procedures

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were determined with BASF method No L0179/02. Residues were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.

Table 6.3.1-4: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in sunflowers

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		BAS 656 PH			M23			M26		
Seeds	0.01, 0.10, 1.0	101	5.9	18	103	4.8	18	102	5.0	18
Method No L0179/02		M27			M30					
Seeds	0.01, 0.10, 1.0	103	4.2	18	103	11	18			

II. RESULTS AND DISCUSSION

No residues above the respective method LOQ for BAS 656 PH and its metabolites were found in any of the samples or the control specimens. Therefore total dimethenamid-P was <0.02 mg/kg. A summary is given in Table 6.3.1-5. Details can be found in Table 6.3.1-6.

Table 6.3.1-5: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Sunflower	2013	BAS 656 12 H	80-154	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values at or below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30, expressed as parent equivalent

III. CONCLUSION

At harvest, 80-154 days after treatment, no residues of dimethenamid-P and its metabolites above the LOQ were found in sunflower seeds. The total residue was <0.02 mg/kg.

Table 6.3.1-6: Residues of dimethenamid-P treatment after with BAS 656 12 H in Northern Europe

Study details		Crop	Country	Formulation, Application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130186 Yes 2013	Sun-flower	Germany	BAS 656 12 H 1 x 0.864	89	154	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130187 Yes 2013	Sun-flower	Germany	BAS 656 12 H 1 x 0.864	89	145	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130189 Yes 2013	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	141	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130190 Yes 2013	Sun-flower	The Netherlands	BAS 656 12 H 1 x 0.864	89	137	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130191 Yes 2013	Sun-flower	The Netherlands	BAS 656 12 H 1 x 0.864	89	133	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130192 Yes 2013	Sun-flower	United Kingdom	BAS 656 12 H 1 x 0.864	89	145	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: DocID: Trial No.: GLP: Year:	694884 2013/1335422 L130650 Yes 2013	Sun-flower	Belgium	BAS 656 12 H 1 x 0.864	89	141	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30; expressed as parent equivalent
_ Underlined values were used for MRL calculation

Table 6.3.1-7: Residues of dimethenamid-P after treatment with BAS 656 12 H in Southern Europe

Study details		Crop	Country	Formulation, Application rate (kg a.s./ha)[0]	Crop growth stage	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130194 Yes 2013	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	115	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130195 Yes 2013	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	128	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130196 Yes 2013	Sun-flower	Italy	BAS 656 12 H 1 x 0.864	89	132	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130197 Yes 2013	Sun-flower	Italy	BAS 656 12 H 1 x 0.864	89	106	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130198 Yes 2013	Sun-flower	Greece	BAS 656 12 H 1 x 0.864	89	104	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130199 Yes 2013	Sun-flower	Greece	BAS 656 12 H 1 x 0.864	89	101	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130200 Yes 2013	Sun-flower	Spain	BAS 656 12 H 1 x 0.864	89	130	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	694884 2013/1335422 L130201 Yes 2013	Sun-flower	Spain	BAS 656 12 H 1 x 0.864	89	80	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30; expressed as parent equivalent
_ Underlined values were used for MRL calculation

Report: CA 6.3.1/2
Ertus C., 2014a
Study on the residue behaviour of BAS 656 H (Dimethenamid-P) after treatment with BAS 656 12 H in sunflower under field conditions in Northern Europe, 2012
2013/1335405

Guidelines: EC 1107/2009 of the European Parliament, EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 656 12 H (EC)
Lot/Batch #: 0004701751, 720 g/L BAS 656 PH nominal
Purity: Not relevant
CAS#: 163515-14-8
Development code: Not applicable
Spiking levels: 0.01-0.10 mg/kg

2. Test Commodity:

Crop: Sunflower
Type: Oilseeds
Variety: Metharoc
Botanical name: *Helianthus annuus* L.
Crop parts(s) or processed commodity: Seeds
Sample size: 1 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2012 one trial in sunflower was conducted in Germany, in order to determine the magnitude of the residues of dimethenamid-P after treatment with BAS 656 12 H. The trial consisted of tree plots, one untreated control plot, one plot treated once with a target application rate of 0.864 kg dimethenamid-P/ha and one plat treated once with a target application rate of 1.008 kg dimethenamid-P/ha. The application took place at BBCH 09 with a water volume of 200 L/ha. Sampling of seed specimens were 147 days after the application at BBCH 89. All specimens were stored frozen at or below -18°C, including during transportation, until analysis for a maximum period of 182 days.

Table 6.3.1-8: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg/ha)	Water volume (L/ha)	Target date/ timing
2012	1	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	200	BBCH 09
						1.008		

2. Description of analytical procedures

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were determined with BASF method No L0179/02. Residues were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.

Table 6.3.1-9: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in sunflowers

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		BAS 656 PH			M23			M26		
Seeds	0.01 and 0.10	102	N/A	2	105	N/A	2	98.2	N/A	2
Method No L0179/02		M27			M30					
Seeds	0.01 and 0.10	103	N/A	n	106	N/A	2			

N/A Not applicable

II. RESULTS AND DISCUSSION

No residues above the respective method LOQ of BAS 656 PH and its metabolites were found in any of the samples or the control specimens, therefore the total residue was <0.02 mg/kg. A summary is given in the Table 6.3.1-10. Details can be found in Table 6.3.1-11.

Table 6.3.1-10: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Sunflower	2012	BAS 656 12 H	147	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values at or below LOQ (0.010) mg/kg were not converted..
4 Sum of dimethenamid-P and M30 expressed as parent equivalent

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature seed at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.1-11: Residues of dimethenamid-P after treatment with BAS 656 12 H in Northern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DA LA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code:	390502_1	Sun-flower	Germany	BAS 656 12 H 1 x 0.864	89	147	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335405												
Trial No.:	L120044			BAS 656 12 H 1 x 1.008	89	147	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2012												

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
_ Underlined values were used for MRL calculation

Report: CA 6.3.1/3
Gabriel E.J., 2013a
Study on the residue behaviour of Dimethenamid-P (BAS 656 H) in sunflower after treatment with BAS 656 12 H under field conditions in Germany, the Netherlands, Northern France, Southern France, Greece, Italy and Spain, 2012
2012/1272620

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, 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. 9 (March 2011)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 656 12 H (EC)
Lot/Batch #: 0004701751, 720 g/L BAS 656 PH nominal
Purity: Not relevant
CAS#: 163515-14-8
Development code: Not applicable
Spiking levels: 0.01-0.10 mg/kg

2. Test Commodity:

Crop: Sunflower
Type: Oilseeds
Variety: Faro, Durban, PE 64HE01, Tutti, Sikklos, Orasole, Bosfora
Botanical name: *Helianthus annuus* L.
Crop parts(s) or processed commodity: Seeds
Sample size: 1 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2012 eight trials in sunflower were conducted in Northern and Southern Europe (Germany, The Netherlands, Northern and Southern France, Greece, Italy, and Spain) in order to determine the magnitude of the residues of dimethenamid-P after treatment with BAS 656 12 H. The trials consisted of two plots, one untreated control plot and one plot treated once with 0.864 kg dimethenamid-P /ha. The application took place at BBCH 08-09 with a water volume of 200 L/ha.

Sampling of seed specimens were 90-152 days after the application at BBCH 85-89. All specimens were stored frozen at or below -18°C, including during transportation, until analysis, except trials L120278, L120281 and L120282 where the temperature rose up to -10°C. The maximum storage period was 182 days.

Table 6.3.1-12: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg/ha)	Water volume (L/ha)	Target date/ timing
2012	8	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	200	BBCH 09

2. Description of analytical procedures

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were determined with BASF method No L0179/02. Residues were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.

Table 6.3.1-13: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in sunflowers

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		**BAS 656 PH**			**M23**			**M26**		
Seeds	0.01 and 0.10	98.2	N/A	2	102	N/A	2	96.8	N/A	2
Method No L0179/02		**M27**			**M30**					
Seeds	0.01 and 0.10	92.2	N/A	n	93.0	N/A	2			

N/A Not applicable

II. RESULTS AND DISCUSSION

No residues above the respective method LOQ of BAS 656 PH and its metabolites were found in any of the samples or the control specimens, therefore the total residue was <0.02 mg/kg. A summary is given in the Table 6.3.1-14. Details can be found in Table 6.3.1-15 and Table 6.3.1-16.

Table 6.3.1-14: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Sunflower	2012	BAS 656 12 H	90-152	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values at or below LOQ (0.010 mg/kg were not converted..
4 Sum of dimethenamid-P and M30 expressed as parent equivalent

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature seed at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.1-15: Residues of dimethenamid-P after treatment with BAS 656 12 H in Northern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120276 Yes 2012	Sun-flower	Germany	BAS 656 12 H 1 x 0.864	87	138	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120278 Yes 2012	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	152	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120279 Yes 2012	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	151	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120578 Yes 2012	Sun-flower	The Netherlands	BAS 656 12 H 1 x 0.864	85	115	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
_ Underlined values were used for MRL calculation

Table 6.3.1-16: Residues of dimethenamid-P after treatment with BAS 656 12 H in Southern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120280 Yes 2012	Sun-flower	France	BAS 656 12 H 1 x 0.864	89	150	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120281 Yes 2012	Sun-flower	Greece	BAS 656 12 H 1 x 0.864	89	90	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120282 Yes 2012	Sun-flower	Italy	BAS 656 12 H 1 x 0.864	89	118	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: **DocID:** **Trial No.:** **GLP:** **Year:**	423129 2012/1272620 L120583 Yes 2012	Sun-flower	Spain	BAS 656 12 H 1 x 0.864	89	91	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
_ Underlined values were used for MRL calculation

CA 6.3.2 Oilseed rape

Table 6.3.2-1: Critical GAP for the use of BAS 656 PH in/on oilseed rape

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Oilseed rape	1 x 0.6 kg BAS 656 H/ha	100-400 L/ha	n.s.	spray application	BBCH 00-09 BBCH 10-18

PHI = pre-harvest interval

This GAP is currently registered for the product BAS 769 00 H (EC formulation, application rate of 2.5 L product/ha at BBCH 00-09 or BBCH 10-18).

Table 6.3.2-2: GAP information of residue trials conducted in/on oilseed rape in Northern and Southern Europe

Region	Country (trials)	Formulation	Application[0]				DALA[1]	DocID	EU submitted
			Method	Rate[0] kg a.s./ha	Spray conc. (kg a.s./hL)	No			
Northern EU	Germany (2) The Netherlands (1) United Kingdom (1) 2013	BAS 830 01 H (SE)	Spray appl.	0.5	0.25	1	0 10-41 87-143	2013/ 1335420	No
Southern EU	France (1) Italy (1) Spain (1) 2013	BAS 830 01 H (SE)	Spray appl.	0.5	0.25	1	0 18-26 69-103	2013/ 1335420	No

0 Actual application rates varied by 10% at most
1 Days after last application

Report: CA 6.3.2/1
Meyer M., 2014a
Residue behaviour of Quinmerac (BAS 518 H) and Dimethenamid-P (BAS 656 H) in oilseed rape after treatment with BAS 830 01 H under field conditions in Germany, The Netherlands, United Kingdom, Southern France, Italy and Spain, 2013
2013/1335420

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EU Regulation Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 830 01 H (SE)
Lot/Batch #: 451008 (333 g/L dimethenamid-P, 167 g/L quinmerac, nominal)
Purity: Not reported
CAS#: 163515-14-8 (dimethenamid-P); 90717-03-6 (quinmerac)
Development code: Not reported
Spiking levels: 0.01-100 mg/kg

2. Test Commodity:

Crop: Oilseed rape
Type: Oilseeds
Variety: Makro, Expower, Cabernet, Visby, Atenzo, Mosaik, Jura
Botanical name: *Brassica napus*
Crop parts(s) or processed commodity: Whole plant without roots, rest plant without root, seed
Sample size: 0.1-1 kg / min. 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season 7 field trials in oilseed rape were conducted in different representative growing areas in the EU North (Germany, The Netherlands, United Kingdom) and South (France, Italy, Spain) to determine the residue level of dimethenamid-P and quinmerac in or on raw agricultural commodities (RAC). BAS 830 01 H was applied once at a rate equivalent to 0.5 kg dimethenamid-P/ha and 0.25 kg quinmerac/ha in a spray volume of 200 L/ha. The application was performed at growth stage BBCH 18. Specimens of whole plant without roots were collected immediately after the application and at BBCH 51. Specimens of seed and rest of plant without roots were collected at plant maturity (BBCH 89). Samples were stored frozen at or below -18°C for a maximum of 279 days until analysis.

Table 6.3.2-3: Target application rates and timings for oilseed rape

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	7	1	F	BAS 830 01 H (SE)	BAS 656 PH BAS 518 H	0.5* 0.25**	200	BBCH 18

* Dimethenamid-P
** Quinmerac

2. Description of analytical procedures

The specimens were analyzed for dimethenamid-P (BAS 656 PH) and its metabolitesM23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) with BASF method No L0179/02 quantifying each analyte with a limit of quantitation (LOQ) of 0.01 mg/kg and for quinmerac and its metabolites BH 518-2 and BH 518-4 with BASF method No L0050/02 quantifying each analyte with an LOQ of 0.01 mg/kg for seeds and 0.05 mg/kg for plants.

BASF method No L0179/02: Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) metabolites were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by HPLC-MS/MS.

Table 6.3.2-4: Summary of recoveries of dimethenamid-P and its metabolites in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0179/02		**Dimethenamid-P (BAS 656 PH)**			**M23**		
Whole plant*	0.01, 0.1, 1.0, 10, 20, 100**	16	108	5.3	14	105	5.3
Seed	0.01, 0.1, 1.0	12	116	1.7	12	110	4.2
Rest of plant*	0.01, 0.1, 1.0, 10, 20	7	107	2.5	7	106	3.1
Overall		35	111	4.8	33	107	4.8
BASF method No L0179/02		**M26**			**M27**		
Whole plant*	0.01, 0.1, 1.0, 10, 20	14	106	8.3	14	105	3.4
Seed	0.01, 0.1, 1.0	12	112	6.8	12	110	5.7
Rest of plant*	0.01, 0.1, 1.0, 10, 20	7	91	12.2	7	97	4.8
Overall		33	105	11.1	33	105	6.6
BASF method No L0179/02		**M30**					
Whole plant*	0.01, 0.1, 1.0, 10, 20	14	106	12.1			
Seed	0.01, 0.1, 1.0	12	109	10.0			
Rest of plant*	0.01, 0.1, 1.0, 10, 20	7	91	20.2			
Overall		33	104	14.2			

* Without roots
** Only dimethenamid-P

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-5, detailed residue levels are shown in Table 6.3.2-6 and Table 6.3.2-7.

Oilseed rape whole plant without root specimens sampled at the day of the application (BBCH 18) contained 0.36-37.16 mg/kg dimethenamid-P, 0.02-0.98 mg/kg M26 and <0.01-0.28 mg/kg M30 (expressed as parent). In whole plants sampled 10-41 days after the application (BBCH 51) <0.01 mg/kg dimethenamid-P, <0.01-0.05 mg/kg M26 and 0.04-0.45 mg/kg of M30 (expressed as parent) were found.

In rest of plant without roots taken 69-143 DALA (BBCH 89), residues of dimethenamid-P and M26 were <0.01 mg/kg, while residues of M30 in the range of <0.01-0.01 mg/kg were found. In none of the samples residues of M23 and M27 were found. In seed samples at harvest (BBCH 89), no residues of dimethenamid-P at or above the LOQ of 0.01 mg/kg were found, therefore the total residue is <0.02 mg/kg.

No residues at or above the respective LOQ of the analytical method of dimethenamid-P were found in any of the untreated specimens.

Table 6.3.2-5: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Oilseed rape (EU North)	2013	BAS 830 01 H	0	Whole plant*	4.84-15.25	<0.01	0.02-0.23	<0.01	<0.01-0.09	4.92-15.53
			10-41		<0.01	<0.01	<0.01-0.05	<0.01	0.04-0.45	0.05-0.46
			87-143	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01-0.01	<0.02-0.02
			87-143	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Oilseed rape (EU South)	2013	BAS 830 01 H	0	Whole plant*	0.36-37.16	<0.01	0.32-0.98	<0.01	0.17-0.28	0.53-37.39
			18-26		<0.01	<0.01	<0.01	<0.01	0.06-0.20	0.07-0.21
			69-103	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			69-103	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.01 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature seed at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.2-6: Residues of dimethenamid-P in oilseed rape after one application of BAS 830 01 H in Northern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code:	694886	Oilseed rape	Germany	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	7.12	<0.01	0.10	<0.01	0.04	7.16
Doc ID:	2013/1335420				51	10	Whole plant*	<0.01	<0.01	0.02	<0.01	0.25	0.26
Trial No.:	L130132				89	87	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	87	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												
Study code:	694886	Oilseed rape	The Nether-lands	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	4.84	<0.01	0.23	<0.01	0.08	4.92
Doc ID:	2013/1335420				51	11	Whole plant*	<0.01	<0.01	0.05	<0.01	0.45	0.46
Trial No.:	L130133				89	110	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	110	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												
Study code:	694886	Oilseed rape	United Kingdom	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	15.52	<0.01	0.02	<0.01	<0.01	15.53
Doc ID:	2013/1335420				51	28	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.07	0.08
Trial No.:	L130134				89	139	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	139	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												
Study code:	694886	Oilseed rape	Germany	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	8.68	<0.01	0.19	<0.01	0.09	8.77
Doc ID:	2013/1335420				51	41	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05
Trial No.:	L130135				89	143	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02
GLP:	Yes				89	143	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
_ Underlined values were used for MRL calculation

Table 6.3.2-7: Residues of dimethenamid-P in oilseed rape after one application of BAS 830 01 H in Southern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code:	694886	Oilseed rape	France	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	0.36	<0.01	0.98	<0.01	0.17	0.53
Doc ID:	2013/1335420				51	18	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.13	0.14
Trial No.:	L130136				89	103	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	103	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												
Study code:	694886	Oilseed rape	Italy	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	7.56	<0.01	0.33	<0.01	0.28	7.84
Doc ID:	2013/1335420				51	24	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.20	0.21
Trial No.:	L130138				89	69	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	69	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												
Study code:	694886	Oilseed rape	Spain	BAS 830 01 H: 1 x 0.5	18	0	Whole plant*	37.16	<0.01	0.32	<0.01	0.23	37.39
Doc ID:	2013/1335420				51	26	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.06	0.07
Trial No.:	L130139				89	101	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	101	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2013												

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
_ Underlined values were used for MRL calculation

CA 6.3.3 Soybean

Table 6.3.3-1: Critical GAP for the use of BAS 656 PH in/on soybean

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Soybean	1 x 0.864 kg BAS 656 PH/ha	100-400 L/ha	n r.	Spray application	BBCH 00-09

n.r. not reported
PHI pre-harvest interval

This GAP is currently registered for the product BAS 656 12 H (EC formulation, application rate 0.8-1.2 L product/ha at BBCH 00-09).

Table 6.3.3-2: GAP information of residue trials conducted in/on soybean in Northern and Southern Europe

Region	Country (trials)	Formulation	Application[0]				DALA[1]	DocID	EU submitted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Northern EU	Germany (2) Hungary (2) Poland (2) France (2) 2013	BAS 656 12 H (SE)	Spray appl.	0.864	0.864	1	28-90 117-168	2013/ 1335421	No
Southern EU	France (2) Greece (2) Italy (2) Spain (2) 2013	BAS 656 12 H (SE)	Spray appl.	0.864	0.864	1	51-76 112-126	2013/ 1335421	No

0 Actual application rates varied by 10% at most
1 Days after last application

Report: CA 6.3.3/1
Oxspring S., 2014b
Study on the residue behaviour of Dimethenamid-P (BAS 656 H) in soybean after treatment with BAS 656 12 H in Northern and Southern Europe during 2013
2013/1335421

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 656 12 H (EC)
Lot/Batch #: 0004701751, 720 g/L BAS 656 PH nominal
Purity: Not relevant
CAS#: 163515-14-8
Development code: Not applicable
Spiking levels: 0.01, 1.0, 10 mg/kg

2. Test Commodity:

Crop: Soybean
Type: Oilseeds
Variety: Sultana, Silvia, Pannonia, Kincse, Isidor, Augusta, Isidor NT, Atsaffort, Fukui, Blancas, Deka Big, Demetra, PR92B63,
Botanical name: *Glycine max.* L.
Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots and seeds
Sample size: 0.5-1 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2013 a total of 16 field trials in soybean was conducted in Northern and Southern Europe (Germany, Hungary, Poland, France, Greece, Italy and Spain), in order to determine the magnitude of the residues of dimethenamid-P after treatment with BAS 656 12 H. The trials consisted of two plots, one untreated control plot and one plot treated once with 0.864 kg dimethenamid-P/ha. The application took place at BBCH 09 except for trial L130380 application took place at (BBCH 10). Trials L130374 and L130375 were treated on the day of sowing (BBCH 00), with a water volume of 100 L/ha. Treated and untreated whole plant specimens were collected at BBCH 65-69, 51 to 90 days after the last application. Sampling of seed specimens was conducted 112-168 days after the application at BBCH 89. All specimens were generally stored frozen at or below -18°C, including during transportation, until analysis for a maximum period of 232 days. The seed samples were analyzed within 30 days after the sampling.

Table 6.3.3-3: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg/ha)	Water volume (L/ha)	Target date/ timing
2013	16	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	100	BBCH 09

2. Description of analytical procedures

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were determined with BASF method No L0179/02. Residues were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.

Table 6.3.3-4: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in soybean

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		**BAS 656 PH**			**M23**			**M26**		
Whole plant[1]	0.01, 0.1, 1.0, 10	96.5	4.0	4	96.9	4.9	4	86.7	11	4
Seed	0.01, 0.1, 1.0	101	8.9	12	99.5	12	12	102	12	12
Rest of plant[1]	0.01, 0.1, 1.0, 10	99.3	3.3	4	93.6	4.1	4	77.9	5.9	4
Method No L0179/02		**M27**			**M30**					
Whole plant[1]	0.01, 0.1, 1.0, 10	99.6	4.6	4	87.7	2.9	4			
Seed	0.01, 0.1, 1.0	91.6	13	12	89.9	16	12			
Rest of plant[1]	0.01, 0.1, 1.0, 10	90.6	5.1	4	71.1	11	4			

1 Without roots

II. RESULTS AND DISCUSSION

The whole plant no roots specimens of soybean collected at BBCH 65-69 (28-90 DALA) contained no residues of dimethenamid-P and its metabolites M26 and M30 (expressed as parent equivalent) above the LOQ of 0.01 mg/kg. Residues of M23 ranging from <0.01 mg/kg to 0.01 mg/kg, of M27 ranging from <0.01 mg/kg to 0.15 mg/kg were detected. No residues of dimethenamid-P and its metabolite M26 were found in the rest of plant specimens without roots taken at BBCH 89 (112-168 DALA), residues of M23 between <0.01-0.02 mg/kg, of M27 between <0.01-0.04 mg/kg and of M30 between <0.01-0.01 mg/kg were determined.

No residues above the limit of quantitation for dimethenamid-P, M23, M26, M27 and M30 were determined at BBCH growth stage 89 for seed specimens.

No residues above the respective method LOQ of BAS 656 PH and its metabolites were found in any of the control specimens. A summary is given in Table 6.3.1-5. Details can be found in Table 6.3.1-6.

Table 6.3.3-5: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Soybean (EU North)	2013	BAS 656 12 H	28-90	Whole plant*	<0.01	<0.01-0.01	<0.01	0.01-0.15	<0.01	<0.02
			117-168	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			117-168	Rest of plant*	<0.01	<0.01	<0.01	<0.01-0.03	<0.01	<0.02
Soybean (EU South)			51-76	Whole plant*	<0.01	<0.01	<0.01	<0.01-0.03	<0.01	<0.02
			112-126	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			112-126	Rest of plant	<0.01	<0.01-0.02	<0.01	<0.01-0.04	<0.01-0.01	<0.02-0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature soybean seeds at BBCH 89 in Northern and Southern Europe were <0.02 mg/kg, respectively.

Table 6.3.3-6: Residues of dimethenamid-P treatment with BAS 656 12 H in Northern Europe

Study details	Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DA LA[1]	Residues found (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130372 **GLP:** Yes **Year:** 2013	Soybean	Germany	BAS 656 12 H 1 x 0.864	69 89 89	28 122 122	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130373 **GLP:** Yes **Year:** 2013	Soybean	Germany	BAS 656 12 H 1 x 0.864	69 89 89	70 155 155	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.04 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130374 **GLP:** Yes **Year:** 2013	Soybean	Hungary	BAS 656 12 H 1 x 0.864	69 89 89	90 168 168	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130375 **GLP:** Yes **Year:** 2013	Soybean	Hungary	BAS 656 12 H 1 x 0.864	69 89 89	80 164 164	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.06 <0.01 0.02	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130376 **GLP:** Yes **Year:** 2013	Soybean	Poland	BAS 656 12 H 1 x 0.864	69 89 89	72 127 127	Whole plant*† Seed Rest of plant*†	<0.01 <0.01 <0.01	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.15 <0.01 0.03	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130377 **GLP:** Yes **Year:** 2013	Soybean	Poland	BAS 656 12 H 1 x 0.864	69 89 89	71 124 124	Whole plant*† Seed Rest of plant*†	<0.01 <0.01 <0.01	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.05 <0.01 0.02	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130378 **GLP:** Yes **Year:** 2013	Soybean bean	France	BAS 656 12 H 1 x 0.864	65-69 89 89	68 166 166	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02
Study code: 697666 **DocID:** 2013/1335421 **Trial No.:** L130379 **GLP:** Yes **Year:** 2013	Soybean	France	BAS 656 12 H 1 x 0.864	69 89 89	65 117 117	Whole plant*† Seed Rest of plant*	<0.01 <0.01 <0.01	0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.02 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
† Mean of two analyses
_ Underlined values were used for MRL calculation

Table 6.3.3-7: Residues of dimethenamid-P treatment with BAS 656 12 H in Southern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[2]	Total[4]
Study code:	697666	Soybean	France	BAS 656 12 H	69	63	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	112	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130380				89	112	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	France	BAS 656 12 H	69	76	Whole plant*†	<0.01	<0.01	<0.01	0.03	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	125	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130381				89	125	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Greece	BAS 656 12 H	69	53	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	119	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130382				89	119	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Greece	BAS 656 12 H	69	51	Whole plant*	<0.01	<0.01	<0.01	0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	114	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130383				89	114	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Italy	BAS 656 12 H	69	52	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	126	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130384				89	126	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Italy	BAS 656 12 H	69	71	W. plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	126	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130385				89	126	R. plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Spain	BAS 656 12 H	69	75	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	119	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130386				89	119	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes												
Year:	2013												
Study code:	697666	Soybean	Spain	BAS 656 12 H	69	75	Whole plant*†	<0.01	<0.01	<0.01	0.02	<0.01	<0.02
DocID:	2013/1335421			1 x 0.864	89	119	Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L130387				89	119	Rest of plant*†	<0.01	0.02	<0.01	0.04	0.01	0.02
GLP:	Yes												
Year:	2013												

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
† Mean of two analyses
_ Underlined values were used for MRL calculation

CA 6.3.4 Maize

Table 6.3.4-1: Critical GAP for the use of BAS 656 PH in/on maize

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Maize	1 x 0.864 kg BAS 656 H/ha	100-400 L/ha	n.s.	spray application	BBCH 00-09 BBCH 10-16

PHI = pre-harvest interval

This GAP is currently registered for the product BAS 656 12 H (EC formulation, application rate of 0.8-1.2 L product/ha at BBCH 00-09 or BBCH 10-16).

Table 6.3.4-2: GAP information of residue trials conducted in/on maize in Northern and Southern Europe

Region	Country (trials)	Formulation	Application[0]				DALA[1]	DocID	EU submitted
			Method	Rate[0] kg a.s./ha	Spray conc. (kg a.s./hL)	No			
Northern EU	Germany (1) France (1) United Kingdom (1) The Netherlands (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	0 59-61 91-92 111-115 125-149	2012/ 1272621	No
	Germany (1) France (1) United Kingdom (1) The Netherlands (1) 2011	BAS 656 12 H (EC)	Spray appl.	0.864 (1.008)[2]	0.432 (0.504)[2]	1	0 59-61 79 88-93 110-120 128-142	2012/ 1209625	Yes
Southern EU	France (1) Greece (1) Italy (1) Spain (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.864	0.432	1	0 59-60 78-86 89-90 91-117	2012/ 1272621	No
	France (1) Greece (1) Italy (1) Spain (1) 2011	BAS 656 12 H (EC)	spray application	0.864 (1.008)[2]	0.432 (0.504)[2]	1	0 59-61 76-91 91-100 121-138	2012/ 1209625	Yes

0 Actual application rates varied by 10% at most
1 Days after last application
2 Exaggerated application rate on this trial

Report: CA 6.3.4/1
Gabriel E.J.,Meyer M., 2013a
Study on the residue behaviour of Dimethenamid-P (BAS 656 H) in maize after treatment with BAS 656 12 H under field conditions in Germany, Northern France, United Kingdom, the Netherlands, Southern France, Greece, Italy and Spain, 2012
2012/1272621

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, 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. 9 (March 2011)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 656 12 H (EC)
   Lot/Batch #: 0004701751, 720 g/L BAS 656 PH nominal
   Purity: Not relevant
   CAS#: 163515-14-8
   Development code: Not applicable
   Spiking levels: 0.01-10.0 mg/kg

2. **Test Commodity:**
   Crop: Maize
   Type: Cereals
   Variety: Aurelia, DKC 3850, Kougar, P 8057 (Pioneer), DKC 5007, A5 72, Armonico, Castellano
   Botanical name: *Zea mays* L.
   Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots, cobs with husks, grain
   Sample size: 1 kg, 12 pieces/plants

B. STUDY DESIGN

1. Test procedure

During the growing season of 2012 eight trials in maize were conducted in Northern and Southern EU (Germany, Northern France, United Kingdom, The Netherlands, Southern France, Greece, Italy and Spain) in order to determine the magnitude of the residues of dimethenamid-P after treatment with BAS 656 12 H. The trials consisted of two plots, one untreated control plot and one plot treated once with 1.2 L BAS 656 12 H /ha corresponding to an application rate of 0.864 kg dimethenamid-P /ha. The application took place at BBCH 16 with a water volume of 200 L/ha. Sampling of whole plant without roots, rest of plant, cobs with husks or grain was carried out 0, 59-61, 78-115 (BBCH 85), 91, 89-92 and 100-149 (BBCH 89) DALA. All specimens were stored frozen at temperatures of -6.5°C to -18°C, including during transportation, until analysis for a maximum period of 301 days.

Table 6.3.4-3: Target application rates and timings

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg/ha)	Water volume (L/ha)	Target date/ timing
2012	8	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	200	BBCH 16

2. Description of analytical procedures

Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.

Table 6.3.4-4: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in maize

Matrix	Fortification Level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		**BAS 656 PH**			**M23**			**M26**		
Whole plant[1]	0.01 and 10.0	93.1	6.3	4	103	5.1	4	98.3	5.2	4
Cob with husks	0.01 and 0.1	89.8	10.4	4	94.3	2.8	4	88.5	6.7	4
Rest of plant[1]	0.01 and 0.1	100	2.6	8	102	5.0	8	95.7	4.8	8
Grain	0.01 and 0.1	98.4	N/A	2	97.4	N/A	2	92.4	N/A	2
	Overall:	96.1	6.9	18	99.9	5.4	18	94.3	6.1	18
Method No L0179/02		**M27**			**M30**					
Whole plant[1]	0.01 and 10.0	103	6.5	4	93.6	3.5	4			
Cob with husks	0.01 and 0.1	87.3	7.8	4	87.9	13.7	4			
Rest of plant[1]	0.01 and 0.1	96.0	13.1	8	98.3	10.9	8			
Grain	0.01 and 0.1	92.8	N/A	2	97.6	N/A	2			
	Overall:	95.3	11.1	18	94.9	10.2	18			

1 Without roots
N/A Not applicable

II. RESULTS AND DISCUSSION

In Northern Europe directly after the application the residues of BAS 656 PH in whole plant specimens ranged between 21-77 mg/kg and between <0.01-0.06 mg/kg for M26 as well as <0.01-0.02 mg/kg for M30 (expressed as parent). In none of the collected samples residues of the metabolites M23 and M27 were present. In mature maize grain (BBCH 89) and rest of plant commodities residues of dimethenamid-P and its metabolites were blow LOQ, therefore the total residue (sum of parent and M30) was <0.02 mg/kg.

In Southern Europe directly after the application the residues of BAS 656 PH in whole plant specimens ranged between 8.8-48 mg/kg and between <0.01-0.14 mg/kg for M26 as well as <0.01-0.03 mg/kg for M30 (expressed as parent). In none of the collected samples residues of the metabolites M23 and M27 were present. In matured (BBCH 89) maize grain and rest of plant commodities residues of dimethenamid-P and its metabolites were blow LOQ, except for trial L120291 in Spain where 0.021 mg/kg of M30 (expressed as parent) were found in matured rest of plant samples. Therefore the total residue (sum of parent and M30) for maize grain was <0.02 mg/kg.

No residues above the respective method LOQ of BAS 656 PH and its metabolites were found in any of the control specimens.

Table 6.3.4-5: Summarized residue results after treatment with dimethenamid-P

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Maize (EU North)	2012	BAS 656 12 H	0	Whole plant*	21-77	<0.01	<0.01-0.06	<0.01	<0.01-0.01	21.01-77.01
			59-61		<0.01	<0.01	<0.01	<0.01	<0.01-0.02	<0.02-0.03
			59-61	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-92		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			111-115		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-61	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-92		<0.01	<0.01	<0.01	<0.01	<0.01-0.02	<0.02-0.03
			111-115		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			125-149		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			125-149	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Maize (EU South)	2012	BAS 656 12 H	0	Whole plant*	8.8-48	<0.01	<0.01-0.14	<0.01	<0.01-0.03	8.83-48.01
			59-60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			78-86		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			89-90		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-60	Rest of plant*	<0.01	<0.01	<0.01-0.02	<0.01	<0.01-0.10	<0.01-0.11
			78-86		<0.01	<0.01	<0.01-0.04	<0.01	<0.01-0.17	<0.01-0.18
			89-90		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-117		<0.01	<0.01	<0.01-0.04	<0.01	<0.01-0.20	<0.02-0.21
			91-117	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** With husks

III. CONCLUSION

At BBCH 89 no residues of BAS 656 PH and its metabolites above the LOQ were found in maize grain and rest of plant samples. Only for metabolite M30 residues of <0.01-0.02 mg/kg (expressed as parent) in rest of plant samples were found. Residues of total dimethenamid-P (sum of parent and M30) in maize grain at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.4-6: Residues of dimethenamid-P treatment with BAS 656 12 H in Northern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code:	423130	Maize	Germany	BAS 656 12 H	16	0	Whole plant*	46	<0.01	0.04	<0.01	<0.01	46.01
DocID:	2012/1272621			1 x 0.864	73	60	Whole plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
Trial No.:	L120284				79	91	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				79	91	R.pl.	<0.01	<0.01	<0.01	<0.01	0.02	0.03
Year:	2012				85	111	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					85	111	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	125	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	125	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	France	BAS 656 12 H	16	0	Whole plant*	77	<0.01	<0.01	<0.01	<0.01	77.01
DocID:	2012/1272621			1 x 0.864	75	59	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120285				75	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				85	92	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				85	92	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	149	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	149	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	United Kingdom	BAS 656 12 H	16	0	Whole plant*	36	<0.01	0.01	<0.01	<0.01	36.01
DocID:	2012/1272621			1 x 0.864	61	61	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120286				85	91	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				85	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				89	138	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	138	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	The Netherlands	BAS 656 12 H	16	0	Whole plant*	21	<0.01	0.06	<0.01	0.01	21.01
DocID:	2012/1272621			1 x 0.864	69	59	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120287				75	91	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				75	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				85	115	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					85	115	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	140	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	140	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks
_ Underlined values were used for MRL calculation

Table 6.3.4-7: Residues of dimethenamid-P treatment with BAS 656 12 H in Southern Europe

Study details		Crop	Country	Formulation, application rate (kg a.s./ha)[0]	Crop growth stage[2]	DALA[1]	Residues found (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code:	423130	Maize	France	BAS 656 12 H	16	0	Whole plant*	48	<0.01	0.06	<0.01	<0.01	48.01
DocID:	2012/1272621			1 x 0.864	73	60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120288				73	60	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				85	86	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				85	86	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					85	89	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					85	89	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	117	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	117	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	Greece	BAS 656 12 H	16	0	Whole plant*	35	<0.01	<0.01	<0.01	<0.01	35.01
DocID:	2012/1272621			1 x 0.864	73	59	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120289				73	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				85	86	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				85	86	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					87	90	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					87	90	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	99	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	99	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	Italy	BAS 656 12 H	16	0	Whole plant*	37	<0.01	0.05	<0.01	<0.01	37.01
DocID:	2012/1272621			1 x 0.864	85	60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120290				85	60	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP:	Yes				89	91	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				89	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code:	423130	Maize	Spain	BAS 656 12 H	16	0	Whole plant*	8.8	<0.01	0.14	<0.01	0.03	8.83
DocID:	2012/1272621			1 x 0.864	75	60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.:	L120291				75	60	Rest of plant*	<0.01	<0.01	0.02	<0.01	0.10	0.11
GLP:	Yes				85	78	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year:	2012				85	78	Rest of plant*	<0.01	<0.01	0.04	<0.01	0.17	0.18
					89	91	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
					89	91	Rest of plant*	<0.01	<0.01	0.04	<0.01	0.20	0.21

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks
_ Underlined values were used for MRL calculation

Report: CA 6.3.4/2
Perny A., 2013b
Study on the residue behaviour of BAS 656 H (Dimethenamid-P) after treatment with BAS 656 12 H in corn under field conditions in Northern and Southern Europe, 2011
2012/1209625

Guidelines: EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 7

GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 656 12 H (EC)
   Lot/Batch #: FRE-000601, dimethenamid-P: 720 g/L nominal
   Purity: Not relevant
   CAS#: 163515-14-8
   Development code: Not applicable
   Spiking levels: 0.01-45.00 mg/kg

2. **Test Commodity:**
   Crop: Maize
   Type: Cereals
   Variety: Amoroso, Koherence, Podium, Fallove, 33Y74, Sansia, PR32F73, N28
   Botanical name: *Zea mays*
   Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots, cobs with husks, grain
   Sample size: 0.2-1.0 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the growing season of 2011, eight field trials in maize were conducted in different representative growing areas of Northern and Southern Europe to determine the residue levels of dimethenamid-P in or on raw agricultural commodities (RAC).
Each trial consisted of three plots: plot 1 (control), plot 2 and 3 (treated with BAS 656 12 H).
BAS 656 12 H (720 g/L BAS 656 PH, EC) was applied once at a rate equivalent to 1.008 kg a.s./ha in plot 2 and at a rate equivalent to 0.864 kg a.s./ha in plot 3. In all trials the application was made at BBCH growth stage 16-17. The spray volume used was 200 L/ha.
Maize specimens were collected directly after the last application, 59-60 days after the last application (DALA), at reaching BBCH growth stage 85, at 88-92 DALA and at commercial harvest (BBCH growth stage 89). All samples were shipped frozen to the analysis facility and remained frozen until analysis. The maximum storage interval was 248 days.

Table 6.3.4-8: 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
2011	8	1	F	BAS 656 12 H (EC)	BAS 656 PH	0.864	200	BBCH 16
						1.008		

2. Description of analytical procedures

BASF method No L0179/02 was used to analyze the residues of Dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352). BAS 656 PH and its metabolites M23, M26, M27 and M30 were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Table 6.3.4-9: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in maize

Matrix	Fortification Level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		**BAS 656 PH**			**M23**			**M26**		
Whole plant*	0.01, 0.10, 1.0	76.4	4.1	3	74.8	4.5	3	75.3	3.7	3
Cob with husks	0.01, 0.10	105.3	5.2	4	103.8	7.8	4	102.7	6.0	4
Rest of plant*	0.01, 0.10, 1.0, 45	104.3	2.2	12	102.8	4.4	12	102.1	4.2	12
Grain	0.01, 0.1	105.1	N/A	2	96.6	N/A	2	96.7	N/A	2
Method No L0179/02		**M27**			**M30**					
Whole plant*	0.01, 0.10, 1.0	72.1	5.8	3	67.6	3.8	3			
Cob with husks	0.01, 0.10	104.2	3.6	4	101.1	3.8	4			
Rest of plant*	0.01, 0.10, 1.0, 45	101.0	3.5	12	96.6	4.9	12			
Grain	0.01, 0.1	95.4	N/A	2	95.0	N/A	2			

* Without roots
N/A Not applicable

II. RESULTS AND DISCUSSION

Northern Europe:
After one application of 0.864 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant commodity decreased from a range of 15.20-50.00 mg/kg directly after the application to below the LOQ (0.01 mg/kg) after 60-61 days. Residues of its metabolites M26 at 0 DALA in the range of <0.01-0.04 mg/kg were determined in whole plant specimens. No residues of M30 (expressed as parent) above the LOQ were found in whole plant and grain specimens. In the rest of plant residues of M30 (expressed as parent) were found in the range of <0.01-0.02 mg/kg at 59-60 DALA, 0.01 mg/kg at 79 DALA and <0.01-0.01 mg/kg at 88-93 DALA. In cobs and grain no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ, resulting in total dimethenamid-P residue (sum of parent and M30) of <0.02 mg/kg, respectively.

After one application of 1.008 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant commodity decreased from a range of 34.4-64.4 mg/kg directly after the application to below the LOQ (0.01 mg/kg) after 60-61 days. Residues of its metabolites M26 at 0 DALA in the range of <0.01-0.03 mg/kg and of <0.01-0.01 mg/kg at 60-61 DALA were determined in whole plant specimens. No residues of M30 (expressed as parent) above the LOQ were found in whole plant and grain specimens. In the rest of plant residues of M30 (expressed as parent) were found in the range of <0.01-0.01 mg/kg at 59-60 DALA, 0.02 mg/kg at 79 DALA and <0.01-0.01 mg/kg at 88-93 DALA. In cobs and grain no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ. A summary is given in the Table 6.3.4-10. Details can be found in Table 6.3.4-12.

Southern Europe:

After one application of 0.864 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant commodity decreased from a range of 5.00-19.80 mg/kg directly after the application to below the LOQ (0.01 mg/kg) after 59-61 days. Residues of its metabolites M26 at 0 DALA in the range of 0.02-0.04 mg/kg were determined in whole plant specimens. Residues of M30 (expressed as parent) ranged from <0.01-0.09 mg/kg in whole plant at 0 DALA in whole plant. In the rest of plant residues of M30 (expressed as parent) were found in the range of <0.01-0.20 mg/kg at 59-61 DALA, <0.01-0.02 mg/kg at 76-91 DALA and <0.01-0.06 mg/kg at 99-100 DALA. In cobs and grain no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ, resulting in total dimethenamid-P residue (sum of parent and M30) of <0.02 mg/kg, respectively.

After one application of 1.008 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant commodity decreased from a range of 8.72-21.90 mg/kg directly after the application to below the LOQ (0.01 mg/kg) after 59-61 days. Residues of its metabolites M26 at 0 DALA in the range of 0.02-0.32 mg/kg were determined in whole plant and <0.01-0.22 mg/kg at 59-61 DALA in the rest of plant specimens. Residues of M30 (expressed as parent) ranged from <0.01-0.07 mg/kg in whole plant at 0 DALA in whole plant. In the rest of plant residues of M30 (expressed as parent) were found in the range of <0.01-0.08 mg/kg at 59-61 DALA, <0.01-0.12 mg/kg at 91-100 DALA and <0.01-0.02 mg/kg at 121-138 DALA. In cobs and grain no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ.A summary is given in Table 6.3.4-10 and Table 6.3.4-11 below. Details can be found in Table 6.3.4-12 and Table 6.3.4-13.

None of the analyzed untreated specimens showed any residues exceeding the LOQ of 0.01 mg/kg per analyte. The only exception was trial L11045 in South France, where 0.03 mg/kg residues of BAS 656 PH was found directly after the application.

Table 6.3.4-10: Summary of residues in maize after treatment of 1.008 kg dimethenamid-P/ ha

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Maize (EU North)	2012	BAS 656 12 H	0	Whole plant*	34.4-64.4	<0.01	<0.01-0.03	<0.01	<0.01	34.41-64.41
			60-61		<0.01-0.02	<0.01	<0.01-0.01	<0.01	<0.01	<0.02-0.03
			59-60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			79		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			88-93		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			110-120		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-60	Rest of plant	<0.01	<0.01	<0.01	<0.01	<0.01-0.01	<0.02-0.02
			79		<0.01	<0.01	<0.01	<0.01	0.02	0.03
			88-93		<0.01	<0.01	<0.01	<0.01	<0.01-0.01	<0.02-0.02
			110-120		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			128-142		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			128-142	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Maize (EU South)	2012	BAS 656 12 H	0	Whole plant*	8.72-21.90	<0.01	0.02-0.32	<0.01	<0.01-0.07	8.73-21.97
			59-76	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			76-91		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-100		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-61	Rest of plant	<0.01	<0.01	<0.01-0.22	<0.01	<0.01-0.80	<0.02-0.81
			76-87		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-100		<0.01	<0.01	<0.01	<0.01	<0.01-0.12	<0.02-0.13
			121-138		<0.01	<0.01	<0.01	<0.01	<0.01-0.02	<0.02-0.03
			92-138	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks

Table 6.3.4-11: Summary of residues in maize after treatment of 0.864 kg dimethenamid-P/ ha

Crop	Year	Application	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg)					
					BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Maize (EU North)	2011	BAS 656 12 H	0	Whole plant*	15.20-50.00	<0.01	<0.01-0.04	<0.01	<0.01	15.21-50.01
			60-61		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-60	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			79		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			88-93		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			110-120		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-60	Rest of plant	<0.01	<0.01	<0.01	<0.01	<0.01-0.02	<0.02-0.03
			79		<0.01	<0.01	<0.01	<0.01	0.01	0.02
			88-93		<0.01	<0.01	<0.01	<0.01	<0.01-0.01	<0.02-0.02
			110-120		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			128-142		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			128-142	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Maize (EU South)	2011	BAS 656 12 H	0	Whole plant*	5.00-19.80	<0.01	0.02-0.40	<0.01	<0.01-0.09	5.01-19.89
			59-76	Cobs**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			76-91		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			91-100		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			59-61	Rest of plant	<0.01	<0.01	<0.01-0.05	<0.01	<0.01-0.20	<0.02-0.22
			76-91		<0.01	<0.01	<0.01	<0.01	<0.01-0.02	<0.02-0.03
			91-100		<0.01	<0.01	<0.01-0.02	<0.01	<0.01-0.06	<0.02-0.07
			121-138		<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			92-138	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature seed at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.4-12: Residues of dimethenamid-P in maize after application of BAS 656 12 H in Northern Europe

Study details	Crop	Country	Formulation application rate (kg a.s./ha)	Crop growth stage	DALA[1]	Dimethenamid-P residues (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[2]	Total[3]
Study code: 390501	Maize	Germany	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	38.20	<0.01	<0.01	<0.01	<0.01	38.21
Doc ID: 2012/1209625				73	60	Whole plant*	0.02	<0.01	0.01	<0.01	<0.01	0.03
Trial No.: L110444				73	60	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP: yes				73	60	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02
Year: 2011				85	79	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	79	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
				87	88	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				87	88	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	29.30	<0.01	<0.01	<0.01	<0.01	29.31
				73	60	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				73	60	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				73	60	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
				85	79	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	79	Rest of plant*.	<0.01	<0.01	<0.01	<0.01	0.01	0.02
				87	88	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				87	88	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02
Study code: 390501	Maize	France	BAS 656 12 H 1 x 1.008	16/17	0	Whole plant*	34.40	<0.01	0.03	<0.01	<0.01	34.41
Doc ID: 2012/1209625				71/73	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.: L110042				71/73	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP: yes				85	93	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year: 2011				85	93	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			BAS 656 12 H 1 x 0.864	16/17	0	Whole plant*	15.20	<0.01	0.02	<0.01	<0.01	15.21
				71/73	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				71/73	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	93	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	93	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: 390501	Maize	United Kingdom	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	64.40	<0.01	<0.01	<0.01	<0.01	64.41
Doc ID: 2012/1209625				65	60	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.: L110043				71-75	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP: yes				71-75	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year: 2011				85-87	120	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85-87	120	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	142	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	142	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	50.00	<0.01	<0.01	<0.01	<0.01	50.01
				65	60	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				71-75	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				71-75	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85-87	120	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85-87	120	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	142	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	142	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

Table 6.3.4-12: Residues of dimethenamid-P in maize after application of BAS 656 12 H in Northern Europe

Study details	Crop	Country	Formulation application rate (kg a.s./ha)	Crop growth stage	DALA[1]	Dimethenamid-P residues (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[2]	Total[3]
Study code: 390501 **Doc ID:** 2012/1209625 **Trial No.:** L110044 **GLP:** yes **Year:** 2011	Maize	The Nether-lands	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	36.40	<0.01	0.02	<0.01	<0.01	36.41
				71	61	Whole plant*.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	92	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	92	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	110	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	110	Rest of plant*.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	23.60	<0.01	0.04	<0.01	<0.01	23.61
				71	61	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	92	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	92	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	110	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	110	Rest of plant*.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	128	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks
_ Underlined values were used for MRL calculation

Table 6.3.4-13: Residues of dimethenamid-P in maize after application of BAS 656 12 H in Southern Europe

Study details	Crop	Country	Formulation Application Rate (kg a.s./ha)	Crop growth stage	DALA[1]	Matrix	Dimethenamid-P residues (mg/kg) BAS 656 PH	M23	M26	M27	M30[2]	Total[3]
Study code: 390501	Maize	France	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	21.90	<0.01	0.32	<0.01	0.07	21.97
Doc ID: 2012/1209625				83	61	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.: L110045				83	61	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
GLP: yes				85	76	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year: 2011				85	76	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	138	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	138	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	19.80	<0.01	0.40	<0.01	0.09	19.89
				83	61	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				83	61	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	76	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	76	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	138	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	138	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: 390501	Maize	Greece	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	14.40	<0.01	0.14	<0.01	0.01	14.41
Doc ID:				85	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.: L110046				85	59	Rest of plant*	<0.01	<0.01	0.22	<0.01	0.80	0.81
GLP: yes				89	92	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year: 2011				89	92	Rest of plant*	<0.01	<0.01	0.01	<0.01	0.12	0.13
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	16.70	<0.01	0.19	<0.01	0.01	16.71
				85	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	59	Rest of plant*	<0.01	<0.01	0.05	<0.01	0.20	0.21
				89	92	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	92	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05
Study code: 390501	Maize	Italy	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	12.50	<0.01	0.08	<0.01	0.01	12.51
Doc ID: 2012/1209625				71	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Trial No.: L110047				71	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05
GLP: yes				83	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Year: 2011				83	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.06	0.07
				85	100	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	100	Rest of plant*	<0.01	<0.01	0.01	<0.01	0.09	0.10
				89	121	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	121	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	5.00	<0.01	0.03	<0.01	<0.01	5.01
				71	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				71	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
				83	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				83	91	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
				85	100	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	100	Rest of plant*	<0.01	<0.01	0.01	<0.01	0.06	0.07
				89	121	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	121	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

Table 6.3.4-13: Residues of dimethenamid-P in maize after application of BAS 656 12 H in Southern Europe

Study details	Crop	Country	Formulation Application Rate (kg a.s./ha)	Crop growth stage	DALA[1]	Dimethenamid-P residues (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[2]	Total[3]
Study code: 390501 **Doc ID:** 2012/1209625 **Trial No.:** L110048 **GLP:** yes **Year:** 2011	Maize	Spain	BAS 656 12 H 1 x 1.008	16	0	Whole plant*	8.72	<0.01	0.02	<0.01	<0.01	8.73
				75	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02
				85	87	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	87	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Rest of plant*	<0.01	<0.01	0.02	<0.01	0.04	0.05
				89	134	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	134	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			BAS 656 12 H 1 x 0.864	16	0	Whole plant*	6.20	<0.01	0.02	<0.01	<0.01	6.21
				75	59	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				75	59	Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03
				85	87	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	87	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Cob**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				85	91	Rest of plant*	<0.01	<0.01	0.02	<0.01	<0.01	<0.02
				89	134	Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				89	134	Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* Without roots
** Cobs with husks
_ Underlined values were used for MRL calculation

CA 6.3.5 Sugar beet

Table 6.3.5-1: Critical GAP for the use of BAS 656 PH in/on sugar beet

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Sugar beet	1 x 0.864 kg BAS 656 H/ha	100-400 L/ha	n.s.	spray application	BBCH 00-09

PHI = pre-harvest interval

This GAP is currently registered for the product BAS 656 12 H (EC formulation, application rate of 0.8-1.2 L product/ha at BBCH 00-09).

Table 6.3.5-2: GAP information of residue trials conducted in/on sugar beet in Northern and Southern Europe

Region	Country (trials)	Formulation	Application[0]				DALA[1]	DocID	EU submitted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Northern EU	Germany (1) France (1) United Kingdom (1) The Netherlands (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.65	0.325	1	0 103-125	2013/ 1003729	Yes
	Germany (1) France (1) United Kingdom (1) The Netherlands (1) 2011	BAS 656 12 H (EC)	Spray appl.	0.65	0.325	1	0 126-140	2012/ 1182982	Yes
Southern EU	France (1) Italy (1) 2012	BAS 656 12 H (EC)	Spray appl.	0.65	0.325	1	0 125-142	2013/ 1003729	Yes
	France (1) Italy (1) 2011	BAS 656 12 H (EC)	Spray appl.	0.65	0.325	1	0 104-114	2012/ 1182982	Yes

0 Actual application rates varied by 10% at most
1 Days after last application

Report: CA 6.3.5/1
Erdmann H.-P., 2013d
Study on the residue behaviour of BAS 656 H (Dimethenamid-P) in beets after application of BAS 656 12 H under field condition in United Kingdom, Northern and Southern France, The Netherlands, Italy and Germany, 2012
2013/1003729

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 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 656 12 H (EC)
   Lot/Batch #: 0004701751, dimethenamid-P: 720 g/L nominal
   Purity: Not relevant
   CAS#: 163515-14-8
   Development code: Not applicable
   Spiking levels: 0.01-10.0 mg/kg

2. **Test Commodity:**
   Crop: Sugar beet, fodder beet
   Type: Sugar plants (root and tuber vegetables)
   Variety: Klaxon, Vienna, Cayman and Isabella, Coyote, Starmon, Bruna
   Botanical name: *Beta vulgaris*
   Crop parts(s) or processed commodity: Whole plant with roots, leaves with tops, root without tops
   Sample size: 0.1-1.0 kg (12 plants/pieces/roots)

B. STUDY DESIGN

1. Test procedure

During the growing season of 2012, five field trials in sugar beet and on trial fodder beet were conducted in different representative growing areas of Northern and Southern Europe (Germany, The Netherlands, United Kingdom Northern and Southern France and Italy) to determine the residue levels of dimethenamid-P in or on (sugar) beet raw agricultural commodities (RAC). Each trial consisted of two plots: plot 1 (control) and plot 2 (treated with BAS 656 12 H). BAS 656 12 H (720 g/L BAS 656 PH, EC) was applied once at a rate equivalent to 0.65 kg a.s./ha at BBCH growth stage 14-16, or in case of trial L120263 at BBCH growth stage 18. The spray volume used was 200 L/ha.

Beet specimens were collected directly after the last application and 103-142 days thereafter at commercial harvest (BBCH growth stage 49).

All samples were shipped frozen to the analysis facility and remained frozen until analysis. The maximum storage interval was 314 days.

Table 6.3.5-3 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
2012	6	1	F	BAS 656 12 H (EC)	BAS 656 PH	1 x 0.650	200	BBCH 10-16

2. Description of analytical procedures

BASF method No L0179/02 was used to analyze the residues of dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352). BAS 656 PH and its metabolites were extracted with methanol. A portion of the extract was centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Table 6.3.5-4: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in sugar beet

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		BAS 656 PH			M23			M26		
Whole plant[1]	0.01, 1.0, 2.0	99.3	5.9	3	104	4.2	3	100	7.7	3
Leaves	0.01, 0.10, 10.0	98.3	3.2	3	98.2	4.8	3	90.5	7.7	3
Root	0.01, 0.10	97.6	N/A	2	99.3	N/A	2	93.0	N/A	2
Method No L0179/02		M27			M30					
Whole plant[1]	0.01, 1.0, 2.0	105	4.4	3	99.1	5.4	3			
Leaves	0.01, 0.10, 10.0	99.6	5.9	3	94.7	9.5	3			
Root	0.01, 0.10	97.8	N/A	2	96.2	N/A	2			

[1] With roots
N/A Not applicable

II. RESULTS AND DISCUSSION

Northern Europe:
After one application of 0.650 mg/kg BAS 656 12 H, residues of BAS 656 PH in sugar beet whole plant specimens ranged from 22-41 mg/kg directly after the application. At harvest, no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 (expressed as parent) exceeded the LOQ of 0.01 mg/kg in any tested specimens. Therefore the total dimethenamid-P (sum of parent and M30) was <0.02 mg/kg for all tested specimens. A summary is given in Table 6.3.5-5. Details can be found in Table 6.3.5-6.

Southern Europe:
After one application of 0.650 mg/kg BAS 656 12 H, residues of BAS 656 PH sugar beet whole plant specimens was 33 mg/kg directly after the application. At harvest, no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 (expressed as parent) exceeded the LOQ of 0.01 mg/kg in any tested commodity. Therefore the total dimethenamid-P (sum of parent and M30) was <0.02 mg/kg for all tested specimens. A summary is given in Table 6.3.5-5. Details can be found in Table 6.3.5-7.

None of the analyzed untreated specimens showed any residues exceeding the LOQ of 0.01 mg/kg per analyte.

Table 6.3.5-5: Summary of residues in sugar beets after treatment of dimethenamid-P

Region	Year	Application	DALA[1]	Dimethenamid-P residues (mg/kg)						
				Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
EU North†	2011	BAS 656 12	0	Whole plant*	22-41	<0.01	<0.01	<0.01	<0.01	22.01-41.01
			103-125	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			103-125	Roots***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
EU South‡			0	W.plant	22-33	<0.01	<0.01	<0.01	<0.01	22.01-33.01
			125-142	Leaves	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			125-142	Roots	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

1 Days after last application
2 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
3 Sum of dimethenamid-P and M30 expressed as parent equivalent
4 Whole plant without roots
† Sugar beets
‡ Sugar and fodder beets
* With roots
** With tops
*** Without tops

III. CONCLUSION

Residues of total dimethenamid-P (sum of parent and M30) in mature sugar beets at BBCH 49 were <0.02 mg/kg, respectively.

Table 6.3.5-6: Residues of dimethenamid-P in sugar beets after application of BAS 656 12 H in Northern Europe

Study details	Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2]	DALA[1]	Dimethenamid-P residues (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: 4231281 **Doc ID:** 2013/1003729 **Trial No.:** L120262 **GLP:** yes **Year:** 2012	Sugar beet	Germany	BAS 656 12 H 1 x 0.650	16 49 49	0 122 122	Whole plant* Leaves** Roots***	41 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	41.01 <0.02 <0.02
Study code: 4231281 **Doc ID:** 2013/1003729 **Trial No.:** L120263 **GLP:** yes **Year:** 2012	Sugar beet	France	BAS 656 12 H 1 x 0.650	18 49 49	0 125 125	Whole plant* Leaves** Roots***	22 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	22.01 <0.02 <0.02
Study code: 4231281 **Doc ID:** 2013/1003729 **Trial No.:** L120264 **GLP:** yes **Year:** 2012	Sugar beet	United Kingdom	BAS 656 12 H 1 x 0.650	14-16 49 49	0 103 103	Whole plant* Leaves** Roots***	29 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	29.01 <0.02 <0.02
Study code: 4231281 **Doc ID:** 2013/1003729 **Trial No.:** L120265 **GLP:** yes **Year:** 2012	Sugar beet	The Nether-lands	BAS 656 12 H 1 x 0.650	16 49 49	0 117 117	Whole plant* Leaves** Roots***	29 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	29.01 <0.02 0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops
_ Underlined values were used for MRL calculation

Table 6.3.5-7: Residues of dimethenamid-P in beets after application of BAS 656 12 H in Southern Europe

Study details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage	DALA[1]	Dimethenamid-P residues (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[2]	Total[3]
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	4231281 2013/1003729 L120266 yes 2012	Fodder beet	France	BAS 656 12 H 1 x 0.650	16 49 49	0 142 142	Whole plant* Leaves and stem Roots	22 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	22.01 <0.02 <0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	4231281 2013/1003729 L120267 yes 2012	Sugar beet	Italy	BAS 656 12 H 1 x 0.650	14-16 49 49	0 125 125	Whole plant* Leaves** Roots***	33 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	33.01 <0.02 <0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops
_ Underlined values were used for MRL calculation

Report: CA 6.3.5/2
Perny A., 2012a
Study on the residue behaviour of BAS 656 H (Dimethenamid-P) after treatment with BAS 656 12 H in beets (sugar) under field conditions in Northern and Southern Europe, 2011
2012/1182982

Guidelines: EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 7

GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 656 12 H (EC)
   Lot/Batch #: FRE-000601, dimethenamid-P: 720 g/L nominal
   Purity: Not relevant
   CAS#: 163515-14-8
   Development code: Not applicable
   Spiking levels: 0.01-45.0 mg/kg

2. **Test Commodity:**
   Crop: Sugar beet
   Type: Sugar plants
   Variety: Naute, Muraille, Bull Finch, Pyranja, Koala, Massima
   Botanical name: *Beta vulgaris*
   Crop parts(s) or processed:
   commodity: Whole plant with roots, leaves with tops, root without tops
   Sample size: 0.1-1.0 kg (12 plants/pieces/roots)

B. STUDY DESIGN

1. Test procedure

During the growing season of 2011, 6 field trials in sugar beet were conducted in different representative growing areas of Northern and Southern Europe (Germany, United Kingdom, The Netherlands, Northern and Southern France and Italy) to determine the residue levels of dimethenamid-P in or on raw agricultural commodities (RAC). Each trial consisted of two plots: plot 1 (control) and plot 2 (treated with BAS 656 12 H). BAS 656 12 H (720 g/L BAS 656 PH, EC) was applied once at a rate equivalent to 0.65 kg a.s./ha at BBCH growth stage 16. The spray volume used was 200 L/ha.

Sugar beet specimens were collected directly after the last application and 104-140 days thereafter at commercial harvest (BBCH growth stage 49). All samples were shipped frozen to the analysis facility and remained frozen until analysis. The maximum storage interval was 267 days.

Table 6.3.5-8: 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
2011	6	1	F	BAS 656 12 H (EC)	BAS 656 PH	1 x 0.650	200	BBCH 16

2. Description of analytical procedures

BASF analytical method No L0179/02 was used to analyze the residues of dimethenamid-P (BAS 656 PH) and its metabolites M23 (Reg No 360715), M26 (Reg No 360716), M27 (Reg No 360714) and M30 (Reg No 5296352) by means of LC-MS/MS. BAS 656 PH and its metabolites M23, M26, M27 and M30 were extracted with methanol. A portion of the extract wass centrifuged and an aliquot of the supernatant was diluted for determination by LC-MS/MS. The limit of quantitation is 0.01 mg/kg for each analyte.

Table 6.3.5-9: Summary of recoveries of dimethenamid-P (BAS 656 PH) and its metabolites in sugar beets

Matrix	Fortification level [mg/kg]	Summary recoveries								
		Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n	Mean [%]	RSD [%]	n
Method No L0179/02		**BAS 656 PH**			**M23**			**M26**		
Whole plant[1]	0.01, 0.10, 1.0, 45.0	98.4	3.4	4	99.9	6.0	4	100.6	4.5	4
Leaves without tops	0.01, 0.10, 1.0	102.3	2.3	3	99.5	0.8	3	100.0	1.8	4
Roots with tops	0.01, 0.10	106.0	N/A	2	105.5	N/A	2	105.6	N/A	2
Method No L0179/02		**M27**			**M30**					
Whole plant[1]	0.01, 0.10, 1.0, 45.0	99.3	2.7	4	96.0	2.4	4			
Leaves with tops	0.01, 0.10, 1.0	102.9	2.6	3	98.9	2.7	3			
Roots withhout tops	0.01, 0.10	106.8	N/A	2	104.2	N/A	2			

[1] With roots
N/A Not applicable

II. RESULTS AND DISCUSSION

Northern Europe:
After one application of 0.650 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant specimens ranged from 0.71-29.32 mg/kg directly after the application. At harvest, no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ of 0.01 mg/kg in any tested commodity. Therefore the total dimethenamid-P (sum of parent and M30) was <0.02 mg/kg for all tested specimens. A summary is given in Table 6.3.5-10. Details can be found in Table 6.3.5-11.

Southern Europe:
After one application of 0.650 mg/kg BAS 656 12 H, residues of BAS 656 PH in whole plant specimens ranged from 25.20-30.52 mg/kg directly after the application. At harvest, no residues of BAS 656 PH or its metabolites M23, M26, M27 and M30 exceeded the LOQ of 0.01 mg/kg in any tested commodity. Therefore the total dimethenamid-P (sum of parent and M30) was <0.02 mg/kg for all tested specimens. A summary is given in Table 6.3.5-10. Details can be found in Table 6.3.5-12.

None of the analyzed untreated specimens showed any residues exceeding the LOQ of 0.01 mg/kg per analyte.

Table 6.3.5-10: Summary of residues in sugar beets after treatment of dimethenamid-P

Region	Year	Application	DALA[1]	Dimethenamid-P residues (mg/kg)						
				Matrix	BAS 656 PH	M23	M26	M 27	M30[2]	Total[3]
EU North	2011	BAS 656 12	0	Whole plant*	0.71-29.32	<0.01	<0.01	<0.01	<0.01-0.04	<0.75-29.33
			103-125	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
			103-125	Roots***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
EU South			0	Whole plant*	25.20-30.52	<0.01	<0.01	<0.01	<0.01	25.21-30.53
			125-142	Leaves**		<0.01	<0.01	<0.01	<0.01	<0.02
			125-142	Roots***		<0.01	<0.01	<0.01	<0.01	<0.02

Days after last application
Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
Sum of dimethenamid-P and M30 expressed as parent equivalent
Without roots
* With tops
** Without tops

III. CONCLUSION

In all tested commodities collected at commercial harvest, no residues of BAS 656 PH and its metabolites M23, M26, M27 and M30 exceeded the LOQ of 0.01 mg/kg. Residues of total dimethenamid-P (sum of parent and M30) in mature seed at BBCH 89 were <0.02 mg/kg, respectively.

Table 6.3.5-11: Residues of dimethenamid-P in sugar beets after application of BAS 656 12 H in Northern Europe

Study details	Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2]	DA LA[1]	Dimethenamid-P residues (mg/kg)						
						Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: 390503 **Doc ID:** 2012/1182982 **Trial No.:** L110035 **GLP:** yes **Year:** 2011	Sugar beet	Germany	BAS 656 12 H 1 x 0.650	16	0	Whole plant*	29.32	<0.01	<0.01	<0.01	<0.01	29.33
				49	140	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				49	140	Root***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: 390503 **Doc ID:** 2012/1182982 **Trial No.:** L110036 **GLP:** yes **Year:** 2011	Sugar beet	France	BAS 656 12 H 1 x 0.650	15-17	0	Whole plant*	0.71	<0.01	<0.01	<0.01	0.04	0.75
				49	136	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				49	136	Root***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: 390503 **Doc ID:** 2012/1182982 **Trial No.:** L110037 **GLP:** yes **Year:** 2011	Sugar beet	United Kingdom	BAS 656 12 H 1 x 0.650	16	0	Whole plant*	11.40	<0.01	<0.01	<0.01	<0.01	11.41
				49	136	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				49	136	Root***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
Study code: 390503 **Doc ID:** 2012/1182982 **Trial No.:** L110038 **GLP:** yes **Year:** 2011	Sugar beet	The Nether-lands	BAS 656 12 H 1 x 0.650	16	0	Whole plant*	24.68	<0.01	<0.01	<0.01	<0.01	24.69
				49	126	Leaves**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
				49	126	Root***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops
_ Underlined values were used for MRL calculation

Table 6.3.5-12: Residues of dimethenamid-P in sugar beets after application of BAS 656 12 H in Northern Europe

Study details		Crop	Country	Formulation application rate[0] (kg a.s./ha)	Crop growth stage[2]	DALA[1]	Dimethenamid-P residues (mg/kg)						
							Matrix	BAS 656 PH	M23	M26	M27	M30[3]	Total[4]
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	390503 2012/1182982 L110039 yes 2011	Sugar beet	France	BAS 656 12 H 1 x 0.650	16 49 49	0 114 114	Whole plant* Leaves** Root***	25.20 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	25.21 <0.02 <0.02
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	390503 2012/1182982 L110040 yes 2011	Sugar beet	Italy	BAS 656 12 H 1 x 0.650	16 48-49 49	0 104 104	Whole plant* Leaves** Root***	30.52 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	30.53 <0.02 <0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to dimethenamid-P is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of dimethenamid-P and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops
_ Underlined values were used for MRL calculation

CA 6.3.6 Supplementary Information

In addition to the residue data for the representative crops sunflower, oilseed rape, soybean, maize and sugar beet the results of other supervised field trials are shown in this chapter. These data are not meant to be fully evaluated but they provide further data on determination of the metabolites M23, M26, M27 and M30. These data were also considered for determination of metabolite relevance.

The residues for dimethenamid-P and its metabolites M23, M26, M27, and M30 found in supplementary trials are summarized in Table 6.3.6-2.

From the results obtained from specifically analyzing the metabolites M23, M26, M27 and M30 the following conclusions can be drawn:

- In a minor percentage of the samples analyzed metabolites M23 and M27 could be quantified in animal feed items only
- In approximately a quarter of the samples analyzed metabolites M26 and M30 could be quantified (residues above LOQ) but only M30 was dominant at relevant harvest intervals.

Due to the spare distribution of the metabolites of M23 and M27 in analyzed samples and the compared to parent lesser toxicity, it is concluded that both metabolites have no significant impact on the consumer safety. The lower toxicity of M26 compared to parent dimethenamid-P and the direct link between M26 and M30 ~~of residues of M26 and M30~~ supported by the well-known metabolic pathway demonstrate that values of M30 cover the risk for both metabolites. In good approximation, it is therefore proposed to use following residue definition for the risk assessment in plants:

Parent dimethenamid-P and M30 expressed as parent equivalents.

Based on the preliminary results of ongoing residue studies for product registration after Annex I renewal presented below, values of M30 are used for the consumer risk assessment of dimethenamid-P. Dietary risk assessment is performed for all 4 metabolites (see chapter 6.9) to illustrate the non-relevance of M23, M27 and M26 to the plant residue definition. Currently, it is planned to submit the residue trials below as part of an MRL application in the future.

Table 6.3.6-2: Residue results of dimethenamid-P and its metabolites M23, M26, M27 and M30

Report No	Crop#	Commodity	DALA	BBCH	Residues in mg/kg				
					BAS 656 PH	M23	M26	M27	M30
2013/1335409	Apple	Fruit	0	74	<0.01	<0.01	<0.01	<0.01	<0.01
428103		Fruit	27	75	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 656 12 H		Fruit	70	81	<0.01	<0.01	<0.01	<0.01	<0.01
1x 0.864 kg a.s./ha		Fruit	99	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	0	74	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	33	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	59	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	83	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	0	74	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	12	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	79	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	99	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	0	74	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	6	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	50	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	38	89	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1335408	Cherry	Fruit (with stones)	0	73	<0.01	<0.01	<0.01	<0.01	<0.01
428102		Fruit (without stones)	25	75	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 656 12 H		Fruit (without stones)	42	89	<0.01	<0.01	<0.01	<0.01	<0.01
1x 0.864 kg a.s./ha		Fruit (with stones)	0	73	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (with stones)	15	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	21	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	48	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (with stones)	0	73	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (with stones)	29	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	50	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	57	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (with stones)	0	73	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (with stones)	5	75	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	42	81	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit (without stones)	56	89	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1335407	Strawberry#	Fruit	22	71-73	<0.01	<0.01	0.013	<0.01	0.071
428101		Fruit	28	73	<0.01	<0.01	<0.01	<0.01	0.028
BAS 656 12 H		Fruit	43	87	<0.01	<0.01	<0.01	<0.01	0.014
1x 0.864 kg a.s./ha		Fruit	22	67-73	<0.01	<0.01	<0.01	<0.01	0.022
		Fruit	27	67-81	<0.01	<0.01	<0.01	<0.01	0.014
		Fruit	40	87	<0.01	<0.01	<0.01	<0.01	<0.01
		Fruit	22	81	<0.01	<0.01	<0.01	<0.01	0.036
		Fruit	29	81-83	<0.01	<0.01	<0.01	<0.01	0.024
		Fruit	36	87	<0.01	<0.01	<0.01	<0.01	0.016
		Fruit	21	71-73	<0.01	<0.01	0.016	<0.01	0.047
		Fruit	26	81	<0.01	<0.01	<0.01	<0.01	0.020
		Fruit	41	87	<0.01	<0.01	<0.01	<0.01	0.015
2013/1335416	Onion#	Whole plant	0	16	3.8	<0.01	~~0.16~~ 0.15*	<0.01	~~0.17~~ 0.15**
428115		bulb	71	49	<0.01	<0.01	~~0.010~~ <0.01	<0.01	0.020
BAS 659 03 H		Whole plant	0	17-18	1.2	<0.01	0.29	<0.01	~~0.24~~ 0.21
1x 0.85 kg a.s./ha		bulb	42	48-49	<0.01	<0.01	0.013	<0.01	0.089
		Whole plant	0	18	1.3	<0.01	~~0.19~~ 0.18	<0.01	~~0.098~~ 0.086
		bulb	39	49	<0.01	<0.01	0.023	<0.01	0.14
		Whole plant	0	18	3.1	<0.01	0.054	<0.01	~~0.015~~ 0.013
		bulb	63	49	<0.01	<0.01	~~0.010~~ <0.01	<0.01	~~0.010~~ <0.01

Table 6.3.6-2: Residue results of dimethenamid-P and its metabolites M23, M26, M27 and M30

Report No	Crop#	Commodity	DALA	BBCH	Residues in mg/kg				
					BAS 656 PH	M23	M26	M27	M30
2013/1335411 428107 BAS 656 12 H 1x 0.864 kg a.s./ha	Welsh/ Spring onion	Bulb Bulb	0 ~~Earliest harvest~~ 35	13-15 19-41	1.5 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 0.016
		Bulb Bulb	0 ~~Earliest harvest~~ 36	13 49	2.7 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
		Bulb Bulb	0 ~~Earliest harvest~~ 28	13 49	2.6 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 0.034
		Bulb Bulb	0 ~~Earliest harvest~~ 43	13 49	11 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
	Leek	Whole plant with roots Whole plant with roots Whole plant with roots	0 81 132	14 47 49	4.4 <0.01 <0.01	<0.01 <0.01 <0.01	0.074 <0.01 <0.01	<0.01 <0.01 <0.01	0.021 <0.01 <0.01
		Whole plant with roots Whole plant with roots Whole plant with roots	0 79 111	13-15 47 49	3.6 <0.01 <0.01	<0.01 <0.01 <0.01	0.044 <0.01 <0.01	<0.01 <0.01 <0.01	0.018 <0.01 <0.01
		Whole plant with roots Whole plant with roots Whole plant with roots	0 79 99	13-15 47 49	2.6 <0.01 <0.01	<0.01 <0.01 <0.01	0.054 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
		Whole plant with roots Whole plant with roots	0 79	13 49	6.3 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
2013/1335415 428113 BAS 656 12 H 1x 0.864 kg a.s./ha	Cucumber	Fruit Fruit Fruit Fruit	80 64 47 48	89 89 89 89	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
	Zucchini	Fruit Fruit Fruit Fruit	80 36 48 37	89 89 89 89	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
2013/1335406 428112 BAS 656 12 H 1x 0.864 kg a.s./ha	Melon	Fruit Fruit Fruit Fruit	70 67 96 85	85-89 89 89 89	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
	Pumpkin	Fruit Fruit Fruit Fruit	79 84 85 74	89 89 89 89	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
2013/1335410 428106 BAS 769 00 H 1x 0.5 kg a.s./ha	Broccoli	Whole plant without roots Inflorescences	0 69	18 49	4.3 <0.01	<0.01 <0.01	0.044 <0.01	<0.01 <0.01	0.13 <0.01
		Whole plant without roots Inflorescences	0 74	18 49	5.0 <0.01	<0.01 <0.01	0.039 <0.01	<0.01 <0.01	0.18 <0.01
	Cauliflower	Whole plant without roots Inflorescences	0 84	18 49	1.8 <0.01	<0.01 <0.01	0.022 <0.01	<0.01 <0.01	0.20 <0.01
		Whole plant without roots Inflorescences	0 68	18 49	2.7 <0.01	<0.01 <0.01	~~0.010~~ 0.022 <0.01	~~0.018~~ <0.01 <0.01	0.092 <0.01
		Whole plant without roots Inflorescences	0 114	18 49	4.7 <0.01	<0.01 <0.01	0.046 <0.01	<0.01 <0.01	0.11 <0.01
		Whole plant without roots Inflorescences	0 58	18 49	10 <0.01	<0.01 <0.01	0.10 <0.01	<0.01 <0.01	0.12 <0.01
2013/1182806 418108 BAS 769 00 H 1x 0.5 kg a.s./ha	Cauliflower	Whole plant without roots Inflorescences	0 148	18 49	5.32 <0.01	<0.01 <0.01	0.02 <0.01	<0.01 <0.01	0.04 <0.01
		Whole plant without roots Inflorescences	0 49	18 49	2.10 <0.01	<0.01 <0.01	0.06 <0.01	<0.01 <0.01	0.06 <0.01

Table 6.3.6-2: Residue results of dimethenamid-P and its metabolites M23, M26, M27 and M30

Report No	Crop#	Commodity	DALA	BBCH	Residues in mg/kg				
					BAS 656 PH	M23	M26	M27	M30
2013/1335412	White	Whole plant without roots	0	18-19	1.2	<0.01	0.015	~~0.20~~ <0.01	~~<0.01~~ 0.20
428108	cabbage	Head	63	49	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 769 00 H		Whole plant without roots	0	18	4.5	<0.01	0.025	~~0.057~~ <0.01	~~<0.01~~ 0.057
1x 0.5 kg a.s./ha		Head	74	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	4.5	<0.01	0.029	<0.01	~~<0.01~~ 0.23
		Head	68	49	<0.01	<0.01	<0.01	<0.01	<0.01
	***	~~Whole plant without roots~~	~~0~~		~~15~~	~~<0.01~~	~~0.010~~	~~0.019~~	~~<0.01~~
		~~Head~~		~~49~~	~~<0.01~~	~~<0.01~~	~~<0.01~~	~~<0.01~~	~~0.11~~
		Whole plant without roots	0	18	6.2	<0.01	0.047	<0.01	0.12
		Head	52	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	3.7	<0.01	0.029	<0.01	0.32
		Head	70	49	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1182806	White	Whole plant without roots	0	18	1.17	<0.01	0.02	<0.01	0.03
418108	cabbage	Heads	46	49	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 769 00 H		Whole plant without roots	0	18	0.34	<0.01	<0.01	<0.01	0.04
1x 0.5 kg a.s./ha		Heads	124	49	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1335419	Chinese	Whole plant without roots	0	18	9.5	<0.01	0.050	<0.01	0.19
428096	Cabbage	Head with wrapper leaves	45	49	<0.01	<0.01	<0.01	<0.01	0.031
BAS 769 00 H		Whole plant without roots	0	18	2.4	<0.01	0.012	<0.01	0.14
1x 0.5 kg a.s./ha		Head with wrapper leaves	26	49	<0.01	<0.01	<0.01	<0.01	0.020
		Whole plant without roots	0	18	12	<0.01	0.44	<0.01	0.20
		Head with wrapper leaves	64	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	4.5	<0.01	<0.01	<0.01	0.052
		Head with wrapper leaves	26	49	<0.01	<0.01	<0.01	<0.01	0.062
	Curly	Whole plant without roots	0	18	11	<0.01	0.017	<0.01	0.029
	kale	Leaves	35	49	<0.01	<0.01	<0.01	<0.01	0.013
		Whole plant without roots	0	18	20	<0.01	<0.01	<0.01	<0.01
		Leaves	61	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	6.4	<0.01	<0.01	<0.01	0.014
		Leaves	87	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	2.4	<0.01	<0.01	<0.01	0.037
		Leaves	86	49	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1335418	Brussel	Whole plant without roots	0	18	1.0	<0.01	0.04	<0.01	0.16
428125	sprouts	Sprouts	104	49	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 656 12 H		Whole plant without roots	0	18-19	21.0	<0.01	0.23	<0.01	0.22
1x 0.72 kg a.s./ha		Sprouts	143	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18-19	2.0	<0.01	0.026	0.012	0.31
		Sprouts	88	49	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	1.5	<0.01	0.16	<0.01	0.67
		Sprouts	103	49	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1335413	Green	Whole plant without roots	0	14	~~45~~ 44.8	<0.01	<0.01	<0.01	<0.01
428110	bean	Pods with seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 656 12 H		Rest of plant without roots	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
BAS 659 03 H		Seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
1 x 850 g a.s. /ha		Pods without seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	98	89	<0.01	0.019	<0.01	~~0.026~~ 0.025	~~0.015~~ 0.016
		Seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01

Table 6.3.6-2: Residue results of dimethenamid-P and its metabolites M23, M26, M27 and M30

Report No	Crop#	Commodity	DALA	BBCH	Residues in mg/kg				
					BAS 656 PH	M23	M26	M27	M30
		Whole plant without roots	0	14	31.4	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	69	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	98	89	<0.01	<0.01	<0.01	~~0.017~~ 0.018	<0.01
		Seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	14	24.0	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	43	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	43	77-79	<0.01	~~<0.01~~ 0.023	<0.01	~~<0.01~~ 0.020	<0.01
		Seeds	43	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	43	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	58	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	58	89	<0.01	~~0.027~~ 0.026	<0.01	0.027	<0.01
		Seeds	58	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	58	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	13-14	~~59~~ 56.6	<0.01	~~0.015~~ 0.018	<0.01	<0.01
		Pods with seeds	42	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	42	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Seeds	42	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	42	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	62	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	62	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Seeds	62	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	62	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	14	~~62~~ 61.8	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	64	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	64	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Seeds	64	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	64	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	109	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	109	89	<0.01	~~0.014~~ 0.015	<0.01	~~0.029~~ 0.028	<0.01
		Seeds	109	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	109	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	14	~~61~~ 60.8	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	68	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	68	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Seeds	68	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	68	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	98	89	<0.01	0.013	<0.01	~~0.026~~ 0.025	<0.01
		Seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	98	89	<0.01	<0.01	<0.01	<0.01	<0.01

Table 6.3.6-2: Residue results of dimethenamid-P and its metabolites M23, M26, M27 and M30

Report No	Crop#	Commodity	DALA	BBCH	Residues in mg/kg				
					BAS 656 PH	M23	M26	M27	M30
		Whole plant without roots	0	14	~~20~~ 26.0	<0.01	~~0.034~~ 0.036	<0.01	<0.01
		Pods with seeds	49	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	49	77-79	<0.01	<0.01	<0.01	0.014	<0.01
		Seeds	49	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	49	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	70	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	70	89	<0.01	<0.01	<0.01	0.017	<0.01
		Seeds	70	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	70	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	14	19.1	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	74	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	74	77-79	<0.01	<0.01	<0.01	0.010	<0.01
		Seeds	74	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	74	77-79	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods with seeds	95	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Rest of plant without roots	95	89	<0.01	<0.01	<0.01	0.010	<0.01
		Seeds	95	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Pods without seeds	95	89	<0.01	<0.01	<0.01	<0.01	<0.01
2013/1003730 2013/1377012 422462 BAS 769 00 H 1x 0.6 kg a.s./ha	Oilseed rape	Whole plant without roots	0	14-51	27.18	<0.01	0.117	<0.01	0.044
		Whole plant without roots	8	53	<0.01	<0.01	0.231	<0.01	2.92
		Rest of plant without roots	99	89	<0.01	<0.01	<0.01	<0.01	0.086
		Seeds	99	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	15-16	23.41	<0.01	0.206	<0.01	0.104
		Whole plant without roots	5	53-55	<0.01	<0.01	0.130	<0.01	2.84
		Rest of plant without roots	101	89	<0.01	<0.01	<0.01	<0.01	0.016
		Seeds	101	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	30.24	<0.01	0.080	<0.01	0.037
		Whole plant without roots	15	50-53	<0.01	<0.01	0.029	<0.01	2.64
		Rest of plant without roots	71	89	<0.01	<0.01	<0.01	<0.01	0.045
		Seeds	71	89	<0.01	<0.01	<0.01	<0.01	<0.01
		Whole plant without roots	0	18	40.83	<0.01	0.244	<0.01	0.096
		Whole plant without roots	13	50-53	<0.01	<0.01	0.029	<0.01	2.92
		Rest of plant without roots	78	89	<0.01	<0.01	<0.01	<0.01	0.060
		Seeds	78	89	<0.01	<0.01	<0.01	<0.01	<0.01

* 0.053 mg/kg of M26 was found in the untreated control
** 0.067 mg/kg of M30 was found in the untreated control
*** Trial is not considered valid because the cabbage did not reach maturity
# A second year of residue trials is running until 2015. In addition to the above carrot trials are running in 2015. A final business decision on requesting a registration on carrot has not been decided.

CA 6.4 Feeding studies

The requirements for feeding studies are set out according to Commission Regulation (EU) No 283/2013 with data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market as well as the Appendix G (Lundehn document 7031/VI/95 rev.4, July 1996) and OECD guidelines. Feeding studies are required:

(1) if significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to livestock,

and

(2) if metabolism studies indicate that significant residues (above 0.01 mg/kg for each analyte) may occur in any edible animal tissue, taking into account the residue levels in potential feeding stuff obtained at the 1x dose rate.

(3) However, feeding studies shall not be required where intake is below 0.004 mg/kg bw/d, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

Intended uses with the two representative formulations BAS 656 12 H and BAS 830 01 H covered in this submission include the following crops: oilseed rape, sunflower, soybean, maize and sugar beet. All these crops have relevant feed items: rape seed, sunflower seed, soybean seed and their meals as well as rape forage and maize grain and forage as well as sugar beet leaves and roots.

The relevant residue definition for **plants** is proposed as parent dimethenamid-P + M30 metabolite. Based on the data in this chapter, the relevant **residue definition for enforcement** (monitoring) of dimethenamid-P residues in **products of animal origin** is proposed as the sum of parent dimethenamid-P + M30, expressed as dimethenamid-P equivalents. The relevant **residue definition for risk assessment** is proposed as parent dimethenamid-P + M26 +M30 **in liver and kidney** of sheep, cattle, goats, horses and other farm animals. Further discussion will be provided at the end of this chapter and in MCA, Section 6, Chapter 6.7.2.

Additionally, since metabolism studies and residue trials occasionally showed residues of M23, and M27 in feed item matrices, residues and feed burden calculations of those analytes are at times discussed below, to further illustrate their non-relevance to consumers.

In the following chapters the anticipated maximum dietary burden for poultry, pigs and ruminants (dairy cattle and beef cattle) calculated with the EFSA feed burden calculator, as provided in the PROFile (based on Appendix G of the Lundehn document, see above) are presented. Details on the calculation in regards to the proposed residue definition and the selected input values can be found in MCA Section 6, Chapter 6.7 where the proposed EU MRLs for animal products are derived.

CA 6.4.1 Poultry

For completeness, the dietary burden was calculated for parent dimethenamid-P, M23, M26, M27, and M30; only M30 warrants further discussion. The calculated doses in terms of mg per animal and day are derived in Table 6.4.1-1 to Table 6.4.1-2. For dimethenamid-P, M23, M26 and M27 all residues are below the analytical limit of quantitation (LOQ), therefore only one table is presented for this feed burden in Table 6.4.1-1.

Table 6.4.1-1: Estimated maximum dietary burden for poultry that are exposed to residues below LOQ such as dimethenamid-P[1], M23, M26 and M27

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Poultry*	
			% of diet	mg/kg bw/d
I Green forages				
Cabbage	14	0.01	5	0.000226
II Cereal grain				
Maize grain	86	0.01	65	0.000477
V Roots and tubers				
Turnips	10	0.01	20	0.001263
VI Oil seed				
Rape seed meal	86	0.02	10	0.000147
Dietary burden:		mg/kg bw/day	0.00211	
		mg/animal/day	0.004	
		mg/kg total feed (DM)	0.033	

* Feed intake 0.120 kg DM, body weight (bw) 1.9 kg
1 Parent dimethenamid-P or metabolite M23, M26 or M27 using analyte equivalents

Table 6.4.1-2: Estimated maximum dietary burden of M30[1] residues for poultry

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Poultry*	
			% of diet	mg/kg bw/d
I Green forage				
~~Cabbage~~ Kale	14	~~0.11~~ 0.013	5	~~0.002481~~ 0.000293
II Cereal grain				
Maize grain	86	0.01	65	0.000477
V Roots and tubers				
Turnips	10	0.01	20	0.001263
VI Oil seed				
Rape seed meal	86	0.02	10	0.000147
Dietary burden:		mg/kg bw/day	~~0.00437~~ 0.00218	
		mg/animal/day	~~0.0083~~ 0.0041	
		mg/kg total feed (DM)	~~0.069~~ 0.034	

* Feed intake 0.120 kg DM, body weight (bw) 1.9 kg
1 Metabolite M30 using analyte equivalents

Tissue Extrapolations using the Feed Burdens of Parent dimethenamid-P, M23, M26 and M27 in Poultry

For dimethenamid-P and metabolites M23, M26~~ and~~, M27 and M30, the dietary feed burden is below 0.004 mg/kg bw day, which is the exposure trigger for a hen metabolism study. In addition, the feed burdens for ~~these~~ metabolites M23, M26 and M27 are all ~~well~~ below the dietary feed burden for metabolite M30 of ~~0.00437~~ 0.00218 mg/kg bw day or ~~0.069~~ 0.034 mg/kg feed. As such, it can be assumed that any exposure to consumers from residues in animal commodities derived from these metabolites would be lower than the exposure from residues in animal commodities to M30. Therefore, only tissue extrapolations for M30 are shown below.

Tissue Extrapolations using the Feed Burden of Metabolite M30 in Poultry

The calculated feed burden results for metabolite M30 were compared with the above mentioned hen metabolism study for racemic dimethenamid. This metabolism study in laying hens was performed at a dose level of 10 mg/kg body weight (167 mg/kg feed). The results for M30 were converted to dimethenamid-P (CF 0.73) and these values were compared with the results from the metabolism study. These metabolism dose levels are exaggerated by factors ranging from ~~3125~~ 6250 to ~~3340~~ 6680 when compared to the projected residue intakes of Table 6.4.1-2. The overdosing factors are derived in Table 6.4.1-3. The proposed residue definition accounts for the most prominent metabolite M30 and conservatively includes parent even though animals are unlikely to be exposed to parent.

Table 6.4.1-3: Calculation of overdosing factors for hens for metabolite M30

	Dose from hen metabolism study	**Potential intake, hen**
mg/kg bw	10 mg/kg bw	~~0.00437~~ 0.00218 x 0.73 = ~~0.0032~~ 0.0016 mg/kg bw
mg/kg feed	167 mg/kg feed	~~0.069~~ 0.034 x 0.73 = ~~0.050~~ 0.025 mg/kg feed
Overdosing factor = Dose from hen metabolism study / Potential intake		
		Overdosing factor, hen
Dose calculated by body weight		~~3125~~ 6250 = (10 / ~~0.0032~~ 0.0016)
Dose calculated by feed weight		~~3340~~ 6680 = (167 / ~~0.050~~ 0.025)

Based on the overdosing factors, the expected total residues in egg and edible tissues from hens can be extrapolated from the total radioactive residues (TRR) found in the hen metabolism study. This extrapolation is a worst case scenario, since the TRR includes non-extractable and bound residues, along with metabolites not accounted for by the residue analytical method. The extrapolation conservatively shows that no residues for metabolite M30 are expected above the LOQ of the residue analytical method (0.01 mg/kg for tissues and eggs). Extrapolated residues are shown in Table 6.4.1-5.

Table 6.4.1-4: Potential transfer of feed residues to hen tissues and egg

	Total radioactive residue (mg/kg) from hen metabolism study	Extrapolated total residue from potential intake (mg/kg) [Hen ^{14}C-results / Overdosing factor]	
Matrix	**Hen ^{14}C-results**	**Based on intake per body weight**	**Based on projected residues in feed**
Liver	8.33	~~0.002666~~ 0.001333	~~0.002494~~ 0.001247
Muscle	0.58	~~0.000186~~ 0.000093	~~0.000173~~ 0.000087
Fat	0.29	~~0.000093~~ 0.000046	~~0.000087~~ 0.000043
Egg yolk	0.62	~~0.000198~~ 0.000099	~~0.000186~~ 0.000093
Egg white	0.30	~~0.000096~~ 0.000048	~~0.000090~~ 0.000045

Furthermore, highest component from each compartment of the hen metabolism study was compared with the anticipated maximum dietary burden to estimate the anticipated residue in animals. The extrapolation emphasizes that no residues at or above the LOQ are expected in any hen matrix.

Table 6.4.1-5: Tissue extrapolation of feed residues to hen tissues and egg

	Highest component in each compartment		Extrapolated total residue from potential intake (mg/kg) [Hen ^{14}C-results / Overdosing factor]	
Matrix		**[mg/kg]**	**Based on intake per body weight**	**Based on projected residues in feed**
Liver	L1	0.65	~~0.00019~~ 0.000104	~~0.00019~~ 0.000097
Muscle	MU1	0.04	~~0.00001~~ 0.000006	~~0.00002~~ 0.000006
Fat	F1	0.075	~~0.00002~~ 0.000012	~~0.00002~~ 0.000011
Egg yolk	EY4	0.054	~~0.00002~~ 0.000009	~~0.00001~~ 0.000008
Egg white	EW10	0.044	~~0.00001~~ 0.000007	~~0.00002~~ 0.000007

Overall conclusion

From the preceding calculations, it is concluded no feeding study for laying hens is required.

CA 6.4.2 Ruminants

For completeness, the dietary burden was calculated for parent dimethenamid-P, M23, M26, M27, and M30. The calculated doses in terms of mg per animal and day are derived in Table 6.4.2-1 to Table 6.4.2-2. For dimethenamid-P, M23, and M27, all residues are below the analytical limit of quantitation (LOQ), therefore only one table is presented for this feed burden in Table 6.4.2-1. Given the higher observed residues of M26 and M30 in potential animal feeds, a more detailed discussion of M26 and M30 follows.

Table 6.4.2-1: Estimated maximum dietary burden for cattle that are exposed to metabolites below the LOQ such as dimethenamid-P[1], M23, and M27

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Grass (fresh)	20	0.01	70	0.001273	40	0.000857
V Roots and tubers						
Turnips	10	0.01	30	0.001091	60	0.002571
Dietary burden:	mg/kg bw/day		0.002364		0.003429	
	mg/animal/day		1.3		1.2	
	mg/kg total feed (DM)		0.065		0.08	

* Feed intake 20 kg DM, body weight (bw) 550 kg
** Feed intake 15 kg DM, body weight (bw) 350 kg
1 Parent dimethenamid-P or M23 or M27 using analyte equivalents

For dimethenamid-P and metabolites M23, and M27, the dietary feed burden is below 0.004 mg/kg bw day, the exposure that would trigger a goat metabolism study.

Table 6.4.2-2: Estimated maximum dietary burden of M26[1] residues for cattle

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize silage	20	0.05	100	0.009091	-	-
Rape forage	14	0.231	-	-	35	0.024750
V Roots and tubers						
Turnips	10	0.01	-	-	60	0.002571
VI Oil seed						
Rape seed meal	86	0.02	-	-	5	0.000050
Dietary burden:	mg/kg bw/day		0.009091		0.027371	
	mg/animal/day		5.0		9.6	
	mg/kg total feed (DM)		0.25		0.64	

* Feed intake 20 kg DM, body weight (bw) 550 kg
** Feed intake 15 kg DM, body weight (bw) 350 kg
1 Metabolite M26 using analyte equivalents

Table 6.4.2-3: Estimated maximum dietary burden of M30[1] residues for cattle

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Grass (fresh)	20	0.28	100	0.050909	-	-
Rape forage	14	2.92	-	-	35	0.312857
V Roots and tubers						
Turnips	10	0.01	-	-	60	0.002571
VI Oil seed						
Rape seed meal	86	0.02	-	-	5	0.000050
Dietary burden:	mg/kg bw/day		0.050909		0.3154	
	mg/animal/day		28.0		110.4	
	mg/kg total feed (DM)		1.4		7.36	

* Feed intake 20 kg DM, body weight (bw) 550 kg
** Feed intake 15 kg DM, body weight (bw) 350 kg
1 Metabolite M30 using analyte equivalents

Tissue Extrapolations using the Feed Burden of Metabolite M26 in Ruminants as Transferred to M26 in Ruminant Tissue

For metabolite M26, two different comparisons/extrapolations using the M30 goat metabolism study are made. 1) The feed burden calculation for M26 is compared with the residues of M26 derived from the M30 goat metabolism study. 2) The feed burden calculation for M30 will be compared with the residues of M26 derived from the M30 goat metabolism study.

This metabolism study in lactating ruminants was performed at a dose level of M30 at 0.6 mg/kg body weight (12 mg/kg feed). These metabolism dose levels are exaggerated by factors ranging from 19 to ~~48~~ 62 when compared to the projected residue intakes of M26 in Table 6.4.2-2. The overdosing factors are derived in Table 6.4.2-~~3~~4. The results are presented below:

Table 6.4.2-4: Calculation of overdosing factors for beef and dairy cattle from M26 feed burden

	Dose from M30 goat metabolism study	Potential M26 intake, beef cattle	Potential M26 intake, dairy cattle
mg/kg bw	0.5663 mg/kg bw	0.027371 mg/kg bw	0.009091 mg/kg bw
mg/kg feed	12 mg/kg feed	0.64 mg/kg feed	0.25 mg/kg feed
Overdosing factor = Dose from goat metabolism study / Potential intake			
		Overdosing factor, beef cattle	**Overdosing factor, dairy cattle**
Dose Calculated by Body Weight		21 = (0.5663 / 0.027371)	62 = (0.5663/ 0.009091)
Dose Calculated by Feed Weight		19 = (12/ 0.64)	48 = (12 / 0.25)

Table 6.4.2-5: Potential transfer of feed residues to cattle tissues and milk for M26 with feed burden from M26

	Total radioactive M26 residue from goat metabolism study	Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]	
Matrix	**M26 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Beef cattle:			
Muscle	0.002	0.00010	0.00011
Fat	0.002	0.00010	0.00011
Liver	0.011	0.00052	0.00058
Kidney	0.06	0.00286	0.00316
Dairy cattle:			
Milk	0.002	0.00003	0.00004
Muscle	0.002	0.00003	0.00004
Fat	0.002	0.00003	0.00004
Liver	0.011	0.00018	0.00023
Kidney	0.06	0.00097	0.00125

Based on the overdosing factors, the expected total residues in milk and edible tissues from cows can be extrapolated from the total radioactive M26 residues found in the goat metabolism study performed with M30. The extrapolation shows that no M26 residues are expected above the LOQ of the analytical method in any tissue from animals from the feed burden of M26.

Tissue Extrapolations using the Feed Burden of Metabolite M30 in Ruminants as Transferred to M26 in Ruminant Tissues

For metabolite M26, two different comparisons/extrapolations using the M30 goat metabolism study are made. 1) The feed burden calculation for M26 is compared with the residues of M26 derived from the M30 goat metabolism study. 2) The feed burden calculation for M30 will be compared with the residues of M26 derived from the M30 goat metabolism study

As noted above, for metabolite M26, the feed burden calculations from M30 were used and these values were compared with the residues of M26 in tissues derived from the M30 goat metabolism study. This metabolism study in lactating ruminants was performed at a dose level of 0.6 mg/kg body weight (12 mg/kg feed). These metabolism dose levels are exaggerated by factors ranging from 1.6 to 11.1 when compared to the projected M30 residue intake Table 6.4.2-2. The overdosing factors are derived in Table 6.4.2-7. The results are presented below:

Table 6.4.2-6: Potential transfer of feed residues to cattle tissues and milk for M26 with feed burden from M30

	Total radioactive M26 residue from goat metabolism study	**Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]**	
Matrix	**M26 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Beef cattle:			
Muscle	0.002	0.0011	0.0013
Fat	0.002	0.0011	0.0013
Liver	0.011	0.0061	0.0069
Kidney	0.06	0.034	0.038
Dairy cattle:			
Milk	0.002	0.0002	0.0002
Muscle	0.002	0.0002	0.0002
Fat	0.002	0.0002	0.0002
Liver	0.011	0.001	0.0013
Kidney	0.06	0.0054	0.0069

Based on the overdosing factors, the expected total residues in milk and edible tissues from cows can be extrapolated from the total radioactive M26 residues found in the goat metabolism study performed with M30. The extrapolation shows that no M26 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues and milk except kidney).

Tissue Extrapolations using the Feed Burden of Metabolite M30 in Ruminants as Transferred to M30 in Ruminant Tissues

The calculated feed burden results for metabolite M30 were compared with the new goat metabolism study for M30. This metabolism study in lactating ruminants was performed at a dose level of 0.6 mg/kg body weight (12 mg/kg feed). These metabolism dose levels are exaggerated by factors ranging from 1.6 to 11.1 when compared to the projected residue intakes of Table 6.4.2-3. The overdosing factors are derived in Table 6.4.2-7.

Table 6.4.2-7: Calculation of overdosing factors for beef and dairy cattle for M30 with Feed Burden from M30

	Dose from goat metabolism study	Potential intake, beef cattle	Potential intake, dairy cattle
mg/kg bw	0.5663 mg/kg bw	0.3154 mg/kg bw	0.0509 mg/kg bw
mg/kg feed	12 mg/kg feed	7.36 mg/kg feed	1.4 mg/kg feed
Overdosing factor = Dose from goat metabolism study / Potential intake			
		Overdosing factor, beef cattle	**Overdosing factor, dairy cattle**
Dose calculated by body weight		1.8 = (0.5663 / 0.3154)	11.1 = (0.5663 / 0.0509)
Dose calculated by feed weight		1.6 = (12 / 7.36)	8.6 = (12 / 1.4)

Based on the overdosing factors, the expected total residues in milk and edible tissues from cows can be extrapolated from the total radioactive M30 residues found in the goat metabolism study performed with M30. Extrapolated residues are shown in Table 6.4.2-8.

Table 6.4.2-8: Potential transfer of feed residues to cattle tissues and milk for M30

	Total radioactive M30 residue from goat metabolism study	Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]	
Matrix	**M30 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Beef cattle:			
Muscle	0.002	0.001	0.0013
Fat	0.003	0.0016	0.0019
Liver	0.027	0.015	0.017
Kidney	0.048	0.027	0.03
Dairy cattle:			
Milk	0.001	0.0001	0.0001
Muscle	0.002	0.0002	0.0002
Fat	0.003	0.0003	0.0003
Liver	0.027	0.0024	0.0031
Kidney	0.048	0.0043	0.0056

The extrapolation shows that no M30 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues and milk, except kidney and liver).

For the above mentioned calculations and arguments no quantifiable residues would be anticipated in milk and other cattle products as a result of the proposed use except for kidney and liver.

CA 6.4.3 Pigs

A feeding study in pigs is required only when the metabolic pathways differ significantly in pigs as compared to ruminants. Because the overall fate and metabolism of dimethenamid were found to be similar in rats, goats and hens, no significant differences in metabolic pathways are expected in pigs. For dimethenamid-P, M23, and M27 all residues are below the analytical limit of quantitation (LOQ), therefore only one table is presented for this feed burden in Table 6.4.3-1.

Table 6.4.3-1: Estimated maximum dietary burden for residues for pigs that are exposed to residues below the LOQ such as dimethenamid-P[1], M23 and M27

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Pigs**	
			% of diet	mg/kg bw/d
I Green forages				
Sugar beet leaves	16	0.01	25	0.000625
II Cereal grain				
Maize grain	86	0.01	-	-
V Roots and tubers				
Turnips	10	0.01	60	0.0024
VI Oil seed				
Rape seed meal	86	0.02	15	0.00014
Dietary burden:	mg/kg bw/day		0.003165	
	mg/animal/day		0.237	
	mg/kg total feed (DM)		0.079	

** Feed intake 3 kg DM, body weight (bw) 75 kg
1 Parent dimethenamid-P or metabolite M23 or M27 using analyte equivalents

For dimethenamid-P and metabolites M23, and M27, the dietary feed burden is below 0.004 mg/kg bw day, the exposure that would trigger a metabolism study.

Table 6.4.3-2: Estimated maximum dietary burden of M26[1] residues for pigs

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Pigs**	
			% of diet	mg/kg bw/d
I Green forages				
Rape forage	14	0.231	15	0.009900
II Cereal grain				
Maize grain	86	0.01	5	0.000023
V Roots and tubers				
Turnips	10	0.01	60	0.002400
VI Oil seed				
Rape seed meal	86	0.02	20	0.000186
Dietary burden:	mg/kg bw/day		0.012509	
	mg/animal/day		0.938	
	mg/kg total feed (DM)		0.313	

** Feed intake 3 kg DM, body weight (bw) 75 kg
1 Metabolite M26 using analyte equivalents

Table 6.4.3-3: Estimated maximum dietary burden of M30[1] residues for pigs

Crop	Dry matter content (%)	Residue level (HR/STMR mg/kg)	Pigs**	
			% of diet	mg/kg bw/d
I Green forage				
Rape forage	14	2.92	15	0.125143
II Cereal grain				
Maize grain	86	0.01	5	0.000023
V Roots and tubers				
Turnips	10	0.01	60	0.0024
VI Oil seed				
Rape seed meal	86	0.02	20	0.000186
Dietary burden:		mg/kg bw/day	0.1277	
		mg/animal/day	9.58	
		mg/kg total feed (DM)	3.19	

** Feed intake 3 kg DM, body weight (bw) 75 kg
1 Metabolite M30 using analyte equivalents

Tissue Extrapolations using the Feed Burden of Metabolite M26 in Ruminants as Transferred to M26 in Swine Tissues

For metabolite M26, two different comparisons/extrapolations using the M30 goat metabolism study are made. 1) The feed burden calculation for M26 is compared with the residues of M26 derived from the M30 goat metabolism study. 2) The feed burden calculation for M30 is compared with the residues of M26 derived from the M30 goat metabolism study.

In a metabolism study in goats, animals were dosed with M30 at 12 mg/kg diet (see MCA Section 6, Chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (38 to 47 times) with regard to the maximum anticipated feed burden for pigs of 0.313 mg/kg feed DM for metabolite M26 (see Table 6.4.3-4).

Table 6.4.3-4: Calculation of overdosing factors for pig for M26

	Dose from goat metabolism study	Potential intake, pig
mg/kg bw	0.5663 mg/kg bw	0.012 mg/kg bw
mg/kg feed	12 mg/kg feed	0.313 mg/kg feed
Overdosing factor = Dose from goat metabolism study / Potential intake		
		Overdosing factor, pig
Dose calculated by body weight		47 = (0.5663 / 0.012)
Dose calculated by feed weight		38 = (12 / 0.313)

Based on the overdosing factors, the expected total residues in edible tissues from pigs can be extrapolated from the total radioactive M26 residues ~~for~~ found in the goat metabolism study performed with M30. The extrapolation shows that no M26 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues). Extrapolated residues are shown in Table 6.4.3-5.

Table 6.4.3-5: Potential transfer of feed residues to pig tissues for M26

	Total radioactive residue from goat metabolism study	Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]	
Matrix	**M30 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Pig:			
Muscle	0.002	0.00004	0.00005
Fat	0.003	0.00006	0.00008
Liver	0.027	0.00057	0.00071
Kidney	0.048	0.001	0.0013

Tissue Extrapolations using the Feed Burden of Metabolite M30 in Ruminants as Transferred to M26 in Swine Tissues

For metabolite M26, two different comparisons/extrapolations using the M30 goat metabolism study are made. 1) The feed burden calculation for M26 is compared with the residues of M26 derived from the M30 goat metabolism study. 2) The feed burden calculation for M30 will be compared with the residues of M26 derived from the M30 goat metabolism study.

In a metabolism study in goats, animals were dosed with M30 at 12 mg/kg diet (see MCA Section 6, Chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (3.8 to 4.4 times) with regard to the maximum anticipated feed burden for pigs of 3.19 mg/kg feed DM for metabolite M26 (see Table 6.4.3-4).

Table 6.4.3-6: Calculation of overdosing factors for pig for M30

	Dose from goat metabolism study	Potential intake, pig
mg/kg bw	0.5663 mg/kg bw	0.1277 mg/kg bw
mg/kg feed	12 mg/kg feed	3.19 mg/kg feed
Overdosing factor = Dose from goat metabolism study / Potential intake		
		Overdosing factor, pig
Dose calculated by body weight		4.4 = (0.5663 / 0.1277)
Dose calculated by feed weight		3.8 = (12 / 3.19)

Based on the overdosing factors, the expected total residues in edible tissues from pigs can be extrapolated from the total radioactive M26 residues found in the goat metabolism study performed with M30. The extrapolation shows that no M26 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues) except for kidney. Extrapolated residues are shown in Table 6.4.3-7.

Table 6.4.3-7: Potential transfer of feed residues to pig tissues for M26

	Total radioactive residue from goat metabolism study	Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]	
Matrix	**M26 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Pig:			
Muscle	0.002	0.00045	0.00053
Fat	0.002	0.00045	0.00053
Liver	0.011	0.00250	0.00289
Kidney	0.06	0.01364	0.01579

Tissue Extrapolations using the Feed Burden of Metabolite M30 in Ruminants as Transferred to M30 in Swine Tissues

For metabolite M26, two different comparisons/extrapolations using the M30 goat metabolism study are made. 1) The feed burden calculation for M26 is compared with the residues of M26 derived from the M30 goat metabolism study. 2) The feed burden calculation for M30 will be compared with the residues of M26 derived from the M30 goat metabolism study.

In a metabolism study in goats, animals were dosed with M30 at 12 mg/kg diet (see MCA Section 6, Chapter 6.2.3). The dose levels used in the goat metabolism study represent a considerable overdosing (3.8 to 4.4 times) with regard to the maximum anticipated feed burden for pigs of 3.19 mg/kg feed DM for dimethenamid-P (see Table 6.4.3-3).

Table 6.4.3-8: Calculation of overdosing factors for pig for M30

	Dose from goat metabolism study	Potential intake, pig
mg/kg bw	0.5663 mg/kg bw	0.1277 mg/kg bw
mg/kg feed	12 mg/kg feed	3.19 mg/kg feed
Overdosing factor = Dose from goat metabolism study / Potential intake		
		Overdosing factor, pig
Dose calculated by body weight		4.4 = (0.5663 / 0.1277)
Dose calculated by feed weight		3.8 = (12 / 3.19)

Based on the overdosing factors, the expected total residues in edible tissues from pigs can be extrapolated from the total radioactive M30 residues found in the goat metabolism study performed with M30. The extrapolation shows that no M30 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues), except for kidney. Extrapolated residues are shown in Table 6.4.3-9.

Table 6.4.3-9: Potential transfer of feed residues to pig tissues for M30

	Total radioactive residue from goat metabolism study	Extrapolated total residue from potential intake (mg/kg) [Goat ^{14}C-results / Overdosing factor]	
Matrix	**M30 residues (mg/kg)**	**Based on intake per body weight**	**Based on projected residues in feed**
Pig:			
Muscle	0.002	0.00045	0.00053
Fat	0.003	0.0007	0.0008
Liver	0.027	0.0061	0.0071
Kidney	0.048	0.011	0.013

The extrapolation shows that no M30 residues are expected above the LOQ of the analytical method (0.01 mg/kg for tissues and milk except kidney).

Conclusion of Livestock Feeding for Poultry, Cattle and Swine

For poultry, according to EFSA profile feed burdens do not exceed the trigger value of 0.004 mg/kg bw day for any metabolite investigated. Therefore no further metabolism or feeding studies are required.

For cattle and swine, in order to propose the residues for MRLs and risk assessment, the measured residues in ruminants from the goat metabolism study with M30 were used. Ruminants were chosen as the model of extrapolation because the anticipated levels in ruminant tissues were significantly higher than the anticipated residues in swine. For dimethenamid-P, the default MRL value of 0.01 mg/kg was used. However, this significantly overestimates exposure because animals are unlikely to be exposed to parent and previous metabolism studies in rat, goat, and hen have all demonstrated parent dimethenamid-P is rapidly excreted and extensively metabolized. Parent dimethenamid-P has never been measured in any tissue within these studies, and is included as a conservative default only.

For M26, it has been considered that M26 in animal tissues can come from feed burdens of M26 as well as M30. It is important to note, however, that the contribution of M26 in animal tissues from M26 in animal feed items is negligible when compared to the contribution of M26 from animal feed items which contain M30. The use of M26 in these calculations above and in Table 6.4.3-10 below has been included to again demonstrate its non-relevance to the proposed plant residue definition. The results are presented below:

Table 6.4.3-10: Calculation of total residues for dimethenamid-P, M26 and M30 from ruminants

Food of animal origin	Source of feed burden[1]	Residues of M26[1] in ruminants in parent equivalents	Residues of M30[2] in ruminants in parent equivalents	Total residues of parent + M30	Total residues in parent equivalents
Liver	Dimethenamid-P[3]	0.01		0.022	0.028
	M26	0.0006	-		
	M30	0.0069	0.017		
Total liver residues in parent equivalents		0.006	0.012		
Kidney	Dimethenamid-P[3]	0.01		0.032	0.063
	M26	0.0032	-		
	M30	0.038	0.03		
Total kidney residues in parent equivalents		0.03	0.02		

1 Equals residues of M26 in ruminants multiplied by a MWCF of 0.7629.
2 Equals residues of M30 in ruminants multiplied by a MWCF of 0.7306.
3 Included as default MRL only. Animals are not exposed to parent and parent is not a metabolite found in any tissue of any animal tested

For the purposes of **MRLs, monitoring, and enforcement,** the metabolite M30 is the appropriate marker molecule. As shown from the feed burdens above, M30 exposure in animal feed items leads to residues above the LOQ in liver and kidney matrices. However, M26 exposure in feed items only leads to residue above the LOQ in kidney. Therefore the metabolite M30 has the greatest chance of determining misuse. Parent dimethenamid-P is proposed in the residue definition as well, but as a default only. As noted above, it is highly unlikely that an animal or consumer will be exposed to parent dimethenamid-P. Therefore, the final proposed residue definition for MRLs in foods of animal origin is parent dimethenamid-P +M30.

Based on Table 6.4.3-10 above, the following MRLs (rounded up) are proposed for parent + M30 (See MCA Section 6, Chapter 6.7):

0.03 mg/kg for liver of sheep, cattle, goats, horses and other farm animals
0.04 mg/kg for kidney of sheep, cattle, goats, horses and other farm animals.

For the purposes of **risk assessment,** in addition to parent dimethenamid-P + M30, the metabolite M26 must also be considered. As was demonstrated above, exposure to animal feed items containing M30 leads to residues of M26 in kidney. Therefore, the final proposed residue definition for MRLs in foods of animal origin is parent dimethenamid-P + M26 +M30.

Based on the Table 6.4.3-10 above, the following inputs will be used for risk assessment purposes as the sum of parent dimethenamid-P + M26 +M30. They are highly conservative by including dimethenamid-P at the LOQ.

0.03 mg/kg for liver of sheep, cattle, goats, horses and other farm animals
0.07 mg/kg for kidney of sheep, cattle, goats, horses and other farm animals.

Anticipated residues of M26 and M30 in animal commodities can be derived from the M30 goat metabolism study. Extrapolations based on the radioactive residue in the goat metabolism study and the theoretical maximum dietary burden in ruminants originating from residues of both M26 and M30 indicate that measurable residues would be detected in only liver and kidney. No measurable residues are expected in milk or other tissues. Maximum expected theoretical residues in liver and kidney are relatively low (0.02 and 0.05 mg/kg as total residues of M26 and M30, respectively, based on parent equivalents). Chronic dietary risk assessment using PRIMo, version 2, shows that including these residues in the TMDI calculation results in no more than 7.23% ADI utilization for all consumer groups. Based upon the results from this conservative approach and in an effort to protect animal welfare, it is proposed no feeding study in cattle is necessary for the metabolite M26.

CA 6.4.4 Fish

A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation (Commission Regulation (EU) No 283/2013 from March 2013).

Since the log P_{ow} for dimethenamid-P is <3 (log P_{ow} 1.89), dimethenamid-P is not anticipated to accumulate in tissues of fish. Thus a fish metabolism is not required (see Reg (EU) No 283/2013 from March 2013). Additionally, the bio-concentration factor (BCF) for dimethenamid-P in fish, determined in a separate study (evaluated during the EU review of the active substance dimethenamid), was determined very low in edible tissue (20) and moderate in non-edible tissue and whole fish (100 and 57, respectively).

Among the crops covered by this submission, seeds of soybean and sunflower as well as their meal and oilseed rape meal may be considered to serve as feed items for fish. However, in light of the fact that residues of dimethenamid-P in these crop matrices are usually not detectable and that due to the very low lipophilicity of dimethenamid-P residues do not accumulate in fish, no dimethenamid-P derived residues above 0.01 mg/kg can be reasonably expected in edible tissues of fish. Thus, a fish feeding study is not considered necessary.

CA 6.5 Effects of Processing

According to the data requirements as laid down in Commission regulation (EC) 283/2013, such a study shall be provided where residues in products of plant or animal origin subject to processing may occur at a level of or higher than 0.01 mg/kg (based on the residue definition for risk assessment for the raw commodity).

Dimethenamid-P was found to be stable for 31 days in buffer solutions at pH 5, 7 and 9 (cf. Doc. M-II, 7.2.1.1 of the Annex II Dossier) and no metabolites were detected during hydrolysis. However, consumers and animal are not exposed to parent dimethenamid-P at relevant harvest intervals. Therefore this study is supplemental information.

As the metabolite M656PH030 is included in the new modified residue definition, and is the predominant residue at relevant harvest intervals in edible commodities and animal feed items, a new hydrolysis study is presented below.

CA 6.5.1 Nature of the residue

Report:	CA 6.5.1/1 Habeeb S.B., 2014a Simulated processing practices: Hydrolysis of [14-C]-M656PH030 at pH 4 (90°C), pH 5 (100°C) and pH 6 (120°C) 2013/7002635
Guidelines:	OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis, EEC 91/414, EEC 7035/VI/95 rev. 5
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	M656PH030 (C^{14}, C^{13} and unlabeled)
Lot/Batch #:	1089-1003 (C^{14}), 1088-1005 (C^{13}), L74-138 (unlabeled)
Purity:	Radiochemical: 98% (C^{14}) Chemical: 95.8% (C^{14}), 92.6% (C^{13}), 83.7% (unlabeled)
Spiking level:	10 mg/mL

B. STUDY DESIGN AND METHODS

1. Test procedure

pH Buffers of 4.0, 5.0 and 6.0 were prepared according and tested for accuracy to the protocol. Individual test systems (in triplicate) were prepared in triplicate in amber glass vials containing [^{14}C]-M656PH030 test material in buffered aqueous solutions. Triplicate test vessels were placed in the water bath set at 90±5°C for the pH 4 buffer for 20 minutes and 100±5°C for the pH 5 buffer for 60 minutes. Triplicate test vessels were placed in the autoclave set at 120±5°C for the pH 6 buffer for 20 minutes. Each test system was sterilized prior to the use in this study.

2. Description of analytical procedures

At sampling for each buffer spiked with [^{14}C]-M656PH030, triplicate test samples for pH 4, pH 5 and pH 6 were removed from the constant temperature water bath. Triplicate aliquots were analyzed by LSC to determine the radioactivity in the sample. An aliquot of each sample was transferred into an amber sterile HPLC vial and analyzed by HPLC with radio-detection for distribution of radioactivity.

II. RESULTS AND DISCUSSION

Total ^{14}C recovery (based on the applied radioactivity) for the pH 4 hydrolysis samples at 90±5°C ranged from 94.5-109% TAR, for the pH 5 hydrolysis samples at 100±5°C ranged from 102-110% TAR, and for pH 6 hydrolysis samples at 120±5°C ranged from 103-108% TAR.

Table 6.5.1-1: Recovery after processing simulation tests with ^{14}C-M656PH030

Process represented	Test condition	Recovery of ^{14}C-M656PH030 (%)		
		Average	Range	RSD
Pasteurization	pH 4, 90°C, 0 min	97.8	90.2-105	7.6
	pH 4, 90°C, 20 min	96.3	90.8-102	5.8
Baking/brewing/boiling	pH 5, 100°C, 60 min	97.7	96.8-98.3	0.8
	pH 5, 100°C, 60 min	98.0	95.4-102	3.7
Sterilization	pH 6, 120°C, 20 min	100	98-102	2.0
	pH 6, 120°C, 20 min	95.1	94.4-96.3	1.1

III. CONCLUSION

This study demonstrated that M656PH030 does not hydrolyze at pH 4, pH 5 and pH 6 after 20 min, 60 min and 20 min of incubation of the samples at 90±5, 100±5, and 120±5°C.
Only minor hydrolysis products were observed at pH 4, pH 5, and pH 6 and none of the products were greater than 5% TAR and were not identified. The test compound M656PH030 is hydrolytically stable at the pH and the temperature levels tested in the study. On the basis of these results, M656PH030 is not expected to hydrolyze during the pasteurization (90°C), baking/brewing/boiling (100°C), and sterilization (120°C).

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Since dimethenamid-P is intended to be used on sunflower, oilseed rape, soybean, maize and sugar beet information on the distribution behavior between peel and pulp is not relevant.

CA 6.5.3 Magnitude of residues in processed commodities

The investigation of effects of industrial processing and/or household preparation and on the nature of the residue is not required for dimethenamid-P since no residues above the respective limit of quantitation were found in any relevant plant or plant product to be processed destined for human consumption. Therefore it is concluded that no processing studies are necessary.

In addition, the contribution of the commodities under consideration to the theoretical maximum daily intake (TMDI) is <10% of the ADI and the estimated daily intake is <10% of the ARfD.

CA 6.6 Residues in Rotational Crops

CA 6.6.1 Metabolism in rotational crops

The magnitude of dimethenamid-P residues in rotational crops was investigated using [3-^{14}C-thienyl]-labeled dimethenamid (Germany, 2000) and ^{14}C-labeled dimethenamid-P (France, 2013). These studies were also reviewed by EFSA under Article 12 in 2013 (DocID 2013/1414385). The characteristics of these studies are summarized in Table 6.6.1-1.

Table 6.6.1-1: Summary of metabolism studies in rotational crops available

Crop group	Crop	Label position	Application and sampling details					Author, Year	DocID
			Method, F or G [(a)]	Rate (kg a.s./ha)	Sowing intervals (DAT)	Harvest intervals (DAT)	Remarks		
Leafy vegetables	Lettuce	[3-^{14}C-tienyl]-labeled R,S-dimethenamid	Soil app. [(b)], G	Maize: 1.68/4.40 Soybean: 1.68/3.36	332	364	Sown after maize and soybean (Germany 2000)	Pierotti 1992	1992/12425
Root and tuber vegetables	Carrot				332	408	Sown after maize and soybean (Germany 2000)	Pierotti 1992	1992/12425
Cereals	Winter wheat				141	387	Sown after maize and soybean (Germany 2000)	Pierotti 1992	1992/12425
	Spring wheat				322	396	Sown after maize and soybean (Germany 2000)	Pierotti 1992	1992/12425
Leafy vegetables	Spinach	^{14}C-labeled dimethenamid-P	Bare soil app. by spraying, G	1.008	30, 120, 365	27-30 [(c)], at maturity	(France, 2013)	Wenzel, 2012	2012/1162137
Root and tuber vegetables	White radish					at maturity	(France, 2013)	Wenzel, 2012	2012/1162137
Cereals	Spring wheat					43-52 [(d)], at maturity	(France, 2013)	Wenzel, 2012	2012/1162137

(a): Outdoor/field application (F) or glasshouse/protected/indoor application (G)
(b): Pre-emergence application on primary crops (maize and soybean)
(c): Immature samples
(d): Forage samples

The following was summarized from the EFSA Reasoned Opinion 2013:

"In the first study (Germany, 2000), racemic dimethenamid was applied directly on primary crops (maize and soya beans) and the degradation products were present in winter wheat, spring wheat, lettuce and carrots. Their total amount ranged from 0.013 mg/kg in carrots to 0.17 mg/kg in winter wheat straw, but all individual compounds were present at levels below 0.01 mg/kg. Three metabolites were identified that were also identified in primary crops: M23, M27 and the sulfoxide of the thiolactic acid conjugate (M30). They were all present below 0.01 mg/kg."

"Based on both studies on rotational crops, it can be generally considered that the metabolism in rotational crops is similar to that in primary crops (EFSA, 2005).

Considering the confined rotational crop studies and the cGAP, dimethenamid specific residues in rotational crops are not expected to exceed 0.01 mg/kg; particular risk mitigating measures for rotational crops are not necessary."

The second study presented in the EFSA Conclusion 2013 is summarized below.

This study was requested by and submitted to France in 2012 and reviewed by EFSA in 2013 under Article 12 (DocID 2013/1414385). EFSA concluded the study was valid, did not trigger any plant back interval, and did not trigger a higher tier field rotational crop study. However, it has never been directly submitted to the RMS. Therefore it is included below for completeness. It should be noted that the original confined rotational crop (1992) was conducted with racemic dimethenamid-P and did not meet current agricultural practices. Therefore, a new study with dimethenamid-P following the current guidelines and agricultural practices was performed for Annex I renewal.

Report:	CA 6.6.1/1 Wenzel N. et al., 2012a Nachbaustudie mit 14C-BAS 656 H (14C-markiertes Dimethenamid-P) - Confined rotational crop study with 14C-BAS 656 H (14C-labelled Dimethenamid-P) 2012/1162137
Guidelines:	EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), BBA IV 3-10, EEC 7524/VI/95 rev. 2 (July 22 1997), OECD 502 Metabolism in Rotational Crops (January 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 6.6.1/2 Wenzel N., 2014a Amendment No. 1 - Nachbaustudie mit 14C-BAS 656 H (14C-markiertes Dimethenamid-P) - Confined rotational crop study with 14C-BAS 656 H (14C-labelled Dimethenamid-P) 2014/1046558
Guidelines:	EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997), BBA IV 3-10, EEC 7524/VI/95 rev. 2 (July 22 1997), 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:

Description:	^{14}C-thienyl-labeled (5 position) dimethenamid-P, unlabeled dimethenamid-P
Lot/Batch #:	824-4024 (^{14}C-labeled) L74-174 (unlabeled)
Purity:	Radiochemical purity: 99.3% (^{14}C-labeled) Chemical purity a.s.: 99.4% (unlabeled) Specific activity: 7.63 MBq/mg
CAS#:	163515-14-8

Stability of test compound: Test compound was stable over investigation time.

2. Test Commodity:

Crop:	Spring wheat	white radish	spinach
Type:	Cereals	root & tuber vegetables	leafy vegetables
Variety:	Thassos	April Cross	Corvette F1
Botanical name:	*Triticum aestivum*	*Raphanus sativus*	*Lactuca sativa*
Crop part/processed commodity:	Wheat forage, wheat hay, wheat straw, wheat grain Radish top, radish root Immature spinach, mature spinach		
Sample size:	0.023-3.054 kg		

3. Soil:

A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.6.1-2).

Table 6.6.1-2: Soil physicochemical properties

Soil series	Soil type	pH	TOC %[2]	Sand %	Silt %	Clay %	Max. water holding capacity	CEC[1] meq/100 g
Bruch West	Sandy loam*	7.0**	1.36	65.9*	22.1*	11.9*	27.2*	9.6

1 Cation exchange capacity
2 Total organic carbon
* USDA scheme
** $CaCl_2$

B. STUDY DESIGN

The study was conducted during the period of May 2010 to August 2012 at the BASF Agricultural Center Limburgerhof, in Limburgerhof, Germany.

1. Test procedure

Plastic containers with sandy loam were used for the plant back intervals of 30, 120 and 365 days. One single spray application of ^{14}C-dimethenamid-P was performed on the soil. After 30 days and simulated ploughing, three crops were sowed (spinach, white radish and spring wheat). After harvest of the mature crops and completion of the respective plant back interval, the top layer of the soil was mixed before replanting (individual plant back intervals of 120 days and 365 days). The maintenance of the growing crops was performed using normal agricultural practice. For preparation of the test item ^{14}C-dimethenamid-P (dissolved in acetonitrile) and unlabelled dimethenamid-P were mixed at a ratio of about 1:1 and dissolved in a mixture of blank formulation of BAS 656 AB H and water. The homogenous emulsion was then applied to the containers at a rate of 1,006 g a.s./ha in a spray volume of 214 L/ha. Considering losses during the application process 99.31% of the application solution was actually applied.

2. Sampling

Immature and mature spinach leaves were sampled 28-37 days after treatment (DAT). Mature white radishes were harvested at 71 DAT and separated into the root and green parts (tops). Immature green plants of spring wheat (wheat forage) were sampled and partly dried to wheat hay at 50 DAT. In addition, mature wheat ears and straw were collected at 119 DAT. The ears were separated into grain and chaff and the chaff was mixed to the straw. Soil samples were taken after the individual plant back intervals and after harvest of the mature crops. All samples were stored in a freezer at -18°C or below until analysis. The extracts were stored in a refrigerator or, for longer periods, in a freezer.

3. Description of analytical procedures

Homogenization/TRR determination: Plant matrices with a sufficient level of radioactivity were extracted with solvents, while for samples with low concentrations of radiolabeled compounds the amount of the total radioactive residues (TRR) was obtained only by combustion analysis. Prior to extraction, the plant and soil sample materials were frozen in liquid nitrogen and chopped/ground to obtain homogenizates. Aliquots of the homogenized samples were dried and combusted using a sample oxidizer.

Extraction/TRR determination: Aliquots of homogenized plant material were extracted three times with methanol. After each extraction step, the liquid phase was separated from the solid by centrifugation, decanted and filtered, while the remaining plant material was subjected to the next extraction step. The methanol extracts of the three steps were combined, adjusted to volume, and aliquots of the combined extract were measured by LSC. The residue was further extracted in the same way with appropriate volumes of water twice. The water extracts were also combined and aliquots of both extracts were radio-assayed by LSC. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR).

The residues after solvent extraction were dried and combusted for the determination of the residual radioactive residue (RRR). The total radioactive residues (TRR calculated) were obtained by calculating the sum of ERR and RRR values and additionally by combustion of sample aliquots (TRR measured). All calculations throughout the present study were based on the TRR calculated.
Partitioning/ERR determination: Subsamples of methanol extracts of plant matrices after a plant back interval of 30 DAT (with exception of white radish root and spring wheat grain) were evaporated to the water phase and partitioned three times with dichloromethane. Afterwards, the remaining water phases of the respective plant samples were partitioned three times with ethyl acetate. Aliquots of the dichloromethane, ethyl acetate and water phases were analyzed by LSC measurement and the ERR was calculated.

Solubilization/RRR determination: The residual radioactive residues after extraction with methanol and water (RRR) with a sufficient level of radioactivity were subsequently extracted twice with 1% ammonia. After ammonia extraction, the residues were solubilized with different carbohydrate and protein cleaving enzymes. For selected wheat samples, additional acidic (boiling for 3 h with 6 M HCl) and alkaline hydrolyses (boiling for 3 h with 6 M NaOH) were performed. The amount of radioactivity in the final residue was determined by combustion.

Radioanalysis: Aliquots of homogenized solid plant and soil samples were combusted by means of a sample oxidizer. ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly (recovery values between 91% and 97%). For determination of the background radioactivity, aliquots of untreated wheat straw samples and white radish root samples were combusted under the same conditions.

Identification of components: The identification of the metabolites was based on yeast fermentation procedures (investigation of the highly polar components observed in the HPLC analyses of white radish root), on co-chromatography experiments with a blend of carbohydrates (fructose, glucose and sucrose), the two metabolites M81 and M51 identified in a dimethenamid-P metabolism study in soybean and on HPLC retention time comparison. HPLC with LSC detection was used for the analysis of extracts, partition phases, solubilizates and reference items. Identification/structure elucidation and quantification of metabolites was accomplished by mass spectrometry (ESI-MS/MS).

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Plant

All control samples were subjected to combustion analysis and no ^{14}C above background was detected in these samples. The treated samples were subjected to combustion analysis and the results are presented in Table 6.6.1-3 for plants and in Table 6.6.1-4 for soil. The calculated TRR of all extracted rotational crop matrices showed no major differences to the TRR values obtained by combustion.

For the representative leafy crop, spinach, total radioactive residues (TRR calculated) in both immature and mature plants sowed 30 days after soil treatment with ^{14}C-dimethenamid-P were determined. The residue level in immature spinach accounted for 0.084 mg/kg, while the residues in mature spinach (30 DAT) amounted to 0.095 mg/kg. Lower residue levels for both immature and mature spinach were found at 120 DAT (both 0.008 mg/kg, TRR combusted). After a year of soil aging (365 DAT, TRR combusted), the residue concentrations in immature and mature spinach had decreased further, down to 0.005 mg/kg and 0.004 mg/kg, respectively. For the representative root crop, white radish, total radioactive residues in roots and in the top part of the mature crop were determined. The total radioactive residues in the top part of mature crops were 0.089 mg/kg at a plant back interval of 30 DAT, decreasing to 0.012 mg/kg and to 0.002 mg/kg (TRR combusted) after 120 DAT and 365 days of soil aging, respectively.
The total radioactive residues in roots of white radish decreased from 0.028 mg/kg (30 DAT), to 0.009 mg/kg (120 DAT, TRR combusted) and finally to 0.001 mg/kg (365 DAT, TRR combusted).

For the representative cereal crop, spring wheat, the matrix with the highest residue levels was hay with an initial TRR of 0.818 mg/kg (30 DAT), which decreased to 0.121 mg/kg (120 DAT), and finally to 0.032 mg/kg (365 DAT) followed by straw where the levels deceased as follows: 0.514 mg/kg (30 DAT), 0.137 mg/kg (120 DAT) and 0.036 mg/kg (365 DAT). Residue levels in grain were 0.193 mg/kg (30 DAT), 0.083 mg/kg (120 DAT) and 0.035 mg/kg (365 DAT).
Spring wheat forage was the matrix with the lowest residue levels. Again, the total radioactive residues after the different soil aging intervals decreased from 0.128 mg/kg (30 DAT) to 0.019 mg/kg (120 DAT) and to 0.008 mg/kg (365 DAT, TRR combusted).

The limit of quantitation (LOQ), determined for spring wheat straw and white radish root was found to be approximately 0.0002 mg/kg and below 0.00005 mg/kg, respectively.

Table 6.6.1-3: TRR, ERR and RRR in rotational crop samples after treatment with ^{14}C-BAS 656 PH

Matrix	Days after sowing	TRR[1]	TRR[2]	Methanol extract		Water extract		ERR[3]		RRR[4]	
		[mg/kg]	[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT[5]											
Immature spinach	28	0.090	0.084	0.054	63.8	0.003	3.9	0.057	67.7	0.027	32.3
Mature spinach	37	0.094	0.095	0.061	63.8	0.007	7.7	0.068	71.5	0.027	28.5
White radish top	71	0.106	0.089	0.057	64.3	0.007	8.2	0.065	72.5	0.025	27.5
White radish root	71	0.028	0.028	0.018	63.2	0.001	4.3	0.019	67.5	0.009	32.5
Spring wheat forage	50	0.142	0.128	0.056	44.0	0.004	3.2	0.060	47.2	0.067	52.8
Spring wheat hay	50	0.925	0.818	0.284	34.7	0.084	10.3	0.368	44.9	0.451	55.1
Spring wheat straw	119	0.537	0.514	0.159	31.0	0.074	14.3	0.233	45.4	0.280	54.6
Spring wheat grain	119	0.201	0.193	0.010	5.1	0.013	6.5	0.022	11.5	0.171	88.5
Plant back interval: 120 DAT[5]											
Immature spinach	30	0.008	-	-	-	-	-	-	-	-	-
Mature spinach	42	0.008	-	-	-	-	-	-	-	-	-
White radish top	65	0.013	0.012	0.007	55.8	0.001	9.3	0.008	65.1	0.004	34.9
White radish root	65	0.009	-	-	-	-	-	-	-	-	-
Spring wheat forage	52	0.023	0.019	0.008	40.8	0.001	4.2	0.009	45.0	0.011	55.0
Spring wheat hay	52	0.137	0.121	0.034	27.9	0.014	11.2	0.047	39.1	0.073	60.9
Spring wheat straw	107	0.156	0.137	0.048	35.5	0.021	15.4	0.070	50.9	0.067	49.1
Spring wheat grain	107	0.076	0.083	0.005	5.6	0.012	13.9	0.016	19.5	0.067	80.5
Plant back interval: 365 DAT[5]											
Immature spinach	27	0.005	-	-	-	-	-	-	-	-	-
Mature spinach	34	0.004	-	-	-	-	-	-	-	-	-
White radish top	64	0.002	-	-	-	-	-	-	-	-	-
White radish root	64	0.001	-	-	-	-	-	-	-	-	-
Spring wheat forage	43	0.008	-	-	-	-	-	-	-	-	-
Spring wheat hay	43	0.039	0.032	0.007	20.5	0.004	13.0	0.011	33.5	0.021	66.5
Spring wheat straw	100	0.030	0.036	0.010	27.6	0.005	13.2	0.015	40.8	0.021	59.2
Spring wheat grain	100	0.036	0.035	0.002	5.6	0.003	7.4	0.005	13.0	0.031	87.0

1 Total radioactive residue (TRR): was calculated as the sum of ERR + RRR
2 Total radioactive residue (TRR): was measured after combustion
3 Extractable radioactive residue (ERR) calculated as sum of methanol and water extract
4 Residual radioactive residue
5 Days after treatment

Soil
For all soil samples, the residue levels (TRR) were determined by direct combustion analysis of subsamples (see Table 6.6.1-4). Measurements were carried out before as well as after soil aging and ploughing. Additionally, soil was sampled after harvest of the individual mature crops for each plant back interval.
The residue concentration in the top soil layer after application accounted for 7.589 mg/kg. After aging and ploughing, the residue concentration decreased from 0.152 mg/kg at 30 DAT to 0.085 mg/kg at 120 DAT and 0.096 mg/kg at 365 DAT. After harvest of the mature crops, the residue levels in soil remained more or less stable for the plant back intervals of 30, 120 and 365 DAT (except for a slightly higher value for the soil after harvest of spinach at plant back interval 30 DAT).

Table 6.6.1-4: Total radioactive residues in soil after treatment with ^{14}C-BAS 656 PH

Soil samples	Time after sowing [days]	TRR determined by direct combustion [mg/kg]
Plant back interval: 30 DAT		
After application (petri dishes)		
0 DAT	-	7.589
Plant back interval: 30 DAT		
After ploughing		
30 DAT	0	0.152
After harvest of mature crops		
Spinach (67 DAT)	37	0.119
White radish (101 DAT)	71	0.063
Spring wheat (149 DAT)	119	0.095
Plant back interval: 120 DAT		
After ploughing		
120 DAT	0	0.085
After harvest of mature crops		
Spinach (162 DAT)	42	0.057
White radish (185 DAT)	65	0.075
Spring wheat (227 DAT)	107	0.080
Plant back interval: 365 DAT		
After ploughing		
365 DAT	0	0.096
After harvest of mature crops		
Spinach (399 DAT)	34	0.057
White radish (429 DAT)	64	0.087
Spring wheat (465 DAT)	100	0.077

B. EXTRACTION AND CHARACTERIZATION OF RESIDUES

1. Extraction and characterization of residues in rotational crops

The extractability of the radioactive residues with methanol and water ranged from 33.5% to 72.5% TRR for spinach, white radish and spring wheat forage, hay and straw (see Table 6.6.1-3). For spring wheat grain, the extractability was relatively low with approximately 12 to 20%. The major portions of the radioactive residues were generally extracted with methanol, except for spring wheat grain where almost similar portions were extracted with methanol and water.

In order to characterize the methanol extractable radioactive residues as organosoluble or water soluble fractions, liquid/liquid partition of the rotational crop matrices harvested 30 DAT (except for white radish root and spring wheat grain) was carried out using dichloromethane and ethyl acetate as organic solvents (data not shown). In most cases, major portions of the radioactive residues extracted with methanol were water soluble, and only lower portions were found in the organic fractions. In the case of spring wheat forage, comparable portions were found in the organic phases (sum) and in the water phase.

In most of the rotational crop matrices analyzed, considerable amounts of the radioactive residues were not extractable with methanol and water. The residual radioactive residues after solvent extraction of particular matrices of the plant back intervals 30, 120 and 365 DAT were further characterized using an individual combination of sequential solubilization steps including treatment with ammonia, hydrochloric acid and sodium hydroxide and solubilization with enzymes. The solubilized residues had possibly been associated with or embedded/incorporated insoluble plant material such as proteins, cell wall polymers and starch. The most effective solubilization steps were treatments with macerozyme and glucosidase, followed by hydrochloric acid and sodium hydroxide treatment and solubilization with aqueous ammonia. In the case of wheat grain, incubation with amylases also released substantial amounts of radioactivity.

2. Identification and quantification of extractable residues in rotational crops

The identification of the metabolites was based on yeast fermentation procedures, on co-chromatography experiments with a blend of carbohydrates (fructose, glucose and sucrose) and the two metabolites M81 and M51 identified in a dimethenamid-P metabolism study in soybean and on HPLC retention time comparison.

The radioactive residues in the extracts and solubilizates of all rotational crop matrices consisted of a highly polar fraction (6.8 to 81.2% TRR), which is mainly composed of carbohydrates. In addition, the metabolites M81 (glucose conjugate of a demethoxylated and twofold S-oxidized methanethiol conjugate of the dechlorinated parent compound) and M51 (twofold S-oxidized 2-mercapto-acetic acid conjugate of the dechlorinated parent compound) were identified in individual rotational crop matrices and accounted for a maximum of 11.2 and 4.2% TRR, respectively. Furthermore, degradation products in minor concentrations were characterized by their chromatographic properties (ranging from <0.1 to 7.6% TRR, each below 0.023 mg/kg). The parent molecule BAS 656 PH was neither detected in the extracts nor in the solubilizates.

The residual radioactive residues after solvent extraction with methanol and water (Table 6.6.1-3) ranged from a minimum of 27.5% TRR (white radish top, 30 DAT) to a maximum of 88.5% TRR (spring wheat grain, 30 DAT). The residual radioactive residues were further characterized using an individual combination of sequential solubilization steps applying ammonia treatment, incubations with macerozyme, glucosidase, amylases, tyrosinase and treatments with hydrochloric acid and sodium hydroxide (data not shown). The most effective solubilization steps were the treatments with macerozyme, hydrochloric acid and/or sodium hydroxide, glucosidase and solubilization with aqueous ammonia releasing up to 35.7% TRR (spring wheat hay, 365 DAT), up to 26.6% TRR (spring wheat forage, 120 DAT), up to 22.3% TRR (spring wheat grain, 30 DAT) and up to 10.9% TRR (spring wheat grain, 30 DAT), respectively (data not shown). In addition, wheat grain incubation with amylases released up to 18.4% TRR (spring wheat grain, 30 DAT). The high solubilization of residual radioactive residues after enzyme treatments indicates extensive metabolization of dimethenamid-P to C1 and C2 units with subsequent incorporation into natural products.

Table 6.6.1-5: Summary of identified components in rotational crop matrices

Crop matrix	Polar fraction		M81		M51	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
30 DAT						
Immature spinach	0.026	31.4	n.d.		n.d.	
Mature spinach	0.022	22.7	n.d.		n.d.	
White radish top	0.016	18.0	0.10	11.2	n.d.	
White radish root	0.021	74.8	n.d.		n.d.	
Spring wheat forage	0.030	23.8	0.006	4.4	0.005	4.2
Spring wheat hay	0.273	33.3	0.026	3.2	0.021	2.6
Spring wheat straw	0.098	19.2	0.014	2.8	0.008	1.5
Spring wheat grain	0.157	81.2	n.d.		n.d.	
120 DAT						
White radish top	0.002	18.0	<0.001	1.7	<0.001	1.3
Spring wheat forage	0.003	13.5	0.001	5.3	0.001	2.8
Spring wheat hay	0.016	13.5	0.004	3.4	0.003	2.9
Spring wheat straw	0.009	6.8	0.005	3.3	0.002	1.5
Spring wheat grain	0.012	13.9	n.d.		n.d.	
365 DAT						
Spring wheat straw	0.004	10.7	n.d.		n.d.	

n.d. Not detected

3. Proposed metabolic pathway

The radioactive residues in the extracts and solubilizates of all rotational crop matrices consisted of a highly polar fraction primarily containing carbohydrates (6.8 to 81.2% TRR). In addition, the metabolites M81 and SES16802 were identified in individual rotational crop matrices and accounted for a maximum of 11.2 and 4.2% TRR, respectively. Furthermore, degradation products in minor concentrations were characterized by their chromatographic properties (ranging from <0.1 to 7.6% TRR, each below 0.023 mg/kg). These components probably were intermediates in the degradation of BAS 656 PH to C1 or C2 units which finally entered anabolic biosynthetic pathways to natural products. The solubilization and characterization of considerable parts of these non-extractable residues by enzymatic cleavage of natural macromolecules might indicate a subsequent incorporation into plant polysaccharides. The identified metabolic pathway is shown in Figure 6.6.1-1.

4. Storage stability

All samples were stored in a freezer at approximately -18 C or below during the course of the study. A comparison of the extractabilities and of the metabolite patterns obtained at the beginning and at the end of the investigation period showed that there was no relevant change in the nature of the radioactive residues of BAS 656 PH during storage of the plant samples over the period of investigation. The stability in stored extracts was demonstrated over periods of up to 21 months.

III. CONCLUSION

The objective of this study was to determine the total carbon-14 labeled dimethenamid-P (BAS 656 PH) derived residues taken up from the soil by spinach, white radish and spring wheat grown as rotational crops at plant back intervals of 30, 120 and 365 days following the application of dimethenamid-P on bare soil.

Moderate translocation of radioactive residues from soil into the plants was observed for the plant back interval of 30 DAT. Total radioactive residues (TRR) in spinach (immature and mature samples) did not exceed 0.095 mg/kg for all plant back intervals. The TRR in white radish top ranged from 0.002 (TRR combusted, 365 DAT) to 0.089 mg/kg, whereas in white radish root, the residue levels were slightly lower (ranging from 0.001 (TRR combusted, 365 DAT) to 0.028 mg/kg). In spring wheat, the highest residue levels were measured in hay (ranging from 0.032 to 0.818 mg/kg) and straw (0.036 to 0.514 mg/kg). Total radioactive residues in forage and grain accounted for 0.008 (TRR combusted, 365 DAT) to 0.128 mg/kg and for 0.035 to 0.193 mg/kg, respectively. The residue concentration in the top soil layer after aging and ploughing slightly decreased for the plant back interval of 120 DAT and remained more or less stable after 365 DAT.

The extractability of the radioactive residues with methanol and water ranged from 33.5% to 72.5% TRR for spinach, white radish and spring wheat forage, hay and straw. For spring wheat grain, the extractability was relatively low with approximately 12 to 20%. The major portions of radioactive residues were extracted with methanol, except for spring wheat grain where almost similar portions were extracted with methanol and water. In most of the rotational crop matrices analyzed, considerable amounts of the radioactive residues were not extractable with methanol and water. The residual radioactive residues after solvent extraction of particular matrices of plant back intervals 30, 120 and 365 DAT were further characterized using an individual combination of sequential solubilization steps including treatment with ammonia, hydrochloric acid and sodium hydroxide and solubilization with enzymes. The solubilized residues had possibly been associated with or embedded/incorporated insoluble plant material such as proteins, cell wall polymers and starch. The most effective solubilization steps were treatments with macerozyme and glucosidase, followed by hydrochloric acid and sodium hydroxide treatment and solubilization with aqueous ammonia. In the case of wheat grain, incubation with amylases also released substantial amounts of radioactivity.

Quantification of the extracts and solubilizates after solvent extraction using HPLC resulted in an early eluting polar fraction for all crops, matrices and plant back intervals. This fraction was identified to primarily contain carbohydrates. In addition, the metabolites M81 and SES16802 were identified by co-chromatography analyses in selected rotational crop matrices.

Dimethenamid-P was extensively metabolized in rotational crop matrices after application to soil and translocation into the plants. Soil metabolites of dimethenamid-P were taken up and transformed in rotational crops primarily into carbohydrates which were identified in all matrices examined. In addition, the metabolites M81 (glucose conjugate of a demethoxylated and twofold S-oxidized methanethiol conjugate of the dechlorinated parent compound) and M51 (twofold S-oxidized 2-mercapto-acetic acid conjugate of the dechlorinated parent compound) were identified in rotational crop matrices. Unchanged parent compound was neither detected in extracts nor in solubilizates. The solubilization and characterization of considerable parts of the non-extractable residues by enzymatic cleavage of natural macromolecules indicates a subsequent incorporation of ^{14}C labeled C1 or C2 units into plant polysaccharides.

Figure 6.6.1-1: Metabolic pathway of dimethenamid-P (BAS 656 PH) in rotational crops

Dimethenamid-P
(BAS 656 H)

Glutathione
Conjugation

M24

- Glu, -Gly

M25

1. Deamination
2. Decarboxylation
3. Oxidation

M32

S-Oxidation

M31

S-Oxidation

M51

Decarboxylation
Ether Cleavage

M68 /
M69
or isomer

Glycosylation

M81
or isomer

Decomposition to C1 and / or C2 entities

Natural compounds (e.g. carbohydrates, amino acids)

CA 6.6.2 Magnitude of residues in rotational crops

According to the data requirement as laid down in Commission Regulation (EC) 283/2013, the magnitude of residues in rotational crops has to be investigated if the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (>0.01 mg/kg).

Since the confined rotational crop study demonstrated that residues of dimethenamid-P and the metabolite M30, the two components constituting the residue definition, are not detected at all in all investigated succeeding crops and the two further components occurring in amounts above 0.01 mg/kg are not relevant in residue trials and was supported by EFSA in 2013, the conduct of a field rotational crop study is not considered necessary.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Plant matrices

For proposing a suitable residue definition in plant and animal matrices, multiple investigations were performed. As presented in MCA, Section 6, Chapters 6.02, 6.03, 6.05 and 6.06, plant and animal studies were performed in which dimethenamid or dimethenamid-P was applied according to the intended use patterns.

For deriving a suitable **residue definition for food of plant origin**, the following studies were considered:

- Crop metabolism studies in three different crop categories
- Confined rotational crop study
- New supervised field trials with separate analysis for metabolites in all representative crops as well as supplementary residue trials which will be submitted in the future for MRL Evaluation
- Hydrolysis at exaggerated temperatures simulating processing

Three **crop metabolism studies** metabolism studies (maize, sugar beet, and soybean) have been previously submitted and reviewed under Annex I and Article 12 evaluation. These studies were considered acceptable and resulted in the current residue definition of Dimethenamid-p (dimethenamid-p including other mixtures of constituent isomers (sum of isomers)). All previously submitted residue studies supported this residue definition, but it should be noted that only parent dimethenamid-P was analyzed in these residue studies.

Two new metabolism studies (in 2006 maize and 2012 soybean), conducted with dimethenamid-P, provide the same picture of the metabolic fate of dimethenamid in plants and are thus congruent with the previous understanding of dimethenamid-P metabolism in crops. Metabolism of dimethenamid-P has been confirmed to proceed via cleavage of the amino acids glutamic acid and glycine from the glutathione side-chain of M24, followed by deaminiation and reduction, leading to the 3-mercapto-lactic acid derivative M26. Oxidation of the thioether group of M26 (S-oxidation) yields the sulfoxide M30. Decarboxylation of M30 followed by oxidation leads to metabolite M31. Oxidation of the sulfoxide group of M31 results in the sulfone M51. Further decarboxylation and oxidation (C- and S-oxidation) steps on M51 yield the sulfonic acid derivative M27. The main metabolite present in residue trials (see MCA Section 6, Chapter 6.3) was the sulfoxide of the thiolactic acid conjugate, M30.

The metabolic profile was essentially identical for sugar beet, soybean and maize treated with dimethenamid and this pathway is common to several chloroacetamide herbicides. It should be noted that M23 and M27 were present in soybean metabolism. However, this is a quantifiable difference and not a difference in metabolic pathway. Essentially all metabolic processes occur after glutathione conjugation and are related to the glutathione moiety of the conjugate. Metabolic modification at other sites on the thiophene ring apparently does not occur.

In the **confined rotational crop study**, none of the above mentioned metabolites arose. Only M81 and M51 were at levels which allowed for identification. Further degradation products in minor concentrations were characterized as possible intermediates of the degradation of dimethenamid-P to C1 or C2 units, which finally enter the biosynthetic pathway of carbohydrates/ polysaccharides and proteins.

To simulate processing, **hydrolysis studies** were performed at exaggerated temperatures using ^{14}C-M656PH030 (also called M30). Radiolabeled metabolite M30 was incubated under the conditions representative of boiling, baking, brewing, sterilization and pasteurization. Since M30 was the only metabolite present in all crop metabolism studies and is the only metabolite present in edible commodities in supervised residue trials at relevant harvest intervals, it was selected as the most appropriate compound for testing of hydrolysis.

This study demonstrated that M30 does not hydrolyze at pH 4, pH 5 and pH 6 after 20 min, 60 min and 20 min of incubation of the samples at 90±5, 100±5, and 120±5°C. Only minor hydrolysis products were observed at pH 4, pH 5, and pH 6 and none of the products were greater than 5% TAR and were not identified. The test compound M30 is hydrolytically stable at the pH and the temperature levels tested. On the basis of these results, M30 is not expected to hydrolyze during the pasteurization (90°C), baking/brewing/boiling (100°C), and sterilization (120°C).

In **supervised field trials,** analysis considered parent dimethenamid-P, and metabolites M23, M27, M26 and M30. In representative commodities of sunflower, soybean, maize, oilseed rape, and sugar beet, a total of 44 trials were run in in the Northern zone and 35 trials were run in the Southern zone of the European Union. In all of these, representative commodities, all analytes were below LOQ in all consumable matrices. However, M30 was found in animal feed items of all matrices tested. Occasionally, metabolites M23, M26 and M27 were measurable as well in animal feed items (See MCA Chapter 6, Section 6.03). In supplementary field trials on Onions, Leafy Cabbages, and Head Cabbage, the metabolite M30 was also observed in edible matrices at relevant harvest intervals (See MCA Chapter 6, Section 6.03).

In summary, the only metabolite present in all commodities tested (particularly in edible matrices) at relevant harvest intervals is the sulfoxide of the thiolactic acid conjugate M30, While parent dimethenamid-P is often present in day 0 samples, at relevant harvest intervals the dominant residue is M30. Metabolites M23, M26 and M27 were sporadically present at low levels in animal feed items but were not present in all commodities groups or any edible matrix, except for M26 measured in one trial of onions.

Therefore, the following new residue definition is proposed for MRL setting and dietary risk assessment:

For MRL Setting:
Sum of parent dimethenamid-P + metabolite M30 (Reg. No 5296352; sulfoxide of thiolactic acid conjugate), as a sum of all isomers, expressed as dimethenamid-P equivalents

For Risk Assessment:
Sum of parent dimethenamid-P + metabolite M30 (Reg. No 5296352; sulfoxide of thiolactic acid conjugate), as a sum of all isomers, expressed as dimethenamid-P equivalents

Animal matrices

As no residues of dimethenamid-P arise in metabolism studies or residue trials in any feed item at relevant harvest intervals, it is unlikely that livestock animals will be exposed to the parent compound. Despite this fact, two metabolism studies were conducted in the past in livestock with parent. The general metabolic pathway in rodents, hens and ruminants was found to be comparable: Dimethenamid-P is rapidly and extensively metabolized, with the majority of the radioactivity being rapidly excreted. Metabolism studies showed that there was no accumulation of dimethenamid-P residues in any edible tissues, poultry eggs, ruminant milk as the parent compound was never observed in any measured animal matrix.

Since livestock animals may be exposed *via* animal feeds to residues of the sulfoxide of the thiolactic acid conjugate M30, a new goat metabolism study with this metabolite was included in this submission. During this study, the thiolactic acid conjugate M26 and sulfoxide of thiolactic acid conjugate M30 were found in significant amounts in liver and kidney.

Thus, for livestock animals, the metabolites M26 and M30 are the metabolites which could be detected; the following residue definitions are proposed for MRL setting and risk assessment purposes:

For Enforcement (and MRL Setting):
Sum of parent dimethenamid-P + metabolite M30 (Reg. No 5296352; sulfoxide of thiolactic acid conjugate), as a sum of all isomers, expressed as dimethenamid-P equivalents in liver and kidney of sheep, cattle, goats, horses and other farm animals

For Risk Assessment:
Sum of parent dimethenamid-P + metabolite M26 (Reg. No 5886781; sulfoxide of thiolactic acid conjugate) + metabolite M30 (Reg. No 5296352; sulfoxide of thiolactic acid conjugate), as a sum of all isomers, expressed as dimethenamid-P equivalents in liver and kidney of sheep, cattle, goats, horses and other farm animals

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the existing EU MRLs, the tentative MRLs that have recently been published by EFSA according to Article 12(2) of Regulation (EC) No 396/2005 as well as the MRLs proposed in this dossier for the intended uses.

Code number	Commodity	Existing EU MRL (mg/kg)	MRL [Art. 12]# (mg/kg)	MRL proposed by BASF (mg/kg)
Plant matrices				
Existing enforcement residue definition: Dimethenamid-p (dimethenamid-p including other mixtures of constituent isomers (sum of isomers)) **Proposed residue definition:** sum dimethenamid-P parent + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents)				
401050	Sunflower	0.02*	0.01*	0.02*
401060	Oilseed rape	0.02*	0.01*	0.02*
401070	Soybean	0.02*	0.01*	0.02*
500030	Maize	0.01*	0.01*	0.02*
900010	Sugar beet	0.02	0.01*	0.02*
Animal tissues				
No existing enforcement residue definition **Proposed residue definition:** **MRL Setting for liver and kidney[2]:** sum dimethenamid-P parent + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents) **Risk Assessment for liver and kidney[2]:** sum dimethenamid-P parent + M26 + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents)				
	Liver	--	--	0.03[1] mg/kg
	Kidney	--	--	0.04[1] mg/kg

\# according to 'Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for dimethenamid-P according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(4):3216,53 pp. doi:10.2903/j.efsa.2013.3216'

* indicates the lower limit of analytical determination

1 As the sum of dimethenamid-P + M30. For Risk Assessment calculations 0.03 mg/kg in liver and 0.07 mg/kg in kidney will be used. See Table 6.7.2-10.

2 MRLs are only proposed in liver and kidney of sheep, cattle, goats, horses and other farm animals as no other residues above the LOQ are predicted in any animal matrix.

MRLs in this dossier are based on residue trials performed after the EU Review of dimethenamid-P with application rates according to the current cGAPs. In these more recent magnitude of residue studies not only parent dimethenamid-P but also metabolites M23, M26, M27 and M30 were analyzed. MRLs are proposed based on the rounded MRLs of total dimethenamid-P (sum of parent dimethenamid-P + metabolite M30, expressed as dimethenamid-P equivalents) derived with the OECD calculator (OECD calculator spreadsheet: http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html).

The residue data for dimethenamid-P in the present dossier was analyzed using the analytical method BASF method No L0179/01 or L0179/02. The method analyses the parent compound dimethenamid-P and the metabolites M23, M26, M27 and M30 with an LOQ of 0.01 mg/kg. Thus, according to the residue definition, the LOQ for the sum of dimethenamid-P and its metabolite M30 is 0.02 mg/kg. Therefore an overall LOQ of 0.02 mg/kg is considered suitable for all plant commodities. For animal matrices new MRLs for kidney and liver are proposed in this submission. The detailed feed burden calculation and MRL proposals are presented below.

Therefore it is proposed that:

1. MRL calculations for plants should be done using an LOQ of 0.02 mg/kg according to the proposed residue definition for plant and the LOQ of the analytical method.
2. A new MRL of 0.03 mg/kg for liver and 0.04 mg/kg for kidney should apply for liver and kidney of sheep, cattle, goats, horses and other farm animals according to the proposed residue definition. For risk assessment, 0.03 mg/kg in liver and 0.07 mg/kg in kidney of sheep, cattle, goats, horses and other farm animals will be used.

Sunflower

For sunflower 24 trials were performed in the EU, 12 in the EU North and 12 in the EU South in the period from 2012 to 2013. All trials were conducted according to the cGAP (0.864 kg a.s./ha). These trials are considered suitable for MRL derivation since they were conducted according to the cGAP and relevant guidelines. In the trials the following total residues of the parent dimethenamid-P and metabolite M30 (sulfoxide of thiolactic acid conjugate) were found at harvest:

<0.02 (12x) mg/kg	Northern Europe
<0.02 (12x) mg/kg	Southern Europe

MRL calculations using the OECD calculator are not meaningful in this case. When all residues are below the LOQ the recommended procedure is to set the EU MRL to the LOQ, in the case of dimethenamid-P to 0.02 mg/kg.

All residues were below the LOQ of the analytical methods applied (0.02 mg/kg) and thus the number of trials for each EU region is sufficient for MRL derivation.

The tentative EU MRL was set to 0.01 mg/kg according to the residue definition of parent dimethenamid-P only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.01 mg/kg).

An

EU MRL of **0.02 mg/kg** for **sunflower seed**

is proposed according to the proposed residue definition (sum of dimethenamid-P and metabolite M30) and the present residue data and based on the LOQ for total dimethenamid-P residues.

Oilseed rape

For oilseed rape 7 trials were performed in the EU, 4 in the EU North and 3 in the EU South in 2013. All trials were conducted according to the cGAP (0.5 kg a.s./ha). The number of trials is considered sufficient since all residues were blow LOQ. The following total residues of parent dimethenamid-P and metabolite M30 (sulfoxide of thiolactic acid conjugate) were found at harvest in rape seed:

<0.02 (4x) mg/kg	Northern Europe
<0.02 (3x) mg/kg	Southern Europe

MRL calculations using the OECD calculator are not meaningful in this case. When all residues are below the LOQ the recommended procedure is to set the EU MRL to the LOQ, in the case of dimethenamid-P to 0.02 mg/kg.

The tentative EU MRL was set to 0.01 mg/kg according to the residue definition of parent dimethenamid-P only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.01 mg/kg).

An

EU MRL of **0.02 mg/kg** for **rape seed**

is proposed according to the proposed residue definition (sum of dimethenamid-P and metabolite M30) and the present residue data and based on the highest LOQ for total dimethenamid-P residues.

Soybean

For soybean 16 trials were performed in the EU, 8 in the EU North and 8 in the EU South in 2013. All trials were conducted according to the cGAP (0.864 kg a.s./ha). All trials are considered suitable for MRL derivation. The following total residues of parent dimethenamid-P and metabolite M30 (sulfoxide of thiolactic acid conjugate) were found at harvest:

<0.02 (8x) mg/kg	Northern Europe
<0.02 (8x) mg/kg	Southern Europe

MRL calculations using the OECD calculator are not meaningful in this case. When all residues are below the LOQ the recommended procedure is to set the EU MRL to the LOQ, in the case of dimethenamid-P to 0.02 mg/kg.

The tentative EU MRL was set to 0.01 mg/kg according to the residue definition of parent dimethenamid-P only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.01 mg/kg).

An

EU MRL of **0.02 mg/kg** for **soybean seed**

is proposed according to the proposed residue definition (sum of dimethenamid-P and metabolite M30) and the present residue data and based on the LOQ for total dimethenamid-P residues.

Maize

For maize 16 trials were performed in the EU, 8 in the EU North and 8 in the EU South in the period from 2011 to 2012. All trials were conducted according to the cGAP (0.864 kg a.s./ha±25%). In 8 comparative trials (4 in EU North and 4 in EU South), an application rate of 1.008 kg a.s./ha was used on a second plot. All trials are considered suitable for MRL derivation. The following total residues of parent dimethenamid-P and metabolite M30 (sulfoxide of thiolactic acid conjugate) were found at harvest:

<0.02 (8x)	Northern Europe
<0.02 (8x)	Southern Europe

MRL calculations using the OECD calculator are not meaningful in this case. When all residues are below the LOQ the recommended procedure is to set the EU MRL to the LOQ, in the case of dimethenamid-P to 0.02 mg/kg.

The tentative EU MRL was set to 0.01 mg/kg according to the residue definition of parent dimethenamid-P only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.01 mg/kg).

An

EU MRL of **0.02 mg/kg** for **maize grain**

is proposed according to the proposed residue definition (sum of dimethenamid-P and metabolite M30) and the present residue data and based on the highest LOQ for total dimethenamid-P residues.

Sugar beet

For sugar beet 12 trials were performed in the EU, 8 in the EU North and 4 in the EU South in the period from 2011 to 2012. All trials were conducted according to the cGAP (0.65 kg a.s./ha). The following total residues of the parent dimethenamid-P and metabolite M30 (sulfoxide of thiolactic acid conjugate) were found at harvest:

<0.02 (8x)	Northern Europe
<0.02 (4x)	Southern Europe

MRL calculations using the OECD calculator are not meaningful in this case. When all residues are below the LOQ the recommended procedure is to set the EU MRL to the LOQ, in the case of dimethenamid-P to 0.02 mg/kg.

The tentative EU MRL was set to 0.01 mg/kg according to the residue definition of parent dimethenamid-P only and the residue values used for MRL derivation being all below the LOQ of the respective analytical method (<0.01 mg/kg).

An

EU MRL of **0.02 mg/kg** for **sugar beet roots**

is proposed according to the proposed residue definition (sum of dimethenamid-P and metabolite M30) and the present residue data and based on the highest LOQ for total dimethenamid-P residues.

Animal matrices

Estimation of residues in livestock feed

A worst case diet was derived for different livestock species with the EFSA dietary burden calculator (based on Appendix G of the Lundehn guidance document 7031/VI/95 rev.4, July 1996). 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:

$$\text{Uptake [mg/kg bw/day]} = \frac{\text{Total intake of dry matter [kg/ animal/day]} \times \text{\% of diet} \times \text{Residue in feed item [mg/kg]}}{\text{Dry matter content of feed item [\%]} \times \text{Bodyweight [kg]}}$$

The following tables show the calculations of the maximum dietary burden for each relevant livestock species, which are based on the highest or median residue levels of total dimethenamid-P residues, depending on the commodity. These maximum dietary burdens are then used to derive suitable MRLs for products of animal origin.

Animals are not expected to be exposed to parent dimethenamid-P based on the results of residue and metabolism studies when used according to the GAP. However, the parent compound is included as a default in calculations of feed burden and the residue definition. Since livestock animals may be exposed to residues of the plant metabolite M30, and since separate livestock metabolism studies were conducted for M30, separate feed burden calculations were carried out for parent dimethenamid-P, M26, and M30.

The estimated maximum feed burdens (for parent and M30) were then compared to the dose levels of the respective metabolism studies with dimethenamid-P in hen and goats and for M30 in goats and expected residues in animal tissues liver and kidney were extrapolated for proposed MRLs. Following the usual practice, highest residues (HR) were used as input values for forages and median residues (STMR / $STMR_P$) for bulk or processed commodities.

For further crops (apples, turnips, swedes, cotton) MRLs may be proposed in the future, but residues below the LOQ are anticipated. For turnips and swedes, residues were extrapolated from sugar beets. Cotton seed residues were extrapolated from soybean and oilseed rape trials. Grass was extrapolated from maize forage. These crops were included to provide the most conservative worst case scenario. It should be noted that the doses assume that the diet completely consists of plant material which had been treated with dimethenamid-P considering worst case assumptions such as intake of the highest residue (HR). Feed burden calculations and doses to be used when estimating maximum dietary burden are included in Table 6.7.2-1 and Table 6.7.2-2 inputs for proposing the residue definition are included first followed by justification for proposed residue definitions and MRLs.

Table 6.7.2-1: Residue values used for calculation of the feed burden for parent dimethenamid-P

Crop	STMR/ STMR$_P$ [mg/kg]	HR/ HR$_P$ [mg/kg]	Origin
Grass (fresh) [1]	0.01	0.01	Extrapolation from maize forage
Grass silage[1]	0.01	0.01	Extrapolation from maize forage
Grass (hay)[1]	0.035	0.035	STMR/HR from grass x drying factor from silage to hay 3.5
Cabbage	0.01	0.01	Based on supplementary trials in cabbage
Kale	0.01	0.01	Based on supplementary trials in curly kale
Apple pomace	0.025	-	Based on supplementary trials in apples and including a default processing factor according to EFSA Profile of 2.5
Peas, beans and lupins (dry)	0.01	0.01	Based on supplementary trials in beans and extrapolation to peas and lupins
Turnips	0.01	0.01	Extrapolation from sugar beets
Swedes	0.01	0.01	Extrapolation from sugar beets
Sunflower seed	0.01	-	Based on all sunflower trials in EU (=LOQ)
Sunflower seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Rape forage	0.01	0.01	Based on all oilseed rape trials in EU (=LOQ)
Rape seed	0.01	-	Based on all oilseed rape trials in EU (=LOQ)
Rape seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Soybean seed	0.01	-	Based on all soybean trials in EU (=LOQ)
Soybean meal	0.013	-	STMR x PF $_{default}$ 1.3
Cotton seed	0.01	-	Extrapolation from oilseed rape and soybean
Cotton seed meal	0.013	-	STMR x PF $_{default}$ 1.3
Linseed	0.01	-	Extrapolation from oilseed rape
Linseed meal	0.02	-	STMR x PF $_{default}$ 2
Maize grain	0.01	-	Based on all maize trials in EU (=LOQ)
Maize silage	0.01	0.01	Based on all maize trials in EU (=LOQ)
Sugar beet ~~tops~~ roots*	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Sugar beet leaves	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Fodder beet ~~tops~~ roots*	0.01	0.01	Extrapolation from sugar beets
Fodder beet leaves	0.01	0.01	Extrapolation from sugar beets

1 Pasture use is currently being considered for future submissions, therefore it is included here to produce the most conservative estimate

* Correction of a typing error

Table 6.7.2-2: Estimated maximum dietary burden of dimethenamid-P[1] residues for poultry and pigs

Crop	Dry matter content (%)	Residue level[2] (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forages						
Cabbage	14	0.01	5	0.000226	-	-
Sugar beet leaves	16	0.01	-	-	25	0.000625
II Cereal grain						
Maize grain	86	0.01	65	0.000477	-	-
V Roots and tubers						
Turnips	10	0.01	20	0.001263	60	0.0024
VI Oilseed						
Rape seed meal	86	0.02	10	0.000147	15	0.00014
Dietary burden:	mg/kg bw/day		0.002113		0.003165	
	mg/animal/day		0.004		0.237	
	mg/kg total feed (DM)		0.033		0.079	

* Feed intake 0.120 kg DM (dry matter) per day, body weight (bw) 1.9 kg
** Feed intake 3 kg DM per day, body weight (bw) 75 kg
1 Parent dimethenamid-P
2 For derivation of residue values see Table 6.7.2-1

Table 6.7.2-3: Estimated maximum dietary burden of dimethenamid-P[1] residues for cattle

Crop	Dry Matter Content (%)	Residue Level[2] (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Grass (fresh)	20	0.01	70	0.001273	40	0.000857
V Roots and tubers						
Turnips	10	0.01	30	0.001091	60	0.002571
Dietary burden:	mg/kg bw/day		0.002364		0.003429	
	mg/animal/day		1.3		1.2	
	mg/kg total feed (DM)		0.065		0.08	

* Feed intake 20 kg DM (dry matter) per day, body weight (bw) 550 kg
** Feed intake 15 kg DM per day, body weight (bw) 350 kg
1 Parent dimethenamid-P
2 For derivation of residue values see Table 6.7.2-1

Thus, the doses to be used when estimating the maximum residues in products of animal origin are:

for parent dimethenamid-P residues

dairy cattle	0.0023 mg/kg bw/d (0.065 mg/kg DM)
beef cattle	0.0034 mg/kg bw/d (0.08 mg/kg DM)
poultry	0.0021 mg/kg bw/d (0.033 mg/kg DM)
pigs	0.0032 mg/kg bw/d (0.079 mg/kg DM)

Table 6.7.2-4: Residue values used for calculation of the feed burden for metabolite M26

Crop	STMR/ STMR$_P$ [mg/kg]	HR/ HR$_P$ [mg/kg]	Origin
Grass (fresh)[1]	0.01	~~0.01~~ 0.05*	Extrapolation from maize forage
Grass silage[1]	0.01	~~0.01~~ 0.05*	Extrapolation from maize forage
Grass (hay) [1]	0.035	~~0.035~~ 0.175*	STMR/HR from grass x drying factor from silage to hay 3.5
Cabbage	0.01	0.01	Based on supplementary trials in cabbage
Kale	0.01	0.01	Based on supplementary trials in curly kale
Apple pomace	0.025	-	Based on supplementary trials in apples and including a default processing factor according to EFSA Profile
Peas, beans and lupins (dry)	0.01	0.01	Based on supplementary trials in beans and extrapolation to peas and lupins
Turnips	0.01	0.01	Extrapolation from sugar beets
Swedes	0.01	0.01	Extrapolation from sugar beets
Sunflower seed	0.01	-	Based on all sunflower trials in EU (=LOQ)
Sunflower seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Rape forage	~~0.01~~ 0.016*	~~0.023~~ 0.231*	Based on all oilseed rape trials in EU (=LOQ)
Rape seed	0.01	-	Based on all oilseed rape trials in EU (=LOQ)
Rape seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Soybean seed	0.01	-	Based on all soybean trials in EU (=LOQ)
Soybean meal	0.013	-	STMR x PF $_{default}$ 1.3
Cotton seed	0.01	-	Extrapolation from soybean and oilseed rape
Cotton seed meal	0.013	-	STMR x PF $_{default}$ 1.3
Linseed	0.01	-	Extrapolation from oilseed rape
Linseed meal	0.02	-	STMR x PF $_{default}$ 2
Maize grain	0.01	0.01	Based on all maize trials in EU (=LOQ)
Maize silage	0.01	0.05	Based on all maize trials in EU (=LOQ)
Sugar beet ~~tops~~ roots*	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Sugar beet leaves	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Fodder beet ~~tops~~ roots*	0.01	0.01	Extrapolation from sugar beets
Fodder beet leaves	0.01	0.01	Extrapolation from sugar beets

* Correction of a typing error though the corresponding calculation was performed with the correct values

1 Pasture use is currently being considered for future submissions, therefore it is included here to produce the most conservative estimate

Table 6.7.2-5: Estimated maximum dietary burden of M26[1] residues for poultry and pigs

Crop	Dry matter content (%)	Residue Level[2] (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forages						
Cabbage	14	0.01	5	0.000226	-	-
Rape forage	14	0.231	-	-	15	0.009900
II Cereal grain						
Maize grain	86	0.01	65	0.000477	5	0.000023
V Roots and tubers						
Turnips	10	0.01	20	0.001263	60	0.002400
VI Oilseed						
Rape seed meal	86	0.02	10	0.000147	20	0.000186
Dietary burden:	mg/kg bw/day		0.00211		0.012509	
	mg/animal/day		0.004		0.938	
	mg/kg total feed (DM)		0.033		0.313	

* Feed intake 0.120 kg DM (dry matter) per day, body weight (bw) 1.9 kg
** Feed intake 3 kg DM, body weight (bw) 75 kg
1 Metabolite M26
2 For derivation of residue values see Table 6.7.2-4

Table 6.7.2-6: Estimated maximum dietary burden of M26[1] residues for cattle

Crop	Dry matter content (%)	Residue level[2] (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize silage	20	0.05	100	0.009091	-	-
Rape forage	14	0.231	-	-	35	0.024750
V Roots and tubers						
Turnips	10	0.01	-	-	60	0.002571
VI Oilseed						
Rape seed meal	86	0.02	-	-	5	0.000050
Dietary burden:	mg/kg bw/day		0.009091		0.027371	
	mg/animal/day		5.0		9.6	
	mg/kg total feed (DM)		0.25		0.64	

* Feed intake 20 kg DM, body weight (bw) 550 kg
** Feed intake 15 kg DM, body weight (bw) 350 kg
1 Metabolite M26
2 For derivation of residue values see Table 6.7.2-4

Thus, the doses to be used when estimating the maximum residues in products of animal origin are:

for M26 Residues

dairy cattle	0.0091 mg/kg bw/d (~~1.4~~ 0.25* mg/kg DM)
beef cattle	0.0274 mg/kg bw/d (~~7.36~~ 0.64* mg/kg DM)
poultry	0.0021 mg/kg bw/d (~~0.069~~ 0.033* mg/kg DM)
pigs	0.0125 mg/kg bw/d (~~3.19~~ 0.31* mg/kg DM)

* Correction of a typing error though the corresponding calculation was performed with the correct values

Table 6.7.2-7: Residue values used for calculation of the feed burden for metabolite M30

Crop	STMR/ STMR$_P$ [mg/kg]	HR/ HR$_P$ [mg/kg]	Origin
Grass (fresh) [1]	0.01	0.28	Extrapolation from maize forage
Grass silage[1]	0.01	0.28	Extrapolation from maize forage
Grass (hay) [1]	~~0.022~~ 0.035*	~~0.62~~ 0.98*	STMR/HR from grass x drying factor from silage to hay 3.5
Cabbage	0.01	~~0.11~~ 0.01	Based on supplementary trials in cabbage
Kale	0.01	0.013	Based on supplementary trials in curly kale
Apple pomace	0.025	-	Based on supplementary trials in apples and including a default processing factor according to EFSA Profile
Peas, beans and lupins (dry)	0.01	0.01	Based on supplementary trials in beans and extrapolation to peas and lupins
Turnips	0.01	0.01	Extrapolation from sugar beets
Swedes	0.01	0.01	Extrapolation from sugar beets
Sunflower seed	0.01	-	Based on all sunflower trials in EU (=LOQ)
Sunflower seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Rape forage	0.34	2.92	Based on all oilseed rape trials in EU (=LOQ)
Rape seed	0.01	-	Based on all oilseed rape trials in EU (=LOQ)
Rape seed meal	0.02	-	STMR seed x PF $_{default}$ 2
Soybean seed	0.01	-	Based on all soybean trials in EU (=LOQ)
Soybean meal	0.013	-	STMR x PF $_{default}$ 1.3
Cotton seed	0.01	-	Extrapolation from soybean and oilseed rape
Cotton seed meal	0.013	-	STMR x PF $_{default}$ 1.3
Linseed	0.01	-	Extrapolation from oilseed rape
Linseed meal	0.02	-	STMR x PF $_{default}$ 2
Maize grain	0.01	0.01	Based on all maize trials in EU (=LOQ)
Maize silage	0.01	0.28	Based on all maize trials in EU (=LOQ)
Sugar beet ~~tops~~ roots*	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Sugar beet leaves	0.01	0.01	Based on all sugar beet trials in EU (=LOQ)
Fodder beet ~~tops~~ roots*	0.01	0.01	Extrapolation from sugar beets
Fodder beet leaves	0.01	0.01	Extrapolation from sugar beets

* Correction of a typing error though the corresponding calculation was performed with the correct values

1 Pasture use is currently being considered for future submissions, therefore it is included here to produce the most conservative estimate

Table 6.7.2-8: Estimated maximum dietary burden of M30[1] residues for cattle

Crop	Dry matter content (%)	Residue level[2] (HR/STMR mg/kg)	Dairy cattle*		Beef cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Grass (fresh)	20	0.28	100	0.050909	-	-
Rape forage	14	2.92	-	-	35	0.312857
V Roots and tubers						
Turnips	10	0.01	-	-	60	0.002571
VI Oil seed						
Rape seed meal	86	0.02	-	-	5	0.00005
Dietary burden:	mg/kg bw/day		0.050909		0.3154	
	mg/animal/day		28.0		110.4	
	mg/kg total feed (DM)		1.4		7.36	

* Feed intake 20 kg DM (dry matter) per day, body weight (bw) 550 kg
** Feed intake 15 kg DM per day, body weight (bw) 350 kg
1 Metabolite M30
2 For derivation of residue values see Table 6.7.2-7

Table 6.7.2-9: Estimated maximum dietary burden of M30[1] residues for poultry and pigs

Crop	Dry matter content (%)	Residue level[2] (HR/STMR mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
~~Cabbage~~ Kale	14	~~0.11~~ 0.013	5	~~0.002481~~ 0.000293	-	-
Rape forage	14[#]	2.92[#]	-	-	15	0.125143
II Cereal grain						
Maize grain	86	0.01	65	0.000477	5	0.000023
V Roots and tubers						
Turnips	10	0.01	20	0.001263	60	0.0024
VI Oil seed						
Rape seed meal	86	0.02	10	0.000147	20	0.000186
Dietary burden:	mg/kg bw/day		~~0.00437~~ 0.00218		0.1277	
	mg/animal/day		~~0.0083~~ 0.0041		9.58	
	mg/kg total feed (DM)		~~0.069~~ 0.034		3.19	

* Feed intake 0.120 kg DM (dry matter) per day, body weight (bw) 1.9 kg
** Feed intake 3 kg DM per day, body weight (bw) 75 kg
1 Metabolite M30
2 For derivation of residue values see Table 6.7.2-7
\# Correction of a typing error

Thus, the doses to be used when estimating the maximum residues in products of animal origin are:

for M30-derived residues

dairy cattle	0.051 mg/kg bw/d (1.4 mg/kg DM)
beef cattle	0.315 mg/kg bw/d (7.36 mg/kg DM)
poultry	~~0.0044~~ 0.0022 mg/kg bw/d (~~0.069~~ 0.034 mg/kg DM)
pigs	0.128 mg/kg bw/d (3.19 mg/kg DM)

Cattle products

In the two goat metabolism studies with dimethenamid-P and M30 which can serve as feeding studies in this case (see MCA Section 6, Chapter 6.4), the nominal dose levels were 8.9 mg/kg bw/day for dimethenamid and 0.57 mg/kg bw/day for M30, respectively. Thus, overdosing factors range from 2596 to 2788 x for beef cattle and from 3431 to 3765 x for dairy cattle for dimethenamid-P and overdosing factors of 1.6 to 1.8x for beef cattle and from 8.6 to 11.1x for dairy cattle for M30 were calculated.

Parent dimethenamid-P

When samples of the goat metabolism study were analyzed, overall residues in milk were 0.65 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for dimethenamid-P of 0.0001 mg/kg in milk.

In tissues, residues of 0.97, 0.97, 16.62 and 9.92 mg/kg were found in muscle, fat, liver and kidney for dimethenamid-P, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for dimethenamid-P. Since feed burden calculations are conducted under various worst-case assumptions extrapolations give a very conservative estimate of the residues.

Metabolite M26 from feed burden of M26 (used for risk assessment only)

The overall residues of M26 in milk in the M30 goat metabolism study were 0.001 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for M26 of 0.0002 mg/kg in milk. In tissues, residues of M26 were 0.002, 0.002, 0.011 and 0.06 mg/kg were found in muscle, fat, liver and kidney, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M26 in milk, muscle, fat, liver, and kidney. Therefore residues in animal tissues which occur from the exposure of feed burden from M26 are negligible compared to the residues of M26 which occur from feed burdens of M30.

Metabolite M26 from feed burden of M30 (used for risk assessment only)

The overall residues of M26 in milk in the M30 goat metabolism study were 0.001 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for M26 of 0.0002 mg/kg in milk. In tissues, residues of M26 were 0.002, 0.002, 0.011 and 0.06 mg/kg were found in muscle, fat, liver and kidney, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M26 in milk, muscle, fat and liver, and a residue of 0.038 mg/kg in kidney for M26.

Metabolite M30
The overall residues in milk in the M30 goat metabolism study were 0.001 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for M30 of 0.0001 mg/kg in milk. In tissues, residues of 0.002, 0.003, 0.027 and 0.048 mg/kg were found in muscle, fat, liver and kidney for M30, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M30 in muscle and fat, and a residue of 0.02 mg/kg in liver and 0.03 mg/kg in kidney for M30.

Poultry products
A feeding study in poultry is not required as the estimated maximum feed burden (see Table 6.7.2-2, Table 6.7.2-5 and Table 6.7.2-9) for poultry and based on the metabolism studies in laying hens, residues in poultry products are predicted to be below 0.01 mg/kg for dimethenamid-P as well as the metabolites M27 and M30.

Parent dimethenamid-P
In a metabolism study in poultry, animals were dosed with racemic dimethenamid-P at 167 mg/kg diet. The dose level used in the metabolism study represents a considerable overdosing with regard to the highest feed burden of 0.033 mg/kg feed DM calculated for poultry (see Table 6.7.2-2). No residues of dimethenamid-P at or above the LOQ of 0.01 mg/kg are expected in poultry eggs and edible tissues (fat, muscle and liver).

Metabolite M26 (for risk assessment only)
Results of the feed burden calculations for M26 were compared with the above mentioned metabolism study. The estimated maximum feed burden in poultry for M26 is also much lower than the anticipated dose used in the metabolism study, and no residues of the metabolite M26 at or above the LOQ (0.01 mg/kg) are expected in eggs and tissues.

Metabolite M30
Results of the feed burden calculations for M30 were compared with the above mentioned metabolism study. The estimated maximum feed burden in poultry for M30 is also much lower than the anticipated dose used in the metabolism study, and no residues of the metabolite M30 at or above the LOQ (0.01 mg/kg) are expected in eggs and tissues.

Pig products
A feeding study in pigs is only required, if the metabolic pathways differ significantly in rats as compared to ruminants. Since the metabolism of dimethenamid-P in rat and ruminants is similar, no separate metabolism study for pigs has been conducted and extrapolation is based on the metabolism studies with ruminants.

Metabolite M26 from Feed Burden M26
In the M30 metabolism study in tissues, residues of 0.002, 0.003, 0.027 and 0.048 mg/kg were found in muscle, fat, liver and kidney for M30, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M26 in muscle, fat, liver and kidney in animals that are exposed to M26 in animal feed items.

Metabolite M26 from Feed Burden M30

In the M30 metabolism study in tissues, residues of 0.002, 0.003, 0.027 and 0.048 mg/kg were found in muscle, fat, liver and kidney for M30, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M26 in muscle, fat, liver and a residue of 0.01579 mg/kg in kidney.

Metabolite M30

In the M30 metabolism study in tissues, residues of 0.002, 0.003, 0.027 and 0.048 mg/kg were found in muscle, fat, liver and kidney for M30, respectively. Application of the overdosing factors leads to anticipated residues below the LOQ of 0.01 mg/kg for M30 in muscle, fat, liver and a residue of 0.013 mg/kg in kidney.

Justification for MRLs and Residue Definitions based on Animal Data

In order to propose the residues for MRL setting and risk assessment the measured residues in ruminants from the goat metabolism study with M30 was used. Ruminants were chosen as the model of extrapolation because the anticipated levels in ruminant tissues were significantly higher than the anticipated residues in swine. For dimethenamid-P, the default MRL of 0.01 mg/kg used. However, this significantly overestimates exposure because animals will not be exposed to parent and previous metabolism studies in rat, goat, and hen have all demonstrated that parent dimethenamid-P is rapidly excreted and extensively metabolized. The parent has never been measured in any tissue within these studies; it is included as a default only.

For M26, it has been considered that M26 in animal tissues can come from feed burdens of M26 as well as M30. It is important to note, however, that the contribution of M26 in animal tissues from M26 in animal feed items is assumed negligible when compared to the contribution of M26 from animal feed items which contain M30. The use of M26 in these calculations below has been included to again demonstrate its non-relevance to the proposed plant residue definition. The results are presented below:

Table 6.7.2-10: Calculation of total residues for dimethenamid-P, M26 and M30 from ruminants

<table>
<tr><th>Food of animal origin</th><th>Source of feed burden[1]</th><th>Residues of M26[1] in ruminants in parent equivalents</th><th>Residues of M30[2] in ruminants in parent equivalents</th><th>Total residues of parent + M30</th><th>Total residues in parent equivalents</th></tr>
<tr><td rowspan="3">Liver</td><td>Dimethenamid-P[3]</td><td colspan="2">0.01</td><td rowspan="4">0.022</td><td rowspan="4">0.028</td></tr>
<tr><td>M26</td><td>0.0006</td><td>-</td></tr>
<tr><td>M30</td><td>0.0069</td><td>0.017</td></tr>
<tr><td colspan="2">Total liver residues in parent equivalents</td><td>0.006</td><td>0.012</td></tr>
<tr><td rowspan="3">Kidney</td><td>Dimethenamid-P[3]</td><td colspan="2">0.01</td><td rowspan="4">0.032</td><td rowspan="4">0.063</td></tr>
<tr><td>M26</td><td>0.0032</td><td>-</td></tr>
<tr><td>M30</td><td>0.038</td><td>0.03</td></tr>
<tr><td colspan="2">Total kidney residues in parent equivalents</td><td>0.03</td><td>0.02</td></tr>
</table>

1 Equals residues of M26 in ruminants multiplied by a MWCF of 0.7629.
2 Equals residues of M30 in ruminants multiplied by a MWCF of 0.7306.
3 Included as default MRL only. Animals are not exposed to parent and parent is not a metabolite found in any tissue related to foods of animal origin.

For the purposes of **MRLs, monitoring, and enforcement,** the metabolite M30 is the appropriate marker molecule. As shown from the feed burdens above, M30 exposure in animal feed items leads to residues above the LOQ in liver and kidney matrices. However, M26 exposure in feed items only leads to residue above the LOQ in kidney. Therefore the metabolite M30 has the greatest chance of determining misuse. Parent dimethenamid-P is proposed in the residue definition as well, but as a default only. As noted above, it is highly unlikely that an animal or consumer will be exposed to parent dimethenamid-P.

Based on Table 6.7.2-10 above, the following MRLs are proposed for parent + M30:

0.03 mg/kg for liver of sheep, cattle, goats, horses and other farm animals
0.04 mg/kg for kidney of sheep, cattle, goats, horses and other farm animals.

For the purposes of **risk assessment,** in addition to parent dimethenamid-P + M30, the metabolite M26 must also be considered. As was demonstrated above, exposure to animal feed items containing M30 leads to residues of M26 in kidney. Extrapolations based on the radioactive residue in the goat metabolism study and the theoretical maximum dietary burden in ruminants originating from residues of both M26 and M30 indicate that measurable residues would be detected in only liver and kidney. No measurable residues are expected in milk or other tissues. Maximum expected theoretical residues in liver and kidney are relatively low (0.03 and 0.06 mg/kg as total residues of M26 and M30, respectively, based on parent equivalents). Chronic dietary risk assessment using PRIMo, version 2 show that including all relevant plant and animal residues in the TMDI calculation results in no more than 7.3% ADI utilization for all consumer groups. Based upon the results from this conservative approach and in an effort to protect animal welfare, no feeding study in cattle is necessary.

Based on Table 6.7.2-10 above, the following inputs will be used for risk assessment purposes as the sum of parent dimethenamid-P + M26 +M30. They are highly conservative by including dimethenamid-P at the LOQ.

0.03 mg/kg for liver of sheep, cattle, goats, horses and other farm animals
0.07 mg/kg for kidney of sheep, cattle, goats, horses and other farm animals.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Plant Products

According to the proposed residue definition for MRL setting of

Sum parent dimethenamid-P + M30 expressed as parent equivalents;

and referring to MRL derivations in MCA Section 6, Chapter 6.7.2.1 it is proposed to establish EU MRLs of:

0.02 mg/kg for sunflower seed
0.02 mg/kg for oilseed rape seed
0.02 mg/kg for soybean seed
0.02 mg/kg for maize
0.02 mg/kg for sugar beet

Animal products

According to the proposed residue definition for MRL setting of

Sum parent dimethenamid-P + M30 expressed as parent equivalents;

and referring to MRL derivations in MCA Section 6, Chapter 6.7.2.2 it is proposed to establish EU MRLs of:

0.03 mg/kg for liver of sheep, cattle, goats, horses and other farm animals.
0.04 mg/kg for kidney of sheep, cattle, goats, horses and other farm animals.

CA 6.8 Proposed safety intervals

Dimethenamid-P is being recommended for pre to post emergence use of the crop (BBCH 00-09 for sunflower and soybean and BBCH 10-18 for oilseed rape, maize and sugar beet). The timing of use is governed by the growth stage of the crop and the weeds and is not in relation to the time of harvest. Residue trials have been conducted with applications made at the latest recommended crop growth stage with harvest taking place at the time of crop maturity following good agricultural practice.

Pre-harvest interval
In sunflower and soybean one post-emergence application at the growth stage BBCH 00-09 and in oilseed rape, maize and sugar beet at BBCH 10-18 is intended, with the pre-harvest interval being fixed by the conditions of use.

Re-entry period for livestock to areas to be grazed
No re-entry period for livestock has to be defined because dimethenamid-P is not intended to be used in areas to be grazed.

Re-entry period for man to treated crops
Dimethenamid-P will be applied pre-emergence or early post-emergence to oilseed rape, sunflower, soybean, maize and sugar beet. Because of the plant size at the growth stages for the intended use patterns, most of the active substance will be applied to soil and interception by crop foliage is minimal. Also, the result of the risk assessments indicate that re-entry of treated field crops is possible after the spray solution has dried. The respective assessments are detailed under MCP Chapter 7.2.3 in the supplemental Dossiers for the representative formulations BAS 656 12 H and BAS 830 01 H.

Withholding period for animal feed stuffs
Treated sunflower, oilseed rape, soybean, maize and sugar beet may be used as fodder for livestock. Dimethenamid-P derived residues in those feed items are assessed in this dossier by providing updated calculations of livestock dietary burdens and deriving suitable MRLs for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to dimethenamid-P derived residues.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since dimethenamid-P containing products are intended for both pre-emergence and post emergence uses.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended.

Waiting period between last application and sowing or planting succeeding crops
No waiting period is necessary. Due to the fact that no relevant accumulation of dimethenamid-P or its degradation products was observed in the confined rotational crop study (see document MCA, Chapter 6.6), no limitation concerning the succeeding crops is necessary.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

CA 6.9.1 Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI calculations

The MRL values used in the dietary risk assessments in this chapter are listed in Table 6.9.1-1 and Table 6.9.1-2 below.

The chronic consumer risk assessment for dimethenamid-P was performed using the EU MRLs proposed in this Annex I Renewal Dossier for oilseed rape, sunflower, soybean, maize and sugar beet (Table 6.9.1-1) as well as for further crops where an MRL application will be requested in the future (Table 6.9.1-2). Since no MRL has been proposed at this time in supplementary crops, and further field trials may be needed, the Highest Residue was used in these supplementary crops in place of an MRL. This would provide the most conservative risk assessment available at this time. For further commodities which are not registered, the default MRL of 0.02 mg/kg was included. For animal matrices, a default MRL of 0.02 mg/kg (parent + M30) was used except for liver (0.03 mg/kg) and kidney (0.04 mg/kg), as MRLs are only proposed for kidney and liver in this Dossier. For risk assessment, 0.03 mg/kg in liver and 0.07 mg/kg in kidney was used based on the proposed risk assessment definition of parent + M26 + M30. In all cases for animal dietary inputs, the values are highly conservative. First, because animals and consumers are not exposed to parent dimethenamid-P based on metabolism and residue data. Second, because feed burden calculations have shown no residues above the LOQ for any matrix other than liver and kidney. The risk assessment is presented in Table 6.9.1-7. For all crops listed in the residues section of this dossier, MRLs have been derived on the basis of the conducted residue trials. MRL values for animal products are based on extrapolation from the residue levels as observed in metabolism studies on goats at the theoretical worst case feed burden calculations (see MCA Section 6, Chapter 6.04 and 6.7.2). For the chronic risk assessment, the ADI of dimethenamid-P of 0.02 mg/kg bw/d (as derived in the last peer review process and laid down in the European Commission Review Report for the active substance dimethenamid-P SANCO/1402/2001 – Final, 3 July 2003) was applied.

Additionally, an exposure assessment was performed for the four main plant metabolites of dimethenamid-P, M23, M26, M27 and M30. The exposure assessments for M23, M26, and M27 were included to support their non-relevance as part of the residue definition. Furthermore, M23, M26, and M27 have been toxicologically tested, M30 is covered by M26, and are less toxic than parent dimethenamid-P (See MCA Section 5, Chapter 5.8). While M30 is not toxicologically relevant, it is the metabolite most present in all crops tested. Therefore, it is included as part of the residue definition.

The residue values used for the individual exposure assessments are shown in Table 6.9.1-8 to Table 6.9.1-11.

Table 6.9.1-1: Existing and proposed maximum residue levels for dimethenamid-P in the EU

Code Number	Commodity	Existing EU MRL (mg/kg)	MRL [Art. 12]# (mg/kg)	MRL Proposed by BASF (mg/kg)
Plant matrices				
Existing enforcement residue definition: Dimethenamid-p (dimethenamid-p including other mixtures of constituent isomers (sum of isomers)) **Proposed residue definition:** sum dimethenamid-P parent + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents)				
401050	Sunflower	0.02*	0.01*	0.02*
401060	Oilseed rape	0.02*	0.01*	0.02*
401070	Soybean	0.02*	0.01*	0.02*
500030	Maize	0.01*	0.01*	0.02*
900010	Sugar beet	0.02	0.01*	0.02*
Animal tissues				
No existing enforcement residue definition **Proposed residue definition:** **MRL Setting for liver and kidney[2]:** sum dimethenamid-P parent + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents) **Risk Assessment for liver and kidney[2]:** sum dimethenamid-P parent + M26 + M30 (dimethenamid-p including other mixtures of constituent isomers (sum of isomers) in parent equivalents)				
	Liver for all animals (except poultry)	-	-	0.03[1] mg/kg
	Kidney for all animals (except poultry)	-	-	0.04[1] mg/kg

\# according to 'Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for dimethenamid-P according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(4):3216,53 pp. doi:10.2903/j.efsa.2013.3216'

* indicates the lower limit of analytical determination

1 As the sum of dimethenamid-P + M30. For Risk Assessment calculations 0.03 mg/kg in liver and 0.07 mg/kg in kidney will be used.

2 MRLs are only proposed in liver and kidney of liver and kidney of sheep, cattle, goats, horses and other farm animals as no other residues above the LOQ are predicted in any animal matrix.

Table 6.9.1-2: Further total dimethenamid-P[1] residue values used for dietary risk assessment[2]

Crop	Input values (Total) [mg/kg]	Remarks
Apple	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Cherries	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Strawberry	0.022	HR of supplementary trials[3]
Onion	0.11	HR of supplementary trials[3]
Welsh/spring onion	0.03	HR of supplementary trials[3]
Leek	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Cucumber	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Zucchini	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Melon	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Pumpkin	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Broccoli	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Cauliflower	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Brussels sprouts	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Head cabbage	~~0.09~~ 0.02	~~HR of supplementary trials[3]~~ Total LOQ (0.02 mg/kg) of supplementary trials
Chinese cabbage	0.06	HR of supplementary trials[3]
Curly kale	0.02	Total LOQ (0.02 mg/kg) of supplementary trials
Green bean	0.02	Total LOQ (0.02 mg/kg) of supplementary trials

1 Dimethenamid-P plus M30 expressed as parent equivalent .The conversion factor from M30 to dimethenamid-P is 0.7306.
2 Supplementary trials presented in MCA Section 6, Chapter 6.3.6
3 In the absence of proposed MRLs, the HR was used to give the absolute worst case scenario and show there is no risk to the consumer. MRLs will be proposed on these commodities via an MRL application in the future.

For the exposure assessment of metabolites all relevant supported uses were considered and with the selected residue levels a worst case approach was followed. The risk assessments for M23, M26 and M27 are presented to illustrate their non-relevance for the plant residue definition.

Table 6.9.1-3: Residue values used for the exposure assessment of M23[1]

Crop	M23 input values [mg/kg]	Remarks
Apple	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cherries	0.01	LOQ (0.01 mg/kg) of supplementary trials
Strawberry	0.01	LOQ (0.01 mg/kg) of supplementary trials
Sunflower	0.01	LOQ (0.01 mg/kg) of all available trials
Oilseed rape	0.01	LOQ (0.01 mg/kg) of all available trials
Soybean	0.01	LOQ (0.01 mg/kg) of all available trials
Maize	0.01	LOQ (0.01 mg/kg) of all available trials
Sugar beet	0.01	LOQ (0.01 mg/kg) of all available trials
Onion	0.01	LOQ (0.01 mg/kg) of supplementary trials
Welsh/spring onion	0.01	LOQ (0.01 mg/kg) of supplementary trials
Leek	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cucumber	0.01	LOQ (0.01 mg/kg) of supplementary trials
Zucchini	0.01	LOQ (0.01 mg/kg) of supplementary trials
Melon	0.01	LOQ (0.01 mg/kg) of supplementary trials
Pumpkin	0.01	LOQ (0.01 mg/kg) of supplementary trials
Broccoli	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cauliflower	0.01	LOQ (0.01 mg/kg) of supplementary trials
Brussels sprouts	0.01	LOQ (0.01 mg/kg) of supplementary trials
Head cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Chinese cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Curly kale	0.01	LOQ (0.01 mg/kg) of supplementary trials
Green bean	0.01	LOQ (0.01 mg/kg) of supplementary trials
Further plant matrices	0.01	LOQ (0.01 mg/kg)
Animal matrices	0.01	LOQ (0.01 mg/kg)

Max: 3.6% of 0.02 mg/kg bw/d = 0.72 µg/kg bw/d

1 Supplementary trials presented in MCA Section 6, Chapter 6.3.6. No residues above the LOQ were observed.

Table 6.9.1-4: Residue values used for the exposure assessment of M26[1]

Crop	M26 input values [mg/kg]	Remarks
Apple	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cherries	0.01	LOQ (0.01 mg/kg) of supplementary trials
Strawberry	0.01	LOQ (0.01 mg/kg) of supplementary trials
Sunflower	0.01	LOQ (0.01 mg/kg) of all available trials
Oilseed rape	0.01	LOQ (0.01 mg/kg) of all available trials
Soybean	0.01	LOQ (0.01 mg/kg) of all available trials
Maize	0.01	LOQ (0.01 mg/kg) of all available trials
Sugar beet	0.01	LOQ (0.01 mg/kg) of all available trials
Onion	0.023	HR of supplementary trials[2]
Welsh/spring onion	0.01	LOQ (0.01 mg/kg) of supplementary trials
Leek	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cucumber	0.01	LOQ (0.01 mg/kg) of supplementary trials
Zucchini	0.01	LOQ (0.01 mg/kg) of supplementary trials
Melon	0.01	LOQ (0.01 mg/kg) of supplementary trials
Pumpkin	0.01	LOQ (0.01 mg/kg) of supplementary trials
Broccoli	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cauliflower	0.01	LOQ (0.01 mg/kg) of supplementary trials
Brussels sprouts	0.01	LOQ (0.01 mg/kg) of supplementary trials
Head cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Chinese cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Curly kale	0.01	LOQ (0.01 mg/kg) of supplementary trials
Green bean	0.01	LOQ (0.01 mg/kg) of supplementary trials
Further plant matrices	0.01	LOQ (0.01 mg/kg)
Animal matrices except kidney	0.01	LOQ (0.01 mg/kg)
Kidney	0.04[3]	Results from feed burden calculations

Max: 3.6% of 0.02 mg/kg bw/d = 0.72 µg/kg bw/d

1 Supplementary trials presented in MCA Section 6, Chapter 6.3.6. All residues are below the LOQ of 0.01 mg/kg, except for onion.

2 Since MRLs will not be proposed for M26, the HR was used to give the absolute worst case scenario, show there is no risk to the consumer and illustrate the non-relevance of M26 to the consumer.

3 See MCA Chapter 6.4

Table 6.9.1-5: Residue values used for the exposure assessment of M27[1]

Crop	M27 input values [mg/kg]	Remarks
Apple	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cherries	0.01	LOQ (0.01 mg/kg) of supplementary trials
Stawberry	0.01	LOQ (0.01 mg/kg) of supplementary trials
Sunflower	0.01	LOQ (0.01 mg/kg) of all available trials
Oilseed rape	0.01	LOQ (0.01 mg/kg) of all available trials
Soybean	0.01	LOQ (0.01 mg/kg) of all available trials
Maize	0.01	LOQ (0.01 mg/kg) of all available trials
Sugar beet	0.01	LOQ (0.01 mg/kg) of all available trials
Onion	0.01	LOQ (0.01 mg/kg) of supplementary trials
Welsh/spring onion	0.01	LOQ (0.01 mg/kg) of supplementary trials
Leek	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cucumber	0.01	LOQ (0.01 mg/kg) of supplementary trials
Zucchini	0.01	LOQ (0.01 mg/kg) of supplementary trials
Melon	0.01	LOQ (0.01 mg/kg) of supplementary trials
Pumpkin	0.01	LOQ (0.01 mg/kg) of supplementary trials
Broccoli	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cauliflower	0.01	LOQ (0.01 mg/kg) of supplementary trials
Brussels sprouts	0.01	LOQ (0.01 mg/kg) of supplementary trials
Head cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Chinese cabbage	0.01	LOQ (0.01 mg/kg) of supplementary trials
Curly kale	0.01	LOQ (0.01 mg/kg) of supplementary trials
Green bean	0.01	LOQ (0.01 mg/kg) of supplementary trials
Further plant matrices	0.01	LOQ (0.01 mg/kg)
Animal matrices	0.01	LOQ (0.01 mg/kg)

Max: 3.6% of 0.02 mg/kg bw/d = 0.72 µg/kg bw/d

1 Supplementary trials presented in MCA Section 6, Chapter 6.3.6. No residues above the LOQ were observed.

Table 6.9.1-6: Residue values used for the exposure assessment of M30[1]

Crop	M30 input values [mg/kg]	Remarks
Apple	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cherries	0.01	LOQ (0.01 mg/kg) of supplementary trials
Strawberry	0.016	HR of supplementary trials[2]
Sunflower	0.01	LOQ (0.01 mg/kg) of all available trials
Oilseed rape	0.01	LOQ (0.01 mg/kg) of all available trials
Soybean	0.01	LOQ (0.01 mg/kg) of all available trials
Maize	0.01	LOQ (0.01 mg/kg) of all available trials
Sugar beet	0.01	LOQ (0.01 mg/kg) of all available trials
Onion	0.14	HR of supplementary trials[2]
Welsh/spring onion	0.034	HR of supplementary trials[2]
Leek	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cucumber	0.01	LOQ (0.01 mg/kg) of supplementary trials
Zucchini	0.01	LOQ (0.01 mg/kg) of supplementary trials
Melon	0.01	LOQ (0.01 mg/kg) of supplementary trials
Pumpkin	0.01	LOQ (0.01 mg/kg) of supplementary trials
Broccoli	0.01	LOQ (0.01 mg/kg) of supplementary trials
Cauliflower	0.01	LOQ (0.01 mg/kg) of supplementary trials
Brussels sprouts	0.01	LOQ (0.01 mg/kg) of supplementary trials
Head cabbage	~~0.11~~ 0.01	~~HR of supplementary trials[2]~~ LOQ (0.01 mg/kg) of supplementary trials
Chinese cabbage	0.062	HR of supplementary trials[2]
Curly kale	0.013	HR of supplementary trials[2]
Green bean	0.01	LOQ (0.01 mg/kg) of supplementary trials
Further plant matrices	0.01	LOQ (0.01 mg/kg)
Animal matrices except liver and kidney	0.01	LOQ (0.01 mg/kg)
Liver	0.02	LOQ (0.01 mg/kg)
Kidney	0.03	Results from feed burden calculation

Max: 3.7% of 0.02 mg/kg bw/d = 0.74 µg/kg bw/d

1 Supplementary trials presented in MCA Section 6, Chapter 6.3.6

2 In the absence of proposed MRLs, the HR was used to give the absolute worst case scenario and show there is no risk to the consumer. MRLs will be proposed on these commodities via an MRL application in the future.

Table 6.9.1-7: TMDI calculation for total dimethenamid-P with PRIMo Model (rev 2.0) using MRLs proposed by BASF

dimethenamid-P + M30

Status of the active substance:	included	Code no.	BAS 656 PH
LOQ (mg/kg bw):	0.02	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.02	ARfD (mg/kg bw):	0.25
Source of ADI:	EFSA	Source of ARfD:	EFSA
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum
1 7

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)
7.26	FR toddler	3.96	Milk and cream,	1.18	FRUIT (FRESH OR FROZEN)	0.80	Root and tuber vegetables	7.17
6.92	NL child	2.93	Milk and cream,	1.50	FRUIT (FRESH OR FROZEN)	0.66	Root and tuber vegetables	6.57
6.88	UK Infant	3.87	Milk and cream,	1.01	Sugar beet (root)	0.55	FRUIT (FRESH OR FROZEN)	6.70
6.38	UK Toddler	2.29	Sugar beet (root)	2.07	Milk and cream,	0.64	FRUIT (FRESH OR FROZEN)	6.20
5.48	FR infant	2.57	Milk and cream,	1.52	FRUIT (FRESH OR FROZEN)	0.71	Root and tuber vegetables	5.45
5.44	DE child	2.30	FRUIT (FRESH OR FROZEN)	1.43	Milk and cream,	0.41	Wheat	5.30
5.08	WHO Cluster diet B	0.85	Wheat	0.72	FRUIT (FRESH OR FROZEN)	0.52	Root and tuber vegetables	4.48
3.81	DK child	1.26	Milk and cream,	0.55	Wheat	0.51	FRUIT (FRESH OR FROZEN)	3.60
3.78	SE general population 90th percentile	1.24	Milk and cream,	0.58	FRUIT (FRESH OR FROZEN)	0.55	Root and tuber vegetables	3.19
3.63	ES child	1.25	Milk and cream,	0.56	FRUIT (FRESH OR FROZEN)	0.44	Wheat	3.49
3.51	E adult	1.07	FRUIT (FRESH OR FROZEN)	0.40	Root and tuber vegetables	0.28	Milk and cream,	3.32
3.24	WHO cluster diet E	0.58	FRUIT (FRESH OR FROZEN)	0.52	Root and tuber vegetables	0.39	Wheat	2.92
3.15	WHO cluster diet D	0.65	Wheat	0.50	Milk and cream,	0.45	Root and tuber vegetables	2.73
3.01	WHO regional European diet	0.48	Milk and cream,	0.46	Root and tuber vegetables	0.35	FRUIT (FRESH OR FROZEN)	2.60
2.74	WHO Cluster diet F	0.44	Root and tuber vegetables	0.40	Milk and cream,	0.38	FRUIT (FRESH OR FROZEN)	2.47
2.48	NL general	0.66	Milk and cream,	0.49	FRUIT (FRESH OR FROZEN)	0.31	Root and tuber vegetables	2.24
2.09	ES adult	0.50	Milk and cream,	0.41	FRUIT (FRESH OR FROZEN)	0.23	Wheat	1.97
1.91	UK vegetarian	0.38	Sugar beet (root)	0.34	FRUIT (FRESH OR FROZEN)	0.33	Milk and cream,	1.74
1.90	FR all population	0.62	FRUIT (FRESH OR FROZEN)	0.33	Wheat	0.27	Milk and cream,	1.83
1.76	LT adult	0.40	Milk and cream,	0.35	Root and tuber vegetables	0.23	FRUIT (FRESH OR FROZEN)	1.58
1.73	PT General population	0.64	FRUIT (FRESH OR FROZEN)	0.39	Wheat	0.19	Onions	1.53
1.72	DK adult	0.54	Milk and cream,	0.33	FRUIT (FRESH OR FROZEN)	0.20	Wheat	1.59
1.70	IT kids/toddler	0.66	Wheat	0.35	FRUIT (FRESH OR FROZEN)	0.16	Solanacea	1.63
1.67	UK Adult	0.40	Sugar beet (root)	0.30	Milk and cream,	0.29	FRUIT (FRESH OR FROZEN)	1.55
1.43	FI adult	0.57	Milk and cream,	0.25	FRUIT (FRESH OR FROZEN)	0.15	Root and tuber vegetables	1.31
1.27	IT adult	0.41	Wheat	0.28	FRUIT (FRESH OR FROZEN)	0.13	Solanacea	1.20
1.25	PL general population	0.41	Root and tuber vegetables	0.34	FRUIT (FRESH OR FROZEN)	0.18	Onions	0.90

dimethenamid-P + M30

Status of the active substance:	included	Code no.	BAS 656 PH
LOQ (mg/kg bw):	0.02	proposed LOQ:	

Toxicological end points

ADI (mg/kg bw/day):	0.02	ARfD (mg/kg bw):	0.25
Source of ADI:	EFSA	Source of ARfD:	EFSA
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum
1 7

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)
6.84	FR toddler	3.96	Milk and cream,	0.80	Root and tuber vegetables	0.29	Pome fruit	6.72
6.84	UK Infant	3.87	Milk and cream,	1.01	Sugar beet (root)	0.47	Root and tuber vegetables	6.69
6.81	NL child	2.93	Milk and cream,	0.68	Pome fruit	0.66	Root and tuber vegetables	6.58
6.34	UK Toddler	2.29	Sugar beet (root)	2.07	Milk and cream,	0.41	Root and tuber vegetables	6.19
5.42	DE child	1.43	Milk and cream,	1.27	Pome fruit	0.46	Citrus fruit	5.26
5.00	WHO Cluster diet B	0.85	Wheat	0.52	Root and tuber vegetables	0.43	Onions	4.49
4.50	FR infant	2.57	Milk and cream,	0.71	Root and tuber vegetables	0.28	Pome fruit	4.43
3.78	DK child	1.26	Milk and cream,	0.55	Wheat	0.44	Rye	3.60
3.61	ES child	1.25	Milk and cream,	0.44	Wheat	0.23	Citrus fruit	3.49
3.56	SE general population 90th percentile	1.24	Milk and cream,	0.55	Root and tuber vegetables	0.32	Wheat	3.24
3.46	IE adult	0.40	Root and tuber vegetables	0.32	Miscellaneous fruit	0.28	Milk and cream,	3.31
3.10	WHO cluster diet E	0.52	Root and tuber vegetables	0.39	Wheat	0.30	Milk and cream,	2.89
3.07	WHO cluster diet D	0.65	Wheat	0.50	Milk and cream,	0.45	Root and tuber vegetables	2.73
2.88	WHO regional European diet	0.48	Milk and cream,	0.46	Root and tuber vegetables	0.30	Wheat	2.63
2.64	WHO Cluster diet F	0.44	Root and tuber vegetables	0.40	Milk and cream,	0.36	Wheat	2.48
2.41	NL general	0.66	Milk and cream,	0.31	Root and tuber vegetables	0.21	Wheat	2.26
2.08	ES adult	0.50	Milk and cream,	0.23	Wheat	0.14	Citrus fruit	1.96
1.89	FR all population	0.41	Table and wine grapes	0.33	Wheat	0.27	Milk and cream,	1.83
1.88	UK vegetarian	0.38	Sugar beet (root)	0.33	Milk and cream,	0.20	Wheat	1.74
1.73	PT General population	0.39	Wheat	0.28	Table and wine grapes	0.19	Onions	1.53
1.70	IT kids/toddler	0.66	Wheat	0.16	Solanacea	0.15	Other cereal	1.62
1.70	DK adult	0.54	Milk and cream,	0.20	Wheat	0.19	Root and tuber vegetables	1.59
1.65	UK Adult	0.40	Sugar beet (root)	0.30	Milk and cream,	0.17	Wheat	1.56
1.62	LT adult	0.40	Milk and cream,	0.35	Root and tuber vegetables	0.20	Pome fruit	1.61
1.40	FI adult	0.57	Milk and cream,	0.15	Root and tuber vegetables	0.11	Citrus fruit	1.31
1.26	IT adult	0.41	Wheat	0.13	Solanacea	0.10	Pome fruit	1.20
1.12	PL general population	0.41	Root and tuber vegetables	0.23	Pome fruit	0.18	Onions	0.93

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of dimethenamid-P + M30 is unlikely to present a public health concern.

Table 6.9.1-8: Exposure assessment for metabolite M23 with PRIMo Model (rev 2.0) against the ADI for parent dimethenamid-P of 0.02 mg/kg

M23			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.02**	ARfD (mg/kg bw):	
Source of ADI:	**Parent**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.
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	
1	4
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)
3.6	FR toddler	2.0	Milk and cream,	0.6	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
3.5	UK Infant	1.9	Milk and cream,	0.5	Sugar beet (root)	0 3	FRUIT (FRESH OR FROZEN)	
3.3	NL child	1.5	Milk and cream,	0.7	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
3.2	UK Toddler	1.1	Sugar beet (root)	1.0	Milk and cream,	0 3	FRUIT (FRESH OR FROZEN)	
2.7	FR infant	1.3	Milk and cream,	0.8	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
2.7	DE child	1.2	FRUIT (FRESH OR FROZEN)	0.7	Milk and cream,	0 2	Wheat	
2.3	WHO Cluster diet B	0.4	Wheat	0.4	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
2.0	DK child	0.6	Milk and cream,	0.3	Wheat	0 3	FRUIT (FRESH OR FROZEN)	
1.9	SE general population 90th percentile	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
1.8	ES child	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 2	Wheat	
1.7	IE adult	0.5	FRUIT (FRESH OR FROZEN)	0.2	Root and tuber vegetables	0.1	Meat, preparations of meat,	
1.5	WHO cluster diet E	0.3	FRUIT (FRESH OR FROZEN)	0.3	Root and tuber vegetables	0 2	Wheat	
1.4	WHO cluster diet D	0.3	Wheat	0.3	Milk and cream,	0 2	Root and tuber vegetables	
1.3	WHO regional European diet	0.2	Milk and cream,	0.2	Root and tuber vegetables	0 2	Meat, preparations of meat,	
1.3	WHO Cluster diet F	0.2	Root and tuber vegetables	0.2	Milk and cream,	0 2	FRUIT (FRESH OR FROZEN)	
1.1	NL general	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0 2	Root and tuber vegetables	
1.0	ES adult	0.2	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Meat, preparations of meat,	
0.9	FR all population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Milk and cream,	
0.9	UK vegetarian	0.2	Sugar beet (root)	0.2	FRUIT (FRESH OR FROZEN)	0 2	Milk and cream,	
0.9	DK adult	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Wheat	
0.8	UK Adult	0.2	Sugar beet (root)	0.1	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	
0.8	IT kids/toddler	0.3	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	Solanacea	
0.8	LT adult	0.2	Milk and cream,	0.2	Root and tuber vegetables	0.1	FRUIT (FRESH OR FROZEN)	
0.8	PT General population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Solanacea	
0.7	FI adult	0.3	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	0.1	Root and tuber vegetables	
0.6	IT adult	0.2	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	Solanacea	
0.5	PL general population	0.2	Root and tuber vegetables	0.2	FRUIT (FRESH OR FROZEN)	0 0	Solanacea	

Table 6.9.1-9: Exposure assessment for metabolite M26 with PRIMo Model (rev 2.0) against the ADI for parent dimethenamid-P of 0.02 mg/kg

M26			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.02**	ARfD (mg/kg bw):	
Source of ADI:	**Parent**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.
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	
1	4
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)
3.6	FR toddler	2.0	Milk and cream,	0.6	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
3.5	UK Infant	1.9	Milk and cream,	0.5	Sugar beet (root)	0 3	FRUIT (FRESH OR FROZEN)	
3.3	NL child	1.5	Milk and cream,	0.7	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
3.2	UK Toddler	1.1	Sugar beet (root)	1.0	Milk and cream,	0 3	FRUIT (FRESH OR FROZEN)	
2.7	FR infant	1.3	Milk and cream,	0.8	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
2.7	DE child	1.2	FRUIT (FRESH OR FROZEN)	0.7	Milk and cream,	0 2	Wheat	
2.4	WHO Cluster diet B	0.4	Wheat	0.4	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
2.0	DK child	0.6	Milk and cream,	0.3	Wheat	0 3	FRUIT (FRESH OR FROZEN)	
1.9	SE general population 90th percentile	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
1.8	ES child	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 2	Wheat	
1.7	IE adult	0.5	FRUIT (FRESH OR FROZEN)	0.2	Root and tuber vegetables	0.1	Meat, preparations of meat,	
1.5	WHO cluster diet E	0.3	FRUIT (FRESH OR FROZEN)	0.3	Root and tuber vegetables	0 2	Wheat	
1.4	WHO cluster diet D	0.3	Wheat	0.3	Milk and cream,	0 2	Root and tuber vegetables	
1.4	WHO regional European diet	0.2	Milk and cream,	0.2	Root and tuber vegetables	0 2	Meat, preparations of meat,	
1.3	WHO Cluster diet F	0.2	Root and tuber vegetables	0.2	Milk and cream,	0 2	FRUIT (FRESH OR FROZEN)	
1.1	NL general	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0 2	Root and tuber vegetables	
1.0	ES adult	0.2	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Meat, preparations of meat,	
0.9	FR all population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Milk and cream,	
0.9	UK vegetarian	0.2	Sugar beet (root)	0.2	FRUIT (FRESH OR FROZEN)	0 2	Milk and cream,	
0.9	DK adult	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Wheat	
0.9	UK Adult	0.2	Sugar beet (root)	0.1	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	
0.8	IT kids/toddler	0.3	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	Solanacea	
0.8	LT adult	0.2	Milk and cream,	0.2	Root and tuber vegetables	0.1	FRUIT (FRESH OR FROZEN)	
0.8	PT General population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Solanacea	
0.7	FI adult	0.3	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	0.1	Root and tuber vegetables	
0.6	IT adult	0.2	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	Solanacea	
0.5	PL general population	0.2	Root and tuber vegetables	0.2	FRUIT (FRESH OR FROZEN)	0 0	Solanacea	

Table 6.9.1-10: Exposure assessment for metabolite M27 with PRIMo Model (rev 2.0) against the ADI for parent dimethenamid-P of 0.02 mg/kg

M27			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.02**	ARfD (mg/kg bw):	
Source of ADI:	**Parent**	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.
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	
1	4
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)
3.6	FR toddler	2.0	Milk and cream,	0.6	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
3.5	UK Infant	1.9	Milk and cream,	0.5	Sugar beet (root)	0 3	FRUIT (FRESH OR FROZEN)	
3.3	NL child	1.5	Milk and cream,	0.7	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
3.2	UK Toddler	1.1	Sugar beet (root)	1.0	Milk and cream,	0 3	FRUIT (FRESH OR FROZEN)	
2.7	FR infant	1.3	Milk and cream,	0.8	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
2.7	DE child	1.2	FRUIT (FRESH OR FROZEN)	0.7	Milk and cream,	0 2	Wheat	
2.3	WHO Cluster diet B	0.4	Wheat	0.4	FRUIT (FRESH OR FROZEN)	0 3	Fruiting vegetables	
2.0	DK child	0.6	Milk and cream,	0.3	Wheat	0 3	FRUIT (FRESH OR FROZEN)	
1.9	SE general population 90th percentile	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
1.8	ES child	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 2	Wheat	
1.7	IE adult	0.5	FRUIT (FRESH OR FROZEN)	0.2	Root and tuber vegetables	0.1	Meat, preparations of meat,	
1.5	WHO cluster diet E	0.3	FRUIT (FRESH OR FROZEN)	0.3	Root and tuber vegetables	0 2	Wheat	
1.4	WHO cluster diet D	0.3	Wheat	0.3	Milk and cream,	0 2	Root and tuber vegetables	
1.3	WHO regional European diet	0.2	Milk and cream,	0.2	Root and tuber vegetables	0 2	Meat, preparations of meat,	
1.3	WHO Cluster diet F	0.2	Root and tuber vegetables	0.2	Milk and cream,	0 2	FRUIT (FRESH OR FROZEN)	
1.1	NL general	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0 2	Root and tuber vegetables	
1.0	ES adult	0.2	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Meat, preparations of meat,	
0.9	FR all population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Milk and cream,	
0.9	UK vegetarian	0.2	Sugar beet (root)	0.2	FRUIT (FRESH OR FROZEN)	0 2	Milk and cream,	
0.9	DK adult	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Wheat	
0.8	UK Adult	0.2	Sugar beet (root)	0.1	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	
0.8	IT kids/toddler	0.3	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	Fruiting vegetables	
0.8	LT adult	0.2	Milk and cream,	0.2	Root and tuber vegetables	0.1	FRUIT (FRESH OR FROZEN)	
0.8	PT General population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Fruiting vegetables	
0.7	FI adult	0.3	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	0.1	Root and tuber vegetables	
0.6	IT adult	0.2	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	Fruiting vegetables	
0.5	PL general population	0.2	Root and tuber vegetables	0.2	FRUIT (FRESH OR FROZEN)	0.1	Fruiting vegetables	

Table 6.9.1-11: Exposure assessment for metabolite M30 with PRIMo Model (rev 2.0) against the ADI for parent dimethenamid-P of 0.02 mg/kg

M30

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.02	ARfD (mg/kg bw):	
Source of ADI:	Parent	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Explain choice of toxicological reference values.

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
1 4

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)
3.7	FR toddler	2.0	Milk and cream,	0.6	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
3.7	NL child	1.5	Milk and cream,	0.7	FRUIT (FRESH OR FROZEN)	0 3	Root and tuber vegetables	
3.6	UK Infant	1.9	Milk and cream,	0.5	Sugar beet (root)	0 3	FRUIT (FRESH OR FROZEN)	
3.3	UK Toddler	1.1	Sugar beet (root)	1.0	Milk and cream,	0 3	FRUIT (FRESH OR FROZEN)	
3.0	WHO Cluster diet B	0.5	Onions	0.4	Wheat	0.4	FRUIT (FRESH OR FROZEN)	
2.8	DE child	1.2	FRUIT (FRESH OR FROZEN)	0.7	Milk and cream,	0 2	Wheat	
2.8	FR infant	1.3	Milk and cream,	0.8	FRUIT (FRESH OR FROZEN)	0.4	Root and tuber vegetables	
2.3	SE general population 90th percentile	0.6	Milk and cream,	0.3	Head cabbage	0 3	Onions	
2.1	DK child	0.6	Milk and cream,	0.3	Wheat	0 3	FRUIT (FRESH OR FROZEN)	
1.9	ES child	0.6	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	0 2	Wheat	
1.9	WHO cluster diet D	0.4	Onions	0.3	Wheat	0 3	Milk and cream,	
1.9	WHO cluster diet E	0.3	FRUIT (FRESH OR FROZEN)	0.3	Root and tuber vegetables	0 3	Onions	
1.9	IE adult	0.5	FRUIT (FRESH OR FROZEN)	0.2	Root and tuber vegetables	0.1	Milk and cream,	
1.8	WHO regional European diet	0.3	Onions	0.2	Milk and cream,	0 2	Root and tuber vegetables	
1.6	WHO Cluster diet F	0.2	Root and tuber vegetables	0.2	Milk and cream,	0 2	FRUIT (FRESH OR FROZEN)	
1.4	NL general	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0 2	Onions	
1.1	ES adult	0.2	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Onions	
1.1	UK vegetarian	0.2	Sugar beet (root)	0.2	FRUIT (FRESH OR FROZEN)	0 2	Milk and cream,	
1.0	PT General population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Onions	0 2	Wheat	
1.0	LT adult	0.2	Head cabbage	0.2	Milk and cream,	0 2	Root and tuber vegetables	
1.0	FR all population	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Milk and cream,	
1.0	DK adult	0.3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.1	Onions	
0.9	UK Adult	0.2	Sugar beet (root)	0.1	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	
0.9	IT kids/toddler	0.3	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	Onions	
0.9	PL general population	0.2	Onions	0.2	Root and tuber vegetables	0 2	Head cabbage	
0.8	FI adult	0.3	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	0.1	Onions	
0.7	IT adult	0.2	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	Onions	

M30

Status of the active substance:	included	Code no.	BAS 656 PH
LOQ (mg/kg bw):	0.02	proposed LOQ:	

Toxicological end points

ADI (mg/kg bw/day):	0.02	ARfD (mg/kg bw):	
Source of ADI:	Parent	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum
1 4

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)
3.5	NL child	1.5	Milk and cream,	0.3	Pome fruit	0 3	Root and tuber vegetables	
3.5	UK Infant	1.9	Milk and cream,	0.5	SUGAR PLANTS	0 2	Root and tuber vegetables	
3.5	FR toddler	2.0	Milk and cream,	0.4	Root and tuber vegetables	0.1	Pome fruit	
3.3	UK Toddler	1.1	SUGAR PLANTS	1.0	Milk and cream,	0 2	Root and tuber vegetables	
2.8	WHO Cluster diet B	0.5	Onions	0.4	Wheat	0 3	Root and tuber vegetables	
2.8	DE child	0.7	Milk and cream,	0.6	Pome fruit	0 2	Citrus fruit	
2.3	FR infant	1.3	Milk and cream,	0.4	Root and tuber vegetables	0.1	Pome fruit	
2.0	DK child	0.6	Milk and cream,	0.3	Wheat	0 2	Rye	
2.0	SE general population 90th percentile	0.6	Milk and cream,	0.3	Onions	0 3	Root and tuber vegetables	
1.9	ES child	0.6	Milk and cream,	0.2	Wheat	0.1	Onions	
1.8	IE adult	0.2	Root and tuber vegetables	0.2	Miscellaneous fruit	0.1	Milk and cream,	
1.8	WHO cluster diet D	0.4	Onions	0.3	Wheat	0 3	Milk and cream,	
1.7	WHO cluster diet E	0.3	Root and tuber vegetables	0.3	Onions	0 2	Wheat	
1.6	WHO regional European diet	0.3	Onions	0.2	Milk and cream,	0 2	Root and tuber vegetables	
1.4	WHO Cluster diet F	0.2	Root and tuber vegetables	0.2	Milk and cream,	0 2	Wheat	
1.3	NL general	0.3	Milk and cream,	0.2	Onions	0 2	Root and tuber vegetables	
1.1	ES adult	0.2	Milk and cream,	0.1	Onions	0.1	Wheat	
1.0	UK vegetarian	0.2	SUGAR PLANTS	0.2	Milk and cream,	0 2	Onions	
1.0	PT General population	0.2	Onions	0.2	Wheat	0.1	Table and wine grapes	
1.0	FR all population	0.2	Table and wine grapes	0.2	Wheat	0.1	Milk and cream,	
0.9	DK adult	0.3	Milk and cream,	0.1	Onions	0.1	Wheat	
0.9	IT kids/toddler	0.3	Wheat	0.1	Onions	0.1	Solanacea	
0.9	UK Adult	0.2	SUGAR PLANTS	0.1	Milk and cream,	0.1	Onions	
0.8	LT adult	0.2	Milk and cream,	0.2	Root and tuber vegetables	0.1	Pome fruit	
0.8	FI adult	0.3	Milk and cream,	0.1	Onions	0.1	Root and tuber vegetables	
0.7	PL general population	0.2	Onions	0.2	Root and tuber vegetables	0.1	Pome fruit	
0.7	IT adult	0.2	Wheat	0.1	Onions	0.1	Solanacea	

The chronic consumer risk assessment (see Table 6.9.1-7) based on the newly proposed EU MRLs and the ADI of 0.02 mg/kg for the sum of parent dimethenamid-P plus M30, yielded ADI utilization rates of ~~7.26%~~ 6.84% (~~7.26-1.25%~~ 6.84-1.12%) with the EFSA PRIMo (input values see Table 6.9.1-1 and Table 6.9.1-2 as well as default MRLs of 0.02 mg/kg). According to the presented TMDI calculations, a long-term intake of dimethenamid-P residues (sum of parent dimethenamid-P and M30) is unlikely to present a public health concern.

IEDI calculations

For all models included in the EFSA model, the use of STMR or $STMR_P$ values in the estimation of the chronic dietary consumer risk is up to this point in time not necessary since the crude overestimated TMDI of total dimethenamid-P was ~~7.3%~~ 6.84% of the ADI.

CA 6.9.2 Acute Reference Dose (ARfD) and Dietary Exposure Calculation

IESTI calculations

The acute consumer risk assessment (see Table 6.9.2-1) based on the newly proposed EU MRLs (Table 6.9.1-1) and the residues shown in Table 6.9.1-2 and the ARfD of 0.25 mg/kg for the sum of parent dimethenamid-P plus M30, yielded ARfD utilization rates of ~~1.9%~~ 1.8% for children and ~~1.1%~~ 0.9% for adults with the EFSA PRIMo.

Table 6.9.2-1: IESTI calculation for total dimethenamid-P with PRIMo Model (rev 2.0) using proposed MRLs by BASF

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 ESTI 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)
1.9	Head cabbage	0.09 / -	1.3	Onions	0.11 / -	1.1	Head cabbage	0.09 / -	0.9	Chinese cabbage	0.06 / -
1.8	Onions	0.11 / -	1.2	Melons	0.02 / -	0.9	Chinese cabbage	0.06 / -	0.7	Head cabbage	0.09 / -
1.2	Potatoes	0.02 / -	1.1	Head cabbage	0.09 / -	0.7	Onions	0.11 / -	0.5	Onions	0.11 / -
1.2	Melons	0.02 / -	1.0	Watermelons	0.02 / -	0.4	Pumpkins	0.02 / -	0.4	Pumpkins	0.02 / -
1.1	Oranges	0.02 / -	0.9	Chinese cabbage	0.06 / -	0.3	Watermelons	0.02 / -	0.3	Watermelons	0.02 / -
No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		

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)
0.4	Apple juice	0.02 / -	0.1	Orange juice	0.02 / -
0.4	Orange juice	0.02 / -	0.1	Apple juice	0.02 / -
0 3	Carrot, juice	0.02 / -	0.0	Bread/pizza	0.02 / -
0 3	Grape juice	0.02 / -	0.0	Wine	0.02 / -
0.1	Peach juice	0.02 / -	0.0	Pineapples preserved	0.02 / -

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)
1.8	Onions	0.11 / -	1.3	Onions	0.11 / -	0.9	Chinese cabbage	0.06 / -	0 9	Chinese cabbage	0.06 / -
1.2	Potatoes	0.02 / -	1.2	Melons	0.02 / -	0.7	Onions	0.11 / -	0 5	Onions	0.11 / -
1.2	Melons	0.02 / -	1.0	Milk and milk	0.02 / -	0.4	Pumpkins	0.02 / -	0.4	Pumpkins	0.02 / -
1.1	Oranges	0.02 / -	1.0	Watermelons	0.02 / -	0.3	Watermelons	0.02 / -	0 3	Watermelons	0.02 / -
1.0	Milk and milk	0.02 / -	0.9	Chinese cabbage	0.06 / -	0.3	Melons	0.02 / -	0 3	Melons	0.02 / -
No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		

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)			
0.4	Apple juice	0.02 / -				0.1	Orange juice	0.02 / -			
0.4	Orange juice	0.02 / -				0.1	Apple juice	0.02 / -			
0.3	Carrot, juice	0.02 / -				0.0	Bread/pizza	0.02 / -			
0.3	Grape juice	0.02 / -				0.0	Wine	0.02 / -			
0.1	Peach juice	0.02 / -				0.0	Pineapples preserved	0.02 / -			

CA 6.10 Other studies

No other/special studies were deemed necessary. The studies and information provided under previous sections are considered adequate and sufficient.

Effect on the residue level in pollen and bee products

The objective of these studies shall be to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

Dimethenamid-P is a herbicide with applications intended either pre-emergence or at early post-emergence and residue trials showed a very favorable residue profile, with often a no residue situation. Thus, no significant residues are likely to be expected in the blossoms and thus no residues of dimethenamid-P in pollen, honey or other bee products are expected.

Also, in the absence of valid test guidelines for such investigations at the time of submission of this dossier, the type and conditions of the studies to be performed (if considered necessary) may be discussed with the national competent authorities upon application for authorization of plant protection products containing the active substance dimethenamid-P.

Appendix 1 Tier 1 Summaries of the Supervised Field Residue Trials

Sunflower

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No Location (Trial No)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s /hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
390502_1 2013/1335405 Goch-Kessel 47574 / Germany L120044	SO 0702 Metharoc	1. 22.04.2012 2. 25.06.-16.07.2012 3. 21.09.2012	Spray	0.432	200	0.870	1 27.04.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	147	Method L0179/02 LOQ 0.01 mg/kg
				0.504	200	1.022	1 27.04.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	147	
423129 2012/1272620 49685 Garthe Germany L120276	SO 0702 Faro	1. 18.05.2012 2. 25.06.-10.07.2012 3. N/A (wild grown)	Spray	0.432	200	0.864	1 25.05.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	Method L0179/02 LOQ 0.01 mg/kg
423129 2012/1272620 Chemillé-sur-Dême 37370 / France L120278	SO 0702 Durban	1. 17.05.2012 2. 15.07.-30.07.2012 3. 25.10.-28.10.2012	Spray	0.432	200	0.864	1 25.05.2012	08-09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	152	Method L0179/02 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No Location (Trial No)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s /hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
423129 2012/1272620 02190 Amifontaine France L120279	SO 0702 PE 64HE01	1. 26.03.2012 2. 26.06.-10.07.2012 3. 17.09.2012	Spray	0.432	200	0.864	1 19.04.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	151	Method L0179/02 LOQ 0.01 mg/kg
423129 2012/1272620 9541 XH Vlagtwedde The Netherlands L120578	SO 0702 Faro	1. 12.06.2012 2. 14.07.-30.07.2012 3. N/A(wild grown)	Spray	0.432	200	0.864	1 19.06.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	115	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 16818 Reinsberg Germany L130186	SO 0702 Alisson	1. 19.04.2013 2. 08.07.-23.07.2013 3. 30.09.2013	Spray	0.432	200	0.864	1 23.04.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	154	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 49685 Höltingshausen Germany L130187	SO 0702 Maestro	1. 08.05.2013 2. 26.06.-10.08.2013 3. 09.10.2013	Spray	0.432	200	0.864	1 17.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	145	Method L0179/02 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No Location (Trial No)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s /hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
694884 2013/1335422 08190 Vieux les Asfeld France L130189	SO 0702 PE64HE01	1. 16.04.2013 2. 10.07.-20.07.2013 3. 24.09.2013	Spray	0.432	200	0.864	1 06.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	141	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 9541 XH Vlagtwedde The Netherlands L130190	SO 0702 Maestro	1. 16.05.2013 2. 30.06.–14.08.2013 3. 08.10.2013	Spray	0.432	200	0.864	1 24.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	137	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 Rekken The Netherlands L130191	SO 0702 Mestro	1. 30.05.2013 2. 14.07.–30.08.2013 3. 16.10.2013	Spray	0.432	200	0.864	1 05.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	133	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 Banbury OX15 6EP United Kingdom L130192	SO 0702 ES Paulina	1. 04.05.2013 2. 10.08.–15.09.2013 3. 10.10.–15.10.2013	Spray	0.432	200	0.864	1 23.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	145	Method L0179/02 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No Location (Trial No)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s /hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
694884 2013/1335422 6221 Saint-Amand Belgium L130650	SO 0702 P64HE01	1. 28.05.2013 2. N/A 3. 29.10.2013	Spray	0.432	200	0.864	1 10.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	141	Method L0179/02 LOQ 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent

Southern Europe
RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
423129 2012/1272620 84100 Orange France L120280	SO 0702 Tutti	1. 28.04.2012 2. 15.07.-30.07.2012 3. 22.09.2012	Spray	0.432	200	0.864	1 07.05.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	150	Method L0179/02 LOQ 0.01 mg/kg
423129 2012/1272620 59010 Platanos Greece L120281	SO 0702 Sikklos	1. 20.06.2012 2. 25.07-05.08 2012 3. 15.09.-30.09.2012	Spray	0.432	200	0.864	1 22.06.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	90	Method L0179/02 LOQ 0.01 mg/kg
423129 2012/1272620 Lusurasco di Alseno 29010 / Italy L120282	SO 0702 Orasole	1. 28.04.2012 2. 16.07.-25.07.2012 3. 06.09.2012	Spray	0.432	200	0.864	1 11.05.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	118	Method L0179/02 LOQ 0.01 mg/kg
423129 2012/1272620 18128 Zafarraya Spain L120283	SO 0702 Bosfora	1. 13.06.2012 2. 15.08.-25.08.2012 3. 15.10.2012	Spray	0.432	200	0.864	1 16.07.2012	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	Method L0179/02 LOQ 0.01 mg/kg

Southern Europe
RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	720 g/L	(common name and content)	
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
694884 2013/1335422 84420 Piolenc France L130194	SO 0702 Olencia	1. 06.06.2013 2. 01.08.–14.08.2013 3. 07.10.2013	Spray	0.432	200	0.864	1 14.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	115	Method L0179/02 LOQ 0.01 mg/kg

Southern Europe
RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
694884 2013/1335422 47120 ST Pierre sur Dropt France L130195	SO 0702 ES-ETHIC	1. 08.06.2013 2. 19.08.–03.09.2013 3. 25.10.-30.10.2013	Spray	0.432	200	0.864	1 19.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	128	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 kg25030 Pompiano Italy L130196	SO 0702 Orasole	1. 14.05.2013 2. 16.07.–25.07.2013 3. 01.10.2013	Spray	0.432	200	0.864	1 22.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	132	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 20090 Caleppio di Settala Italy L130197	SO 0702 Orasole	1. 15.06.2013 2. 12.08.–20.08.2013 3. 02.10.2013	Spray	0.432	200	0.864	1 18.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	106	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 GR-59032 Platanos Greece L130198	SO 0702 Sikklos	1. 02.05.2013 2. 20.06.–30.06.2013 3. 15.08.–30.08.2013	Spray	0.432	200	0.864	1 09.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	104	Method L0179/02 LOQ 0.01 mg/kg

Southern Europe
RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	Commercial Product (name)	-
Crop/crop group:	Sunflower (Oilseeds)	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	Residues calculated as:	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	Commodity / Variety	Date of 1. Sowing/Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment[0]			No of treatm. and last date	Growth stage (BBCH)[2]	Portion analysed	Residues (mg/kg)						DALA[1] (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 656 PH	M23	M26	M27	M30[3]	Total[4]		
694884 2013/1335422 GR-57200 Profitis Greece L130199	SO 0702 Sikklos	1. 06.05.2013 2. 05.07.–15.07.2013 3. 15.08.–30.08.2013	Spray	0.432	200	0.864	1 13.05.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	101	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 29700 Velez Malaga Spain L130200	SO 0702 LG5520	1. 03.04.2013 2. 16.06.–05.07.2013 3. 20.08.2013	Spray	0.432	200	0.864	1 12.04.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	130	Method L0179/02 LOQ 0.01 mg/kg
694884 2013/1335422 29700 Velez Malaga Spain L130201	SO 0702 LG5520	1. 07.06.2013 2. 22.07.–02.08.2013 3. 06.09.2013	Spray	0.432	200	0.864	1 18.06.2013	09	Seeds	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	80	Method L0179/02 LOQ 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent

Oilseed rape

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P (BAS 656 PH)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape (Oilseeds)	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	Quinmerac (BAS 518 H, 167 g/L)
Content of active substance (g/kg or g/L)	333 g/L BAS 656 PH		
Formulation (e.g. WP)	SE (BAS 830 01 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report No location (trial No)	**Commodity/ Variety**	**1. Date of Sowing / planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues found (mg/kg)**						**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[2]**	**Total[3]**		
694886	SO 0495	1. 15.04.13		0.25	200	0.50	1	18	Whole plant*	7.12	<0.01	0 10	<0.01	0.04	7.16	0	Method
2013/1335420	Spring	2. 14.06.-09.07.13	Spray				31.05.13		Whole plant*	<0.01	<0.01	0.02	<0.01	0.25	0.26	10	L0179/02
49456 Bakum	Oilseed rape	3. 26.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	87	LOQ
Germany	Makro								Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	87	0.01 mg/kg
L130132																	
694886	SO 0495	1. 07.09.12		0.25	200	0.50	1	18	Whole plant*	4.84	<0.01	0 23	<0.01	0.08	4.92	0	Method
2013/1335420	Winter	2. 15.05.-04.06.13	Spray				18.04.13		Whole plant*	<0.01	<0.01	0.05	<0.01	0.45	0.46	11	L0179/02
9695 AK Belingwohlde	Oilseed rape	3. 09.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	LOQ
The Netherlands	Expower								Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	0.01 mg/kg
L130133																	
694886	SO 0495	1. 13.08.12		0.25	200	0.50	1	18	Whole plant*	15.52	<0.01	0.02	<0.01	<0.01	15.53	0	Method
2013/1335420	Oilseed rape	2. 18.05.-28.06.13	Spray				02.04.13		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.07	0.08	28	L0179/02
Banbury, OX17 IDX	Cabernet	3. 20.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	139	LOQ
United Kingdom									Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	139	0.01 mg/kg
L130134																	
694886	SO 495	1. 01.09.12		0.25	200	0.50	1	18	Whole plant*	8.68	<0.01	0 19	<0.01	0.19	8.77	0	Method
2013/1335420	Winter	2. 30.04.-14.05.13	Spray				08.03.13		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05	41	L0179/02
51377 Leverkusen	Oilseed rape	3. 03.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02	143	LOQ
Germany	Visby								Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	143	0.01 mg/kg
L130135																	

0 Actual application rates varied by 10% at most

1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots

Oilseed rape

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P (BAS 656 PH)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape (Oilseeds)	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	Quinmerac (BAS 518 H, 167 g/L)
Content of active substance (g/kg or g/L)	333 g/L BAS 656 PH	**(common name and content)**	
Formulation (e.g. WP)	SE (BAS 830 01 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report No location (trial No)	**Commodity/ Variety**	**1. Date of Sowing / planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues found (mg/kg)**						**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
694886	SO 0495	1. 27.09.13		0.25	200	0.50	1	18	Whole plant*	0.36	<0.01	0.98	<0.01	0.17	0.53	0	Method
2013/1335420	Oilseed rape	2. 15.04.-10.05.13	Spray				29.03.13		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.13	0.14	18	L0179/02
47120 Levignac de	Atenzo	3. 10.07.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	103	LOQ
Guyenne, France									Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	103	0.01 mg/kg
L130136																	
694886	SO 0495	1. 22.04.13		0.25	200	0.50	1	18	Whole plant*	7.56	<0.01	0.33	<0.01	0.28	7.84	0	Method
2013/1335420	Oilseed rape	2. 29.06.-04.07.13	Spray				28.05.13		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.20	0.21	24	L0179/02
25030 Pompiano	Mosaik	3. 05.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	69	LOQ
Italy									Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	69	0.01 mg/kg
L130138																	
694886	SO 0495	1. 26.03.13		0.25	200	0.50	1	18	Whole plant*	37.16	<0.01	0.32	<0.01	0.23	37.39	0	Method
2013/1335420	Oilseed rape	2. 05.06.-06.07.13					26.04.13		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.06	0.07	26	L0179/02
29700 Velez Malaga	Jura	3. 05.08.13							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	101	LOQ
Spain									Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	101	0.01 mg/kg
L130139																	

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at sampling
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots

Soybean

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Soybean (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
697666	VD 0541	1. 15.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	0.01	<0.01	0.02	<0.01	<0.02	28	Method
2013/1335421	Sultana	2. Jun-Jul					02.06.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	122	L0179/02
71665 Vaihingen /Enz		3. 02.10.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	122	LOQ
Germany L130372																	0.01 mg/kg
697666	VD 0541	1. 16.04.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	<0.01	<0.01	0.04	<0.01	<0.02	70	Method
2013/1335421	Silvia	2. Jul-Aug					02.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	155	L0179/02
74349 Ingersheim		3. 04.10.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	155	LOQ
Germany L130373																	0.01 mg/kg
697666	VD 0541	1. 30.04.2013	Spray	0.864	100	0.864	1	00	Whole plant*†	<0.01	<0.01	<0.01	0.01	<0.01	<0.02	90	Method
2013/1335421	Pannonia/	2. 15.07.-30.07.2013					30.04.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	168	L0179/02
H-8143 Sarszentmihaly	Kinsce	3. N.r.							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	168	LOQ
Hungary L130374																	0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Soybean (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
697666	VD 0541	1. 10.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	<0.01	<0.01	0.06	<0.01	<0.02	80	Method
2013/1335421	Isidor	2. 15.07.-30.07.2013					10.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	164	L0179/02
H8710 Balatons-zentgyprgy		3. N.r.							Rest of plant*	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	164	LOQ
Hungary																	0.01 mg/kg
L130375																	
697666	VD 0541	1. 13.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	0.01	<0.01	0.15	<0.01	<0.02	72	Method
2013/1335421	Augusta	2. 05.07.-01.08.2013					21.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	127	L0179/02
64-500 Szamotuly		3. 25.09.2013							Rest of plant*†	<0.01	<0.01	<0.01	0.03	<0.01	<0.02	127	LOQ
Poland																	0.01 mg/kg
L130376																	
697666	VD 0541	1. 15.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	0.01	<0.01	0.05	<0.01	<0.02	71	Method
2013/1335421	Augusta	2. 08.07.-07.08.2013					27.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	124	L0179/02
88-400 Znin		3. 28.09.2013							Rest of plant*†	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	124	LOQ
Poland																	0.01 mg/kg
L130377																	
697666	VD 0541	1. 10.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*†	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	68	Method
2013/1335421	Isidor NT	2. 15. 07.-05.08.2013					22.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	166	L0179/02
45130 Baule		3. 04.11.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	166	LOQ
France																	0.01 mg/kg
L130378																	
697666	VD 0541	1. 08.06.2013	Spray	0.864	100	0.864	1	00	Whole plant*†	<0.01	0.01	<0.01	0.02	<0.01	<0.02	65	Method
2013/1335421	Sultana	2. 01.08.-20.08.2013					12.06.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	117	L0179/02
91150 Mespuits		3. 07.10.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	117	LOQ
France																	0.01 mg/kg
L130379																	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots
† Mean of two analyses of the same samples
N.r. Not reported

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Soybean (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
697666	VD 0541	1. 09.06.2013	Spray	0.864	100	0.864	1	10	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	63	Method
2013/1335421	Atsaffort	2. 07.08.-21.08.2013					17.06.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	112	L0179/02
82400 St Paul d'Epsis		3. 07.10.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	112	LOQ
France																	0.01 mg/kg
L130380																	
697666	VD 0541	1. 13.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*[†]	<0.01	<0.01	<0.01	0.03	<0.01	<0.02	76	Method
2013/1335421	Fukui	2. 10.08.-20.08.2013					04.06.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	125	L0179/02
82500 Dugeres		3. 07.10.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	125	LOQ
France																	0.01 mg/kg
L130381																	
697666	VD 0541	1. 02.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	53	Method
2013/1335421	Blancas	2. 20.06.-05.07.2013					09.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	119	L0179/02
59032 Imathia		3. 05.09.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	119	LOQ
Greece																	0.01 mg/kg
L130382																	
697666	VD 0541	1. 06.05.2013	Spray	0.864	100	0.864	1	09	Whole plant*	<0.01	<0.01	<0.01	0.01	<0.01	<0.02	51	Method
2013/1335421	Blancas	2. 25.06.-05.07.2013					13.05.2013		Seed	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	114	L0179/02
57200 Thessaloniki		3. 04.09.2013							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	114	LOQ
Greece																	0.01 mg/kg
L130383																	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Soybean (Oilseeds)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
697666 2013/1335421 44012 Finale Emilia Italia L130384	VD 0541 Deka Big	1. 15.05.2013 2. July 3. 30.09.2013	Spray	0.864	100	0.864	1 27.05.2013	09	Whole plant* Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02	52 126 126	Method L0179/02 LOQ 0.01 mg/kg
697666 2013/1335421 40051 Malalbergo Italia L130385	VD 0541 Demetra	1. 29.05.2013 2. July 3. 11.09.2013	Spray	0.864	100	0.864	1 08.05.2013	09	Whole plant* Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02	71 126 126	Method L0179/02 LOQ 0.01 mg/kg
697666 2013/1335421 06180 Gevora Badajoz Spain L130386	VD 0541 PR92B63	1. 21.05.2013 2. 26.07.-12.08.2013 3. .09.2013	Spray	0.864	100	0.864	1 30.05.2013	09	Whole plant* Seed Rest of plant*	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.02 <0.02 <0.02	75 119 119	Method L0179/02 LOQ 0.01 mg/kg
697666 2013/1335421 06184 Pueblo Noevo del Guadiana Spain L130386	VD 0541 PR92B63	1. 21.05.2013 2. 26.07.-12.08.2013 3. 26.09.2013	Spray	0.864	100	0.864	1 30.05.2013	09	Whole plant*† Seed Rest of plant*†	<0.01 <0.01 <0.01	<0.01 <0.01 0.02	<0.01 <0.01 <0.01	0.02 <0.01 0.04	<0.01 <0.01 0.01	<0.02 <0.02 0.02	75 119 119	Method L0179/02 LOQ 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application

3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots
† Mean of two analyses of the same samples

Maize

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 18.05.2011	Spray	0.504	200	1.008	1	16	Whole plant*	38.2	<0.01	<0.01	<0.01	<0.01	38.21	0	Method
2012/1209625	Amoroso	2. 15.07.-22.07.2011					04.07.2011		Whole plant*	0.02	<0.01	<0.01	<0.01	<0.01	0.03	60	L0179/02
67114 Rehhütte		3. 06.10.2011							Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	LOQ
Germany									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02	60	0.01 mg/kg
L110444									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	79	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	79	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	88	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.07	88	
			Spray	0.432	200	0.864	1	16	Whole plant*	29.3	<0.01	<0.01	<0.01	<0.01	29.31	0	Method
							04.07.2011		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	LOQ
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	60	0.01 mg/kg
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	79	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	79	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	88	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	88	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 20.04.2011	Spray	0.504	200	1.008	1	16-17	Whole plant*	34.4	<0.01	0.03	<0.01	<0.01	34.41	0	Method
2012/1209625	Koherence	2. 07.07.-18.07.2011					23.05.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
67160 Seebach		3. 03.10.2011							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	LOQ
Northern France									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		0.01 mg/kg
L110042									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		
			Spray	0.432	200	0.864	1	16-17	Whole plant*	15.2	<0.01	0.02	<0.01	<0.01	15.21		Method
							23.05.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		L0179/02
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		LOQ
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		0.01 mg/kg
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 29.04.2011	Spray	0.504	200	1.008	1	16	Whole plant*	64.4	<0.01	<0.01	<0.01	<0.01	64.41	0	Method
2012/1209625	Podium	2. 28.07.-11.08.2011					06.06.2011		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
Stratton,/Audley OX279AS		3. 10.10.2011							Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	LOQ
United Kingdom									Rest of plant*.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
L110043									Cob	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	120	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	120	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	
			Spray	0.432	200	0.864	1	16	Whole plant*	50.0	<0.01	<0.01	<0.01	<0.01	50.01	0	Method
							06.06.2011		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	LOQ
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	120	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	120	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 13.05.2011	Spray	0.504	200	1.008	1	16	Whole plant*	36.4	<0.01	<0.01	<0.01	<0.01	36.41	0	Method
2012/1209625	Fallove	2. 18.07.-01.08.2011					08.06.2011		Whole plant*	<0.01	<0.01	0.02	<0.01	<0.01	<0.02	61	L0179/02
5856 AB Wellerlooi		3. 14.10.2011							Cob	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	LOQ
The Netherlands									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	0.01 mg/kg
L110044									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	128	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	128	
			Spray	0.432	200	0.864	1	16	Whole plant*	23.6	<0.01	0.04	<0.01	<0.01	23.61	0	Method
							08.06.2011		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	L0179/02
									Cob	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	LOQ
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	0.01 mg/kg
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	110	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	128	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	128	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
423130	GC 0645	1. 02.05.2012	Spray	0.432	200	0.864	1	16	Whole plant*	46	<0.01	0.04	<0.01	<0.01	46.01	0	Method
2012/1272621	Aurelia	2. 20.07.-07.08.2012					21.06.2012		Whole plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	60	L0179/02
49685 Garthe		3. 23.10.2012							Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	LOQ
Germany									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	91	0.01 mg/kg
L120284									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	111	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	111	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	125	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	125	
423130	GC 0645	1. 01.04.2012	Spray	0.432	200	0.864	1	16	Whole plant*	77	<0.01	<0.01	<0.01	<0.01	77.01	0	Method
2012/1272621	DKC 3850	2. 15.07.-15.08.2012					12.06.2012		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
37380 Reugny		3. 06.11.-08.11.2012							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	LOQ
France									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	0.01 mg/kg
L120285									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02	92	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	149	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	149	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s /hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
423130	GC 0645	1. 14.04.2012	Spray	0.432	200	0.864	1	16	Whole plant*	36	<0.01	0.01	<0.01	<0.01	36.01	0	Method
2012/1272621	Kougar	2. 10.08.-24.08.2012					10.06.2012		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	L0179/02
OX27 75L Tusmore		3. 26.10.2012							Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	LOQ
United Kingdom									Rest of plant*.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
L120286									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	
423130	GC 0645	1. 09.05.2012	Spray	0.432	200	0.864	1	16	Whole plant*	21	<0.01	0.06	<0.01	0.01	21.01	0	Method
2012/1272621	P 8057	2. 30.07.-17.08.2012					19.06.2012		Whole plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
9541 XH Vlagtwedde	(Pioneer)	3. 15.10.2012							Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	LOQ
The Netherlands									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
L120287									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	115	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	115	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	140	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	140	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots
Cobs Cobs with husks

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 25.04.2011	Spray	0.504	200	1.008	1	16	Whole plant*	21.9	<0.01	0.032	<0.01	0.07	21.97	0	Method
2012/1209625	33Y74	2. 05.07.-12.07.2011					27.05.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	L0179/02
31330 Grenade		3. 12.10.2011							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	LOQ
Sur Garonne									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	76	0.01 mg/kg
France									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	76	
L110045									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	
			Spray	0.432	200	0.864	1	16	Whole plant*	19.8	<0.01	0.40	<0.01	0.09	19.89	0	Method
							04.07.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	L0179/02
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	61	LOQ
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	76	0.01 mg/kg
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	76	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	138	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390501	GC 0645	1. 25.05.2011	Spray	0.504	200	1.008	1	16	Whole plant*	14.4	<0.01	0.14	<0.01	0.01	14.41	0	Method
2012/1209625	Sansia	2. n.r.					12.07.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
59100 Kavasila		3. 12.10.2011							Rest of plant*	<0.01	<0.01	0.22	<0.01	0.80	0.81	59	LOQ
Greece									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	0.01 mg/kg
L110046									Rest of plant*	<0.01	<0.01	0.01	<0.01	0.11	0.12	92	
			Spray	0.432	200	0.864	1	16	Whole plant*	16.7	<0.01	0.19	<0.01	0.01	16.71	0	Method
							12.07.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
									Rest of plant*	<0.01	<0.01	0.05	<0.01	0.20	0.21	59	LOQ
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	92	0.01 mg/kg
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05	92	
390501	GC 0645	1. 07.04.2011	Spray	0.504	200	1.008	1	16	Whole plant*	12.5	<0.01	0.08	<0.01	0.02	12.52	0	Method
2012/1209625	PR32F73	2. 15.06.-03.07.2011					17.05.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
27050 Corana		3. 15.09.-16.09.2011							Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.04	0.05	59	LOQ
Italy									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
L110047									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.06	0.07	91	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	100	
									Rest of plant*	<0.01	<0.01	0.01	<0.01	0.09	0.10	100	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	121	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	121	
			Spray	0.432	200	0.864	1	16	Whole plant*	5.0	<0.01	0.03	<0.01	<0.01	5.01	0	Method
							17.05.2011		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	59	LOQ
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	91	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	100	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.06	0.07	100	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	121	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	121	
390501 2012/1209625 4317257 / Torroella de Montgri Spain L110048	GC 0645 N28	1. 11.04.2011 2. June 2011 3. 28.09.2011	Spray	0.504	200	1.008	1 17.05.2011	16	Whole plant*	8.72	<0.01	0.02	<0.01	<0.01	8.73	0	Method L0179/02 LOQ 0.01 mg/kg
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02	59	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	87	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.01	0.02	87	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Rest of plant*	<0.01	<0.01	0.02	<0.01	0.04	0.05	91	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	134	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	134	
			Spray	0.432	200	0.864	1 17.05.2011	16	Whole plant*	6.2	<0.01	0.02	<0.01	<0.01	6.21	0	Method L0179/02 LOQ 0.01 mg/kg
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	0.02	0.03	59	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	87	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	87	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	134	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	134	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
423130	GC 0645	1. 04.05.2012	Spray	0.432	200	0.864	1	16	Whole plant*	48	<0.01	0.06	<0.01	<0.01	48.01	0	Method
2012/1272621	DKC 5007	2. 15.07.-28.07.2012					09.06.2012		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
26730 Hostun		3. 04.10.2012							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	LOQ
France									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	86	0.01 mg/kg
L120288									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	86	
									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	89	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	89	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	117	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	117	
423130	GC 0645	1. 29.04.2012	Spray	0.432	200	0.864	1	16	Whole plant*	35	<0.01	<0.01	<0.01	<0.01	35.01	0	Method
2012/1272621	A5 72	2. 30.06.-15.07.2012					22.05.2012		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	L0179/02
59032 Platanos,		3. 01.09.-20.09.2012							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	59	LOQ
Imathia									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	86	0.01 mg/kg
Greece									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	86	
L120289									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	90	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	90	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	99	
									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	99	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Maize (Cereals)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
423130	GC 0645	1. 13.04.2012	Spray	0.432	200	0.864	1	16	Whole plant*	37	<0.01	0.05	<0.01	<0.01	37.01	0	Method
2012/1272621	Armonico	2. 28.06.-04.07.2012					01.06.2012		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
20080 Zibido San Giacomo		3. 31.08.2012							Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	LOQ
/ Italy									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	0.01 mg/kg
L120290									Rest of plant*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
423130	GC 0645	1. 13.06.2012	Spray	0.432	200	0.864	1	16	Whole plant*	8.8	<0.01	0.14	<0.01	0.03	8.83	0	Method
2012/1272621	Castellano	2. 17.08.-24.08.2012					16.07.2012		Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	60	L0179/02
18128 Zafarraya		3. 15.10.2012							Rest of plant*	<0.01	<0.01	0.02	<0.01	0.10	0.11	60	LOQ
Spain									Cobs	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	78	0.01 mg/kg
L120291									Rest of plant*	<0.01	<0.01	0.04	<0.01	0.17	0.18	78	
									Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91	
									Rest of plant*	<0.01	<0.01	0.04	<0.01	0.20	0.21	91	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* Without roots
Cobs Cobs with husks

Sugarbeet

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Sugarbeet (Sugar plants)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment0**			**No of treatm. and last date**	**Growth stage (BBCH)[2].**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390503 2012/1182982 47574 Goch-Pfalzdorf Germany L110035	VR0596 Naute	1. 23.03.2011 2. - 3. 20.09.2011	Spray	0.325	200	0.650	1 03.05.2011	16	Whole plant* Leaves** Roots***	29.32 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	29.33 <0.02 <0.02	0 140 140	Method L0179/02 LOQ 0.01 mg/kg
390503 2012/1182982 67160 Seebach France L110036	VR0596 Muraille	1. 02.04.2011 2. - 3. 30.09.2011	Spray	0.325	200	0.650	1 10.05.2011	15-17	Whole plant* Leaves** Roots***	0.71 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	0.04 <0.01 <0.01	0.75 <0.02 <0.02	0 136 136	Method L0179/02 LOQ 0.01 mg/kg
390503 2012/1182982 Wood Enderby PE22 7PQ United Kingdom B1019 UK1	VR0596 Bull Finch	1. 25.03.2011 2. - 3. 23.09.2011	Spray	0.325	200	0.650	1 10.05.2011	16	Whole plant* Leaves** Roots***	11.4 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	11.41 <0.02 <0.02	0 136 136	Method L0179/02 LOQ 0.01 mg/kg
390503 2012/1182982 6595 ME Ottersum The Netherlands B1019 NL1	VR0596 Pyranja	1. 15.04.2011 2. - 3. 21.09.2011	Spray	0.325	200	0.650	1 18.05.2011	16	Whole plant* Leaves** Roots***	24.68 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	24.69 <0.02 <0.02	0 126 126	Method L0179/02 LOQ 0.01 mg/kg
423128 2013/1003729	VR 0596 Klaxon	1. 21.03.2012 2. N/A	Spray	0.325	200	0.650	1 07.05.2012	16	Whole plant* Leaves**	41 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	41.01 <0.02	0 122	Method L0179/02

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Sugarbeet (Sugar plants)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment0**			**No of treatm. and last date**	**Growth stage (BBCH)[2].**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
16833 Lentzke Germany L120262		3. 06.09.2012							Roots***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	122	LOQ 0.01 mg/kg
423128 2013/1003729 51420 Witry les Reims France L120263	VR 0596 Vienna	1. 20.03.2012 2. N/A 3. 20.10.2012	Spray	0.325	200	0.650	1 16.05.2012	18	Whole plant* Leaves** Roots***	22 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	22.01 <0.02 <0.02	0 125 125	Method L0179/02 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Sugarbeet (Sugar plants)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment0**			**No of treatm. and last date**	**Growth stage (BBCH)[2].**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
423128 2013/1003729 CO11 2NF Essex United Kingdom L120264	VR 0596 Cayman, Isabela	1. 25.03.2012 2. N/A 3. 03.09.2012	Spray	0.325	200	0.650	1 23.05.2012	14-16	Whole plant* Leaves** Roots***	29 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	29.01 <0.02 <0.02	0 103 103	Method L0179/02 LOQ 0.01 mg/kg
423128 2013/1003729 6599 CJ Ven-Zelderheide The Netherlands L120265	VR 0596 Coyote	1. 09.04.2012 2. N/A 3. 17.09.-18.09.12	Spray	0.325	200	0.650	1 23.05.2012	16	Whole plant* Leaves** Roots***	29 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	29.01 <0.02 <0.02	0 117 117	Method L0179/02 LOQ 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at last application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Sugarbeet (Sugar plants)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[3]**	**Total[4]**		
390503 2012/1182982 64300 Castetis France B1019 SA1	VR0596 Koala	1. 27.05.2011 2. - 3. 20.10.2011	Spray	0.325	200	0.650	1 28.08 2011	16	Whole plant* Leaves** Roots***	25.20 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	25.21 <0.02 <0.02	0 114 114	Method L0179/02 LOQ 0.01 mg/kg
390503 2012/1182982 Pontecurone AL Italy B1019 IT1	VR0596 Massima	1. 07.03.2011 2. - 3. 10.08.2011	Spray	0.325	200	0.650	1 28.04.2011	16	Whole plant* Leaves** Roots***	30.52 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	30.53 <0.02 <0.02	0 104 104	Method L0179/02 LOQ 0.01 mg/kg
423128 2013/1003729 40018 San Pietro in Casale Italy L120267	VR 0596 Bruna	1. 27.02.2012 2. N/A 3. 20.08.2012	foliar Spray	0.325	200	0.650	1 17.04.2012	14-16	Whole plant* Leaves** Roots***	33 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	33.01 <0.02 <0.02	0 125 125	Method L0179/02 LOQ 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at last application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops

Beet

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Dimethenamid-P	**Commercial Product (name)**	-
Crop/crop group:	Beet (Root and tuber vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	720 g/L		
Formulation (e.g. WP)	EC (BAS 656 12 H)	**Residues calculated as:**	Dimethenamid-P (BAS 656 PH), M23, M26, M27, M30

1	2	3	4	5			6	7	8	9						10	11
Report-No. Location (Trial No.)	**Commodity / Variety**	**Date of 1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate per treatment[0]**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 656 PH**	**M23**	**M26**	**M27**	**M30[2]**	**Total[3]**		
423128	AM 1051	1. 26.03.2012	Spray	0.325	200	0.650	1	16	Whole plant*	22	<0.01	<0.01	<0.01	<0.01	22.01	0	Method
2013/1003729	Starmon	2. N/A					23.05.2012		Leaves and stem	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	L0179/02
17330 Bernay		3. 27.11.2012							Root***	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	142	LOQ
Saint-Martin																	0.01 mg/kg
France																	

0 Actual application rates varied by 10% at most
1 Days after last application
2 Growth stage at last application
3 Expressed as parent equivalent. The conversion factor from M30 to BAS 656 H is 0.7306. Values below LOQ (0.010 mg/kg) were not converted.
4 Sum of BAS 656 H and M30 expressed as parent equivalent
* With roots
** With tops
*** Without tops

Dimethenamid-P

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

Regulatory Manager
BASF Nederland B.V.,
P O Box 1019
6801 MC Arnhem,
The Netherlands

Telephone +31 26 3717286
Telefax +31 26 3717122
e-mail basf.com

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 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Dimethenamid-P is a soil herbicide used to control annual grasses and broadleaf weeds in several arable crops, such as corn, sugarbeet, oil seed rape, sunflower and soybean, on beans, on leafy-, stem- and bulb-vegetables, on “berry fruits”, orchards, as well as ornamentals and nursery stock. It is applied pre-emergence and early post-emergence.

The active substance is taken up by the coleoptiles of annual grasses or the roots of annual dicots. This takes place when the seeds germinate and emerge through the treated surface. The active substances act by the inhibition of cell mitosis, thereby retarding growth and leading to dying off.

BASF AG submitted a dossier to include Dimethenamid-P in Annex I on 16 April 1999. The Rapporteur Member State was Germany and the coRapporteur Member State was the Netherlands.

All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the monograph of Dimethenamid-P and in the SANCO/1402/2001-Final document.

For the current registration renewal under Directive 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 / evaluations were initiated where considered necessary.

The data used in this characterization was obtained from two major sources. The first of these was the open literature, which included papers in peer-reviewed journals and reports from government and other agencies in the EU and several other countries. The literature search was done via databases such as PubMed, Agricola, and SciFinder using the key-word “Dimethenamid” or “Dimethenamid-P” and the CAS Numbers 87674-68-8 and 163515-14-8 respectively. This initial search was a net cast as wide as possible to ensure complete coverage of the literature. These references were then reviewed and, on the basis of the title and the abstract, a subset was retained for use in the characterization. Priority was given to papers published since 2003 and, where possible, copies of these were obtained for more detailed review. Hence, adequate summaries are provided in the appropriate dossier chapters.

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.

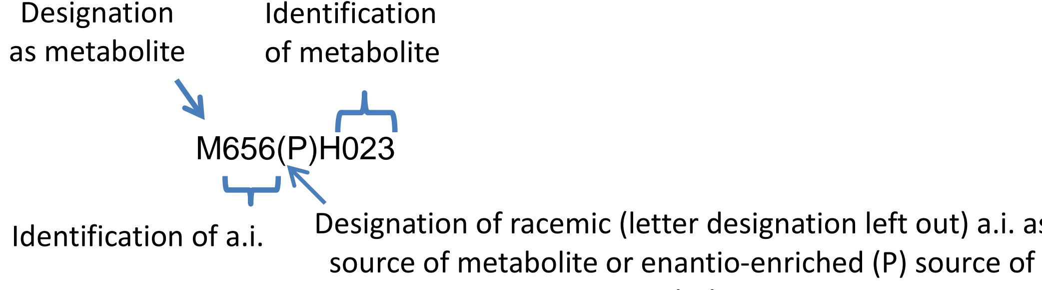
For the implementation of the uniform principles of Annex VI, the conclusions of the review report on Dimethenamid-P and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 28/Nov./2003, shall be taken into account. In this overall assessment:

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.
- New metabolism and degradation studies (laboratory and field) because of new developments and 1107/2009 requirements.
- New adsorption/desorption studies for metabolites
- New non-guideline studies to generate data used for higher Tier exposure assessment.
- Newly elucidated metabolites from lysimeter leachate to be considered for the groundwater risk assessments; assessments conducted according to current guidance.
- Monitoring data
- Additional bridging argumentation for enantioenriched a.s.
- New analytical methods for soil/sediment, water and air according to current guidance requirements and including the new metabolites, if required.

General explanation on metabolite nomenclature in relation to stereoisomers

Dimethenamid-P is the S-enantiomer of the racemic Dimethenamid. For the active ingredient, a data-package conducted with the racemic mixture was taken into consideration and a bridging concept was applied and accepted for the Annex I inclusion of Dimethenamid-P. A comparable situation exists for the metabolite evaluation that partly relies on information where either the source of the metabolite was based on studies conducted with the racemic mixture or where the metabolite evaluated was based on racemic synthesis pathway. Consequently metabolites where the source was the racemic compound and/or where the synthesis could not clearly be attributed to the chiral synthesis pathway were assigned with a code that has the following structure as given for the example **M23**, in the old metabolite naming code, now presented in the new system:



Hence, a metabolite code derived from a known **racemic** source of Dimethenamid would be M656H023 and the corresponding metabolite code from a known **enantio-enriched** source (Dimethenamid-P) would therefore be M656**P**H023 (bold lettering only for emphasis in this instance). Because data was generated over years in which both naming conventions were in place, both will be observed in the dossier from time to time.
Due to the extensive metabolism of Dimethenamid-P a full list of metabolites is not included in this portion of the dossier. For a full list of metabolites and their respective compartments please see document N3.

CA 7.1 Fate and behaviour in soil

While endpoints did not change for several studies, new data was generated for the aerobic soil metabolism of Dimethenamid-P. These data further confirm that racemic endpoints can be extrapolated to the enantio-enriched product.

New endpoints were generated regarding metabolites of Dimethenamid-P (new kinetic evaluations after FOCUS). New studies on the DT50 of metabolites M43, M47, M54 were performed. New K_{oc} data was generated for M23, M27, M31, M43, M47 and M54.

New field studies address the degradation behaviour of Dimethenamid-P in soil when applied in a new representative formulation at the current application rate. Furthermore, the newest studies cover also the new requirements of 1107/2009, ie dissipation processes are excluded to investigate only the degradation of Dimethenamid-P and M656PH027 in soil.

A previously submitted lysimeter study was re-evaluated to add further information to the complex metabolism picture of Dimethenamid-P. This yielded conservative estimates for groundwater concentration in addition to further structure elucidation efforts. A strategic grouping strategy was developed to enable further environmental exposure profiling for refinements in the risk assessment. The metabolites of Dimethenamid-P, for the most part, consist of key intermediates involved in modifications of the chloroacetamide side chain. These key intermediates then fracture in to multiple metabolites as well. The observed metabolic pattern drove the formation of our grouping strategy.

CA 7.1.1 Route of degradation in soil

A brief summary of the degradation of Dimethenamid-P in soil as evaluated during the Annex I listing process is provided below.

Both Dimethenamid and Dimethenamid-P are extensively metabolised in a variety of different soils. In aerobic soil studies, with most soils, mineralisation is rapid and CO_2 was formed at levels up to 36% of the TAR after 120 days. The same metabolites (M23, M27 and M31) were found in all studies. Only M23 (the oxalamide) and M27 (the sulfonate) were ever isolated at levels greater than 10% TAR, but these were not persistent and degraded from peak levels. M31 was found with up to 6.9% TAR, also showing a clear decline of residues from peak level. Bound residues increased with time and a major portion of the radioactivity was found in the fulvic and humic acid fractions. The degradation of Dimethenamid and Dimethenamid-P were, as expected, virtually identical. Anaerobic degradation of Dimethenamid-P was similar albeit slower than aerobic degradation rate.

Photolytic degradation occurred to produce CO_2, but the degradation was observed to be slower than the aerobic processes. Photolysis is not expected to be a major route of degradation in a natural environment.

New data has also been generated for this review. New data was generated as per the outline above to provide a complete data package up to date with the most current guidelines and requirements.

Aerobic metabolism studies, using EU and US soils, were re-analyzed (including metabolites M23 and M27, metabolite M31) according to the latest kinetic guidance documents where applicable. All the results given indicate that neither Dimethenamid-P nor its metabolites, M23, M27 and M31, are stable in an active soil environment and therefore, no risk of persistence exists.

Further studies were conducted to demonstrate the similarity of the degradation profiles between racemic Dimethenamid and Dimethenamid-P. Dimethenamid-P was used in soil metabolism studies and chiral analysis performed on the samples. Metabolites, observed metabolite levels, mineralization and formation of non-extractable residues were all observed to be well within range of the previously gathered racemic data. This information validates that data between DMTA and DMTA-p are interchangeable.

Endpoints used in the evaluation are presented under the relevant section where the endpoints are used and also may be found in the relevant sections of document N-2.

A proposed pathway for the degradation of Dimethenamid/Dimethenamid-P in soil is presented below. No significant change in the enantiomeric ratio was observed during the degradation of Dimethenamid-P in any study. Considering this constant ratio, it is concluded that both enantiomers are degraded in soil with the same rate and no interconversion between the enantiomers occurs.The information gained in this body of work show that the pathways are virtually identical.

Dimethenamid-P

M656PH032

M656PH026

M656PH011

M656PH031

M656PH30

M656PH23

M656PH027

CO_2

CA 7.1.1.1 Aerobic degradation

Two new studies were set up to investigate the chiral stability and potential stereoselectivity in the degradation of Dimethenamid-P in soil. In the first study existing samples from a previous study were subjected to chiral analysis. The second study was set up to investigate qualitatively the stereoselectivity of the degradation of Dimethenamid-P in a second soil.

Report:	CA 7.1.1.1/1 Staudenmaier H., 2013a Chiral analysis of Dimethenamid-P after incubation in soil 2012/1073064
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

EXECUTIVE SUMMARY

Dimethenamid-P is a chiral compound containing mainly the S-enantiomer. The purpose of the present study was the investigation of the stereochemistry of Dimethenamid-P during degradation in soil using chiral separation techniques.

The study was performed with soil extracts which were obtained from a previous study (*BASF DocID 2009/1011362 - summarized under CA 7.1.4.2/2*) in which soil samples were treated with 2.7 mg Dimethenamid-P/kg dry soil, corresponding to a field application rate of 1 kg test item/hectare. Within the latter study the soil samples were incubated in the dark at 20°C with a soil moisture of 40% of the maximum water holding capacity for up to 119 days. Soil extracts obtained from samples after 0, 28, 58, 89 and 119 DAT were transferred to the present study.

As was found in the preceding study (BASF DocID 2009/1011362), Dimethenamid-P degraded from approximately 100% TAR to 8.0% TAR within 119 days.
The ratio of the enantiomers of Dimethenamid-P (S-enantiomer and R-enantiomer) during the degradation of the active substance was investigated by analysis on a chiral column. The ratio of S- and R-enantiomers was about 97.4 % to 2.6 % at the beginning and almost no change of this ratio was observed during the degradation of Dimethenamid-P within 119 days of incubation. These results show that there is no selective degradation of the enantiomers as well as no interconversion between the enantiomers. The degradation rate of Dimethenamid-P is the same as for racemic Dimethenamid.

The S-enantiomer was further separated into two peaks, termed isomer 1 and isomer 2. The ratio of these two peaks was 35.4 % to 62.0 % at the beginning and this ratio remained practically unchanged during the experiment. In order to obtain information on the behaviour of the two isomers of the S-enantiomer, both isomers were enriched separately from a solution of non-radiolabeled Dimethenamid-P. Additional investigations indicated that these isomers tend to interconvert already under mild conditions at room temperature with this interconversion being accelerated at higher temperature.

Degradation times of the sum of both enantiomers, as well as of the S- enantiomer and of the R-enantiomer of Dimethenamid-P were estimated following the recommendations of the FOCUS Kinetics workgroup. DT_{50} values of 30.9 days, 30.9 days and 31.6 days were estimated for the sum of both enantiomers, the S- and the R-enantiomer, respectively using the SFO model. The kinetic evaluation showed that there is no significant difference between the degradation of the total sum of isomers, as well as the R- and S-enantiomer individually. All substances degrade with the same rate, i.e. the rate of degradation is within the confidence intervals of all three kinetic analyses.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code: BAS 656 H
CAS Number.: 163515-14-8
Chemical name (IUPAC): (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamid
Molecular weight: 275.8 g/mol (unlabeled)
Position of radiolabel: thienyl-5-C14
Specific radioactivity: 6.53 MBq/mg (391800 dpm/µg)
Radiochemical purity: 99.4%

2. Soils

No soil samples or extracts were generated in the current study. The test item was analyzed in samples generated in a preceding study (BASF DocID 2009/1011362) by incubation of Dimethenamid-P under aerobic conditions in soil. The soil was from Borstel, Lower Saxony, Germany. The soil characteristics are summarised in Table 7.1.1.1-1.

Table 7.1.1.1-1: Soil Characteristics

Parameter	**Borstel, Lower Saxony, Germany**
Soil type	Sand (USDA)
Particle size distribution [%]	
Sand (50 - 2000 µm)	91.1
Silt (2 - 50 µm)	6.3
Clay (< 2 µm)	2.7
Organic carbon [%]	0.75
Microbial biomass [mg C/kg dry soil]	14.5
CEC [cmol/kg]	2.7
pH ($CaCl_2$)	5.9
pH (H2O)	6.5

B. STUDY DESIGN

1. Experimental conditions

In study BASF DocID 2009/1011362 soil was treated with Dimethenamid-P at a nominal application rate of 2.7 mg/kg dry soil, corresponding to a field application rate of 1 kg test item, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³.

Twenty soil portions of 100 g dry soil equivalents were filled into test vessels. The test vessels were incubated in the dark at 20 ± 2°C in an incubator with an open gas flow system.
The soil samples (100 g dry weight aliquots) were extracted once with methanol by shaking on a laboratory shaker and the phases were separated by centrifugation. The supernatant was decanted, filtered, made up to volume and aliquots were measured by LSC. The extraction procedure was repeated three additional times with methanol/water (80/20).
The methanol extract and the corresponding methanol/water extracts were pooled and concentrated using a rotary evaporator. The dry precipitates were redissolved in methanol/water (80/20) before analysis.

Extracts of samples from days 0, 28, 58, 89 and 119 DAT were further worked up and investigated by chiral HPLC analysis in the present study

2. Sampling

Samples were taken at several time points up to 119 days after treatment. At each sampling time two vessels were removed from the incubator and were either worked up directly or stored in the freezer. Further details of treatment and sampling are given in study BASF DocID 2009/1011362.

3. Description of analytical procedures

<u>Isolation and investigation of Dimethenamid-P from soil extracts</u>

Since the entire soil extracts were not suitable for analysis on the chiral separation column, the peak of the active substance was first isolated by achiral HPLC fractionation of soil extracts from study 148199. The HPLC run was fractionated and the peak containing Dimethenamid-P was further worked up. The collected material for Dimethenamid-P was adjusted to volume, mixed and three times partitioned against of ethyl acetate. All extracts were measured by LSC. The ethyl acetate extracts were combined and concentrated to dryness at 40°C and redissolved in the mobile phase for HPLC analysis. Aliquots of the solution were analyzed by LSC and HPLC on a chiral column ((S,S)-Whelk-O-1-Pirkle) to investigate the composition of stereoisomers.

Further Investigation of isomers

The S-enantiomer was found to separate into two peaks (isomers 1 and 2 in chromatographic order). These were assumed to be conformational isomers which normally interconvert rapidly. In order to investigate whether the two isomers can be interconverted, the peaks were isolated separately and incubated in organic solvent or aqueous solution under various temperature and pH conditions followed by HPLC analysis.
The single isomers were isolated by HPLC fractionation using a chiral column. An aliquot of a solution of non-radiolabeled Dimethenamid-P was dissolved in n-heptane/isopropanol/ tetrahydrofuran (970/20/10, v/v/v) and aliquots thereof were fractionated by HPLC. Corresponding fractions containing isomers 1 and 2 of the S-enantiomer were combined and partly further cleaned up by chiral HPLC fractionation, finally resulting in two fractions containing predominantly isomer 1 or predominantly isomer 2 dissolved in methanol.

Each of the isolated isomers was investigated further by incubation under various conditions: The methanol solutions were diluted with water or buffer (1:10 dilution of the Titrisol buffer concentrate, Merck). Solutions in buffer of pH 4, pH 5, pH 7 and pH 9 were incubated for 5 h, 24 h and 4 days at room temperature. Furthermore, solutions in water were incubated at 50°C and 70°C for 1 h, 5.5 h and 24 h. After the incubations the solutions were partitioned three times against ethyl acetate. The organic phase was concentrated to dryness, redissolved in n-heptane/ isopropanol/ tetrahydrofuran and analyzed by chiral HPLC. In a further experiment, the fractions were evaporated to dryness and taken up in organic solvent (HPLC mobile phase: n-heptane/ isopropanol/tetrahydrofuran). Aliquots were transferred into HPLC vials, sealed and incubated at 70°C for 1, 2 or 3 h. After that time the fractions were analyzed by chiral HPLC.

The results of HPLC analysis are given in Table 7.1.1.1-4 to Table 7.1.1.1-9.

4. Calculation of the degradation rate

Kinetic analysis and calculations of DT50 and DT90 values were performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was conducted by non-linear regression methods employing the software tool KinGUI 2.

II. RESULTS AND DISCUSSION

A. Investigation of the ratio of enantiomers of Dimethenamid-P during degradation in soil

The results of the chiral analysis are given in Table 7.1.1.1-2 (in relative percent of the HPLC analyses - %ROI) and Table 7.1.1.1-3 (in percent of applied - %TAR). The ratio of S- and R-enantiomers was about 97.4 % to 2.6 % at the beginning and almost no change of this ratio was observed during the degradation of Dimethenamid-P within 119 days of incubation (Table 7.1.1.1-2). Considering this constant ratio, it is concluded that both enantiomers are degraded in soil with the same rate and no conversion of the S-enantiomer to the R-enantiomer or vice versa occurs. The S-enantiomer was further separated into two peaks, termed isomer 1 and isomer 2. The ratio of these two peaks was 35.4 % to 62.0 % at the beginning and - considering the analytical uncertainty of these not baseline separated peaks - also this ratio remained practically unchanged during degradation (Table 7.1.1.1-2).
For the R-enantiomer no separation into isomers was observed. However this is considered to be a specific feature of the chiral (S,S)-Whelk-O 1 Pirkle column: In the meantime another chiral HPLC system became available which separates also 2 isomers of the R-enantiomer of Dimethenamid-P whereas the S-enantiomer is only poorly separated into isomers (non-GLP information). Since the R-enantiomer remains very low in this study this was not further investigated.

B. Additional investigations on the isomers 1 and 2 of the S-enantiomer

Slow, but visible changes of the ratio of the proposed conformational isomers were found during incubation in aqueous solution at room temperature at different pH values in the range of pH 4 to pH 9. This indicates that the isomers tend to interconvert already under mild conditions (Table 7.1.1.1-4 and Table 7.1.1.1-5) thus giving supporting evidence that the isomers are conformers.
The changes were slightly accelerated at 50°C and - after 1 day at a further increase of the temperature to 70°C - approached from either side a ratio of the isomers similar to that in the original parent compound (Table 7.1.1.1-6 and Table 7.1.1.1-7).
Fast interconversion of the isomers was observed in organic solvent (n-heptane/ isopropanol/tetrahydrofuran) at an elevated temperature of 70°C (Table 7.1.1.1-8 and Table 7.1.1.1-9). Thermodynamic dependency gives evidence that the observed isomers are conformational in nature.

Table 7.1.1.1-2: Proportion of enantiomers in soil extracts obtained from soil samples incubated with Dimethenamid-P [% ROI in HPLC]

DAT	R-enantiomer [%]	S-enantiomer isomer 1 [%]	S-enantiomer isomer 2 [%]	S-enantiomer isomers 1 + 2 [%]
0	2.6	35.4	62.0	97.4
28	2.7	34.7	62.6	97.3
58	2.5	34.8	62.7	97.5
89	2.4	34.4	63.3	97.7
119	2.3	34.4	63.3	97.7

Table 7.1.1.1-3: Proportion of enantiomers in soil extracts obtained from soil samples incubated with Dimethenamid-P [% TAR]

DAT	Dimethenamid % TAR	R-enantiomer [%TAR]	S-enantiomer isomer 1 [%TAR]	S-enantiomer isomer 2 [%TAR]	S-enantiomer isomers 1 + 2 [%TAR]
0	100.5	2.6	35.6	62.2	97.8
28	51.4	1.4	17.8	32.2	50.0
58	26.8	0.7	9.3	16.8	26.1
89	15.7	0.4	5.4	9.9	15.3
119	7.9	0.2	2.7	5.0	7.7

Table 7.1.1.1-4: Proportion of isomers after incubation of enriched isomer 1 of the S-enantiomer of Dimethenamid-P in aqueous solutions of different pH

pH	Incubation time	Isomer 1	Isomer 2
4	5 h	91.2	8.8
	24 h	89.7	10.3
	4 d	87.5	12.5
5	5 h	91.1	8.9
	24 h	87.4	12.6
	4 d	89.7	10.3
7	5 h	91.4	8.6
	24 h	89.2	10.8
	4 d	88.4	11.6
9	5 h	90.9	9.1
	24 h	n r.*	n r.*
	4 d	88.6	11.4

*not reported, sample was dried completely by evaporation

Table 7.1.1.1-5: Proportion of isomers after incubation of enriched isomer 2 of the S-enantiomer of Dimethenamid-P in aqueous solutions of different pH

pH	Incubation time	Isomer 1	Isomer 2
4	5 h	12.7	87.3
	24 h	13.6	86.4
	4 d	13.2	86.8
5	5 h	12.6	87.4
	24 h	13.4	86.6
	4 d	13.1	86.9
7	5 h	12.4	87.6
	24 h	14.2	85.8
	4 d	13.2	86.8
9	5 h	12.5	87.5
	24 h	14.3	85.7
	4 d	13.7	86.3

Table 7.1.1.1-6: Proportion of isomers after incubation of enriched isomer 1 of the S-enantiomer of Dimethenamid in aqueous solution at 50°C and 70°C

Temperature [°C]	Incubation time	Isomer 1	Isomer 2
-*	0	93.6	6.4
50	1 h	90.3	9.7
	5 h	88.9	11.1
	24 h	86.2	13.8
70	1 h	89.2	10.8
	5 h	84.2	15.8
	24 h	50.1	49.9

*before incubation

Table 7.1.1.1-7: Proportion of isomers after incubation of enriched isomer 2 of the S-enantiomer of Dimethenamid in aqueous solution at 50°C and 70°C

Temperature [°C]	Incubation time	isomer 1	isomer 2
-*	0	12.2	87.8
50	1 h	13.9	86.1
	5 h	13.8	86.2
	24 h	16.0	84.0
70	1 h	14.4	85.6
	5 h	18.7	81.3
	24 h	40.8	59.2

*before incubation

Table 7.1.1.1-8: Proportion of isomers after incubation of enriched isomer 1 of the S-enantiomer of Dimethenamid-P in organic solution at 70°C

Incubation time [h]	Isomer 1 [%]	Isomer 2 [%]
0	96.8	3.2
1	62.1	38.0
2	49.8	50.2
3	47.7	52.3

Table 7.1.1.1-9: Proportion of isomers after incubation of enriched isomer 2 of the S-enantiomer of Dimethenamid-P in organic solution at 70°C

Incubation time [h]	Isomer 1 [%]	Isomer 2 [%]
0	6.0	94.1
1	34.2	65.8
2	43.6	56.4
3	45.4	54.6

C. Degradation times

Both enantiomers separately and the total sum of enantiomers have been evaluated following the steps in the flowcharts proposed by the FOCUS Kinetics guidance document. SFO resulted in statistically very good fits for all evaluations and is the most appropriate kinetic model. A summary of the DT50/DT90 is provided in Table 7.1.1.1-10.
The kinetic evaluation shows that there is no significant difference between the degradation of racemic Dimethenamid and the R- and S-enantiomers of Dimethenamid-P individually. All substances degrade at the same rate, i.e. the rate of degradation is within the confidence intervals of all three kinetic analyses. Similar degradation rates also demonstrate that there is no interconversion between isomers. Interconversion would lead to differing kinetics between the enantiomers. This is also backed-up analyzing the ratio of S- and R-enantiomer over time, which does not show a significant and continuous increase/decrease over time.

Table 7.1.1.1-10: DT_{50}/DT_{90} of the enantiomers of Dimethenamid-P in soil

Chemical	Kinetic model	DT_{50} [d]	DT_{90} [d]
Sum of enantiomers (= Dimethenamid-P)	SFO	30.9	102.7
R-enantiomer	SFO	31.6	104.9
S-enantiomer	SFO	30.9	102.8

III. CONCLUSION

The ratio of enantiomers of Dimethenamid-P was investigated in soil extracts from a preceding soil degradation study by analysis on a chiral column.
No significant change in the enantiomeric ratio was observed during the degradation of Dimethenamid-P within 119 days of incubation. Considering this constant ratio, it is concluded that both enantiomers are degraded in soil with the same rate and no interconversion between the enantiomers occurs.
The S-enantiomer was further separated into two peaks, called isomer 1 and isomer 2 (conformational isomers). Additional investigations indicated that these isomers tend to interconvert with heating.

Report: CA 7.1.1.1/2
Unsworth R., 2014a
Dimethenamid-P: Chiral separation after degradation in soil
2013/1412031
Guidelines: EU Regulation 1107/2009 with Regulation 283/2013, OECD 307 (2002)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The enantiomeric composition of Dimethenamid-P and its degradation products was studied in a sandy loam soil incubated in the laboratory under aerobic conditions. Soil samples were set up and allowed to acclimatise before being treated with [14C]-Dimethenamid-P. The test substance was radiolabelled in the [thienyl-5-14C] position. Dimethenamid-P was applied at a nominal rate of 2.67 mg/kg, equivalent to a field rate of 1000 g a.i./ha assuming a uniform incorporation in the top 2.5 cm depth of soil having a bulk density of 1.5 g/cm3. The samples were incubated under aerobic conditions in the dark at about 20°C and a moisture content equivalent to pF 2 for periods of up to 120 days after application. Samples of soil were extracted with methanol/water and the extracts analysed by HPLC and chiral HPLC.

The overall recoveries of radioactivity from the soil samples were in the range 92.8% to 103.0% of applied radioactivity.

The proportion of radioactivity extracted from soil decreased with time with a corresponding increase in the levels of non-extractable radioactivity. Immediately after application of test substance, 97.6 – 99.8% applied radioactivity was extracted from soil declining to 30.0 - 30.5% applied radioactivity after 120 days.

Non-extractable radioactivity increased to a mean value of 43.0% after 120 days. Volatile radioactivity, associated with 14CO2, increased to a mean of 23.1% by 120 days.

The amount of Dimethenamid-P in the soil extracts declined from a mean of 97.3% applied radioactivity at the time of application to a mean of 4.7% at 120 days.

Dimethenamid-P degraded to M23 (up to 6.0% applied radioactivity), M27 (up to 3.8% applied radioactivity), M31 (up to 2.2% applied radioactivity) and up to 27 unidentified degradation products (≤3.5% applied radioactivity).

The chiral chromatography showed that the proportions of the enantiomers of Dimethenamid-P in soil extracts at T0 (approximately 97% (S) enantiomer : 3% (R) enantiomer) remained similar throughout the 120 days.

In addition degradates of Dimethenamid-P were incorporated into the soil (bound residues) and subsequently mineralised to carbon dioxide.

I. MATERIAL AND METHODS

A. MATERIALS

Name:	Dimethenamid-P
Code:	BAS 656 H or BAS 656-PH
Chemical Name (CAS):	2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-((1S)-2-methoxy-1- methylethyl)acetamide
CAS number:	163515-14-8
Molecular Weight:	275.8
Physical state:	Liquid
Position of radiolabel (*):	[thienyl-5-^{14}C]
Specific activity:	7.58 MBq/mg
Radiochemical purity:	>97%

Test soil
The test soil (Calke) was a sandy loam with an organic carbon content of 3.9% and a pH of 4.6 (0.01M CaCl2). The microbial biomass of the soil was a mean of 1.7% (674.5 mgC/kg) of the total organic carbon after 62 days of incubation and 1.4% (540 mgC/kg) at the end of the incubation period, demonstrating that the soil was microbiologically viable throughout. The physical and chemical characteristics of the soil are shown in the table below.

Table 7.1.1.1-11:Soil Characteristics

Parameter	**Calke, Derbyshire, UK**
Pesticide use	No pesticide sprays have been used since year 2000
Particle size distribution:	
UK classification:	
0.063 mm – 2 mm (%):	68
0.002 mm – 0.063 mm (%):	17
<0.002 mm (%):	15
USDA classification:	
0.05 mm – 2 mm (%):	71
0.002 mm – 0.05 mm (%):	15
<0.002 mm (%):	14
Texture class (UK and USDA):	Sandy Loam
pH in water	5.5
pH in 0.01 M $CaCl_2$	4.6
Organic carbon (%)	3.9
Cation exchange capacity (meq/100 g)	13.9
Soil moisture content (g/100g dry wt)	22.2
Water content at pF 2 (g/100g dry wt)	33.0
Microbial biomass (mgC/kg)	
At 62 day incubation	775 and 574
At end of incubation (120 day)	498 and 582
Microbial biomass (%)	
At 62 day incubation	1.99 and 1.47
At end of incubation (120 day)	1.28and 1.49

B. STUDY DESIGN

The enantiomeric composition of Dimethenamid-P and its degradation products was studied in soil incubated under aerobic conditions in the laboratory. Samples of soil adjusted to a moisture content equivalent to pF 2 were treated with [thienyl-5-14C]-Dimethenamid-P at a nominal concentration of 2.67 mg/kg, equivalent to a field rate of 1000 g a.i./ha assuming a uniform incorporation in the top 2.5 cm depth of soil having a bulk density of 1.5 g/cm3. Samples were arranged in flow-through systems and incubated in darkness at 20 ± 2°C for periods of up to 120 days. Radiolabelled volatile metabolites including CO2 were trapped and quantified. At appropriate time intervals, samples were taken for analysis and the soil extracted with aqueous/organic solvents. Extracts of soil were analysed by HPLC. Non-extractable radioactivity was quantified and a material balance obtained for each sample.

Test methods

Preparation and incubation of the test systems
Portions of soil, equivalent to 100 g dry weight, were added to glass test vessels and distilled water added to adjust the moisture content to that at pF2. Twenty-two vessels were established for [14C]-Dimethenamid-P.

Air was drawn through each system at a flow rate of approximately 60 mL/minute. Flow rates were checked and adjusted throughout the incubation period. During the acclimatisation period (11 days) all traps except the humidifying water bottle were empty. At intervals, vessels were weighed and water added as necessary to maintain the moisture content equivalent to that at pF 2.

In addition, six vessels containing 500 g (dry weight equivalent) of soil were established for the determination of soil microbial biomass. The samples were incubated in series under the same conditions as above.
All test systems were maintained in darkness at nominally 20 ± 2°C in a temperature-controlled room. The temperature in the room was monitored throughout the incubation period.

Preparation and application of the test substance

The [14C]-Dimethenamid-P was radiodiluted for application. The [14C]-Dimethenamid-P application solution was prepared by combining a portion (12.25 mg) of non-radiolabelled Dimethenamid-P with a portion (1.35 mg, 10.2 MBq) of [14C]-Dimethenamid-P and diluting to volume (5 mL) with acetonitrile. The concentration of the application solution was 2.72 mg/mL and the radiodiluted specific activity was 45000 dpm/µg. Aliquots (100 µL) of the application solution was applied to the surface of the soil samples. Following application the solvent was allowed to evaporate and the soil mixed by gently shaking. Each vessel was weighed and water added as necessary to maintain the moisture content at pF 2. Following application, each vessel (with the exception of those taken for zero-time analysis) was reincorporated into its respective flow-through system.

To accurately determine the amount of radioactivity (and Dimethenamid-P) added to each sample, further aliquots (100 µL) of the application solution were taken for radioassay before and after the treatment process. Soil samples were prepared and treated at the exaggerated rate of 27 mg/kg. The [14C] Dimethenamid-P application solution was prepared by combining a portion (53.27 mg) of non-radiolabelled Dimethenamid-P with a portion (0.54 mg, 4.1 MBq) of [14C] Dimethenamid-P and diluting to volume (2 mL) with acetonitrile. Aliquots of the application solution (100 µL) were applied to the soil sample as described for the main experiment. The samples were incubated as described for the main experiment.

Sampling
Duplicate samples of soil treated with [14C]-Dimethenamid-P were taken immediately after application and after 3, 7, 14, 30, 45, 59, 90 and 120 days of incubation.

The following samples were analysed; duplicate replicates for zero-time and 120 days and a single replicate for 14, 30 and 59 days. The remaining samples were stored frozen.

Trapping solutions were taken for analysis when the associated sample was taken for analysis. Additionally, all remaining traps were taken for analysis and replaced with fresh media as necessary at 7, 14 days and subsequently at approximately two weekly intervals after application. Two vessels, established for the determination of microbial biomass, were taken for analysis during incubation (after approximately 60 days). The second set of vessels was taken for analysis after 120 days of incubation.

For the determination of radioactivity in soil extracts and residues, total sample weights, replicate weights and liquid scintillation counting data were recorded and processed using the DEBRA automated laboratory data capture and processing system (V5.5.4.49), LabLogic Systems Ltd, Sheffield, UK).

Preparation of samples for chromatographic analysis
For each sample, portions of all of the extract solutions were combined in proportion to their total volumes and duplicate aliquots of the pools taken for radioassay. Extract pools were concentrated under nitrogen gas at approximately 40°C prior to analysis. The recovery was monitored through the concentration procedure and was quantitative. Concentrated extracts were analysed by HPLC.

Incubation conditions
The temperature data recorded in the incubation room remained generally within the range 20 ± 2°C throughout, except during the first few days when the temperature increased for short periods. This was not considered to have affected the outcome or integrity of the study Achieved application rates and radiochemical puritiesThe amount of [14C]-Dimethenamid-P applied per sample was 273 µg (0.2 MBq). This equated to an application rate of 2.73 mg/kg. The radiochemical purity of [14C]-Dimethenamid-P was measured by HPLC prior to application and was 97.7%. The chiral purity of [14C]-Dimethenamid-P was measured and was (S) 96.6%: (R) 3.4% for the fortification solution.

II. RESULTS AND DISCUSSION

Recovery of radioactivity
All total recoveries of radioactivity (‘mass balances’, i.e. the sum of extractable and non-extractable radioactivity in soil, and volatile radioactivity) were in the range 92.8 to 103.0% of the amount of applied radioactivity (%AR)
In soil treated with [14C]-Dimethenamid-P, extractable radioactivity declined with time, from a mean of 98.7% at the time of application to a mean of 30.3% after 120 days. There was a corresponding increase with time in non-extractable radioactivity with a mean value of 43.0% after 120 days. Volatile radioactivity increased to a mean of 23.1% after 120 days.
Radioactivity trapped by potassium hydroxide solution was shown to be associated with 14CO2.

Chromatographic analysis
The amount of Dimethenamid-P declined from a mean of 97.3% of applied radioactivity at T0 to a mean of 4.7% after 120 days. Dimethenamid-P was degraded to M23, M27, M31 (≤6.0% AR) and up to 27 unidentified metabolites (≤3.5% AR) several of which were polar in nature.

Chiral chromatographic analysis
The proportions of the enantiomers of Dimethenamid-P in soil extracts at T0 (approximately 97% (S) : 3% (R) remained similar throughout the 120 days.
The chiral analyses of the metabolites (M23, M27 and M31) were based on methods supplied by the sponsor, which were not developed using enantiomer pure reference standards. Therefore, the chiral analysis results for the metabolites should be treated with caution. More specifically, it is unclear whether the separated peaks do represent the enantiomers or other types of isomers (eg rotamers) that were also observed with metabolites of Dimethenamid-P.
In the soil extracts at 59 days the proportions of the isomers of M23 were 52.9% (isomer 1): 47.1% (isomer 2). For M27 the proportions of the isomers were 98.3% (isomer 1): 1.7% (isomer 2). For M31 the proportions of the isomers were 67.3% (isomer 1): 32.7% (isomer 2).

Biotransformation pathway
Under aerobic conditions Dimethenamid-P was degraded to M23, M27, M31 and up to 27 unidentified degradates including some which were polar in nature. Degradates were incorporated into the soil (bound residues) and subsequently mineralised to carbon dioxide.

Table 7.1.1.1-12: Distribution and recovery of radioactivity in soil treated with [14C]-Dimethenamid-P

	% TAR									
Days after treatment	**MeOH**	**MeOH**	**MeOH / H2O**	**MeOH / H2O**	**MeOH / H2O**	**non-extractable residues**	**total**	**Volatiles (organics)**	**Volatiles (CO_2)**	**material balance**
0	75.23	-	22.38	-		3.98	101.59	na	na	101.59
	83.75	-	16.05	-		3.15	102.95	na	na	102.95
0 mean	79.49	-	19.22	-		3.57	102.27	na	na	102.27
14	55.13	-	11.85	-	3.62	21.77	92.37	nd	4.08	96.45
30	27.03	12.71	7.49	4.36	3.88	35.29	90.76	nd	6.94	97.70
59	18.55	8.53	5.24	3.85	3.19	45.98	85.34	nd	13.08	98.42
120	12.29	5.08	6.54	4.23	1.90	43.58	73.62	nd	19.18	92.80
	12.77	4.94	6.60	4.24	1.99	42.42	72.96	nd	26.97	99.93
120 mean	12.53	5.01	6.57	4.24	1.95	43.00	73.29	nd	23.08	96.37

Results expressed as % applied radioactivity
na (not applicable)
nd (not detected)
- (not analyzed)

Table 7.1.1.1-13: Proportions of radioactive components in soil treated with [14C]-Dimethenamid-P

DAT	% TAR									
		ukn	ukn	ukn	ukn	ukn	ukn	ukn	ukn	ukn
	t_R[a]	3.0	4.5	5.0	6.0	7.0	8.5	10.0	12.0	13.0
	^{14}C total									
0	97.61	ND	ND	ND	ND	ND	ND	ND	ND	ND
0	99.80	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	70.60	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	55.47	ND	ND	ND	ND	ND	0.9	ND	ND	ND
59	39.36	0.4	0.4	ND	0.4	ND	2.1	0.2	ND	0.3
120	30.04	0.5	0.2	0.7	1.1	0.3	3.5	0.4	ND	0.4
120	30.54	0.6	0.9	ND	1.0	0.7	3.5	0.5	0.8	0.2

DAT	% TAR									
		ukn	ukn	ukn	ukn	ukn	ukn	**M27**	ukn	ukn
	t_R[a]	14.0	14.5	16.0	16.5	17.0	18.2	19.5	23.0	24.0
	^{14}C total									
0	97.61	ND	ND	ND	ND	ND	ND	ND	ND	ND
0	99.80	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	70.60	ND	ND	ND	ND	ND	ND	1.5	ND	ND
30	55.47	ND	ND	ND	ND	ND	ND	2.4	ND	ND
59	39.36	0.7	0.2	ND	ND	ND	ND	3.2	ND	0.2
120	30.04	0.7	0.5	0.1	0.1	0.3	0.1	3.8	ND	0.2
120	30.54	0.7	0.5	0.4	ND	0.5	ND	3.7	0.2	0.2

DAT	% TAR									
		ukn	ukn	ukn	ukn	**M23**	**M31**	ukn	ukn	ukn
	t_R[a]	25.0	25.5	27.0	28.5	30.0	32.5	34.5	35.5	36.5
	^{14}C total									
0	97.61	ND	ND	ND	ND	ND	ND	ND	ND	ND
0	99.80	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	70.60	ND	ND	ND	ND	4.1	2.0	0.4	ND	ND
30	55.47	ND	ND	ND	ND	5.3	2.1	1.0	ND	ND
59	39.36	0.2	0.4	0.6	0.2	6.0	2.2	0.5	0.6	0.3
120	30.04	ND	0.2	0.4	0.1	4.3	1.8	0.5	0.7	0.1
120	30.54	ND	0.5	0.6	ND	4.1	2.1	0.3	0.7	ND

DAT	% TAR					
		ukn	ukn	ukn	**BAS 656 H**	**others**
	t_R[a]	37.5	38.5	40.0		
	^{14}C total					
0	97.61	ND	ND	ND	95.8	1.9
0	99.80	ND	ND	ND	98.7	1.1
14	70.60	0.6	0.9	ND	60.5	0.6
30	55.47	0.7	1.2	ND	39.1	2.8
59	39.36	0.8	1.8	0.5	15.2	1.8
120	30.04	0.6	2.3	0.3	4.8	1.3
120	30.54	0.9	1.9	0.3	4.6	1.1

Results expressed as % applied radioactivity
a approximate retention time (min)
ND not detected
Others low level radioactivity not considered to constitute a discrete region of interest

Table 7.1.1.1-14: Proportions of Dimethenamid-P isomers in extracts from soil treated with [14C]-Dimethenamid-P

Component	Sampling time (days)							
	T_0	T_0	7	14	30	59	120	120
Isomer (S)	97.2	97.3	97.3	99.1	98.3	97.2	96.0	97.4
Isomer (R)	2.8	2.7	2.7	0.9	1.7	2.8	4.0	2.6

Results are expressed as % proportion
Proportions were derived from Laura integration for T_0 to 30 days
Proportions were derived from fraction collection integration for 59 to 120 days

Table 7.1.1.1-15: Proportions of metabolite isomers in extracts from soil at 59 days treated with [14C]-Dimethenamid-P

Component	Metabolite		
	M23	M27	M31
Isomer (1)	52.9	98.3	67.3
Isomer (2)	47.1	1.7	32.7

Results are expressed as % proportion
Values were derived from fraction collection integration.

III. CONCLUSION

Dimethenamid-P was degraded, in aerobic Calke soil at 20°C and pF 2, to M23, M27, M31 and up to 27 unidentified degradates (≤3.5%) including some which were polar in nature. None of the observed metabolites exceeded 6.0% TAR. In addition, degradates were incorporated into the soil (bound residues) and subsequently mineralised to carbon dioxide.
The enantiomeric ratio of Dimethenamid-P remained unchanged throughout the study.

CA 7.1.1.2 Anaerobic degradation

Data from the previous review has not been updated and is still valid. A brief description follows for the reviewer's convenience. Under anaerobic conditions volatile breakdown products in the form of $^{14}CO_2$ accounted for 3.3% TAR at termination (after 93 days). Non-extractable residues increased with time and reached an average of 33.9% TAR at termination. The metabolic profile of Dimethenamid under anaerobic conditions is very similar to that found under aerobic conditions, although the processes were slower. The fractions that could be isolated under aerobic conditions, including M23 and M27, were also found in this study.

CA 7.1.1.3 Soil photolysis

The soil photolysis of Dimethenamid-P was previously evaluated and peer reviewed during the last Annex-I inclusion. No new data has been generated.

Photolytic degradation occurred to produce CO_2, but the degradation was slower than the aerobic processes and photolysis is not expected to be a major route of degradation in a natural environment. A previously submitted photolysis study had shown a single metabolite above 5% at 3 time points (Sabat and Yu, BASF Doc ID 1992/12387) in M656H09. This study though was done at a highly exaggerated dose rate (500 mg a.i./kg soil). A more recent study (Nietschmann and Yu, BASF Doc ID 1997/5181) was performed using a more realistic dose rate of 1.9 mg a.i./kg soil. Both racemic and enantio-enriched Dimethenamid-P were used for this comparative study. In this more realistic scenario no metabolic product other than CO_2 was observed in appreciable amounts in either racemic nor enantio-enriched a.i.'s

CA 7.1.2 Rate of degradation in soil

The rate of degradation in soil of Dimethenamid-P ((*S*)-Dimethenamid) and the racemic mixture Dimethenamid ((*R,S*)-Dimethenamid) was evaluated in the context of the Annex I inclusion process. Soil laboratory half-lives of Dimethenamid-P show that the compound readily degrades in to a variety of metabolites as shown by the complex metabolic scheme.

Dimethenamid-P and Dimethenamid are rapidly degraded in soil under laboratory conditions with DT_{50} values ranging from 8 to 38 days. There is no difference in the degradation rate between the compounds. These results were confirmed by field soil dissipation studies. The laboratory DT_{50} values for the two metabolites, M23 and M27, are in the range of 24 to 41 days and 40 to 140 days, respectively. It is obvious that the presence of M23 and M27 is transient.

Kinetic re-evaluation was performed for the already peer-reviewed aerobic soil degradation studies under consideration of the parent substance and the main identified soil metabolites M23, M27 and M31. Three newly identified metabolites (M43, M47 and M54) from the re-evaluation of the lysimeter leachates were selected for the determination of DT50 values.

New field studies were run with Dimethenamid-P as well as metabolite M27. Latter study was used to elucidate the degradation rate of M27 in case data from the former study were not suitable. The field studies were run in accordance with current guidelines 1107/2009 to exclude surface processes.

Metabolite Grouping Strategy

The degradation pathway of Dimethenamid-P is extensive and leads to a complex array of observed metabolites in varying levels. In order to provide a more comprehensive exposure assessment of the metabolic pathway, compounds were selected for further testing to generate more data for use in exposure modeling. The compounds were selected to be in line with the grouping strategy for Dimethenamid-P. The grouping strategy stems mainly from the results of the lysimeter study, but is meant to align with toxicological testing strategies. With respect to further toxicity testing, it is proposed – in order to avoid unnecessary animal studies – not to test all degradates, but only a selection of representative key structures. The objective of this is to propose a strategy for testing of selected key structures. For this purpose the detected metabolites are compiled into groups and the key metabolites are identified within each group. The identification of these metabolites is based on their chemical structure and/or their expected transformation to other metabolites within the same group in mammals. Overlap exists between the metabolism of Dimethenamid-p in soil, plant and animal, which was also be taken into consideration for the grouping proposal. The grouping proposed in this document is mainly referring to soil and ground-water metabolites, but also includes testing strategies for crop metabolites. The grouping strategy is based on the fact that, while the proposed metabolic scheme of Dimethenamid-P is complex and contains many metabolites, most are the result of step-wise transformations of Dimethenamid-P. The grouping strategy should provide logical and scientific based representation of the metabolites while minimizing animal testing.

This grouping strategy led us to three additional metabolites for further testing. In addition to possessing ads/des and DT50 data on Dimethenamid-P and metabolites M656PH023, M656PH027 and M656031 (all observed in soil metabolism studies) we wished to gather more data on minor metabolites M656PH043, M656PH047 and M656PH054 as supporting information. Rate of degradation data (DT50) as well as adsorption/desorption data was generated for these molecules in addition to being aligned with the overall grouping strategy proposed for toxicological testing, these (six) metabolites represent all observed functionality, cover the main metabolites from sol metabolism, and also cover the highest observed metabolite concentrations estimated from the lysimeter studies.

Based on this grouping strategy attempts were made to synthesize key metabolites from the proposed scheme. Structure elucidation of the metabolites from lysimeter studies have been discussed elsewhere in this dossier [see 2014/1031599 Staudenmaier H. 2014 b] as well as strategic grouping of the metabolites for testing (See Document N4 of this dossier). Representative compounds were chosen from each group for use in toxicology studies as well as for standards in chromatography. A team of expert synthetic chemists were employed to devise and carry out synthesis of the target metabolites. Successful synthesis of metabolites M30, M45/M46, M47/M48 and M54/M58 were carried out. Much knowledge of these molecular systems was gained during the preparation and synthesis.

This knowledge was applied to the remaining metabolites. A prioritization of the remaining molecules was done ranking the difficulty of synthesis of each based on fundamental synthetic principles and retrosynthetic analysis.
This gave the order of increasing difficulty: **M62<M49<M52<M53/57<M59/60/61**.
The synthetic efforts towards M49, M52, M53/57, M59/60/61 and M62 are summarized here and are also presented in order of increasing difficulty. Despite being thorough and well planned, these strategies yielded no compounds adequate for testing. The attempts demonstrate the limitations of technical feasibility inherent in attempting to mimic natural processes. The series of transformations were challenging to conduct in a laboratory setting. Natural systems possess many tools which we do not for molecular transformations.

Synthesis of M62

The ethyl ester of Metabolite M62 was successfully synthesized for testing. The structure was confirmed by analytical techniques (NMR and MS). The molecule, though, is unstable under various conditions of saponification. During shipment, the compound quickly decarboxylated.

$-CO_2$

M62

An ethyl ester derivative of M62 has been synthesized as a surrogate for M62 for testing.

Attempts towards M49 and M52

Metabolites M49 and M52 have similar structural moieties and therefore the synthesis was designed to be convergent as the molecules could be made from a common intermediate. Once the key (common) intermediate was synthesized, the side chains could be installed to give either desired structure. It was envisioned that both of these target molecules could be easily transformed in to parent by saponification of the existing methyl or ethyl esters.

M49 M52

Synthesis of the common intermediate started with a Wittig reaction to give the unsaturated keto-ester shown. This molecule was then subjected to various conditions (10 different conditions) for halogenation with varying degrees of success.

"halogenation"

Cross coupling was then attempted with the halogenated product which was isolated in low yield. Halogenation was first attempted with the amine as the coupling partner (7 attempts under Buchwald conditions) in addition to 6 different sets of conditions with the amide derivative as the coupling partner. None of these attempts yielded the desired products. Protection of the ketone as the glycol ketal (2 attempts) or the TMS/TBS enol ether (5 attempts) also resulted in decomposition of the materials. A separate strategy involving amine substitution of the bis brominated derivative (3 attempts) also gave poor results.

A separate strategy was developed only to target M49. It was felt that if this molecule could be made, M52 could be made via a similar strategy. This route involved condensation of the amine with a diketone and subsequent carbonylation of a vicinal halide to give the methyl ester of the desired compound, M49. The reactions to for the vicinal halide (3 attempts) again yielded no product.

[CO]

A final linear route was devised to reach M49. This route had three alternative derivations (11 different failed iterations) none of which yielded any useable material.

Attempts towards M53

The isomeric mixture of M53 contained as a key structural component, a 2-thiopyranone ring. Therefore, the key step in this 11 step linear synthesis involved a cyclo-condensation to provide the ring.

M53

The first step involved Boc protection of the primary amine. Condensation under basic and neutral conditions was attempted. Decomposition of the starting material or unreacted starting materials were the only products observed after several iterations. The sequence was also tried with unprotected amine to no avail.

$(Boc)_2O$

Three separate routes were also devised for M53 but none produced any material of use.

Retrosynthetic analysis of M59

As mentioned in the introduction to this summary this brings the most difficult of molecules to synthesize. Many theoretical schemes and reactions were proposed during the retrosynthetic analysis of this molecule. Based on the similarity in structure to the other molecules in this summary no further feasible synthetic pathways could be elucidated.

Attempts at Biosynthesis

Classic synthetic methods proved fruitless towards the desired metabolites. BASF sought to try to synthesize the molecules with biological transformations. Whole cell cultures as well as isolated enzyme reactions on various substrates (DMTA-p, M11, M23 and M27) and under various conditions were attempted. Some of the reactions produced complex mixtures, but none of the desired metabolites were observed.

Despite success and knowledge generated in synthesizing the previous metabolites the remaining molecules presented many synthetic challenges. Multiple schemes, reactions, pathways and attempts were made but the remaining metabolites (other than the ester derivative of M62) could not be produced.

CA 7.1.2.1 Laboratory studies

New laboratory studies were performed with Dimethenamid-P to meet new data requirements as well as provide further bridging data on chiral stability.

CA 7.1.2.1.1 Aerobic degradation of the active substance

The previous Annex I review demonstrated that both Dimethenamid and Dimethenamid-P are extensively metabolised in a variety of different soils. In the aerobic studies, with most soils, mineralisation is rapid and CO_2 was formed at levels up to 36% of the TAR after 120 days. The same metabolites (M23, M27 and M31) were found in all studies. Only M23 (the oxalamide) and M27 (the sulfonate) were ever isolated at levels greater than 10% TAR, but these were not persistent and degraded from peak levels. M31 was found with up to 6.9% TAR, also showing a clear decline of residues from peak level. Bound residues increased with time and a major portion of the radioactivity was found in the fulvic and humic acid fractions. The degradation of Dimethenamid and Dimethenamid-P were, as expected, identical.

Dimethenamid-P and Dimethenamid are rapidly degraded in soil under laboratory conditions with DT_{50} values ranging from 8 to 38 days. There is no difference in the degradation rate between the compounds. These results were confirmed by field soil dissipation studies (see chapter 9.2). The laboratory DT_{50} values for the two metabolites, M23 and M27, are in the range of 24 to 41 days and 40 to 140 days, respectively. It is obvious that the presence of M23 and M27 is transient.

Two aerobic metabolism studies, considering EU soils, are re-analyzed (CA 7.1.2.1.2/1) including, besides parent and metabolite M23 and M27, metabolite M31 according to latest kinetic guidance.
Two additional aerobic metabolism studies, considering US soils, are re-evaluated as well. Re-analysis similarly to the EU soils could be performed (see CA 7.1.2.1.2/2) for one study (Wendt, 1997) [*see EU Annex II, section 7.1.1.1.1, 7.1/01, Wendt, D.R. (1997): Comparative aerobic soil metabolism of SAN-1289H and SAN 582 H. - BASF DocID 1997/5257*]. The second study (Krueger, 1990) [*see EU Annex II, section 7.1.1.1.1, 7.1/02, Krueger, J. P.: Aerobic Soil Metabolism of SAN-582H – BASF DocID 1990/11105*] does not meet the actual requirements for metabolite evaluation and was therefore not considered for kinetic re-evaluation. The data is not considered adequate for evaluation since 1) the material balance is at 5 of 9 sampling times in at least 1 replicate below 90%, 2) only two metabolites are investigated M23 and M27, M31 was not and 3) three out of nine sampling times are after the recommended limit of 120d after which the biological activity cannot be guaranteed any more. If the results of these 3 samplings are not included then no decrease for any of the two metabolites is observed which is in contradiction to the fate information already known of these metabolites. Kinetic evaluation of the parent (including the total mass balance) produced a DT50 slightly below the present DT50 of 38 days. Since this evaluation was performed for all sampling points (including the three samples after the 120d limit) and the DT50 is only marginally different from the original DT50, no kinetic re-evaluation for this study was performed in depth. As such, no new kinetic evaluation was reported in this dossier and the conservative DT50 of 38 days of the original study was kept.

All the results given indicate that neither Dimethenamid nor its metabolites, M23, M27 and M31, are stable in an active soil environment and therefore, no risk of persistence exists.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Report: CA 7.1.2.1.2/1
Platz K., 2008a
Kinetic evaluation of different laboratory soil degradation experiments of Dimethenamid (BAS 656 H) for derivation of modeling endpoints of the parent compound and its metabolites M23, M27 and M31
DocID 2008/1048056
Guidelines: FOCUS Kinetics (2006)
GLP: no

Executive Summary

Aerobic degradation in soil of Dimethenamid and formation and degradation of its metabolites M23, M27 and M31 have been studied in three laboratory soil experiments. The aim of this study was to evaluate the experimental data to derive degradation parameters (DT_{50}) for modeling the environmental fate of Dimethenamid and formation and degradation endpoints for its metabolites.

The data sets were evaluated following the stepwise estimation approach to derive modeling endpoints for parent and metabolites as outlined by FOCUS kinetics [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434pp.*]. The software package KinGUI version 1.1 was used for parameter estimation.

The results of the kinetic evaluation showed that the 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.

I. MATERIAL AND METHODS

The degradation behavior in soil of Dimethenamid and its metabolites M23, M27 and M31 was evaluated using 3 data sets of two aerobic degradation studies.

Table 7.1.2.1.2-1: Overview of data sets from laboratory soil degradation studies for the kinetic evaluation of the degradation behavior of Dimethenamid and its metabolites

Data set	Site	Soil type	Study type	Temp [°C] / MWHC [%]	Reference
1	BBA soil 2.2	Loamy sand	Aerobic degradation	20 / 40	*König (1995)*
2	BBA soil 2.3	Sandy loam	Aerobic degradation	20 / 40	*König (1995)*
3	Flaach	Sandy clay	Aerobic degradation	20 / 40	*König (1996)*

The observed residue data is prepared as recommended by FOCUS (*FOCUS (2006)*, p. 61, chapter 6.1).

Kinetic analysis for deriving most appropriate kinetic models

The compartment model used for the estimation approach is described in **Figure 7.1.2.1.2-1**. The different flow rates between the compartments are based on Single First Order Kinetics (SFO kinetic, Box 5-1, p. 51 in *FOCUS (2006)*). The compartment scheme and the underlying differential equations were implemented in the software package KinGUI version 1.1 [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923; BASF DocID 2007/1062781*]. Due to software restrictions, metabolites M23, M31 and M27 were termed A1, B1, C1 in the KinGUI tool.

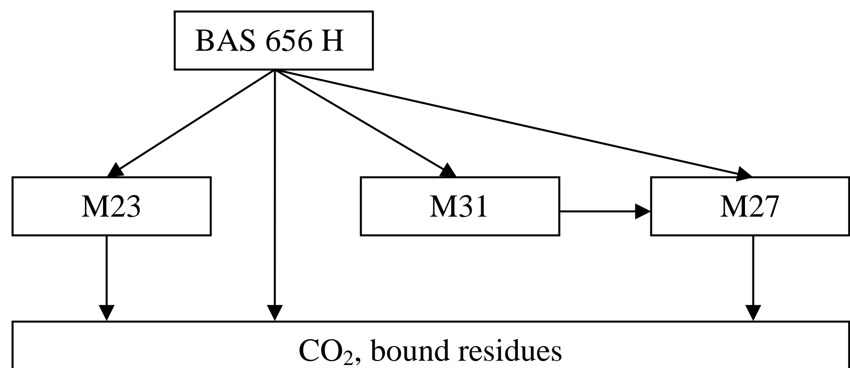


Figure 7.1.2.1.2-1: Compartment model considered in the kinetic evaluation

The KinGUI tool was used for parameter fitting. The error tolerance and the number of iterations of the optimisation tool were set to 0.00001 and 100, respectively.

The different data sets were evaluated following the stepwise procedure to derive modeling endpoints for parent compounds and their metabolites that is outlined by FOCUS (*FOCUS (2006)*, p. 156).

The initial (start) parameters settings employed in the optimisation are provided in the KinGUI report files.

The appropriateness of a distinct kinetic model to describe soil degradation can be tested with the following checks recommended by FOCUS (*FOCUS (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; p. 89 in *FOCUS (2006)*)
- t-test to evaluate whether estimated degradation parameters differ from zero (Equation 6-3; p. 93 in *FOCUS (2006)*)

A kinetic model is deemed appropriate if the residuals are randomly distributed, the χ^2 - error value is < 15 % and the estimated degradation parameters differ from zero as outlined by FOCUS (*FOCUS (2006)*, chapter 6.3.1).

II. RESULTS AND DISCUSSION

The different data sets were investigated in a stepwise procedure.

Step 1: Run (Fit) parent only with a Single First Order kinetic model (SFO)

In all data sets, the parent compound could be excellently described by a SFO kinetic as indicated by very low χ^2 error values (< 5 %) and a visual assessment of the residual plots. The residuals are randomly scattered around the zero line. For simplification, the results of the step 1 runs were not reported as the goodness of fit of the parent compound is proved as reported in step 2 and 3. (Criterion step 1 of the decision tree fulfilled).

Step 2: Run (Fit) parent and all metabolites with SFO (Table 7.1.2.1.2-2**:** - Table 7.1.2.1.2-4**:**)

The χ^2 error values of the step 2 fits of the parent compound were 3.5 %, 4.6 % and 2.3 % for data set BBA soil 2.2, BBA soil 2.3 and Flaach soil, respectively.

Table 7.1.2.1.2-2: DT_{50} and formation value and χ^2 error of data set BBA soil 2.2 (step 2)

Compartment	DT_{50} [d]	p-value	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	12.8	< 0.001	-	3.5
A1 (M23)	41.0	< 0.001	From Parent: 0.1435	9.3
B1 (M31)	61.3	< 0.001	From Parent: 0.1007	10.8
C1 (M27)	60.6	0.220	From Parent: 0.1251 From M31: 0.9990	10.4
Sink	-		From Parent: 0.6307 From M31: 0.0010 From M23/M27: 1.0000	-

Table 7.1.2.1.2-3: DT_{50} and formation values and χ^2 error of data set BBA soil 2.3 (step 2)

Compartment	DT_{50} [d]	p-value	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	13.3	< 0.001	-	4.6
A1 (M23)	23.8	< 0.001	From Parent: 0.1891	14.7
B1 (M31)	39.3	0.010	From Parent: 0.0572	11.1
C1 (M27)	43.5	0.245	From Parent: 0.1711 From M31: 0.9952	10.5
Sink	-		From Parent: 0.5826 From M31: 0.0048 From M23/M27: 1.0000	-

Table 7.1.2.1.2-4: DT_{50} and formation values and χ^2 error of data set Flaach soil (step 2)

Compartment	DT_{50} [d]	p-value	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	7.69	< 0.001	-	2.3
A1 (M23)	24.1	< 0.001	From Parent: 0.1282	11.6
B1 (M31)	37.7	< 0.001	From Parent: 0.0425	20.0
C1 (M27)	37.7	0.274	From Parent: 0.1336 From M31: 0.5140	4.7
Sink	-		From Parent: 0.6957 From M31: 0.4860 From M23/M27: 1.0000	-

The observations of the metabolites are excellently described by the model data as indicated by the visual fit and the χ^2 error values of the different fits (visual fit not shown in this step; though at step 3). The χ^2 error values range between 4.7 % and 14.7 %, except of metabolite M31 (B1) in experiment Flaach soil, which showed an error value of 20 %. The increased error level can be explained by the relatively high scattering of the model data compared to the observed low concentrations of < 5 %. However, the overall fit of metabolite M31 in experiment Flaach soil is still considered acceptable regarding the visual fit.

The formation and degradation parameters of metabolite M23 and M27 were estimated with low standard deviations and type I-error rates. In contrast, the formation fractions of metabolite M31 (B1) to metabolite M27 (C1) were estimated with very high standard deviations (variation coefficients of B1_FFC1 > 200 %) in all data sets. This uncertainty of the formation of the metabolite cause very high type I-error rates of the DT_{50} values of M27 (C1) (type I-error rate > 20 %), leading to a failure of the t-test of the degradation rate constants of M27 despite the evident visual decline of the compound in the experiments. As the criterion at step 2 to meet the goodness-of-fit criteria for each metabolite was not fulfilled, a further "case-by-case decision" was established in step 3.

Step 3: Case-by-case decision

If the formation fraction and degradation rate of a metabolite could not be estimated reliably FOCUS kinetics (*FOCUS (2006)*, chapter 8.4.2, page 153) recommends using a conservative formation fraction of 1 in combination with a conservative DT_{50} value estimated from the decline curve or a default value. Alternative conservative estimates should be allowed if there is a clear overestimation of observed metabolite residues using the default assumptions.

The formation fractions for the pathway from M31 to M27 (B1_FFC1) estimated in step 2 were 0.9990, 0.9952 and 0.5140 for the data sets BBA soil 2.2, BBA soil 2.3 and Flaach soil, respectively. The corresponding DT_{50} values of M27 (C1_k) were 60.6 d, 43.5 d and 37.7 d. Hence, it is evident that an artificial conservative DT_{50} value of 1000 d for M27 would lead to a clear overestimation of the observations of M27.

Therefore, an alternative approach was considered and only the formation fraction for the pathway from M31 to M27 was fixed to 1, while the degradation rate for M27 was estimated. The visual fit for this step are given in **Figure 7.1.2.1.2-2** to **Figure 7.1.2.1.2-4** for BBA soil 2.2, BBA soil 2.3 and Flaach, respectively.

The approach obtains the same quality of visual fit and statistical goodness-of-fit in terms of χ^2 error values (2.3 % - 19.6 %) as the previous step. However, the respective standard deviations and type I-error rates of the formation and degradation parameters are improved and statistically significant.

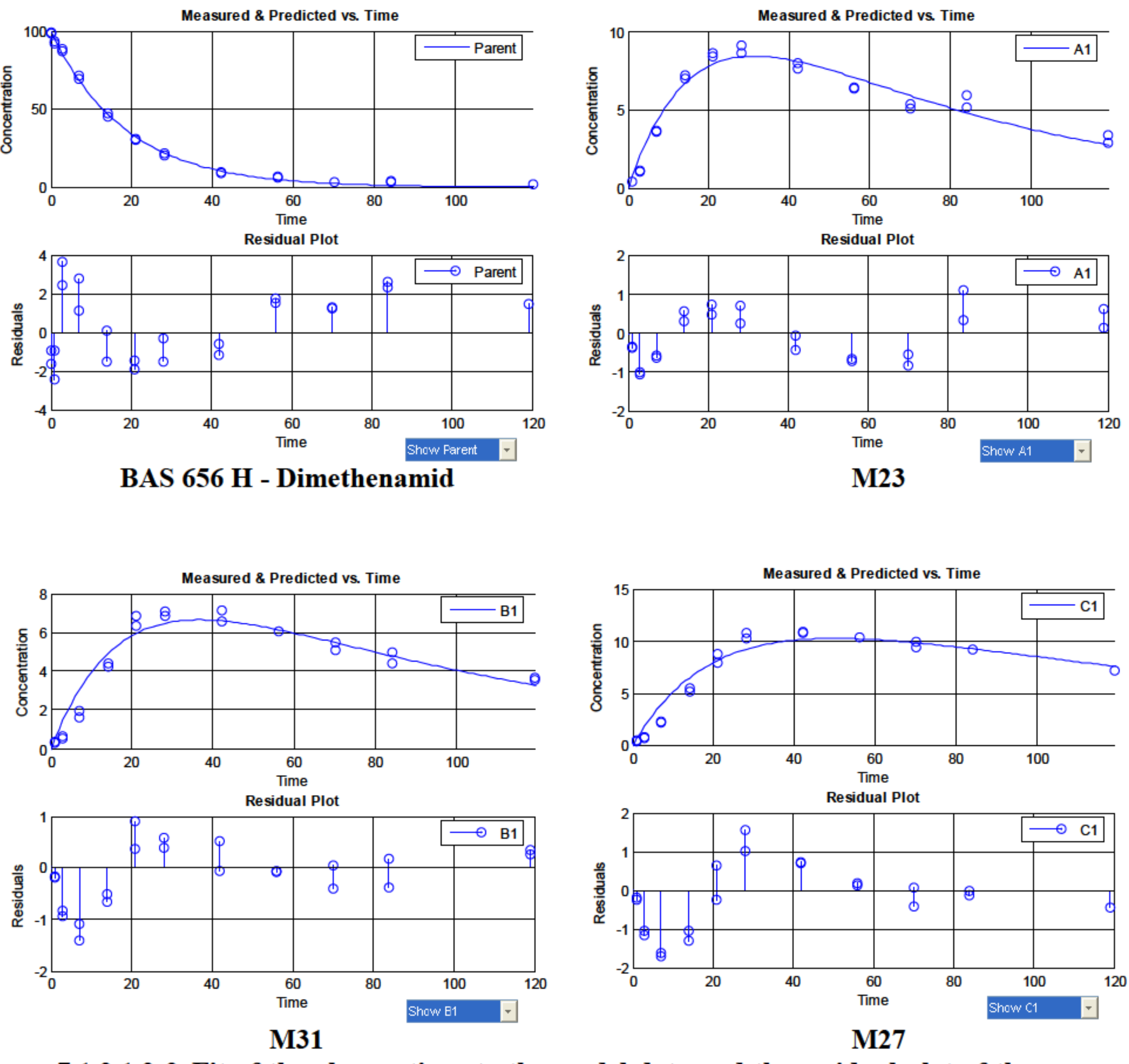


Figure 7.1.2.1.2-2: Fit of the observations to the model data and the residual plot of the parent and the metabolites of data set BBA soil 2.2 (step 3: formation fraction B1_FFC1 fixed to 1)

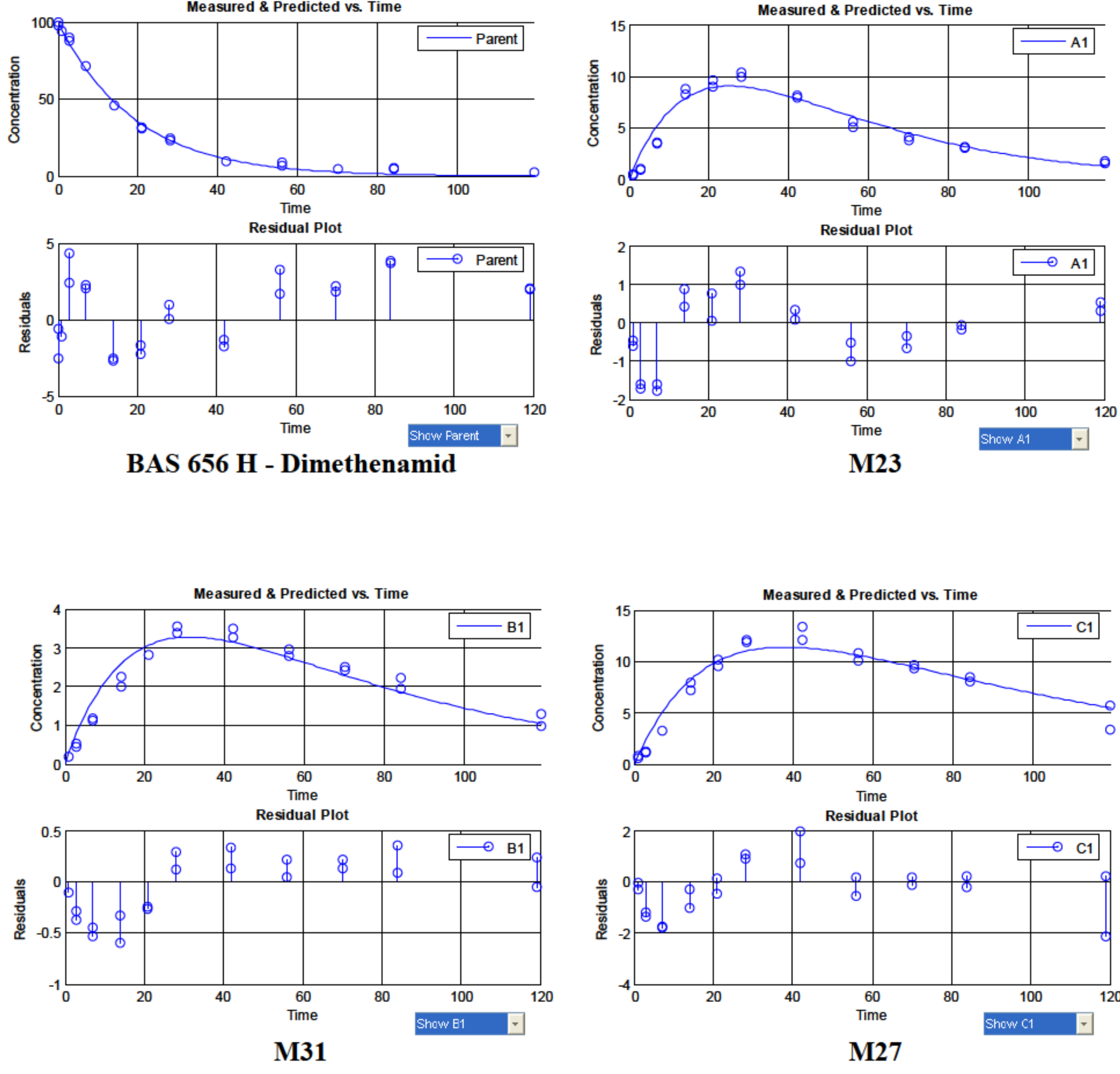


Figure 7.1.2.1.2-3: Fit of the observations to the model data and the residual plot of the parent and the metabolites of data set BBA soil 2.3 (step 3: formation fraction B1_FFC1 fixed to 1)

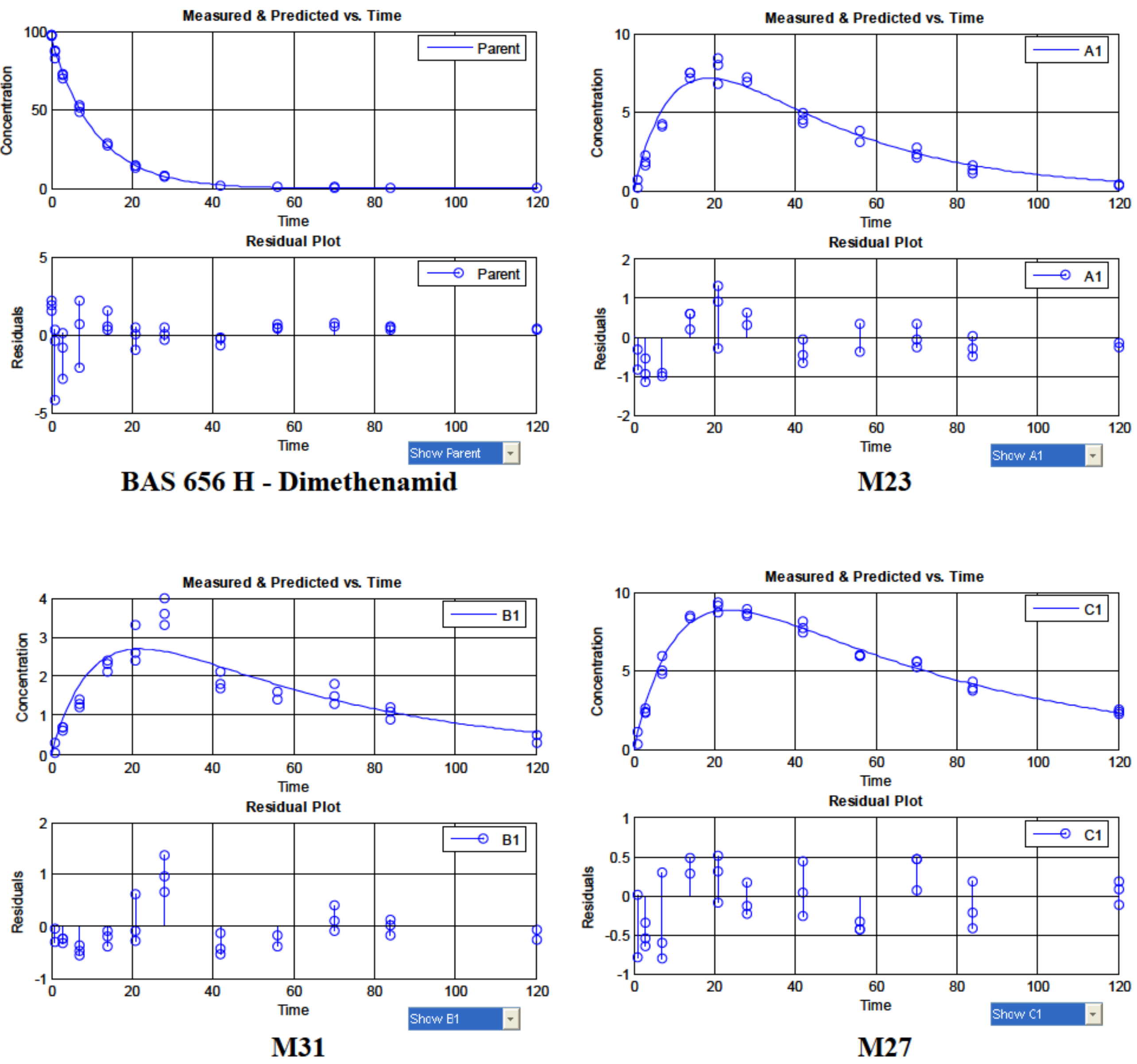


Figure 7.1.2.1.2-4: Fit of the observations to the model data and the residual plot of the parent and the metabolites of data set Flaach soil (step 3: formation fraction B1_FFC1 fixed to 1)

Apart from the excellent visual and statistical fit, the step 3 approach may also be considered conservative regarding the evaluation of mobility of the compounds in soil. The parent shows stronger sorption in soil than metabolite M31, resulting in a higher mobility of M31. In this case, higher formation of M27 due to M31 than due to the parent compound will result in higher predicted groundwater concentrations of M27.

An overview of the DT_{50} endpoints is given in **Table 7.1.2.1.2-5** and the formation fractions are summarized in **Table 7.1.2.1.2-7**.

Table 7.1.2.1.2-5: Overview of the DT_{50} endpoints of BAS 656 H (estimation approach step 3)

soil / experiment	BAS 656 H DT_{50} [d]	M23 DT_{50} [d]	M31 DT_{50} [d]	M27 DT_{50} [d]
BBA 2.2	12.8	41	61.3	60.6
BBA 2.3	13.3	23.8	39.4	43.5
Flaach	7.7	24.1	37.7	33.1

Table 7.1.2.1.2-6: Overview of the DT_{90} endpoints of BAS 656 H and its metabolites (estimation approach step 3)

soil / experiment	BAS 656 H DT_{90} [d]	M23 DT_{90} [d]	M31 DT_{90} [d]	M27 DT_{90} [d]
BBA 2.2	42.5	136.1	203.5	201.2
BBA 2.3	44.1	79.1	131.0	144.4
Flaach	25.6	80.2	125.1	110.1

Table 7.1.2.1.2-7: Overview of the formation fractions of the metabolites and the arithmetic mean values (estimation approach step 3)

soil / experiment	Parent→M23 [-]	Parent→M31 [-]	Parent→M27 [-]	Parent→Sink [-]	M31→M27 [-]
BBA 2.2	0.144	0.101	0.125	0.631	1
BBA 2.3	0.189	0.057	0.171	0.583	1
Flaach	0.128	0.043	0.133	0.696	1
Arithmetic Mean	**0.154**	**0.067**	**0.143**	**0.637**	**1**

III. CONCLUSION

Latest guidance by the FOCUS work group was considered to describe the observed degradation of Dimethenamid and formation and degradation of its metabolites. Statistical valid degradation and formation rates were obtained.

Report: CA 7.1.2.1.2/2
Bronner G., 2010a
Determination of kinetic parameters for the degradation in US-soil of BAS 656 H and its metabolites M23, M27 and M31 in laboratory incubation studies DocID 2010/1135818
Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP: no

Executive Summary

Aerobic degradation in soil of the herbicidal substance BAS 656 - Dimethenamid-P ((S)-enantiomer Dimethenamid), the racemic mixture BAS 656 - Dimethenamid ((R,S)-Dimethenamid) and the formation and degradation of their metabolites M23, M27 and M31 have been studied in two laboratory soil experiments (Wendt, 1997). The aim of the present kinetic modeling study is to evaluate the laboratory results following the latest guidance by the FOCUS work group on degradation kinetics (FOCUS, 2006) in order to derive degradation endpoints (DT_{50} values) for modelling the fate of the parent compound and formation and degradation endpoints of its metabolites.

I. MATERIAL AND METHODS

The degradation behavior in soil of BAS 656 H - Dimethenamid and its metabolites M23, M27 and M31 was evaluated using two data sets of aerobic degradation studies.

Table 7.1.2.1.2-8: Overview of data sets from laboratory soil degradation studies for the kinetic evaluation of the degradation behavior of BAS 656 H - Dimethenamid and its metabolites

Experiment (data set)	Soil designation/ Site	Soil type	Study type	Temp [°C] / [%] of MWHC	Reference
DS 1 racemic mixture ((R,S)-Dimethenamid	Elliot Clay Loam/ Champaign County, IL (USA)	Clay loam	Aerobic degradation	23 / 75% of field capacity (at 0.33 bar)	Wendt (1997)
DS 2 Dimethenamid-P ((S)-enantiomer Dimethenamid)	Elliot Clay Loam/ Champaign County, IL (USA)	Clay loam	Aerobic degradation	23 / 75% of field capacity (at 0.33 bar)	Wendt (1997)

The compartment model used for the estimation approach was also described in [PLATZ, K. (2008); CA 7.1.2.1.2/1].

II. RESULTS AND DISCUSSION

The different data sets were investigated in a stepwise procedure:

Step 1: Run (Fit) parent only with a Single First Order kinetic model (SFO)
In all data sets, the parent compound could be well described by an SFO kinetic as indicated by very low χ^2 error values (< 10 %) and a visual assessment of the residual plots. The residuals are randomly scattered around the zero line. For simplification, the results of the step 1 runs were not reported as the goodness of fit of the parent compound is proved as reported in step 2 (Criterion step 1 of the decision tree fulfilled).

Step 2: Run (Fit) parent and all metabolites with SFO
As mentioned in step 1 the parent compound could be well described by an SFO kinetic. The χ^2 error values of the step 2 fits of the parent compound were 8.7% and 8.5% for data set DS 1 and DS 2, respectively.

Table 7.1.2.1.2-9: DT_{50}, formation fractions and χ^2 error of data DS 1 (racemic mixture of Dimethenamid) for SFO kinetic at step 2

Compartment	DT_{50} [d]	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	9.4	-	8.7
A1 (M23)	30.2	From Parent: 0.130	12.9
B1 (M31)	62.0	From Parent: 0.101	26.9
C1 (M27)	59.8	From Parent: 0.111 From M31: 0.661	13.2
Sink	-	From Parent: 0.658 From M31: 0.339 From M23/M27: 1.000	-

Table 7.1.2.1.2-10: DT_{50}, formation fractions and χ^2 error of data set DS 2 ((S)-enantiomer of Dimethenamid) for SFO kinetic at step 2

Compartment	DT_{50} [d]	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	9.3	-	8.5
A1 (M23)	26.1	From Parent: 0.117	19.6
B1 (M31)	54.8	From Parent: 0.121	12.1
C1 (M27)	57.2	From Parent: 0.112 From M31: 0.643	7.21
Sink	-	From Parent: 0.649 From M31: 0.358 From M23/M27: 1.000	-

The observations of the metabolites are well described by the model data as indicated by the visual fit and the χ^2 error values of the different fits. The χ^2 error values range between 7.21 % and 13.2 % except of metabolite M31 (B1) in experiment DS 1 and metabolite M23 (A1) in experiment DS 2, which showed an error value of 19.6 and 26.9 %, respectively. The increased error level can be explained by the relatively high scattering of the model data compared to the observed low concentrations of < 5 %. However, in one of the two experiments these two metabolites were fitted with χ^2 error values below 15%. Hence the poor fit is likely due to some random inaccurate values and the overall fit of metabolite M31 in experiment DS 1 and of M23 in experiment DS 2 can still be considered acceptable regarding the visual fit.

The degradation parameters of metabolite M23 and M31 were estimated with low standard deviations and type I-error rates. In contrast, the high uncertainty of the formation of the metabolite M27 from M31 causes very high type I-error rates of the estimated DT_{50} values of M27 (C1) (type I-error rate > 20 %), leading to a failure of the t-test of the degradation rate constants of M27 despite the evident visual decline of the compound in the experiments. As the criterion at step 2 to meet the goodness-of-fit criteria for each metabolite was not fulfilled, a further "case-by-case decision" was established in step 3.

Step 3: Case-by-case decision

If the formation fraction and degradation rate of a metabolite could not be estimated reliably, FOCUS kinetics recommends using a conservative formation fraction of 1 in combination with a conservative DT_{50} value estimated from the decline curve or a default value. Alternative conservative estimates should be allowed if there is a clear overestimation of observed metabolite residues using the default assumptions.

The formation fractions for the pathway from M31 to M27 (B1_FFC1) estimated in step 2 were 0.66 and 0.64 for the data sets DS 1 and DS 2, respectively. The corresponding DT_{50} values of M27 (C1_k) were 59.8 d and 57.2 d. Hence, it is evident that an artificial conservative DT_{50} value of 1000 d for M27 would lead to a clear overestimation of the observations of M27.

Therefore, an alternative approach was considered and only the formation fraction for the pathway from M31 to M27 was fixed to 1, while the degradation rate for M27 was estimated.

With this approach the same quality of visual fit and statistical goodness-of-fit in terms of χ^2 error values (7.12% - 26.9 %) as the previous step are obtained. However, the standard deviations and type I-error rates of the degradation parameters of M27 have decreased and are statistically significant.

Table 7.1.2.1.2-11: DT_{50}, formation parameters and χ^2 error of data set DS 1 (racemic mixture of Dimethenamid) for SFO kinetic at step 3 (formation fraction B1 FFC1 fixed to 1)

Compartment	DT_{50} [d]	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	9.4	-	8.7
A1 (M23)	30.1	From Parent: 0.131	12.9
B1 (M31)	63.6	From Parent: 0.1	26.9
C1 (M27)	49.4	From Parent: 0.109 **From M31: 1.000**	12.8
Sink	-	From Parent: 0.660 **From M31: 0.000** From M23/M27: 1.000	-

Table 7.1.2.1.2-12: DT_{50}, formation fractions and χ^2 error of data set DS 2 ((S)-enantiomer of Dimethenamid) for SFO kinetic at step 3 (formation fraction B1_FFC1 fixed to 1)

Compartment	DT_{50} [d]	Formation fractions [-]	χ^2 error [%]
Parent (BAS 656 H)	9.3	-	8.5
A1 (M23)	26.2	From Parent: 0.117	19.6
B1 (M31)	55.9	From Parent: 0.120	12.1
C1 (M27)	45.6	From Parent: 0.110 **From M31: 1.000**	7.1
Sink	-	From Parent: 0.653 **From M31: 0.000** From M23/M27: 1.000	-

Apart from the good visual and statistical fit, the step 3 approach may also be considered conservative regarding the evaluation of mobility of the compounds in soil. The parent shows stronger sorption in soil than metabolite M31, resulting in a higher mobility of M31. In this case, higher formation of M27 due to M31 results in higher groundwater concentrations of M27 also.

Table 7.1.2.1.2-13: Overview of the DT_{50} endpoints of BAS 656 H and its metabolites (estimation approach step 3)

soil / experiment	BAS 656 H DT_{50} [d]	M23 DT_{50} [d]	M31 DT_{50} [d]	M27 DT_{50} [d]
Elliot Clay Loam/ DS 1	9.40	30.1	63.6	49.4
Elliot Clay Loam/ DS 2	9.32	26.2	55.9	45.6

Table 7.1.2.1.2-14: Overview of the DT_{90} endpoints of BAS 656 H and its metabolites (estimation approach step 3)

soil / experiment	BAS 656 H DT_{90} [d]	M23 DT_{90} [d]	M31 DT_{90} [d]	M27 DT_{90} [d]
Elliot Clay Loam/ DS 1	31.2	99.9	211.4	163.9
Elliot Clay Loam/ DS 2	31.0	87.2	185.8	151.4

Table 7.1.2.1.2-15: Overview of the formation fractions of the metabolites and the arithmetic mean values (estimation approach step 3)

soil / experiment	Parent→M23 [-]	Parent→M31 [-]	Parent→M27 [-]	Parent→Sink [-]	M31→M27 [-]
Elliot Clay Loam/ DS 1	0.131	0.100	0.109	0.660	1
Elliot Clay Loam/ DS 2	0.117	0.120	0.110	0.653	1
Arithmetic Mean	**0.124**	**0.110**	**0.110**	**0.656**	**1**

III. CONCLUSION

Latest guidance by the FOCUS work group was considered to describe the observed degradation of Dimethenamid/Dimethenamid-P and formation and degradation of its metabolites. Statistical valid degradation and formation rates were obtained.

Normalisation of laboratory DT50 to 20°C and pF2

Table 7.1.2.1.2-16: Revised temperature and moisture correction factors according to FOCUS

	Platz (2008)			Krueger (1990)	Bronner (2010)
Soil	Flaach	BBA 2.2	BBA 2.3	Kenyon	Elliott
Soil texture	sandy clay loam	loamy sand	sandy loam	loam	clay loam
Actual temperature (°C)	20	20	20	25	23
Temperature correction factor ($2.58^{(Tact-20)/10}$)	1	1	1	1.606	1.329
Actual moisture	40% MWHC	40% MWHC	40% MWHC	75% FC	75% FC
Moisture at MWHC / FC1/3b* [%] ($g\ g^{-1}$)	28	24	27	32.5	33.4
Moisture of study θact [%] ($g\ g^{-1}$)	11.2	9.6	10.8	24.4	25.0
Moisture at pF2 θpF2 [%] ($g\ g^{-1}$)	22	14	19	25	28
Moisture correction factor $(\theta act/\theta pF2)^{0\ 7}$	0.623	0.768	0.673	0.983	0.924

* FC1/3b (field capacity following US classification) =33.33 kPa

Table 7.1.2.1.2-17: Summary of aerobic degradation rates of Dimethenamid-P / dimethenamid in laboratory soils

Test Substance	Soil	Soil type	pH	Temp. °C / moisture	DT_{50} (d)	DT_{90} (d)	DT_{50} (d) 20 °C pF2/10kPa (Q10 of 2.58)	Method of calcula-tion
Dimethenamid	Flaach	sandy clay loam	7.5	20 / 40%MWHC	7.7	25.6	4.8	SFO
Dimethenamid	BBA 2.2	loamy sand	5.8	20 / 40%MWHC	12.8	42.5	9.8	SFO
Dimethenamid	BBA 2.3	sandy loam	6.6	20 / 40%MWHC	13.3	44.1	9.0	SFO
Dimethenamid	Kenyon	loam	6.0	25/ 75% FC	38	126.2*	60	SFO
Dimethenamid-P	Elliot	clay loam	6.4	23 / 75% FC	9.4	31.2	11.5	SFO
Dimethenamid	Elliot	clay loam	6.4	23 / 75% FC	9.32	31.0	11.4	SFO

* calculated from DT_{50} multiplied by 3.32 after FOCUS kinetics (2006)

Table 7.1.2.1.2-18: Summary of aerobic degradation rates of metabolites of dimethenamid / Dimethenamid-P (M23, M27 and M31) in laboratory soils

Test Substance	Soil	temp. °C / moisture	M23		M27		M31	
			DT_{50} (d)	DT_{50} (d) 20 °C pF2/10kPa (Q10 of 2.58)	DT_{50} (d)	DT_{50} (d) 20 °C pF2/10kPa (Q10 of 2.58)	DT_{50} (d)	DT_{50} (d) 20 °C pF2/10kPa (Q10 of 2.58)
Dimethenamid	Flaach	20 / 40%MWHC	24.1	15.0	33.1	20.6	37.7	23.5
Dimethenamid	BBA 2.2	20 / 40%MWHC	41.0	31.5	60.6	46.5	61.3	47.1
Dimethenamid	BBA 2.3	20 / 40%MWHC	23.8	16.0	43.5	29.3	39.4	26.5
Dimethenamid-P	Elliot	23 / 75% FC	30.1	37.0	49.4	60.7	63.6	78.1
Dimethenamid	Elliot	23 / 75% FC	26.2	32.2	45.6	56.0	55.9	68.7

Report:	CA 7.1.2.1.2/3 Class T.,Heinz N., 2014a Aerobic soil degradation of the three Dimethenamid-P metabolites M656PH054 (Reg.No. 5920718), M656PH047 (Reg.No. 5917260), M656PH043 (Reg.No. 5917262) in three soils (OECD Guideline 307) 2013/1348091
Guidelines:	OECD 307 (2002), BBA VI 4-1 (December 1986), EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of the study was to examine aerobic degradation of the three dimethenamid-P metabolites M656PH054 (Reg No. 5920718), M656PH047 (Reg No. 5917260), M656PH043 (Reg No. 5917262) in three soils.

The following three field-fresh soils were provided: Loamy sand (Li10), loamy sand (LUFA 5M) and loamy sand (LUFA 2.2) (soil class according to DIN). The soils were acclimatized with soil moistures adjusted to 40 % of their maximum water holding capacities (MWHC).

The residues observed for the analytes (expressed as µg/kg) in the incubated soil samples were fitted by using the software package KinGUI (v2). The trigger endpoints (DT50 values) of the dimethenamid-P metabolite M656PH054 range from 22 – 40 days, for M656PH047 from 43 – 95 days and for M656PH043 from 10 – 30 days.

I. MATERIAL AND METHODS

The objective of the study was to examine aerobic degradation of the three dimethenamid-P metabolites M656PH054 (Reg No. 5920718), M656PH047 (Reg No. 5917260), M656PH043 (Reg No. 5917262) in three soils.

A. MATERIALS

The following analytical standards were used:

[chemical structure]	[chemical structure]	[chemical structure]
M656PH054 Reg. No. 5920718	M656PH047 Reg. No. 5917260	M656PH043 Reg. No. 5917262

B. STUDY DESIGN

Three field-fresh soils were provided: Loamy sand Li10, loamy sand LUFA 5M and loamy sand LUFA 2.2 (soil class according to DIN). Soils were kept under aerobic conditions at about 5 °C for about 12 days, then water was adjusted to 40 % of mwhc and the soils were acclimatized 21 days at room temperature in the dark with soil moistures adjusted to 40 % of their maximum water holding capacities.

The nominal application rate (based on dry soil weight) of M656PH054, M656PH047 and M656PH043 dosed to bulk soil was 0.5 mg/kg. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g/cm^3 this corresponds to a theoretical field application rate of about 200 g/ha. Upon correction for the purity of the test item M656PH047, the actual application rate of M656PH047 was 0.127 mg/kg or about 50 g/ha.

Incubation flasks with 50-g of dosed dry soil equivalents were placed in thermostated cabinet(s) set to 20±1°C and thus be kept in the dark. The dosed soil samples were incubated for various intervals up to 118 days prior to extraction.

The analytical method achieved a limit of quantitation (LOQ) of 0.025 mg/kg or 0.006 mg/kg for M656PH047.

Application procedure

The analytes were applied as 500 µg/mL or 127 µg/mL (for M656PH047) solution in methanol in small doses of 250 µL (in total 1.8 mL) to 1.8 kg (dry-weight equivalent) of soil bulk mass, which was measured in an appropriately sized and shaped stainless-steel vessel, while homogenizing the soil with a hand-hold mixer between dosing. After 15 minutes of homogenization, the water content was measured / re-adjusted gravimetrically and the soil was again mixed for another 3 minutes.

Incubation
The 50-g of dry soil mass equivalents in the incubation 500-mL vessels were incubated at 20±1 °C in a thermostated cabinet in the dark. The dosed soil samples were incubated for various intervals up to 118 days prior to extraction. For the soil Lufa 5M the incubations were stopped upon 90 days for Reg. No. 5920718 and 75 days for Reg. No. 5917262, because less than 10 % of the residue applied initially was found.

Soil Extraction
10.0 g soil sample were weighed into a centrifuge tube. For concurrent fortifications, 250 µL of the 1.0 or 0.254 µg/mL (for M656PH047) spike solution or 100 µL of the 50 or 12.7 µg/mL (for M656PH047) spike solution were added to untreated soil samples.

The soil samples were extracted with 25 mL of methanol by shaking for 30 minutes on a horizontal shaker and sonication for 10 minutes in an ultrasonic bath, followed by centrifugation (5 minutes at 4000 rpm). After centrifugation, the supernatant was decanted into a volumetric flask through a glass funnel fitted with silanized glass wool. The extraction was repeated two more times with each 25 mL of methanol/water (1/1;v/v), the extracts were combined and diluted to exactly 100 mL (V_{End}) with methanol. An aliquot of the final extract was diluted by a factor DF of 5 with methanol/water (1/1;v/v) and injected for LC-MS/MS analysis.

LC/MS/MS Method
Soil extracts and calibration solutions were analyzed by liquid chromatography with tandem mass spectrometric detection (LC/MS/MS):

The quantitative determination was carried out by external standardization using calibration standards in solvent. Calibration functions ranging from 0.10 to 100 ng/mL and 0.0254 to 25.4 ng/mL for M656PH047 (≥ 6 levels) were used to quantify M656PH054, M656PH047 and M656PH043 residues.

Calculations of Residues and Recoveries with Example
Calculations were performed by Microsoft Excel with full precision, but were rounded in the tables. Minor discrepancies may arise when recalculated with pocket calculator.

LC/MS/MS determined the concentration c_{End} of the analyte in the soil extracts. c_{End} was used to calculate the residue R (in mg/kg) of the analyte extracted from 10 g (dry soil mass W) of soil with extraction solvent (V_{Ex} = 100 mL).

$$R = c_{End} \times DF \times (V_{Ex} / W) / 1000\ ng/\mu g = c_{End} \times \text{Multiplier } M \times DF$$

Calculation of Degradation Times
The observed results of the analytes in the incubated soil samples (expressed as µg/kg) were fitted using the software package KinGUI version 2. The kinetics analyses followed the “Recommended procedures to derive endpoints for parent compounds” as outlined by FOCUS in Chapter 7. Kinetics analyses were performed as recommended in Chapter 7.1.1 to derive trigger endpoints.

II. RESULTS AND DISCUSSION

The objective of the study was to examine aerobic degradation of M656PH054, M656PH047 and M656PH043 (metabolites of dimethenamid-P) in 3 different soils.

The nominal application rates were 0.5 mg/kg for M656PH054, M656PH043 and 0.127 mg/kg for M656PH043. After given incubation periods, replicate soil incubations were collected and soil was extracted for subsequent LC/MS/MS determination of the analytes.

The analytical method was validated and achieves a limit of quantitation (LOQ) of 0.025 mg/kg for M656PH054 and M656PH043 and 0.006 mg/kg for M656PH047.

Aerobic Soil Degradation

Residues of the analytes remaining and extracted after given incubation periods are summarized in Table 7.1.2.1.2-19 to Table 7.1.2.1.2-27.
Soil results in µg/kg were used for subsequent KinGUI kinetic modelling calculations. The applied residue (AR) was nominally 0.50 mg/kg (based on dry soil weight)

These tables also show the remaining analyte as percentage of the initially applied residue (% AR: nominally 0.5 mg/kg of M656PH054 and M656PH047 and 0.127 mg/kg for M656PH043) at the beginning of the incubation.

The rate of aerobic soil degradation of M656PH054, M656PH047 and M656PH043 is given in the Table 7.1.2.1.2-19 to Table 7.1.2.1.2-27 and visualized in Figure 7.1.2.1.2-5 to Figure 7.1.2.1.2-13.

Table 7.1.2.1.2-19: M656PH054 Aerobic Soil Degradation Results in Soil LUFA 2.2

Days	mg/kg	µg/kg	% AR#
0	0.463	463	92.7
0	0.424	424	84.7
0	0.467	467	93.3
2	0.437	437	87.4
2	0.449	449	89.7
7	0.383	383	76.5
7	0.386	386	77.2
14	0.350	350	69.9
14	0.383	383	76.5
21	0.316	316	63.1
21	0.297	297	59.3
29	0.272	272	54.4
29	0.256	256	51.2
43	0.206	206	41.1
43	0.198	198	39.6
61	0.170	170	33.9
61	0.158	158	31.6
75	0.160	160	32.0
75	0.152	152	30.4
90	0.131	131	26.1
90	0.140	140	28.0
105	0.132	132	26.4
105	0.132	132	26.3
118	0.130	130	26.0
118	0.132	132	26.4

% of Applied Residue

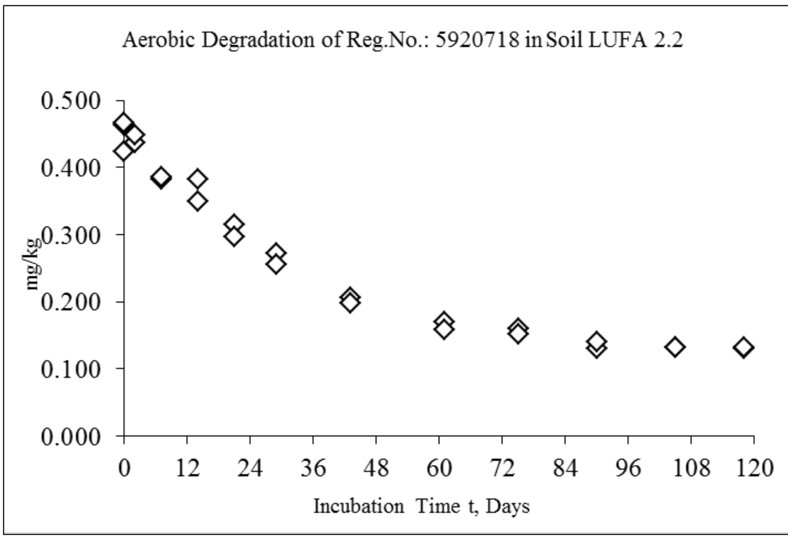


Figure 7.1.2.1.2-5: M656PH054 Aerobic Soil Degradation Results in Soil LUFA 2.2

Table 7.1.2.1.2-20: M656PH054 Aerobic Soil Degradation Results in Soil LUFA 5M

Days	mg/kg	µg/kg	% AR#
0	0.493	493	98.6
0	0.455	455	90.9
0	0.485	485	96.9
2	0.372	372	74.4
2	0.400	400	80.0
7	0.342	342	68.3
7	0.325	325	64.9
14	0.289	289	57.7
14	0.294	294	58.7
21	0.267	267	53.3
21	0.225	225	44.9
29	0.177	177	35.4
29	0.154	154	30.8
43	0.128	128	25.5
43	0.123	123	24.6
61	0.066	66	13.1
61	0.062	62	12.4
75	0.038	38	7.50
75	0.034	34	6.77
90	0.0044	4.4	0.89
90	0.0085	8.5	1.70

% of Applied Residue

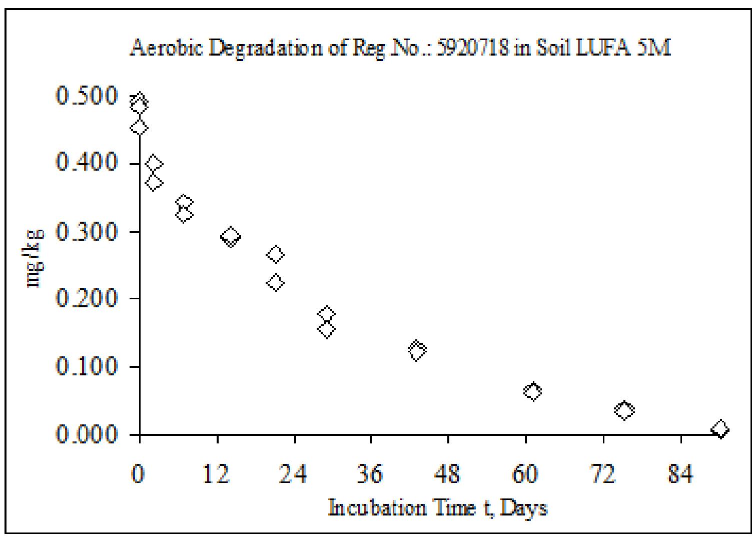


Figure 7.1.2.1.2-6: M656PH054 Aerobic Soil Degradation Results in Soil LUFA 5M

Table 7.1.2.1.2-21: M656PH054 Aerobic Soil Degradation Results in Soil Li10

Days	mg/kg	µg/kg	% AR[#]
0	0.492	492	98.5
0	0.500	500	100.0
0	0.491	491	98.1
2	0.466	466	93.1
2	0.464	464	92.8
7	0.422	422	84.3
7	0.430	430	86.0
14	0.361	361	72.1
14	0.364	364	72.7
21	0.329	329	65.8
21	0.315	315	63.0
29	0.282	282	56.3
29	0.283	283	56.5
43	0.229	229	45.7
43	0.189	189	37.8
61	0.144	144	28.8
61	0.157	157	31.3
75	0.129	129	25.7
75	0.142	142	28.3
90	0.087	87	17.3
90	0.099	99	19.7
105	0.059	59	11.8
105	0.063	63	12.5
118	0.053	53	10.6
118	0.054	54	10.8

% of Applied Residue

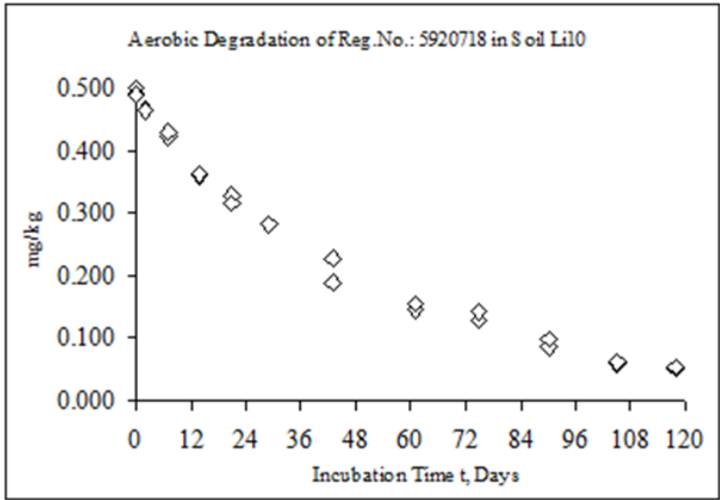


Figure 7.1.2.1.2-7: M656PH054 Aerobic Soil Degradation Results in Soil Li10

Table 7.1.2.1.2-22: M656PH0047 Aerobic Soil Degradation Results in Soil LUFA 2.2

Days	mg/kg	µg/kg	% AR[#]
0	0.479	479	95.8
0	0.449	449	89.8
0	0.500	500	100.0
2	0.469	469	93.8
2	0.477	477	95.3
7	0.474	474	94.8
7	0.472	472	94.3
14	0.455	455	91.0
14	0.490	490	98.0
21	0.448	448	89.5
21	0.418	418	83.6
29	0.390	390	77.9
29	0.386	386	77.2
43	0.357	357	71.4
43	0.364	364	72.8
61	0.270	270	53.9
61	0.259	259	51.8
75	0.254	254	50.8
75	0.245	245	49.0
90	0.229	229	45.7
90	0.243	243	48.5
105	0.222	222	44.4
105	0.225	225	44.9
118	0.217	217	43.3
118	0.217	217	43.4

[#] % of Applied Residue

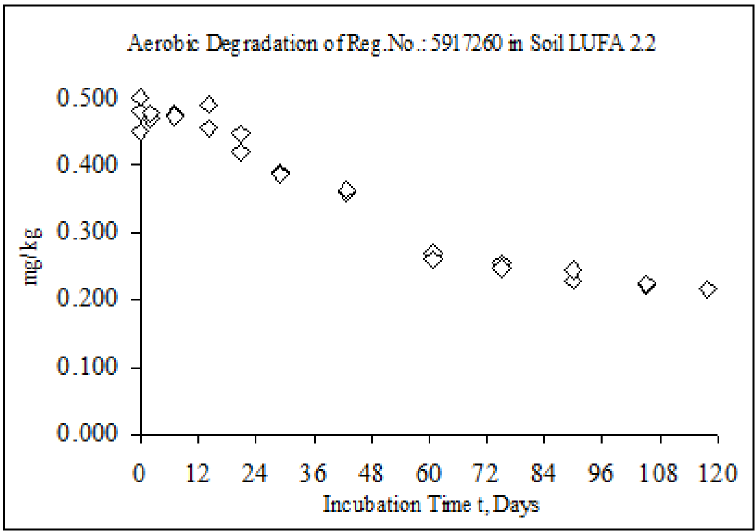


Figure 7.1.2.1.2-8: M656PH047 Aerobic Soil Degradation Results in Soil LUFA 2.2

Table 7.1.2.1.2-23: M656PH0047 Aerobic Soil Degradation Results in Soil LUFA 5M

Days	mg/kg	µg/kg	% AR[#]
0	0.493	493	98.6
0	0.463	463	92.6
0	0.499	499	99.8
2	0.413	413	82.6
2	0.445	445	88.9
7	0.454	454	90.8
7	0.412	412	82.4
14	0.459	459	91.8
14	0.460	460	92.0
21	0.421	421	84.2
21	0.388	388	77.6
29	0.357	357	71.3
29	0.328	328	65.6
43	0.333	333	66.5
43	0.327	327	65.3
61	0.247	247	49.3
61	0.245	245	49.0
75	0.141	141	28.2
75	0.135	135	26.9
90	0.043	43	8.68
90	0.059	59	11.7
105	0.015	15	3.06
105	0.012	12	2.38
118	0.0086	8.6	1.71
118	0.0055	5.5	1.10

[#] % of Applied Residue

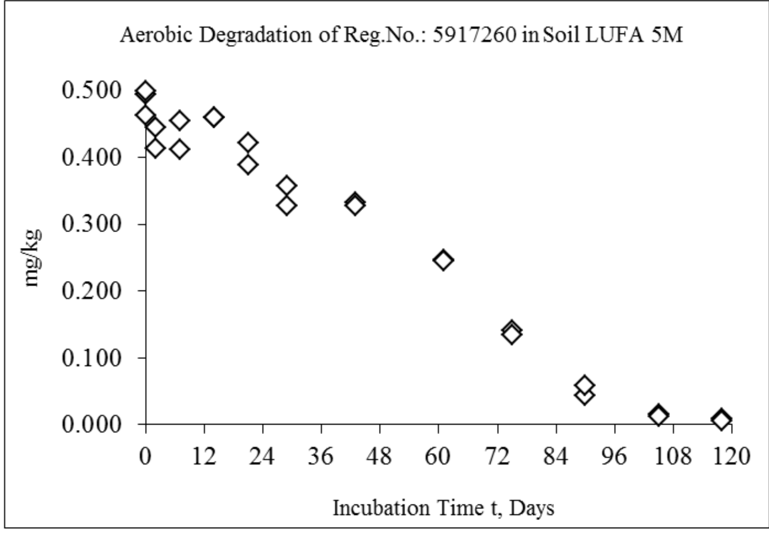


Figure 7.1.2.1.2-9: M656PH047 Aerobic Soil Degradation Results in Soil LUFA 5M

Table 7.1.2.1.2-24: M656PH0047 Aerobic Soil Degradation Results in Soil Li10

Days	mg/kg	µg/kg	% AR[#]
0	0.488	488	97.5
0	0.520	520	104.0
0	0.500	500	99.9
2	0.505	505	101.0
2	0.499	499	99.8
7	0.505	505	101.0
7	0.505	505	101.0
14	0.481	481	96.1
14	0.470	470	94.0
21	0.436	436	87.2
21	0.432	432	86.4
29	0.435	435	86.9
29	0.436	436	87.1
43	0.405	405	80.9
43	0.377	377	75.3
61	0.375	375	74.9
61	0.384	384	76.7
75	0.343	343	68.6
75	0.358	358	71.6
90	0.270	270	53.9
90	0.297	297	59.4
105	0.183	183	36.6
105	0.190	190	38.0
118	0.180	180	36.0
118	0.186	186	37.2

[#] % of Applied Residue

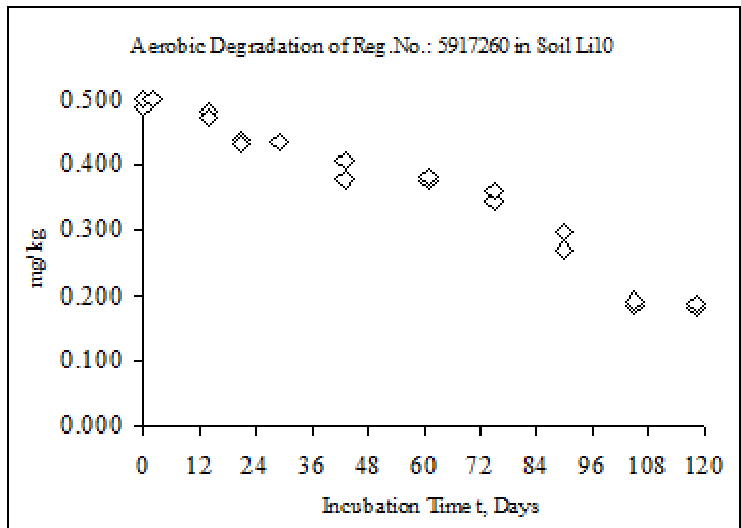


Figure 7.1.2.1.2-10: M656PH047 Aerobic Soil Degradation Results in Soil Li10

Table 7.1.2.1.2-25: M656PH0043 Aerobic Soil Degradation Results in Soil LUFA 2.2

Days	mg/kg	µg/kg	% AR#
0	0.463	463	92.5
0	0.432	432	86.4
0	0.488	488	97.6
2	0.445	445	88.9
2	0.461	461	92.1
7	0.359	359	71.8
7	0.360	360	71.9
14	0.308	308	61.6
14	0.351	351	70.2
21	0.286	286	57.1
21	0.273	273	54.5
29	0.237	237	47.4
29	0.226	226	45.1
43	0.184	184	36.7
43	0.177	177	35.3
61	0.161	161	32.2
61	0.144	144	28.7
75	0.151	151	30.2
75	0.136	136	27.2
90	0.120	120	24.0
90	0.129	129	25.8
105	0.120	120	24.0
105	0.118	118	23.6
118	0.111	111	22.1
118	0.118	118	23.5

% of Applied Residue

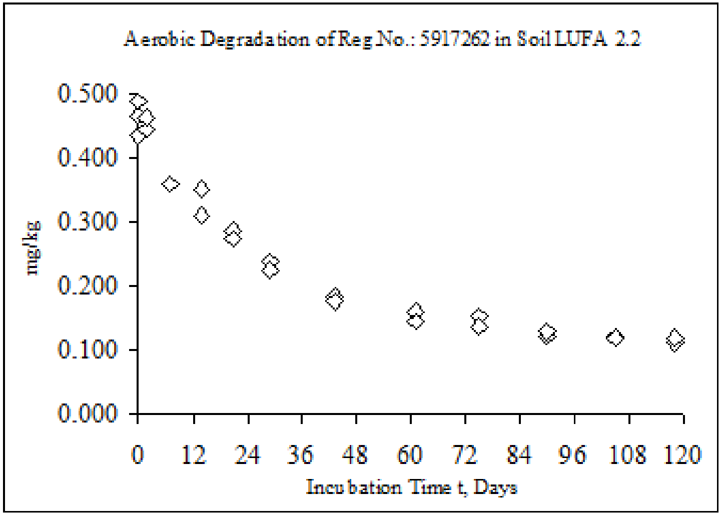


Figure 7.1.2.1.2-11: M656PH043 Aerobic Soil Degradation Results in Soil LUFA 2.2

Table 7.1.2.1.2-26: M656PH0043 Aerobic Soil Degradation Results in Soil LUFA 5M

Days	mg/kg	µg/kg	% AR#
0	0.477	477	95.5
0	0.454	454	90.7
0	0.487	487	97.3
2	0.352	352	70.4
2	0.385	385	76.9
7	0.250	250	49.9
7	0.245	245	49.0
14	0.176	176	35.2
14	0.172	172	34.3
21	0.146	146	29.1
21	0.117	117	23.4
29	0.065	65	13.0
29	0.057	57	11.4
43	0.028	28	5.57
43	0.027	27	5.35
61	0.011	11	2.19
61	0.0078	7.8	1.55
75	0.0058	5.8	1.15
75	0.0057	5.7	1.14

% of Applied Residue

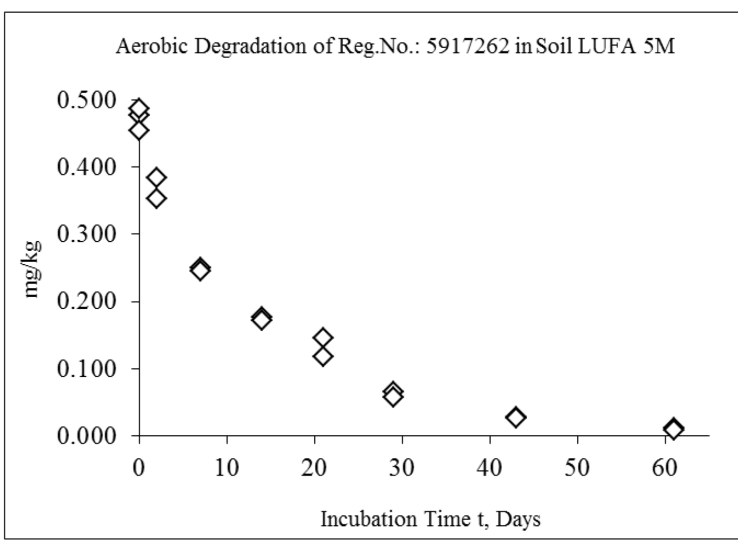


Figure 7.1.2.1.2-12: M656PH043 Aerobic Soil Degradation Results in Soil LUFA 5M

Table 7.1.2.1.2-27: M656PH0043 Aerobic Soil Degradation Results in Soil Li10

Days	mg/kg	µg/kg	% AR[#]
0	0.476	476	95.2
0	0.520	520	104.0
0	0.497	497	99.4
2	0.469	469	93.7
2	0.468	468	93.5
7	0.365	365	73.0
7	0.365	365	72.9
14	0.300	300	60.0
14	0.301	301	60.2
21	0.266	266	53.2
21	0.263	263	52.6
29	0.229	229	45.8
29	0.220	220	44.0
43	0.173	173	34.6
43	0.141	141	28.1
61	0.111	111	22.2
61	0.123	123	24.6
75	0.121	121	24.2
75	0.135	135	26.9
90	0.096	96	19.2
90	0.101	101	20.2
105	0.080	80	16.0
105	0.089	89	17.8
118	0.074	74	14.8
118	0.076	76	15.1

[#] % of Applied Residue

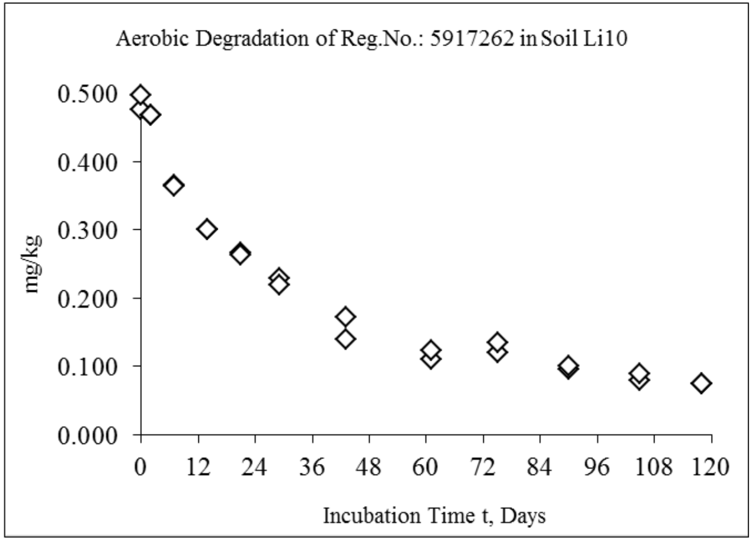


Figure 7.1.2.1.2-13: M656PH043 Aerobic Soil Degradation Results in Soil Li10

Kinetics Analysis of Soil Degradation for M656PH054

The outcome of the kinetic analyses for M656PH054 is summarized in Table 7.1.2.1.2-28**:**
.

Trigger endpoints:
The kinetics analyses were interpreted firstly as recommended in Chapter 7.1.1 (page 110ff) to derive trigger endpoints, with a detailed flow chart (assigned as Figure 7-1) given on page 112 of the FOCUS (SANCO/10058/2005, version 2.0, Jun-06) document.

- Step 1: Is SFO more appropriate than FOMC and gives acceptable fit?
  - Single First Order (SFO) and First-Order Multi-Compartment (FOMC) kinetics were modelled.
  - SFO kinetics with χ^2 errors in the range of 2.35 % to 7.40 %. would be acceptable for all three soils.
  - The t-test applied for the rate constants k resulted with SFO always in type I error rates and probabilities p > t of <0.001.
  - FOMC seems statistically for soil Lufa 2.2 with χ^2 error of 4.11% more appropriate than SFO. This holds true for Lufa 2.2. For Lufa 5M and Li10 the FOMC fits were worse than SFO (larger χ^2 errors, for Lufa 5M t-test not passed). Therefore, FOMC not better than SFO for Lufa 5M and Li10.

- Step 2: Identify best model other than SFO.
  - For soil Lufa 2.2 the DFOP (Double-First-Order in Parallel) has a better visual fit and lower χ^2 error than FOMC, however, the t-test failed on the rate k_2. No reliable fit with DFOP can be obtained.
  - For Lufa 5M and Li10 the t-test failed for more than 1 parameter. No reliable fits with DFOP can be obtained. Statistically they are with χ^2 errors in the range of 2.15 % to 7.40 % more appropriate than FOMC.

Thus one can conclude that for soil Lufa 2.2 FOMC provides the best-fit kinetic evaluation, whereas for soil Lufa 5M and Li10 SFO provides the best-fit kinetics.

Kinetics Analysis of Soil Degradation for M656PH047

The outcome of the kinetic analyses for M656PH047 is summarized in Table 7.1.2.1.2-28:
.

Trigger endpoints:

- Step 1: Is SFO more appropriate than FOMC and gives acceptable fit?
  - Single First Order (SFO) and First-Order Multi-Compartment (FOMC) kinetics were modelled, no biphasic pattern was indicated.
  - SFO is more appropriate than FOMC (lower χ^2 error), and gives acceptable fits
  - The t-test applied for the rate constants k resulted with SFO always in type I error rates and probabilities p > t of <0.001.
    This was not the case for FOMC.
  - SFO is statistically and visually acceptable

- Step 2: Identify best model other than SFO
  - Also, visual inspection of the SFO-fitted lines resulted in acceptable fits.
  - Thus one can conclude that
    SFO kinetics is the best-fit model for all soils and gives an acceptable description of the data statistically as well as visually.

With the SFO model, all soils reached a χ^2 error of < 15 % with the possible exception of soil Lufa 5M resulting in a χ^2 error % of 15.8 %. On the basis of the systematic residuals of the SFO model, the FOMC model fitting is less appropriate as the probability (p-values) corresponding to the calculated t-test values of the estimated degradation rate constants are above the trigger of 0.05.

Kinetics Analysis of Soil Degradation for M656PH043

The outcome of the kinetic analyses for M656PH043 is summarized in Table 7.1.2.1.2-28:
.

Trigger endpoints:

- Step 1: Is SFO more appropriate than FOMC and gives acceptable fit?
  - Single First Order (SFO) and First-Order Multi-Compartment (FOMC) kinetics were modelled.
  - SFO kinetics with χ^2 errors in the range of 8.07 % to 11.4 % would be acceptable for all three soils.
  - The t-test applied for the rate constants k resulted with SFO always in type I error rates and probabilities p > t of <0.001.
  - FOMC seems statistically for all soils with χ^2 errors in the range of 3.42 % to 7.08 % more appropriate than SFO, but for soil Lufa 5M the t-test failed for the parameter β.

- Step 2: Identify best model other than SFO
    - For soil Lufa 2.2 the χ^2 error of the DFOP (Double-First-Order in Parallel) analysis equals 3.19 % and is lower than FOMC, but the t-test failed on the rate k_2.
    - For soil Li10 the χ^2 error of 3.86 % is slightly larger than the FOMC model, all parameters are estimated statistically sound. Since DFOP kinetics can be used to derive modelling endpoints, and there is practically no difference between FOMC and DFOP, DFOP was considered as best-fit model.

Thus one can conclude that for soil Lufa 2.2 FOMC is the best fit model, whereas for soil Lufa 5M SFO kinetics, and for Li10 DFOP is the best-fit model that gives an acceptable description of the data statistically as well as visually.

Trigger endpoints

The results for deriving trigger endpoints (best-fit) DT_{50} and DT_{90} are summarized below:

Table 7.1.2.1.2-28: Trigger endpoints of M656PH054, M656PH047 and M656PH047

Data Set	DT_{50} [d]	DT_{90} [d]	Kinetic model
	M656PH054		
Lufa 2.2	40	334	FOMC
Lufa 5M	22	73	SFO
Li10	37	122	SFO
	M656PH047		
Lufa 2.2	87	289	SFO
Lufa 5M	43	142	SFO
Li10	95	314	SFO
	M656PH043		
Lufa 2.2	30	364	FOMC
Lufa 5M	10	34	SFO
Li10	21	154	DFOP

III. CONCLUSION

The objective of the study was to examine aerobic degradation of M656PH054, M656PH047 and M656PH043 (metabolites of dimethenamid-P) in three different soils.

The residues observed for the analytes (expressed as µg/kg) in the incubated soil samples were fitted by using the software package KinGUI version 2. The trigger endpoints (DT50 values) of the dimethenamid-P metabolite M656PH054 range from 22 – 40 days, for M656PH047 from 43 – 95 days and for M656PH043 from 10 – 30 days.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

See section CA 7.1.1.2

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

See section CA 7.1.1.2

CA 7.1.2.2 Field studies

The laboratory rate studies indicated that Dimethenamid is degraded rapidly in soil and that there is no risk of persistence in the soil under field conditions. The DT_{50} values in the laboratory were all below the trigger value of 60 days given by EEC Directive 91/414 and amended by Directive 95/36/EC. Nevertheless, four field soil dissipation studies were performed to demonstrate the rapid degradation of Dimethenamid and to determine the concentrations of the metabolites M23 (oxalamide) and M27 (sulfonate) in soil under field conditions.

The trials were performed using the formulated product SAN 582 H 900 EC containing 900 g ai/L. The nominal application rate was always 1.6 L of product/ha corresponding to 1440 g Dimethenamid/ha. The formulated product was always sprayed onto uncropped (bare) soil with knapsack sprayers and an attached sprayboom. Despite the highly exaggerated use rate with respect to the current use rate, three major conclusions can be made from this data. First, Dimethenamid is almost exclusively located in the top 10 cm soil layer. Only at early samplings could small amounts of active ingredient be detected below 10 cm. Second, Dimethenamid degrades rapidly in soil. Shortly after application the metabolites M23 and M27 were found in the soil samples. Third, the metabolites M23 and M27 were found at low concentrations and they could be characterized as transient in nature. In addition, they were located mostly in the upper soil layers.

New field studies were run in order to meet current guidelines. The product BAS 656 12 H, formulated as an emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 1008 g a.s./ha. This study was started before the lower application rate was established (864 g a.s./ha) and there fore the absolute values of observed parent and metabolites are higher than will be seen with the new application rate. A second field study was performed with the product BAS 769 00 H with 600 g a.s./ha. The representative formulation BAS 830 01 H (500 g a.s./ha) was selected after starting the field studies. In addition, field studies are triggered due to the exceedance of 60 days of the laboratory half-live of M31 (not evaluated in the former Annex I inclusion). These new studies demonstrate again the overall facile degradation of Dimethenamid-P under field conditions. The data fall in line with previously run field studies giving a (best-fit) DegT50 of 10.2 – 20.4 days for the parent compound. Moreover, metabolite M31 was only found in limited amounts, insufficient to derive degradation rates, indicating the transient nature of M31 in the environment.

In addition, a new field study was run on metabolite M656H027 in order to generate higher tiered degradation endpoints for use in FOCUS modelling scenarios.

The new field studies were normalised to derive modelling DegT50 values at reference temperature (20°C) and reference soil moisture (pF2). The old field dissipation study is not deemed suitable for normalisation since it does not comply with latest requirements.

CA 7.1.2.2.1 Soil dissipation studies

Report: CA 7.1.2.2.1/1
Bayer H.,Marwitz A., 2014a
Field soil dissipation study of BAS 656 H (Dimethenamid-P) in the formulation BAS 656 12 H on bare soil at four different sites in Europe, 2011-2013
2013/1343457

Guidelines: NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SETAC, EFSA Guidance to obtain DT50 values in soil (2010), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of dimethenamid-P (BAS 656 H) and its metabolites M23, M27 and M31 under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. One trial each was performed in Germany, France (North), France (South) and Spain. All sites represent typical regions of agricultural practice representative for growing crops including maize, which is among the most important crops for the use of dimethenamid-P. The trial sites consisted of an untreated and a treated area, the latter being subdivided into 3 subplots that were assigned for replicates.

The product BAS 656 12 H, formulated as an emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 1008 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between early April and late May 2011 using a calibrated boom sprayer. The actual application rates determined by quantifying the amount of spray discharged ranged from 944 to 1048 g a.s./ha for all trials, with an average of 1020 g a.s./ha. Results from spray broth analysis for the individual trial sites revealed concentrations between 58 and 72% of the nominal value with an average of 65% across all sites. Dose verification conducted via application monitors yielded recovery values for the individual trial sites ranging from 72 to 85% of the target rate and an average recovery of 78% over all sites.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 6 mm depth to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted until complete coverage of the soil surface. The layer of sand was controlled up to at least 28 days after application and was renewed when needed. It remained intact until at least 28 days. Within this time period of 28 days, the individual fields received a total precipitation (rain and irrigation) of 85 mm (Germany), 82 mm (France North), 51 mm (France South) and 103 mm (Spain), respectively.

No tillage or fertilisation was performed during the course of the study and no crops were grown throughout any of the trial. The plots were kept free of vegetation via the application of glyphosate or glufosinate ammonium. Rainfall was supplemented with irrigation at sites in Germany (195 mm), Northern France (130 mm), Southern France (313 mm) and Spain (687 mm) and the total water input was at least 102% of the historical average rainfall during the study period at the test sites.

Soil specimens were taken at intervals up to nominal two years and down to a maximum soil depth of 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18°C within a maximum of 8 hours and 30 minutes after sampling and remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and any shipments, shipment verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the field sites with known amounts of dimethenamid-P. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipping verification specimens on dimethenamid-P yielded an average recovery value of 112% across all sites confirming residue stability during all storage and shipment procedures.

Soil specimens and application monitors were analyzed for dimethenamid-P and metabolites M23, M27 and M31 according to BASF method L0109/02. The analytical method involved extraction of the soil with methanol/water (60/40, v/v). The final determination of the analytes was performed by LC-MS/MS with a limit of quantification (LOQ) of 0.005 mg/kg for each analyte. Field soil specimens from the treated plot were analyzed down to a maximum of 50 cm or to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ). Analysis was performed until a maximum of 366 days after treatment (DAT).

No residues above 30% of the LOQ of any analyte were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soils spiked with the four analytes at concentration levels of 0.005, 0.01, 0.05 and 20 mg/kg yielded overall mean recovery rates between 93 and 96% for the individual analytes, confirming the validity of the analytical method used in this study.

Residue values of dimethenamid-P and metabolites M23, M27 and M31 in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the individual field samples, and were summed up for all depths between 0 and 50 cm analyzed. Residue values were not corrected for procedural recoveries except for results obtained from petri dish and shipment verification analysis.

Dimethenamid-P degraded fast at all four European field sites. The total amount of dimethenamid-P residues detected in the soil profiles decreased from an average of 781 g/ha at day 0 to values between 6 and 108 g/ha after 2 months. At sites in Northern France, Southern France and Spain, no residues above the LOQ (0.005 mg/kg) were detectable any longer after 95 days at the latest. At the site in Germany, no residues above the LOQ were left after 1 year.

Considering the distribution of dimethenamid-P residues in the soil profiles, the main proportion was always measured in the top 0-10 cm soil layer and only small amounts of the compound were detected in the 10-20 cm layer. No residues above the LOQ were detected below 20 cm in any sample. Altogether, it can be concluded that dimethenamid-P does not show any significant tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were temporarily detected in small amounts at all sites reaching maximum amounts of 40 g/ha, 48 g/ha, und 45 g/ha, respectively. Thereafter residues declined again and were no longer detected after 151 days at the latest. All metabolites were only found in the top 0-20 cm soil layer, except for 1 single detect of M27 in the 20-30 cm layer at the site in Spain. No residues of the three metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

No calculation of dissipation times is provided in the summarized report. A detailed kinetic evaluation of the degradation behavior of dimethenamid-P in the four European field soils is subject of separate modeling reports.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation): BAS 656 12 H
Active ingredient (a.i.): Dimethenamid-P (BAS 656 H, Reg.No. 363851)
Type of formulation: EC

Batch no.: FRE-000601
Content of a.i.: 722.7 g/L (nominal 720 g/L)
Expiration date: March 31, 2012

2. Test sites

The dissipation of dimethenamid-P under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. Two trials were performed in France (L110062 and L110063) and one trial each was performed in Spain (L110064) and Germany (L110061). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics including soil taxonomy, the basic soil parameters of the corresponding soil horizons as well as soil bulk density in 10-20 cm depth are presented in Table 7.1.2.2.1-1**:** . Soil parameters were determined from soil samples taken from the boundaries of the treated plot following segmentation according to the soil horizons. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-1: Characteristics of the trial sites used to investigate the field dissipation of dimethenamid-P

Trial	L110061			L110062		
Location	Goch-Nierswalde, Germany			Stotzheim, France (North)		
Soil properties	0 - 30 cm	30 - 60 cm	60 - 90 cm	0 - 30 cm	30 – 60 cm	60 - 90 cm
Soil class (DIN 4220)	Sandy silt (Us)	Sandy silt (Us)	Sandy silt (Us)	Medium clay silt (Ut3)	Medium silt clay (Ut3)	Silty loam (Lu)
sand [%]	22.7	19.2	22.1	16.5	12.6	15.1
silt [%]	74.9	74.8	70.7	69.8	70.7	64.3
clay [%]	2.5	6.1	7.3	13.6	16.6	20.6
Soil class (USDA)	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam
sand [%]	33.6	29.0	29.9	15.1	17.6	17.3
silt [%]	64.9	65.7	62.7	66.4	66.2	61.8
clay [%]	1.6	5.3	7.5	18.5	16.1	20.9
Total organic C [%]	1.75	0.50	0.14	1.7	0.71	0.69
Organic matter [%] *	3.02	0.86	0.24	2.93	1.22	1.19
pH [$CaCl_2$]	5.85	5.60	4.44	7.11	7.25	7.01
pH [H_2O]	6.50	6.67	5.55	8.02	8.14	7.91
CEC [mval Ba/100g dry weight]	9.5	4.3	3.7	14.8	13.8	13.2
MWHC [g/100g dry weight]	48.9	52.7	51.2	58.1	51.3	48.2
pF 2.0 [g/100g dry weight]**	23.7	17.7	21.4	25.1	27.2	29.1
pF 2.5 [g/100g dry weight]**	21.8	17.0	16.6	22.7	25.5	25.6
Dry bulk density [g/cm^3]***	1.28	-	-	1.15	-	-
Soil taxonomy	Pseudogley-Cambisol / Pseudogley-Paracambisol			Haplic Calcisol		

Trial	L110063		L110064		
Location	Meauzac, France (South)		Utrera, Spain		
Soil properties	0 - 40 cm	40 - 90 cm	0 - 15 cm	15 – 30 cm	30 – 90 cm
Soil class (DIN 4220)	Silty loamy sand (Slu)	Poor loamy sand (Sl2)	Pure sand (Ss)	Pure sand (Ss)	Sandy clay loam (Lts)
sand [%]	45.7	81.4	86.2	85.6	52.2
silt [%]	43.4	13.5	9.1	9.4	15.3
clay [%]	10.9	5.1	4.6	4.9	32.4
Soil class (USDA)	Sandy loam	Loamy sand	Sand	Sand	Silt clay loam
sand [%]	53.0	84.1	88.1	87.7	53.4
silt [%]	36.2	11.0	7.7	8.0	10.7
clay [%]	10.8	4.8	4.1	4.3	35.9
Total organic C [%]	1.30	0.94	0.48	0.33	0.37
Organic matter [%] *	2.24	1.62	0.83	0.57	0.64
pH [$CaCl_2$]	7.55	7.76	6.93	7.00	6.36
pH [H_2O]	8.50	8.90	7.77	7.90	7.42
CEC [mval Ba/100g dry weight]	9.0	5.0	4.7	4.8	22.8
MWHC [g/100g dry weight]	44.0	34.9	28.0	27.4	45.7
pF 2.0 [g/100g dry weight]**	18.6	8.4	12.0	12.9	33.1
pF 2.5 [g/100g dry weight]**	17.2	8.9	10.0	10.5	30.7
Dry bulk density [g/cm^3]***	1.53	-	1.66	-	-
Soil taxonomy	Endoeutric Albeluvisol		Eutric Planosols		

* organic matter = organic carbon x 1.724
** water retention characteristics, soil moisture at 0.1 or 0.33 bar
*** samples taken at 10-20 cm depth (mean of 3 replicates)
CEC = cation exchange capacity
MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice with soils representative for growing crops including maize, which is among the most important crops for the use of dimethenamid-P. The sites were flat without any significant slope and had been under cultivation for many years. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No dimethenamid-P or product from a similar chemical class had been used on the test plots in the previous three years. The crop and pesticide history of the trial sites is presented in Table 7.1.2.2.1-2.

Table 7.1.2.2.1-2 Management history of the trial sites in the previous years (non-GLP)

Trial	Location	Year	Crops grown	Pesticides used
L110061	Goch-Nierswalde, Germany	2008	green manuring	no pesticides applied
		2009	green manuring	no pesticides applied
		2010	alfalfa	pyridat
		2011*	green manuring	no pesticides applied
L110062	Stotzheim, France (North)	2008	maize	nicosulfuron, mesotrione, dicamba
		2009	vine nursery	oryzalin+diuron, glufosinate+ammonium, oxyfluorfen+propyzamide, fosetyl-aluminium+folpet+cymoxanil, dimethomorph+folpet, chlorpyriphos-methyl, folpet+mandipropamid, sulfur micro, copper oxychloride
		2010	maize	nicosulfuron, mesotrione, dicamba
		2011*	none	glyphosate
L110063	Meauzac, France (South)	2008	maize	bentazone+dicamba, foramsulfuron
		2009	maize	bentazone+dicamba, foramsulfuron
		2010	maize	bentazone+dicamba, foramsulfuron
		2011*	none	no additional pesticide applied
L110064	Utrera, Spain	2008	mustard	no additional pesticide applied
		2009	corn	pendimethalin, abamectin
		2010	sunflower	pendimethalin
		2011*	fallow field	no additional pesticide applied

* until start of trial

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 - 90 m^2) and one treated plot (size: 324 - 630 m^2). The treated plot consisted of three equal sized subplots A, B and C that were assigned for replicates.

The product, formulated as an emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 1008 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between early April and late May 2011 using a calibrated boom sprayer. Treated plots were three-fold replicated with subplot size ranging from 108 to 210 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rates determined by quantifying the amount of spray discharged ranged from 996 to 1036 g a.s./ ha averaged over the three replicates of each treated plot. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil Li 10 (approximately 50 g per dish, sieved to 2 mm). The petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were sealed with a lid, taped up and frozen within 30 minutes, except for trial L110062 (France North) where samples were chilled in a cooled box after collection and placed in freezer storage within less than 3 hours. Further details of application are presented in in Table 7.1.2.2.1-3 below.

Table 7.1.2.2.1-3: Application parameters of field trial sites treated with BAS 656 12 H (EC, 720 g/L)

Trial Country	Application method	No. of appli-cations	Subplot (m²)	Application rate per treatment				Application date
				nominal	actual*	dose verification**		
				[g a.s/ha]	[g a.s./ha]	[g a.s/ha]	% of nominal	
L110061 Germany	broadcast spray to bare soil	1	A (133.5)	1008	1012	717	71	24-May-2011
			B (133.5)	1008	1042	698	69	
			C (133.5)	1008	1037	748	74	
			Average	**1008**	**1030**	**721**	**72**	
L110062 France (North)	broadcast spray to bare soil	1	A (168)	1008	1010	825	82	24-May-2011
			B (168)	1008	992	807	80	
			C (168)	1008	1048	933	93	
			Average	**1008**	**1017**	**855**	**85**	
L110063 France (South)	broadcast spray to bare soil	1	A (210)	1008	944	878	87	20-May-2011
			B (210)	1008	1018	781	77	
			C (210)	1008	1026	629	62	
			Average	**1008**	**996**	**762**	**76**	
L110064 Spain	broadcast spray to bare soil	1	A (108)	1008	1024	823	82	08-Apr-2011
			B (108)	1008	1048	791	78	
			C (108)	1008	1036	808	80	
			Average	**1008**	**1036**	**807**	**80**	

* determined by calculation of spray liquid applied ** determined by means of petri dishes filled with soil

Immediately after application of the test item and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a box spreader until complete coverage of the soil surface. The thickness of the sand layer necessary for complete coverage of the soil was approximately 6 mm. Only at the control plot and at subsubplot 3 of replicate A of trial L110062, a layer of sand of 25-30 mm was applied due to a technical mishap. The affected subsubplot of the treated plot was sampled shortly afterwards (0 DAT) and consequently, there is no adverse effect on the validity of the study. The layer of sand was controlled up to at least 28 days after application and was renewed when needed. It remained intact until at least 28 days. Within this time period of 28 days, the individual fields received a total of 85 mm (Germany), 82 mm (France North), 51 mm (France South) and 103 mm (Spain) precipitation (rain and irrigation), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate or glufosinate ammonium. Rainfall was supplemented with irrigation at sites in Germany (195 mm), Northern France (130 mm), Southern France (313 mm) and Spain (687 mm).

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Summary of climatic conditions at field trial sites used to investigate the dissipation of Dimethenamid-P

Trial	L110061			L110062			L110063			L110064		
Location	Goch-Nierswalde			Stotzheim			Meauzac			Utrera		
	Germany			France (North)			France (South)			Spain		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑		∑	∑
Apr 11	-	-	-	-	-	-	-	-	-	19.6	70.0	7.9
May 11	15.0	10.0	10.0	16.4	7.8	0.0	18.4	2.2	0.0	23.4	28.0	30.5
Jun 11	16.1	89.0	0.0	17.3	102.0	3.0	18.6	49.8	22.6	26.9	3.0	90.0
Jul 11	15.6	112.4	10.0	16.3	97.8	10.9	19.3	81.8	23.0	27.7	0.0	90.3
Aug 11	16.9	136.2	0.0	18.5	95.2	14.9	21.8	0.4	47.0	28.3	0.0	86.1
Sep 11	15.4	54.2	5.0	16.0	75.8	0.0	19.7	14.2	59.0	25.6	15.0	61.1
Oct 11	10.8	78.4	0.0	9.2	35.2	8.7	13.7	17.2	38.0	22.5	70.5	43.3
Nov 11	6.7	8.4	50.0	5.1	2.4	0.0	11.2	19.2	36.0	15.5	88.5	0.0
Dec 11	5.5	146.6	0.0	4.9	101.8	0.0	7.4	66.4	0.0	11.5	14.5	5.1
Jan 12	4.1	122.0	0.0	3.3	58.4	0.0	5.7	33.2	0.0	9.8	40.0	16.3
Feb 12	0.4	24.2	0.0	-2.1	2.8	0.0	0.7	2.4	0.0	7.9	0.5	24.5
Mar 12	8.1	23.8	20.0	8.1	10.2	3.5	10.2	28.4	17.0	13.7	8.0	29.7
Apr 12	8.2	79.8	0.0	8.9	50.6	25.9	10.7	101.0	19.0	15.9	59.5	11.1
May 12	14.4	70.0	10.0	15.4	33.4	0.0	16.6	70.8	0.0	23.6	23.0	23.1
Jun 12	14.6	122.2	0.0	17.2	96.2	0.0	20.0	58.6	0.0	26.6	0.0	43.8
Jul 12	16.9	148.0	10.0	17.9	89.0	10.7	20.1	31.2	18.0	27.4	0.0	46.8
Aug 12	18.4	45.0	25.0	18.9	37.2	17.0	22.6	67.6	0.0	28.1	0.0	46.2
Sept 12	13.5	48.8	30.0	14.0	44.8	21.9	18.0	20.2	17.0	24.6	78.0	31.4
Oct 12	10.0	84.6	10.0	8.9	81.6	0.0	14.4	36.6	16.0	20.0	127.0	-
Nov 12	6.5	34.6	0.0	5.8	93.4	0.0	8.7	57.8	0.0	15.8	135.5	-
Dec 12	4.3	121.0	0.0	3.2	66.2	0.0	6.3	97.0	0.0	12.4	37.0	-
Jan 13	1.8	46.2	0.0	1.3	20.0	0.0	4.6	129.2	0.0	11.9	49.5	-
Feb 13	1.2	37.6	0.0	0.0	37.8	0.0	4.3	61.8	0.0	11.1	55.0	-
Mar 13	2.8	39.8	15.0	2.6	24.6	0.0	8.8	89.0	0.0	14.6	165.5	-
Apr 13	8.4	45.2	0.0	9.6	72.6	13.1	11.5	74.6	0.0	16.2	1.0	-
May 13	12.4	0.2	0.0	13.0	58.4	0.0	14.1	40.8	0.0	-	-	-

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Historical (long-term) weather data on precipitation and average air temperature from at least 10 years were taken from official weather stations located nearby (7-14 km distance to trial site). The historical and actual data each averaged over the complete duration of the individual trials are presented in Table 7.1.2.2.1-5.

Table 7.1.2.2.1-5: Summary of historical and actual weather data at field trial sites averaged over entire trial duration

Trial Country	Tmean Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic	Actual	Historic	Actual			
L110061 Germany	9.9	9.9	1576	1728	195	1923	122
L110062 France (North)	11.0	10.0	1307	1395	130	1525	117
L110063 France (South)	13.1	13.1	1537	1251	313	1564	102
L110064 Spain	17.4	19.2	1164	1069	687	1756	151

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 725 days and down to a maximum soil depth of 90 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-6.

Table 7.1.2.2.1-6: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L110061	Germany	-1, 0, 3, 6, 10, 16, 28, 59, 85, 120, 150, 175, 233, 366, 540, 710
L110062	France (North)	-11, 0, 3, 6, 10, 16, 28, 58, 92, 120, 154, 176, 233, 358, 548, 721
L110063	France (South)	-1, 0, 3, 6, 10, 19, 28, 59, 94, 123, 153, 180, 243, 364, 550, 725
L110064	Spain	-1, 0, 3, 6, 10, 17, 31, 60, 95, 116, 151, 179, 236, 355, 544, 725

Untreated specimens were collected from the control plot at three occasions, one or eleven days before application down to a depth of 90 cm, and after about one and two years to a depth of 10 cm each. The specimens were taken randomly from one part of the untreated plot each time and pooled according to soil depth. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 4.4 to 5.0 cm diameter. The 10 cores taken after about one year and on the final day of sampling were collected with a metal tube of minimum 7.2 and maximum 8.3 cm diameter.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A – C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 7.2 and to maximum 8.3 cm diameter which left a hole contained by a steel or plastic collar. Alternatively, samples were taken by pressing the metal tube described above into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a common soil corer fitted with a plastic liner of diameter 4.3 to 5 cm. Sampling of these cores was conducted in one run or in up to four consecutive steps.

All soil cores collected with the soil probe were sectioned into segments of 10 cm. Soil segments of the same depth were pooled and homogenized. All soil specimens were usually stored at or below -18°C within less than 8 hours and 30 minutes after sampling and remained frozen until analysis.

5. Analytical procedure

Field soil specimens and application monitors (Petri dish samples) were analyzed for dimethenamid-P and its metabolites M23, M27 and M31 according to validated BASF method L0109/02 *[CA 4.1.2/1, Tilting, N. and Sopena-Vazquez, F. 2014a, BASF DocID 2013/1110235]*. The analytical method involved extraction of the soil with methanol/water (60:40, v/v) and final determination of the analytes by HPLC-MS/MS. The limit of quantification (LOQ) was 0.005 mg/kg for each individual analyte. The limit of detection (LOD) was set at 0.0015 mg/kg (30% of LOQ). Generally, a second mass transition was monitored for each analyte.

Analysis of field soil specimens originating from the treated plots was conducted down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg). Analysis was performed until a maximum of 366 days after treatment (DAT).

Spray broth specimens were diluted to the appropriate concentration and analyzed for dimethenamid-P and its metabolites M23, M27 and M31 using HPLC-MS/MS.

The validity of the analytical method was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of dimethenamid-P, M23, M27 and M31 in frozen soil was investigated in a separate study *[CA 7.1.2.2.1/4, Mewis, A. 2014, BASF DocID 2013/1348019]* with soils originating from the individual trial sites of the present terrestrial field dissipation study.

7. Calculation of dissipation times

A calculation of dissipation times is not provided in the summarized report. A detailed kinetic evaluation of the degradation behaviour of dimethenamid-P in the four European field soils is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649]*.

II. RESULTS AND DISCUSSION

A. SPRAY BROTH CONCENTRATION AND APPLICATION VERFICATION

Spray broth homogeneity was confirmed by visual check for all trials. In addition, spray mixtures sampled before and after application of each subplot were analyzed for dimethenamid-P. Procedural recovery experiments conducted along with the analysis of the spray broth samples showed mean recoveries of 98% except for 1 sample where only 78% were obtained. Analyzed concentrations averaged across the individual field sites were in the range of 1.96 to 2.41 g/L corresponding to 58-72% of the target concentration of 3.34 g/L. The analytical results are not corrected for procedural recoveries.

Since spray broth concentrations were lower than expected in all trials, it is assumed that the product had not been properly mixed before bottling or use. However, since the objective of the present study was the determination of the degradation kinetics, and since a very sensitive analytical method (LOQ = 0.005 mg/kg) was used, there is no adverse effect on the validity of the study.

Procedural recovery experiments were conducted with untreated soil along with the analysis of the applied Petri dish samples from the field that served as application monitors. Mean recoveries of each analyzed set of samples for dimethenamid-P ranged from 74% to 105%.

Residue levels of dimethenamid-P achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were corrected for the mean recovery of the respective analytical set and converted into residue rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm²). As a result, the obtained application rates for the individual trials ranged from 721 to 855 g/ha representing 72-85% of the target rate (see Table 7.1.2.2.1-3 for individual figures). The applied amount determined by the application monitors in these trials is below the nominal value of 1008 g/ha, which is in agreement with the results obtained from the spray broth samples. As already stated, no negative impact is anticipated for the validity of the study since the amount of substance applied is close to the reduced application rate of 864 g a.s./ha and more than enough to be able to determine the degradation behaviour of dimethenamid-P in soil.

B. FINDINGS

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of dimethenamid-P and metabolites M23, M27 and M31. No residues above 30% LOQ of any analyte were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the four analytes at concentration levels of 0.005, 0.01, 0.05 and 20 mg/kg yielded overall mean recovery rates for the individual analytes between 93 and 96%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-7.

Table 7.1.2.2.1-7: Method procedural recoveries

Analyte	Fortification level [mg/kg]	n	Mean recovery ± RSD* [%]
dimethenamid-P	0.005, 0.01, 0.05, 20	141	93 ± 10
M23	0.005, 0.01, 0.05, 20	141	96 ± 10
M27	0.005, 0.01, 0.05, 20	141	94 ± 10
M31	0.005, 0.01, 0.05, 20	141	95 ± 10

* mean values are across all soils and soil depths; RSD = relative standard deviation [%]

These data prove that the analytical method applied was suitable to accurately determine residues of dimethenamid-P and its metabolites in soil down to a concentration of 0.005 mg/kg for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg, maximum depth of 50 cm). If samples were analysed in duplicate, the individual numbers were averaged to produce a mean for the respective soil sample. When one of the values was below the LOQ, it was averaged as half of LOQ. For all trials, the 0 DAT double samples of the 0-10 cm soil layer were analysed as well, in order to account for the importance of the day 0 value, and the final data were obtained by averaging the mean values of the respective main and double samples.

All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the four analytes in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field samples, which was calculated based on the dry weight and the volume of the sampled soil specimens. The obtained residue rates in g/ha were summed up for all depths between 0 and 50 cm analyzed. Results are presented in Table 7.1.2.2.1-8 to Table 7.1.2.2.1-11.

Table 7.1.2.2.1-8: Total residues of dimethenamid-P under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110061 Goch-Nierswalde, Germany			L110062 Stotzheim, France		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	**672**	**740**	**722**	**530**	**623**	**888**
3	**590**	**765**	**715**	**752**	**806**	**884**
6	**354**	**727**	**778**	**733**	**832**	**848**
10	**391**	**502**	**499**	**768**	**646**	**623**
16	**276**	**317**	**483**	**458**	**398**	**536**
28	**447**	**420**	**163**	**223**	**155**	**149**
58-59	**108**	**65**	**54**	**7.2**	**7.3**	**5.7**
85-92	**42**	**48**	**42**	0	0	0
120	**19**	**35**	**31**	0	0	0
150-154	**19**	**28**	**17**	0	0	0
175-176	**13**	**27**	**21**	0	0	0
233	**16**	**21**	**12**	0	0	0
358-366	0	0	0	0	0	0
Trial Country	**L110063 Meauzac, France**			**L110064 Utrera, Spain**		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	**858**	**719**	**804**	**937**	**884**	**997**
3	**747**	**761**	**761**	**583**	**896**	**628**
6	**759**	**758**	**603**	**691**	**681**	**876**
10	**538**	**779**	**762**	**348**	**758**	**681**
17-19	**338**	**371**	**217**	**610**	**505**	**457**
28-31	**122**	**159**	**123**	**284**	**155**	**174**
59-60	**14**	**15**	**8.6**	**16**	**17**	**19**
94-95	0	0	0	0	0	0
116-123	0	0	0	0	0	0
151-153	0	0	0	0	0	0
179-180	0	0	0	0	0	0
236-243	0	0	0	0	0	0
355-364	0	0	0	0	0	0

DAT = days after treatment
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero

Table 7.1.2.2.1-9: Total residues of M23 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110061 Goch-Nierswalde, Germany			L110062 Stotzheim, France		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	**5.6**	0	**6.7**
16	0	0	**26**	**7.3**	**15**	**13**
28	**26**	0	0	**11**	**7.9**	**10**
58-59	0	0	0	0	0	0
85-92	0	0	0	0	0	0
120	0	0	0	0	0	0
150-154	0	0	0	0	0	0
175-176	0	0	0	0	0	0
233	0	0	0	0	0	0
358-366	0	0	0	0	0	0
Trial Country	**L110063 Meauzac, France**			**L110064 Utrera, Spain**		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17-19	**17**	**15**	**13**	**12**	**17**	**15**
28-31	**20**	**30**	**26**	**40**	**33**	**26**
59-60	0	0	0	**16**	**16**	**14**
94-95	0	0	0	0	0	0
116-123	0	0	0	0	0	0
151-153	0	0	0	0	0	0
179-180	0	0	0	0	0	0
236-243	0	0	0	0	0	0
355-364	0	0	0	0	0	0

DAT = days after treatment
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero

Table 7.1.2.2.1-10: Total residues of M27 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110061 Goch-Nierswalde, Germany			L110062 Stotzheim, France		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
16	0	0	**8.5**	**6.4**	**6.6**	**7.3**
28	**22**	**8.2**	**10**	**31**	**34**	**29**
58-59	**28**	**36**	**32**	0	**9.7**	0
85-92	**18**	**21**	**13**	0	0	0
120	0	0	0	0	0	0
150-154	0	0	0	0	0	0
175-176	0	0	0	0	0	0
233	0	0	0	0	0	0
358-366	0	0	0	0	0	0
Trial Country	**L110063 Meauzac, France**			**L110064 Utrera, Spain**		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17-19	**22**	**22**	**11**	**10**	**15**	0
28-31	**26**	**45**	**31**	**48**	**37**	**28**
59-60	**12**	0	**10**	**40**	**32**	**23**
94-95	0	0	0	0	**21**	**19**
116-123	0	0	0	**10**	**24**	0
151-153	0	0	0	0	0	0
179-180	0	0	0	0	0	0
236-243	0	0	0	0	0	0
355-364	0	0	0	0	0	0

DAT = days after treatment
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero

Table 7.1.2.2.1-11: Total residues of M31 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110061 Goch-Nierswalde, Germany			L110062 Stotzheim, France		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
16	0	0	**11**	**6.4**	**13**	**15**
28	**12**	**6.9**	0	**36**	**23**	**25**
58-59	0	0	0	0	0	0
85-92	0	0	0	0	0	0
120	0	0	0	0	0	0
150-154	0	0	0	0	0	0
175-176	0	0	0	0	0	0
233	0	0	0	0	0	0
358-366	0	0	0	0	0	0
Trial Country	**L110063 Meauzac, France**			**L110064 Utrera, Spain**		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17-19	**13**	**8.3**	**8.4**	**10**	**13**	**17**
28-31	**16**	**21**	**20**	**45**	**32**	**37**
59-60	0	0	0	**12**	**13**	**14**
94-95	0	0	0	0	0	0
116-123	0	0	0	0	0	0
151-153	0	0	0	0	0	0
179-180	0	0	0	0	0	0
236-243	0	0	0	0	0	0
355-364	0	0	0	0	0	0

DAT = days after treatment
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero

As is evident from the analytical data, dimethenamid-P degraded fast at all four European field sites. The total amount of dimethenamid-P residues detected in the soil profiles decreased from an average of 781 g/ha at day 0 to values between 6 and 108 g/ha after 2 months. At sites in Northern France, Southern France and Spain, no residues above the LOQ (0.005 mg/kg) were detectable any longer after 95 days at the latest. At the site in Germany, no residues above the LOQ were left after 1 year.

Considering the distribution of dimethenamid-P residues in the soil profiles, the main proportion was always measured in the top 0-10 cm soil layer and only small amounts of the compound were detected in the 10-20 cm layer (≤ 0.031 mg/kg). No residues above the LOQ were detected below 20 cm in any sample. Altogether, it can be concluded that dimethenamid-P does not show any significant tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were also monitored during the study. They were temporarily detected in small amounts at all sites.

Metabolite M23 was detected from 10 DAT onwards. It increased to a maximum of 40 g/ha, thereafter declined and was no longer detected after 95 days.

Metabolite M27 was detected from 16 DAT. It increased to a maximum of 48 g/ha, thereafter declined and was no longer detected after 151 days.

Metabolite M31 was detected from 16 DAT. It increased to a maximum of 45 g/ha, thereafter declined and was no longer detected after 95 days.

Metabolites M23, M27 and M31 were exclusively found in the top 0-20 cm soil layer, except for 1 single detect of M27 in the 20-30 cm layer at the site in Spain. No residues of the three metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

A detailed kinetic evaluation of the field trials is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649].*

Shipment verification specimens

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage at the test site and through the shipping process. The samples were prepared at nominal 0, 30 and 90 DAT by fortification of soil with 0.5 mg/kg dimethenamid-P and were subsequently handled in the same manner as the actual residue samples. Concentrations of dimethenamid-P analyzed were corrected for the mean recovery of the respective analytical set.
The analytical results demonstrated no significant losses from the shipping verification samples. The average recovery of dimethenamid-P from the soil samples spiked at the field test sites was 112%. It was concluded that dimethenamid-P was stable in the soils under the shipping and storage conditions used.

Storage stability

The maximum period any sample from the present field soil dissipation study was stored from the time of sampling to analysis was 837 days. Results from a storage stability study performed with the soils from the four field sites *[CA 7.1.2.2.1/4, Mewis, A. 2014, BASF DocID 2013/1348019]* proved that dimethenamid-P and metabolites M23, M27, and M31 are stable in frozen soil for at least 29 months (895 days).

III. CONCLUSION

Dimethenamid-P degraded fast under field conditions in soil at all four European field sites. The total amount of dimethenamid-P residues in the soil profiles decreased from an average of 781 g/ha at day 0 to values between 6 and 108 g/ha after 2 months. At sites in Northern France, Southern France and Spain, no residues above the LOQ (0.005 mg/kg) were detectable any longer after 95 days at the latest. At the site in Germany, no residues above the LOQ were left after 1 year. DT_{50} values are supposed to be low and are subject of separate modeling reports.

Dimethenamid-P residues were exclusively detected in the upper 20 cm of the soils. No residues above the LOQ were detected below 20 cm in any sample. Altogether, it can be concluded that dimethenamid-P does not show any significant tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were temporarily detected in low amounts at all sites reaching maximum amounts of 40 g/ha, 48 g/ha, und 45 g/ha, respectively.

All metabolites were only found in the top 0-20 cm soil layer, except for 1 single detect of M27 in the 20-30 cm layer at the site in Spain. No residues of the three metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

Report: CA 7.1.2.2.1/2
Bayer H.,Marwitz A., 2014b
Field soil dissipation study of M27 (metabolite of BAS 656 H, Dimethenamid) in the formulation EXP 360714 H-AA on bare soil at four different sites in Europe, 2011-2013
2013/1343459

Guidelines: NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 850.6100, SETAC, EFSA Guidance to obtain DT50 values in soil (2010), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of M27, a metabolite of dimethenamid (BAS 656 H) under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. Trials were performed in Germany, Northern France, Southern France and in Spain. All sites represent typical regions of agricultural practice representative for growing crops including maize which is among the most important crops for the use of dimethenamid. The trial sites consisted of an untreated and a treated area, the latter being subdivided into 3 subplots that were assigned for replicates.

The product EXP 360714 H-AA, formulated as a soluble concentrate (SL), was broadcast applied to bare soil in a single application at a nominal rate of 250 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between early May and early June 2011 using a calibrated boom sprayer. The actual application rates determined by quantifying the amount of spray discharged ranged from 249 to 254 g a.s./ha (mean from each trial), with an average of 253 g a.s./ha. Results from spray broth analysis for the individual trial sites revealed concentrations between 90 and 100% of the nominal value with an average of 94% across all sites. Dose verification conducted via application monitors yielded recovery values for the individual sites ranging from 100 to 108% of the target rate and an average recovery of 103% over all sites.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 6 mm depth to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted until complete coverage of the soil surface. The layer of sand was controlled up to at least 28 days after application and was renewed when needed. It remained intact until at least 28 days. Within this time period of 28 days, the individual fields received a total precipitation (rain and irrigation) of 85 mm (Germany), 99 mm (France North), 62 mm (France South) and 51 mm (Spain), respectively.

No tillage or fertilisation was performed during the course of the study and no crops were grown throughout any of the trial. The plots were kept free of vegetation via the application of glyphosate or glufosinate ammonium. Rainfall was supplemented with irrigation at sites in Germany (195 mm), Northern France (120 mm), Southern France (321 mm) and Spain (671 mm) and the total water input was at least 92% of the historical average rainfall during the study period at the test sites.

Soil specimens were taken at intervals up to 710 days after application and down to a maximum soil depth of 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18°C within a maximum of 8 hours 50 minutes after sampling and remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and shipment, shipment verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the field sites with known amounts of M27. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipping verification specimens on M27 yielded an average recovery value of 89% across all sites confirming residue stability during all storage and shipment procedures.

Soil specimens and application monitors were analyzed for M27 and metabolite M23 according to BASF method L0109/02. The analytical method involved extraction of the soil with methanol/water (60/40, v/v). The final determination of the analytes was performed by LC-MS/MS with a limit of quantification (LOQ) of 0.005 mg/kg for each analyte. Field soil specimens from the treated plot were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ). Analysis was performed until a maximum of 248 days after treatment (DAT).

No residues above 30% of the LOQ of any analyte were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with untreated soils spiked with the two analytes at concentrations of 0.005, 0.01, and 0.05 mg/kg yielded overall mean recovery rates of 99 % for each individual analyte, confirming the validity of the analytical method used in this study.

Residue values of M27 and metabolite M23 in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the individual field samples, and were summed up for all depths between 0 and 60 cm analyzed. Residue values were not corrected for procedural recoveries except for results obtained from petri dish and shipment verification analysis.

M27 degraded fast under field conditions in soil at all four European field sites. The total amount of M27 residues detected in the soil profiles decreased from an average of 170 g/ha at day 0 to an average of 55 g/ha after 1 month. No residues above the LOQ (0.005 mg/kg) were detectable any longer after 4 months at the latest.

M27 residues were mainly detected in the top 0-30 cm layer of the soils. No residues above the LOQ were detected below 40 cm in any sample at any site. Altogether, it can be concluded that M27 shows only moderate tendency to move into deeper soil layers indicating moderate potential for M27 residues to leach to groundwater

Metabolite M23 was not present in the soil samples. No residues of M23 above the LOQ were detected in any sample at any site.

No calculation of dissipation times is provided in the summarised report. A detailed kinetic evaluation of the degradation behavior of M27 in the four European field soils is provided in two separate modeling reports.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation):	EXP 360714 H-AA
Active ingredient (a.i.):	M27 (sodium salt, metabolite of dimethenamid (BAS 656 H), Reg.No. 360714)
Type of formulation:	SL
Batch no.:	400003
Content of a.i.:	104.8 g/L (nominal 100 g/L)
Expiration date:	Sep 30, 2011

2. Test sites

The dissipation of M27 (metabolite of dimethenamid) under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. Two trials were performed in France (L110331 and L110332) and one trial each was performed in Germany (L110330) and in Spain (L110333). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics including soil taxonomy, the basic soil parameters of the corresponding soil horizons as well as soil bulk density in 10-20 cm depth are presented in Table 7.1.2.2.1-12**:** . Soil parameters were determined from soil samples taken before application following segmentation according to the soil horizons. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-12: Characteristics of the trial sites used to investigate the field dissipation of M27

Trial	L110330			L110331	
Location	**Goch-Nierswalde, Germany**			**Stotzheim, France (North)**	
Soil properties	0 - 30 cm	30 - 60 cm	60 - 90 cm	0 - 28 cm	28 – 90 cm
Soil class (DIN 4220)	Sandy silt (Us)	Sandy silt (Us)	Medium silty sand (Su3)	Sandy loamy silt (Uls)	Silty loam (Lu)
sand [%]	18.1	25.7	70.3	28.8	27.7
silt [%]	77.1	67.5	25.3	56.4	50.6
clay [%]	4.9	6.7	4.5	14.7	21.7
Soil class (USDA)	Silt loam	Silt loam	Sandy loam	Silt loam	Loam
sand [%]	25.9	33.5	74.0	30.6	28.8
silt [%]	69.3	59.1	20.9	54.9	47.9
clay [%]	4.7	7.3	5.0	14.6	23.3
Total organic C [%]	1.65	0.28	0.12	0.83	0.34
Organic matter [%]*	2.84	0.48	0.21	1.43	0.59
pH [$CaCl_2$]	6.36	5.95	5.03	5.47	6.40
pH [H_2O]	7.07	7.04	6.39	6.40	7.50
CEC [mval Ba/100g dry weight]	9.6	3.5	2.8	8.5	12.1
MWHC [g/100g dry weight]	52.7	47.0	34.3	49.5	47.9
pF 2.0 [g/100g dry weight]**	32.6	17.5	8.2	19.0	24.8
pF 2.5 [g/100g dry weight]**	18.0	15.8	7.3	17.8	18.4
Dry bulk density [g/cm^3]***	1.28	-	-	1.19	-
Soil taxonomy	Anthrosole			Haplic Calcisol	

Trial	L110332		L110333		
Location	**Meauzac, France (South)**		**Utrera, Spain**		
Soil properties	0 - 40 cm	40 - 90 cm	0 - 15 cm	15 – 30 cm	30 – 90 cm
Soil class (DIN 4220)	Silty loamy sand (Slu)	Poor silty sand (Su2)	Poor clay sand (St2)	High loamy sand (Sl4)	Sandy clay loam (Lts)
sand [%]	45.4	85.0	85.5	69.6	51.3
silt [%]	43.7	11.1	8.8	14.5	17.8
clay [%]	11.0	3.9	5.6	15.9	30.9
Soil class (USDA)	Loam	Loamy sand	Loamy sand	Sandy clay loam	Sandy clay loam
sand [%]	51.5	87.0	86.3	70.4	52.8
silt [%]	36.7	8.6	6.7	9.5	15.6
clay [%]	11.9	4.3	6.9	20.3	31.5
Total organic C [%]	1.38	0.80	0.38	0.34	0.30
Organic matter [%]*	2.38	1.38	0.66	0.59	0.52
pH [$CaCl_2$]	7.49	7.01	6.92	6.66	6.72
pH [H_2O]	8.44	7.66	7.93	7.75	7.77
CEC [mval Ba/100g dry weight]	9.0	4.0	5.0	12.2	20.4
MWHC [g/100g dry weight]	42.2	32.1	27.8	36.4	43.0
pF 2.0 [g/100g dry weight]**	18.0	7.2	10.7	35.3	36.7
pF 2.5 [g/100g dry weight]**	14.0	6.0	7.8	19.8	30.2
Dry bulk density [g/cm^3]***	1.59	-	1.61	-	-
Soil taxonomy	Endoeutric Albeluvisol		Planosol eutrico		

* organic matter = organic carbon x 1.724
** water retention characteristics, soil moisture at 0.1 or 0.33 bar
*** samples taken at 10-20 cm depth (mean of 3 replicates)
CEC = cation exchange capacity; MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice with soils representative for growing crops including maize, which is among the most important crops for the use of dimethenamid. The sites were flat without any significant slope and had been under cultivation for many years. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No product containing M27 or dimethenamid or its structural analogues had been used on the test plots in the previous three years. The crop and pesticide history of the trial sites is presented in Table 7.1.2.2.1-13.

Table 7.1.2.2.1-13: Management history of the trial sites in the previous years (non-GLP)

Trial	Location	Year	Crops grown	Pesticides used
L110330	Goch-Nierswalde, Germany	2008	green manuring	no pesticides applied
		2009	green manuring	no pesticides applied
		2010	alfalfa	pyridat
		2011*	green manuring	no pesticides applied
L110331	Stotzheim, France (North)	2008	maize	nicosulfuron, mesotrione, dicamba
		2009	vine nursery	glufosinate-ammonium, oryzalin+diuron, oxyfluorfen+propyzamide, fosetyl-aluminium+folpet+cymoxanil, dimethomorph+folpet, chlorpyriphos-methyl, folpet+mandipropamid, sulfur micro, copper oxychloride
		2010	maize	nicosulfuron, mesotrione, dicamba
		2011*	none	glyphosate
L110332	Meauzac, France (South)	2008	maize	bentazone+dicamba, foramsulfuron
		2009	maize	bentazone+dicamba, foramsulfuron
		2010	maize	bentazone+dicamba, foramsulfuron
		2011*	none	no additional pesticide applied
L110333	Utrera, Spain	2008	fallow field	no additional pesticide applied
		2009	phacelia, tanacetifolia	no additional pesticide applied
		2010	wheat	fenpropimorph
		2011*	fallow field	no additional pesticide applied

* until start of trial

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 - 90 m^2) and one treated plot (size: 324 - 630 m^2). The treated plot consisted of three equal sized subplots A, B and C that were assigned for replicates.

The product, formulated as a soluble concentrate (SL), was broadcast applied to bare soil in a single application at a nominal rate of 250 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between early May and early June 2011 using a calibrated boom sprayer. Treated plots were three-fold replicated with subplot size ranging from 108 to 210 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rates determined by quantifying the amount of spray discharged ranged from 249 to 254 g a.s./ha averaged over the three replicates of each treated plot. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil Li 10 (approximately 50 g per dish, sieved to 2 mm). The petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were sealed with a lid, taped up and frozen within 30 minutes, except for trial L110331 (France North) and trial L110332 (France South) where samples were chilled in a cooled box after collection and placed in freezer storage within less than 2 hours and 15 minutes. Further details of application are presented in Table 7.1.2.2.1-14 below.

Table 7.1.2.2.1-14: Application parameters of field trial sites treated with M27 (SL, 100 g/L)

Trial Country	Application method	No. of appli-cations	Subplot (m²)	Application rate per treatment				Application Date
				nominal [g a.s/ha]	actual* [g a.s./ha]	dose verification** [g a.s/ha]	dose verification** % of nominal	
L110330 Germany	broadcast spray to bare soil	1	A (133.5)	250	251	265	106	24-May-2011
			B (133.5)	250	250	214	86	
			C (133.5)	250	258	280	112	
			Average	250	253	253	101	
L110331 France (North)	broadcast spray to bare soil	1	A (168)	250	239	261	104	07 June-2011
			B (168)	250	249	244	98	
			C (168)	250	258	268	107	
			Average	250	249	258	103	
L110332 France (South)	broadcast spray to bare soil	1	A (210)	250	257	216	86	24-May-2011
			B (210)	250	258	348	139	
			C (210)	250	246	245	98	
			Average	250	254	269	108	
L110333 Spain	broadcast spray to bare soil	1	A (108)	250	259	239	96	10-May-2011
			B (108)	250	255	242	97	
			C (108)	250	249	267	107	
			Average	250	254	250	100	

* determined by calculation of spray liquid applied
** determined by means of petri dishes filled with soil

Immediately after application of the test item and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a box spreader until complete coverage of the soil surface. Fine or medium grained sand was used. The thickness of the sand layer necessary for complete coverage of the soil was approximately 6 mm. The layer of sand was controlled up to at least 28 days after application and was renewed when needed. It remained intact until at least 28 days. Within this time period of 28 days, the individual fields received a total precipitation (rain and irrigation) of 85 mm (Germany), 99 mm (France North), 62 mm (France South) and 51 mm (Spain), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate or glufosinate ammonium. Rainfall was supplemented with irrigation at sites in Germany (195 mm), Northern France (120 mm), Southern France (321 mm) and Spain (672 mm).

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-15.

Table 7.1.2.2.1-15: Summary of climatic conditions at field trial sites used to investigate the dissipation of M27

Trial	L110330			L110331			L110332			L110333		
Location	Goch-Nierswalde			Stotzheim			Meauzac			Utrera		
	Germany			France (North)			France (South)			Spain		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑		∑	∑
May 11	15.0	10.0	10.0	-	-	-	18.1	2.2	0.0	23.4	28.0	30.5
Jun 11	16.1	89.0	0.0	17.2	88.4	0	18.6	49.8	22.0	26.9	3.0	90.9
Jul 11	15.6	112.4	10.0	16.3	97.8	10.5	19.3	81.8	27.5	27.7	0.0	91.7
Aug 11	16.9	136.2	0.0	18.5	95.2	16.7	21.8	0.4	49.5	28.3	0.0	91.0
Sep 11	15.4	54.2	10.0	16.0	75.8	0.0	19.7	14.2	61.1	25.6	15.0	54.6
Oct 11	10.8	78.4	0.0	9.2	35.2	8.7	13.7	17.2	39.2	22.5	70.5	38.6
Nov 11	6.7	8.4	50.0	5.1	2.4	0.0	11.2	19.2	33.3	15.5	88.5	0.0
Dec 11	5.5	146.6	0.0	4.9	101.8	0.0	7.4	66.4	0	11.5	14.5	5.0
Jan 12	4.1	122.0	0.0	3.3	58.4	0.0	5.7	33.2	0	9.8	40.0	17.6
Feb 12	0.4	24.2	0.0	-2.1	2.8	0.0	0.7	2.4	0	7.9	0.5	23.6
Mar 12	8.1	23.8	20.0	8.1	10.2	3.5	10.2	28.4	18.2	13.7	8.0	30.2
Apr 12	8.2	79.8	0.0	8.9	50.6	26.3	10.7	101.0	18.7	15.9	59.5	10.7
May 12	14.4	70.0	10.0	15.4	33.4	0.0	16.6	70.8	0	23.6	23.0	23.1
Jun 12	14.6	122.2	0.0	17.2	96.2	0.0	20.0	58.6	0	26.6	0.0	43.7
Jul 12	16.9	148.0	10.	17.9	89.0	10.1	20.1	31.2	16.9	27.4	0.0	45.5
Aug 12	18.4	45.0	25.0	18.9	37.2	20.8	22.6	67.6	0	28.1	0.0	44.3
Sept 12	13.5	48.8	25.0	14.0	44.8	23.2	18.0	20.2	17.2	24.6	78.0	30.5
Oct 12	10.0	84.6	10.0	8.9	81.6	0.0	14.4	36.6	17.0	20.0	127.0	0.0
Nov 12	6.5	34.6	0.0	5.9	55.8	0.0	9.1	30.4	0	18.1	40.5	0.0
Dec 12	4.3	121.0	0.0	-	-	-	-	-	-	-	-	-
Jan 13	1.8	46.2	0.0	-	-	-	-	-	-	-	-	-
Feb 13	1.2	37.6	0.0	-	-	-	-	-	-	-	-	-
Mar 13	2.8	39.8	15.0	-	-	-	-	-	-	-	-	-
Apr 13	8.4	45.2	0.0	-	-	-	-	-	-	-	-	-
May 13	12.3	0.2	0.0	-	-	-	-	-	-	-	-	-

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

The actual air temperature recorded at the field sites during the study period was similar to the historic values. Whereas the sites in Northern Europe (Germany and Northern France) received more rain during the study period compared to the historical values, rainfall was less than the historic values in Southern Europe (Southern France, Spain). Due to additional irrigation, the total water input at the test sites during the study was at least 92% of the historical average rainfall, which is considered sufficient to allow the cultivation of crops like maize.

Historical (long-term) weather data on precipitation and average air temperature from at least 10 years were taken from official weather stations located nearby (7-14 km distance to trial site). The historical and actual data, each averaged over the complete duration of the individual trials, are presented in Table 7.1.2.2.1-16.

Table 7.1.2.2.1-16: Summary of historical and actual weather data at field trial sites averaged over entire trial duration

Trial Country	Tmean Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic	Actual	Historic	Actual			
L110061 Germany	9.9	9.9	1576	1728	195	1923	122
L110062 France (North)	12.2	10.7	962	1057	120	1177	122
L110063 France (South)	14.4	14.6	1141	732	321	1053	92
L110064 Spain	18.7	20.9	781	596	672	1268	162

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 710 days and down to a maximum soil depth of 90 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-17.

Table 7.1.2.2.1-17: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L110330	Germany	-1, 0, 3, 6, 10, 17, 31, 60, 85, 120, 150, 175, 237, 363, 541, 710
L110331	France (North)	-8, 0, 3, 6, 10, 16, 28, 58, 92, 121, 153, 184/185, 233, 359, 535
L110332	France (South)	0, 3, 6, 10, 16, 29, 59, 93, 125, 153, 183, 248, 367, 541
L110333	Spain	-1, 0, 3, 6, 10, 16, 29, 59, 91, 120, 149, 176, 238, 363, 546

Untreated specimens were collected from the control plot at two to three occasions, one day before application down to a depth of 90 cm, after about one year and, where applicable, after about two years to a depth of 10 cm each. The specimens were taken randomly from one part of the untreated plot each time and pooled according to soil depth. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 4.4 to 5.0 cm diameter. The 10 cores taken after about one and two years were collected with a metal tube of minimum 7.2 and maximum 8.3 cm diameter.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A – C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 7.2 and to maximum 8.3 cm diameter which left a hole contained by a steel or plastic collar. Alternatively, samples were taken by pressing the metal tube described above into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a common soil corer fitted with a plastic liner of diameter 4.4 to 5 cm. Sampling of these cores was conducted in one run or in up to four consecutive steps.

All soil cores collected with the soil probe were sectioned into segments of 10 cm. Soil segments of the same depth were pooled and homogenized. All soil specimens were usually stored at or below -18°C within less than 6 hours after sampling and remained frozen until analysis.

5. Analytical procedure

Field soil specimens were analyzed for M27 and metabolite M23 according to validated BASF method L0109/02 *[CA 4.1.2/1, Tilting, N. and Sopena-Vazquez, F. 2014a, BASF DocID 2013/1110235]*. Petri dish specimens were analysed for M27. The analytical method involved extraction of the soil with methanol/water (60:40, v/v) and final determination of the analytes by HPLC-MS/MS. The limit of quantification (LOQ) was 0.005 mg/kg for each individual analyte. The limit of detection (LOD) was set at 0.0015 mg/kg (30% of LOQ). Generally, a second mass transition was monitored for each analyte.

Analysis of field soil specimens originating from the treated plots was conducted down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg). Analysis was performed up to a maximum of 248 days after treatment (DAT).

Spray broth specimens were diluted to the appropriate concentration and analyzed for M27 using HPLC-MS/MS.

The validity of the analytical method was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of M27 and M23 in frozen soil was investigated in a separate study *[CA 7.1.2.2.1/4, Mewis, A. 2014, BASF DocID 2013/1348019]* with soils originating from fields adjacent to the trial sites of the present terrestrial field dissipation study.

7. Calculation of dissipation times

A calculation of dissipation times is not provided in the present report. A detailed kinetic evaluation of the degradation behaviour of M27 in the four European field soils is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649]*.

II. RESULTS AND DISCUSSION

A. SPRAY BROTH CONCENTRATION AND APPLICATION VERFICATION

Spray broth homogeneity was confirmed by visual check for all trials. In addition, spray mixtures sampled before and after application of each subplot were analyzed for M27. Procedural recovery experiments yielded an average of 103% across all trials.

Analysed spray broth concentrations averaged across the individual field sites were in the range of 0.745 to 0.830 g/L corresponding to 90-100% of the target concentration of 0.826 g/L. The analytical results were not corrected for procedural recoveries. Measured results from spray broth analysis are close to the target concentration confirming the integrity of the test item used in the trials.

Procedural recovery experiments were conducted with untreated soil along with the analysis of the applied Petri dish samples from the field that served as application monitors. Mean recoveries of each analyzed set of samples for M27 ranged from 97% to 103%.

Residue levels of M27 achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were corrected for the mean recovery of the respective analytical set and converted into residue rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm^2). As a result, the obtained rates for the individual trials ranged from 250 to 269 g/ha representing 100-108% of the target application rate (see Table 7.1.2.2.1-14 for individual figures). The applied amount determined via the application monitors in these trials is in good agreement with the nominal value of 250 g/ha and the results from spray broth analysis.

B. FINDINGS

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of M27 and M23. No residues above 30% LOQ of any analyte were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the two analytes at concentration levels of 0.005, 0.01 and 0.05 mg/kg yielded overall mean recovery rates of 99% for the individual analytes confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-18.

Table 7.1.2.2.1-18: Method procedural recoveries

Analyte	Fortification level [mg/kg]	n	Mean recovery ± RSD* [%]
M27	0.005, 0.01, 0.05	130	99 ± 9
M23	0.005, 0.01, 0.05	130	99 ± 9

* mean values are across all soils and soil depths; RSD = relative standard deviation [%]

These data prove that the analytical method applied was able to accurately determine residues of M27 and M23 in soil samples down to a concentration of 0.005 mg/kg for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg, maximum depth of 60 cm). If samples were analysed in duplicate, the individual numbers were averaged to produce a mean for the respective soil sample. When one of the values was below the LOQ, it was averaged as half of LOQ. For all trials, the 0 DAT double samples of the 0-10 cm soil layer were analysed as well, in order to account for the importance of the day 0 value, and the final data were obtained by averaging the mean values of the respective main and double samples.

All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the two analytes in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field samples, which was calculated based on the dry weight and the volume of the sampled soil specimens. The obtained residue rates in g/ha were summed up for all depths between 0 and 60 cm analyzed. Results are presented in Table 7.1.2.2.1-19.

Table 7.1.2.2.1-19: Total residues of M27 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110330 Goch-Nierswalde, Germany			L110331 Stotzheim, France		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	**158**	**140**	**171**	**176**	**176**	**192**
3	**155**	**149**	**212**	**144**	**150**	**141**
6	**182**	**148**	**211**	**111**	**102**	**160**
10	**254**	**136**	**131**	**72**	**91**	**109**
16 - 17	**164**	**101**	**113**	**72**	**65**	**77**
28 - 31	**108**	**49**	**75**	**37**	**44**	**38**
58 - 60	**53**	**48**	**38**	0	0	0
85 - 92	**36**	**21**	0	0	0	0
120 - 121	0	0	0	0	0	0
150 - 153	0	0	0	0	0	0
175 - 185	0	0	0	0	0	0
233 - 237	0	0	0	0	0	0
Trial Country	**L110332 Meauzac, France**			**L110333 Utrera, Spain**		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	**164**	**171**	**216**	**184**	**163**	**127**
3	**180**	**201**	**151**	**164**	**167**	**185**
6	**195**	**132**	**149**	**171**	**121**	**220**
10	**186**	**187**	**128**	**148**	**160**	**137**
16	**142**	**177**	**126**	**153**	**91**	**86**
29	**34**	**31**	**44**	**66**	**63**	**69**
59	0	0	0	**28**	**43**	0
91 - 93	0	0	0	0	**19**	0
120 - 125	0	0	0	0	0	0
149 - 153	0	0	0	0	0	0
176 - 183	0	0	0	0	0	0
238 - 248	0	0	0	0	0	0

DAT = days after treatment
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero

As is evident from the analytical data, M27 degraded quickly at all four European field sites. The total amount of M27 residues detected in the soil profiles decreased from an average of 170 g/ha at day 0 to an average of 55 g/ha (range: 36-77 g/ha) after 1 month. No residues above the LOQ (0.005 mg/kg) were detectable any longer after 4 months at the latest.

Considering the distribution of M27 residues in the soil profiles, residues were exclusively found in the top 0-30 cm layer of the soils, except for 1 single detect at a depth of 30-40 cm at 0.009 mg/kg in the trial conducted in Germany. No residues above the LOQ were detected below 40 cm in any sample at any site. Altogether, it can be concluded that M27 shows only moderate tendency to move into deeper soil layers indicating moderate potential for M27 residues to leach to groundwater.

Metabolite M23 was also monitored during the study. No residues of M23 above the LOQ were detected in any sample.

A detailed kinetic evaluation of the field trials is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649].*

Shipment verification specimens

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through any shipping process. The samples were prepared at nominal 0, 30 and 90 DAT by fortification of soil aliquots with 0.15 mg/kg M27 and were subsequently handled in the same manner as the actual residue samples. Concentrations of M27 analyzed were corrected for the mean recovery of the respective analytical set.
The analytical results demonstrated no significant losses from the shipping verification samples. The average amount of M27 from the soil samples spiked at the field test sites was 89% across all trials. It was concluded that M27 was stable in all soils under the storage and shipping conditions used.

Storage stability

The maximum period any soil sample from the present field soil dissipation study was stored from the time of sampling to extraction was 892 days. Results from a storage stability study performed with four soils soils taken from fields adjacent to those of the present field dissipation study *[CA 7.1.2.2.1/4, Mewis, A. 2014, BASF DocID 2013/1348019]* showed that both, M27 and M23 are stable in frozen soil for at least 29 months (895 days).

III. CONCLUSION

M27, a metabolite of dimethenamid, degraded fast under field conditions in soil at all four European field sites. The total amount of M27 residues detected in the soil profiles decreased from an average of 170 g/ha at day 0 to an average of 55 g/ha after 1 month. No residues above the LOQ (0.005 mg/kg) were detectable any longer after 4 months at the latest. DT_{50} values are supposed to be low and are presented in separate modeling reports.

M27 residues were mainly detected in the top 0-30 cm layer of the soils and no residues above the LOQ were detected below 40 cm in any sample at any site. Altogether, it can be concluded that M27 shows only moderate tendency to move into deeper soil layers indicating moderate potential for M27 residues to leach to groundwater.

Metabolite M23 was also monitored during the study. No residues of M23 above the LOQ were detected in any sample at any site.

Report: CA 7.1.2.2.1/3
Bayer H.,Marwitz A., 2014c
Field soil dissipation study of BAS 656 H (Dimethenamid-P) in the formulation BAS 769 00 H on bare soil at two different sites in Europe, 2011-2013
2013/1343460

Guidelines: NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SETAC Procedures for assessing the environmental fate and behaviour and ecotoxicity of pesticides (March 1995), EFSA Scientific Opinion on field dissipation studies (2010), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of dimethenamid-P (BAS 656 H) and its metabolites M23, M27 and M31 under field conditions was investigated at two sites in Europe representative of Northern EU conditions. One trial each was performed in Germany and the United Kingdom. All sites represent typical regions of agricultural practice representative for growing crops including oilseed rape which is among the most important crops for the use of dimethenamid-P. The trial sites consisted of an untreated and a treated area, the latter being subdivided into three subplots that were assigned for replicates.

The product BAS 769 00 H, formulated as an emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 600 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted in early and in late September 2011 using a calibrated boom sprayer. The actual application rates determined by quantifying the amount of spray discharged ranged from 582 to 626 g a.s./ha for all trials, with an average of 606 g a.s./ha. Results from spray broth analysis revealed a concentration of 102% of the nominal value. Dose verification conducted via application monitors yielded recovery values for the individual trial sites ranging from 81 to 92% of the target rate and an average recovery of 87% over both sites.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 4 mm depth to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted until complete coverage of the soil surface. The layer of sand was controlled up to 27 (Germany) or 29 days after application (UK) and was renewed when needed. It remained intact until at least 27 and 29 days, respectively. Within this time period of 27/29 days, the individual fields received a total precipitation (rain and irrigation) of 25 mm (United Kingdom) and 22 mm (Germany), respectively.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate. Rainfall was supplemented with irrigation at the site in Germany (111 mm). The field in UK was not irrigated due to adequate rainfall. The total water input at trial sites in United Kingdom and Germany was at least 103% of the historical average rainfall during the study period.

Soil specimens were taken at intervals up to 1.5 years after application and down to a maximum soil depth of 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18°C within a maximum of 8 hours after sampling and remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and any shipments, shipment verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the field sites with known amounts of dimethenamid-P. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipping verification specimens on dimethenamid-P yielded an average recovery value of 77% across the two sites confirming residue stability during all storage and shipment procedures.

Soil specimens were analyzed for dimethenamid-P and metabolites M23, M27 and M31 according to BASF method L0109/02. Application monitors (Petri dish samples) were analyzed for dimethenamid P. The analytical method involved extraction of the soil with methanol/water (60/40, v/v). The final determination of the analytes was performed by LC-MS/MS with a limit of quantification (LOQ) of 0.005 mg/kg for each analyte. Field soil specimens from the treated plot were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ). Analysis was performed to a maximum of 367 days after treatment (DAT).

No residues above 30% of the LOQ of any analyte were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soils spiked with the four analytes at concentration levels of 0.005, 0.01 and 0.05 mg/kg yielded overall mean recovery rates between 85 and 93% for the individual analytes, confirming the validity of the analytical method used in this study.

Residue values of dimethenamid-P and metabolites M23, M27 and M31 in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the individual field samples, and were summed up for all depths between 0 and 50 cm analysed. Residue values were not corrected for procedural recoveries except for results obtained from petri dish and shipment verification analysis.

Dimethenamid-P degraded fast under field conditions in soil at both European field sites. The total amount of dimethenamid-P residues in the soil profiles decreased from an average of 518 g/ha at day 0 to an average of 32 g/ha after six months. No residues above the LOQ (0.005 mg/kg) were detectable any longer after 8 or 12 months.

Residues of dimethenamid-P were exclusively detected in the upper 10 cm of the soils. Therefore, it can be concluded that dimethenamid-P does not show any tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were temporarily detected in small amounts only at the site in the United Kingdom reaching maximum amounts of 16 g/ha, 53 g/ha, and 12 g/ha, respectively. Thereafter, residues declined again and were no longer detected after 182 days at the latest. At the site in Germany, no residues of the three metabolites above the LOQ (0.005 mg/kg) were detected in any sample. Metabolites M23 and M31 were exclusively found in the top 0-10 cm soil layer. Metabolite M27 was detected in the 0-30 cm soil layer.

No calculation of dissipation times is provided in this summarized study. A detailed kinetic evaluation of the degradation behavior of dimethenamid-P in the two European field soils is subject of separate modeling reports.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation): BAS 769 00 H
Active ingredient (a.i.): Dimethenamid-P (BAS 656 H, Reg.No. 363851)
Type of formulation: EC

Batch no.: 16830568E0
Content of a.i.: 204.3 g/L (nominal 200 g/L)
Expiration date: August 31, 2011

Besides dimethenamid-P, the formulation also contained metazachlor (200 g/L nominal) which, however, was not of interest for the purpose of the present study.

2. Test sites

The dissipation of dimethenamid-P under field conditions was investigated at two sites in Europe representative of Northern EU conditions. The trials were performed in the United Kingdom (L110481) and in Germany (L110482). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics including soil taxonomy, the basic soil parameters of the corresponding soil horizons as well as the bulk density in 10-20 cm depth are presented in Table 7.1.2.2.1-20. Soil parameters were determined from soil samples taken before application following segmentation according to the soil horizons. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-20: Characteristics of the trial sites used to investigate the field dissipation of dimethenamid-P

Trial	**L110481**		**L110482**		
Location	**Wilson, United Kingdom**		**Lentzke, Germany**		
Soil properties	0 - 30 cm	30 - 90 cm	0 - 38 cm	38 – 66 cm	66 - 90 cm
Soil class (DIN 4220)	Silty loam (Lu)	Poor clay loam (Lt2)	Poor loamy sand (Sl2)	Poor loamy sand (Sl2)	Poor loamy sand (Sl2)
sand [%]	18.7	33.9	72.3	72.3	74.5
silt [%]	56.9	37.7	22.4	21.2	19.7
clay [%]	24.5	28.4	5.2	6.6	5.7
Soil class (USDA)	Silt loam	Silty clay loam	Sandy loam	Sandy loam	Loamy sand
sand [%]	21.2	37.1	75.4	74.2	76.7
silt [%]	56.3	35.5	19.5	18.8	17.3
clay [%]	22.5	27.4	5.1	7.0	6.0
Total organic C [%]	2.48	0.65	0.62	0.17	0.09
Organic matter [%]*	4.28	1.12	1.07	0.29	0.16
pH [$CaCl_2$]	6.84	7.27	5.73	6.04	6.22
pH [H_2O]	7.22	8.31	6.64	7.16	7.22
CEC [mval Ba/100g dry weight]	25.1	17.8	4.2	3.7	3.2
MWHC [g/100g dry weight]	63.8	53.2	43.9	37.9	37.9
pF 2.0 [g/100g dry weight]**	34.9	25.1	14.1	15.5	13.8
pF 2.5 [g/100g dry weight]**	29.0	21.1	12.3	10.8	10.7
Dry bulk density [g/cm^3]***	1.26	-	1.57	-	-
Soil taxonomy	Dystric Cambisol		Albic-Luvisols, Albeluvisol, and Cambisol		

* organic matter = organic carbon x 1.724
** water retention characteristics, soil moisture at 0.1 or 0.33 bar
*** samples taken at 10-20 cm depth (mean of 3 replicates)
CEC = cation exchange capacity
MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice with soils representative for growing crops including oilseed rape, which is among the most important crops for the use of dimethenamid-P. The sites were flat without any significant slope and had been under cultivation for many years. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No dimethenamid-P or any product from a similar chemical class had been used on the test plots in the previous three years. The crop and pesticide history of the trial sites is presented in Table 7.1.2.2.1-21.

Table 7.1.2.2.1-21: Management history of the trial sites in the previous years (non-GLP)

Trial	Location	Year	Crops grown	Pesticides used
L110481	Wilson, United Kingdom	2008	winter wheat	tebuconazole, chlorothalonil, epoxiconazole, trinexapac-ethyl, amidosulfuron, chlormequat, cyproconazole, propiconazole
		2009	winter wheat	iodosulphuron-methyl-sodium, mesosulfuron-methyl, amidosulfuron, chlormequat, chlorothalonil, cyproconazole, propiconazole, epoxiconazole, trinexapac-ethyl, tebuconazole, prothioconazole, lambda-cyhalothrin
		2010	bare soil	glyphosate
		2011*	bare soil	glyphosate
L110482	Lentzke, Germany-East	2008	winter wheat	tritosulfuron, fluroxypyr, flurasulam, pinoxaden, chloquintocet
		2009	winter wheat	glyphosate
		2010	clover	glyphosate
		2011*	carrots	glyphosate, pendimethalin

* until start of trial

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 18 - 36 m^2) and one treated plot (size: 324 m^2). The treated plot consisted of three equal sized subplots A, B and C that were assigned for replicates.

The product, formulated as a emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 600 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between early and late September 2011 using a calibrated boom sprayer. Treated plots were three-fold replicated with subplot size of 108 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rate determined by quantifying the amount of spray discharged was 606 g a.s./ha for both trials averaged over the three replicates of each treated plot. In addition, the dose was verified by means of sampling petri dishes filled with standard soil Li 10 (approximately 50 g per dish, sieved to 2 mm). The petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were sealed with a lid, taped up and frozen within 32 minutes (L110481, UK) or chilled on blue ice after collection and placed in freezer storage within less than 3 hours (L110482, Germany). Further details of application are presented in Table 7.1.2.2.1-22 below.

Table 7.1.2.2.1-22: Application parameters of the field trial site treated with BAS 769 00 H (EC, 200 g/L)

Trial Country	Application Method	No. of appli-cations	Subplot (m²)	Application rate per treatment				Application Date
				nominal	actual*	dose verification**		
				[g a.s/ha]	[g a.s./ha]	[g a.s/ha]	% of nominal	
L110481 Wilson United Kingdom	broadcast spray to bare soil	1	A (108)	600	611	481	80	27-Sep-2011
			B (108)	600	600	470	78	
			C (108)	600	606	499	83	
			Average	**600**	**606**	**484**	**81**	
L110482 Lentzke Germany	broadcast spray to bare soil	1	A (108)	600	610	550	92	9-Sep-2011
			B (108)	600	582	532	89	
			C (108)	600	626	580	97	
			Average	**600**	**606**	**553**	**92**	

* determined by calculation of spray liquid applied
** determined by means of petri dishes filled with soil

Immediately after application of the test item and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a sand spreader until complete coverage of the soil surface. Fine or medium grained sand was used. The thickness of the sand layer necessary for complete coverage of the soil was approximately 4 mm. The layer of sand was controlled up to 27 (Germany) and 29 days (UK) after application and was renewed when needed. It remained intact until 27 and 29 days. Within this time period of 27/29 days, the individual fields received a total precipitation (rain and irrigation) of 25 mm (United Kingdom) and 22 mm (Germany), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate. Rainfall was supplemented with irrigation at the site in Germany (111 mm). The field in UK was not irrigated due to adequate rainfall.

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-23.

Table 7.1.2.2.1-23: Summary of climatic conditions at field trial sites used to investigate the dissipation of Dimethenamid-P

Trial	L110481			L110482		
Location	Wilson			Lentzke		
	United Kingdom			Germany		
Climatic conditions / Month	T_{mean} Air [°C]	Prec. [mm] ∑	Irrigation [mm] ∑	T_{mean} Air [°C]	Prec. [mm] ∑	Irrigation [mm] ∑
Sep 11	18.0	0.0	0.0	13.0	16.6	0.0
Oct 11	12.7	37.6	0.0	7.9	36.8	0.0
Nov 11	9.5	41.0	0.0	2.4	6.0	0.0
Dec 11	6.2	55.6	0.0	2.6	67.8	0.0
Jan 12	5.3	13.8	0.0	-0.1	52.4	0.0
Feb 12	4.2	14.2	0.0	-3.3	23.6	0.0
Mar 12	7.4	26.4	0.0	6.7	10.4	0.0
Apr 12	7.0	104.6	0.0	7.8	24.0	20.2
May 12	11.7	37.2	0.0	13.9	29.0	31.8
Jun 12	13.5	138.4	0.0	14.8	55.4	9.1
Jul 12	15.5	50.0	0.0	17.6	127.4	0.0
Aug 12	16.2	23.6	0.0	17.8	22.4	26.3
Sept 12	12.7	24.8	0.0	13.7	30.0	23.8
Oct 12	9.3	62.6	0.0	8.4	59.8	0.0
Nov 12	6.6	106.4	0.0	4.7	30.0	0.0
Dec 12	4.9	134.2	0.0	-0.1	33.8	0.0
Jan 13	3.7	36.6	0.0	-0.2	63.2	0.0
Feb 13	3.0	31.8	0.0	-0.5	27.2	0.0
Mar 13	2.5	60.8	0.0	2.6	1.0	0.0

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Historical (long-term) weather data on precipitation and average air temperature from at least 10 years were taken from official weather stations located nearby (10-14 km distance to trial site). The historical and actual data, each averaged over the complete duration of the individual trials, are presented in Table 7.1.2.2.1-24.

Table 7.1.2.2.1-24: Summary of historical and actual weather data at field trial sites averaged over entire trial duration

Trial Country	Tmean Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic	Actual	Historic	Actual			
L110481 United Kingdom	8.7	8.9	968	1000	0	1000	103
L110482 Germany	7.0	6.7	775	717	111	828	107

The actual air temperature recorded at the field sites during the study period was similar to the historic values. Whereas the site in UK received slightly more rain during the study period compared to historical values, rainfall was less than historic values at the site in Germany. Due to additional irrigation of the field in Germany, the total water input at both test sites during the study was at least 103% of the historical average rainfall, which is considered sufficient to allow the cultivation of crops like oilseed rape.

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 548 days and down to a maximum soil depth of 90 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-25.

Table 7.1.2.2.1-25: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L110481	United Kingdom	-1, 0, 3, 6, 10, 17, 29, 62, 85, 122, 154, 182, 246, 367, 548
L110482	Germany	-4, 0, 3, 6, 10, 17, 27, 59, 87, 123, [150±5]*, 185, 242, 353, 545

* 150 ± 5 DAT sampling could not be performed due to unfavourable weather conditions

Untreated specimens were collected from the control plot at two occasions, one or four days before application down to a depth of 90 cm, and after about one year to a depth of 10 cm. The specimens were taken randomly from one part of the untreated plot each time and pooled according to soil depth. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 5.0 cm diameter. The 10 cores taken after about one year were collected with a metal tube of minimum 8.2 and maximum 9.2 cm diameter.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A – C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 8.2 and to maximum 9.8 cm diameter which left a hole contained by a steel or plastic collar. Alternatively, samples were taken by pressing the metal tube described above into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar using a common soil corer fitted with a plastic liner of diameter 5 cm. Sampling of these cores was conducted in one run or in up to three consecutive steps.

All soil cores collected with the soil probe were sectioned into segments of 10 cm. Soil segments of the same depth were pooled and homogenized. All soil specimens were usually stored at or below -18°C within less than 8 hours after sampling and remained frozen until analysis.

5. Analytical procedure

Field soil specimens were analysed for dimethenamid-P and metabolites M23, M27 and M31 according to validated BASF method L0109/02 *[CA 4.1.2/1, Tilting, N. and Sopena-Vazquez, F. 2014a, BASF DocID 2013/1110235]*. Petri dish specimens were analysed for dimethenamid-P. The analytical method involved extraction of the soil with methanol/water (60:40, v/v) and final determination of the analytes by HPLC-MS/MS. The limit of quantification (LOQ) was 0.005 mg/kg for each individual analyte. The limit of detection (LOD) was set at 0.0015 mg/kg (30% of LOQ). Generally, a second mass transition was monitored for each analyte.

Analysis of field soil specimens originating from the treated plots was conducted down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg). Analysis was performed to a maximum of 367 days after treatment (DAT).

Spray broth specimens were diluted to the appropriate concentration and analysed for dimethenamid-P using HPLC-MS/MS.

The validity of the analytical method was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of dimethenamid-P, M23, M27 and M31 in frozen soil was investigated in a separate study *[CA 7.1.2.2.1/5, Mewis, A. 2014, BASF DocID 2013/1348029]* with soils originating from the individual trial sites of the present terrestrial field dissipation study.

7. Calculation of dissipation times

A calculation of dissipation times is not provided in this summarized report. A detailed kinetic evaluation of the degradation behaviour of dimethenamid-P in the two European field soils is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649.*

II. RESULTS AND DISCUSSION

A. SPRAY BROTH CONCENTRATION AND APPLICATION VERFICATION

Spray broth homogeneity was confirmed by visual check for both trials. In addition, spray mixtures from trial L110481 (UK) sampled before and after application of each subplot were analyzed for dimethenamid-P. Spray samples from trial L110482 (Germany) could not be analyzed since they got lost at the analytical laboratory. Procedural recovery experiments conducted along with the analysis of the spray broth samples showed a mean recovery of 81% for dimethenamid-P. Analyzed concentrations averaged across the individual subplots of the field site in UK were 2.02 g/L corresponding to 102% of the target concentration of 1.98 g/L. The analytical results are not corrected for procedural recoveries.

Measured results from spray broth analysis of Trial L110481 (UK) are close to the target concentration confirming the integrity of the test item used in the trials.

Procedural recovery experiments were conducted with untreated soil along with the analysis of the applied Petri dish samples from the field that served as application monitors. Mean recovery of the analysed set of samples for dimethenamid-P was 86%.

Residue levels of dimethenamid-P achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were corrected for the mean recovery of the respective analytical set and converted into residue rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm^2). As a result, the obtained application rate averaged across both trials accounted for 519 g/ha representing 87% of the target rate (see Table 7.1.2.2.1-3 for individual figures). The applied amount determined by the application monitors is only slightly below the nominal value of 600 g/ha and hence in good agreement with this value and the results from spray broth analysis.

B. FINDINGS

Untreated soil specimens (control samples) of the respective soil depths from each trial were analysed for residues of dimethenamid-P and metabolites M23, M27 and M31. No residues above 30% LOQ of any analyte were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the four analytes at concentration levels of 0.005, 0.01 and 0.05 mg/kg yielded overall mean recovery rates for the individual analytes between 85 and 93%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-26.

Table 7.1.2.2.1-26: Method procedural recoveries

Analyte	Fortification level [mg/kg]	n	Mean recovery ± RSD* [%]
dimethenamid-P	0.005, 0.01, 0.05	57	85 ± 13
M23	0.005, 0.01, 0.05	57	91 ± 12
M27	0.005, 0.01, 0.05	57	92 ± 12
M31	0.005, 0.01, 0.05	57	93 ± 13

* mean values are across all soils and soil depths; RSD = relative standard deviation [%]

These data prove that the analytical method applied was suitable to accurately determine residues of dimethenamid-P and its metabolites in soil down to a concentration of 0.005 mg/kg for each analyte.

Field soil specimens from the treated plots were analysed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.005 mg/kg, maximum depth of 50 cm). If samples were analysed in duplicate, the individual numbers were averaged to produce a mean for the respective soil sample. When one of the values was below the LOQ, it was averaged as half of LOQ. For all trials, the 0 DAT double samples of the 0-10 cm soil layer were analysed as well, in order to account for the importance of the day 0 value, and the final data were obtained by averaging the mean values of the respective main and double samples.

All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the four analytes in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field samples, which was calculated based on the dry weight and the volume of the sampled soil specimens. The obtained residue rates in g/ha were summed up for all depths between 0 and 50 cm analysed. Results are presented in Table 7.1.2.2.1-27**:** to Table 7.1.2.2.1-30**:**.

Table 7.1.2.2.1-27: Total residues of dimethenamid-P under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110481 Wilson, United Kingdom			L110482 Lentzke, Germany		
	Replicate A	**Replicate B**	**Replicate C**	**Replicate A**	**Replicate B**	**Replicate C**
DAT	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	708	570	496	456	459	416
3	530	251	485	353	293	326
6	588	416	222	283	295	249
10	287	341	395	217	236	305
17	418	348	191	150	153	170
27-29	253	175	212	78	86	81
59-62	125	136	158	50	50	35
85-87	92	147	77	31	42	24
122-123	52	79	49	40	38	31
154	58	69	58	*	*	*
182-185	69	30	43	22	15	13
242-246	0	8.1	13	0	0	0
367	0	0	0			

* no sample taken due to bad weather conditions
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero
DAT = days after treatment

Table 7.1.2.2.1-28: Total residues of M23 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110481 Wilson, United Kingdom			L110482 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17	11	6.6	7.5	0	0	0
27-29	13	6.1	15	0	0	0
59-62	7.2	7.0	16	0	0	0
85-87	0	0	0	0	0	0
122-123	0	0	0	0	0	0
154	0	0	0	*	*	*
182-185	0	0	0	0	0	0
242-246	0	0	0	0	0	0
367	0	0	0			

* no sample taken due to bad weather conditions
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero
DAT = days after treatment

Table 7.1.2.2.1-29: Total residues of M27 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110481 Wilson, United Kingdom			L110482 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17	14	13	7.5	0	0	0
27-29	20	12	23	0	0	0
59-62	38	35	48	0	0	0
85-87	42	30	36	0	0	0
122-123	22	28	12	0	0	0
154	40	31	24	*	*	*
182-185	53	28	26	0	0	0
242-246	0	0	0	0	0	0
367	0	0	0			

* no sample taken due to bad weather conditions
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero
DAT = days after treatment

Table 7.1.2.2.1-30: Total residues of M31 under field conditions in soil calculated to g/ha and summed up for all depths analysed

Trial Country	L110481 Wilson, United Kingdom			L110482 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	0	0	0	0	0	0
10	0	0	0	0	0	0
17	12	0	11	0	0	0
27-29	8.8	0	8.7	0	0	0
59-62	0	0	9.3	0	0	0
85-87	0	0	0	0	0	0
122-123	0	0	0	0	0	0
154	0	0	0	*	*	*
182-185	0	0	0	0	0	0
242-246	0	0	0	0	0	0
367	0	0	0			

* no sample taken due to bad weather conditions
calculations are based on actual dry soil density for individual soil layers
residue values <0.005 mg/kg (<LOQ) were reported and treated as zero
DAT = days after treatment

As is evident from the analytical data, dimethenamid-P degraded fast at both European field sites. The total amount of dimethenamid-P residues detected in the soil profiles decreased from an average of 518 g/ha at day 0 to an average of 32 g/ha (range: 13-69 g/ha) after 6 months. At the site in the United Kingdom, no residues above the LOQ (0.005 mg/kg) were detectable any longer after 1 year at the latest. At the site in Germany, no residues above the LOQ were left after 242 days.

Considering the distribution of dimethenamid-P residues in the soil profiles, residues were exclusively detected in the top 0-10 cm soil layer Therefore, it can be concluded that dimethenamid-P does not show any tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were also monitored during the study. At the site in Germany, no residues of any of the three metabolites above the LOQ (0.005 mg/kg) were detected in any sample. However, they were temporarily detected in small amounts at the site in the United Kingdom.
Metabolite M23 was detected from 17 DAT onwards. It increased to a maximum of 16 g/ha, thereafter declined and was no longer detected after 85 days.
Metabolite M27 was detected from 17 DAT. It increased to a maximum of 53 g/ha, thereafter declined and was no longer detected after 246 days
Metabolite M31 was detected from 17 DAT where it showed a maximum of 12 g/ha. Thereafter it declined and was no longer detected after 85 days.
Metabolites M23 and M31 were exclusively found in the top 0-10 cm soil layer. Metabolite M27 was detected in the 0-30 cm soil layer.

A detailed kinetic evaluation of the field trials is presented in separate modeling reports *[CA 7.1.2.2.1/6, Wiedemann, G. 2014b, BASF DocID 2014/1031648 and CA 7.1.2.2.1/7, Wiedemann, G. 2014a, BASF DocID 2014/1031649].*

Shipment verification specimens

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through the shipping process. The samples were prepared at nominal 0, 30 and 90 DAT by fortification of soil aliquots with 0.5 mg/kg dimethenamid-P and were subsequently handled in the same manner as the actual residue samples. Concentrations of dimethenamid-P analyzed were corrected for the mean recovery of the respective analytical sample set.
The analytical results demonstrated no significant losses from the shipping verification samples. The average recovery of dimethenamid-P from the soil samples spiked at the field test sites was 77%. It was concluded that dimethenamid-P was stable in the soils under the shipping and storage conditions used.

Storage stability

The maximum period any sample from the present field soil dissipation study was stored from the time of sampling to analysis was 710 days. Results from a storage stability study performed with the soils from the two field sites *[CA 7.1.2.2.1/5, Mewis, A. 2014, BASF DocID 2013/1348029]* proved that dimethenamid-P and metabolites M23, M27, and M31 are stable in frozen soil for at least 23 months.

III. CONCLUSION

Dimethenamid-P degraded fast under field conditions in soil at both European field sites. The total amount of dimethenamid-P residues in the soil profiles decreased from an average of 518 g/ha at day 0 to an average of 32 g/ha after six months. No residues above the LOQ (0.005 mg/kg) were detectable any longer after 8 or 12 months. DT_{50} values are supposed to be low and are presented in separate modeling reports.

Dimethenamid-P residues were exclusively detected in the upper 10 cm of the soils. Therefore, it can be concluded that dimethenamid-P does not show any tendency to move into deeper soil layers indicating low potential for dimethenamid-P residues to leach to groundwater.

Metabolites M23, M27 and M31 were temporarily detected only at the site in the United Kingdom reaching maximum amounts of 16 g/ha, 53 g/ha, and 12 g/ha, respectively. At the site in Germany, no residues of the three metabolites above the LOQ (0.005 mg/kg) were detected in any sample.

Metabolites M23 and M31 were only found in the top 0-10 cm soil layer. Metabolite M27 was detected in the 0-30 cm layer.

Report: CA 7.1.2.2.1/4
Mewis A., 2014c
Determination of the storage stability of Dimethenamid-P and its metabolites M23, M27 and M31 in 4 soils under deep frozen conditions
2013/1348019
Guidelines: EPA 860.1380
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of the study was to examine the storage stability of dimethenamid-P and its metabolites M23, M27 and M31 in deep-frozen soil over a storage period of up to 29 months. Storage stability samples were prepared by fortification of untreated samples with the test items and were stored for 0, 1, 2, 4, 8, 12, 18 and 29 months. Four soils were used from different trials of a parallel field study (BASF Doc ID 2013/1343457; trials L110061, L110062, L110063 and L110064). The method L0109/02 (BASF DocID 2013/1110235) was used for determination of the test items. The limit of quantitation (LOQ) was 0.005 mg/kg for all analytes. After 29 months of storage at least 99 % of the initial amount of dimethenamid-P, 84 % of the initial amount of M23, 84 % of the initial amount of metabolite M27 and 77 % of the initial amount of metabolite M31 were determined, indicating that dimethenamid-P and its metabolites are stable in soil for at least 29 months of storage under deep-freeze conditions.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	Dimethenamid-P	M23	M27 (Sodium salt)	M31
Purity:	96.4%	98.8%	97.1% / 97.4% / 97.1% (three different batches were used)	98.7%
CAS #:	163515-14-8	--	--	--

B. STUDY DESIGN

1. Experimental Conditions

Four soil samples from a parallel field study were used. The homogenized samples were stored deep-frozen. 5 g of matrix sample was fortified at 0.1 mg/kg (20 x LOQ) of each analyte (separate systems). The test items were distributed to the entire sample with a solvent volume of maximum 500 µL per sample. For each storage interval and matrix a set of at least 4 samples (2 samples for analysis plus 2 backup samples) was prepared.

The temperatures in the freezer were recorded during the entire storage period; temperatures ranged from a minimum of -25°C to a maximum of -14°C with a mean temperature of -22°C. The samples were analysed at least in duplicate, after fortification and storage for 0, 1, 2, 4, 8, 12, 18 and 29 months. In addition, blank matrix samples and freshly fortified recovery samples were analysed at the analysis dates.

2. Procedure for Determination of the Test Item

For the determination of dimethenamid-P and its metabolites, soil samples were analysed by method L0109/02. The samples were extracted twice with 20 mL of methanol/water (60:40, v/v).
The final determination was performed by HPLC-MS/MS detection using at least two characteristic fragment ions.

II. RESULTS AND DISCUSSION

Residues of dimethenamid-P and its metabolitesM23, M27 and M31 were determined after deep-frozen storage for 0, 1, 2, 4, 8, 12, 18 and 29 months. The results are given in **Table 7.1.2.2.1-31** to **Table 7.1.2.2.1-34**. No significant decline was observed for dimethenamid-P and its metabolites. They were stable for up to 29 months.

Table 7.1.2.2.1-31: Results of the storage stability of dimethenamid-P in soil

Test item	Storage period	Recovery [% of initial amount]			
		Soil L110061	Soil L110062	Soil L110063	Soil L110064
Dimethenamid-P	0 months	100	100	100	100
	1 months	110	110	110	109
	2 months	102	96	97	100
	4 months	98	95	95	94
	8 months	83	85	83	90
	12 months	92	90	93	93
	18 months	87	88	93	90
	29 months	104	103	99	99

Table 7.1.2.2.1-32: Results of the storage stability of M23 in soil

Test item	Storage period	Recovery [% of initial amount]			
		Soil L110061	Soil L110062	Soil L110063	Soil L110064
M23	0 months	100	100	100	100
	1 months	105	105	102	100
	2 months	99	106	96	98
	4 months	104	106	103	101
	8 months	78	92	96	89
	12 months	86	85	77	84
	18 months	84	77	75	85
	29 months	89	85	84	87

Table 7.1.2.2.1-33: Results of the storage stability of M27 in soil

Test item	Storage period	Recovery [% of initial amount]			
		Soil L110061	Soil L110062	Soil L110063	Soil L110064
M27	0 months	100	100	100	100
	1 months	102	98	94	96
	2 months	107	107	101	102
	4 months	110	104	102	101
	8 months	86	91	88	89
	12 months	97	91	93	90
	18 months	96	86	91	91
	29 months	92	88	87	84

Table 7.1.2.2.1-34: Results of the storage stability of M31 in soil

Test item	Storage period	Recovery [% of initial amount]			
		Soil L110061	Soil L110062	Soil L110063	Soil L110064
M31	0 months	100	100	100	100
	1 months	95	101	103	105
	2 months	98	107	102	104
	4 months	99	105	104	103
	8 months	91	104	91	97
	12 months	78	86	77	86
	18 months	79	91	80	90
	29 months	77	95	86	94

III. CONCLUSION

The objective of the current study was to examine the deep-frozen storage stability of dimethenamid-P and its metabolites M23, M27 and M31 in soil over a storage period of up to 29 months. The recovery values in this study did not fall below 70 % of the initial value at each time point and for each analyte. For this reason, residues of dimethenamid-P and its metabolites M23, M27 and M31 can be regarded as stable in the matrix soil for at least 29 months of storage under deep-freeze conditions.

Report: CA 7.1.2.2.1/5
Mewis, F., 2013
Determination of the Storage Stability of Dimethenamid and its metabolites M23, M27 and M31 in 2 soil under deep frozen conditions
2013/1348029

Guidelines:

GLP: no

Executive Summary

The objective of the study was to examine the storage stability of dimethenamid-P and its metabolites M23, M27 and M31 in deep-frozen soil over a storage period of up to 23 months. Storage stability samples were prepared by fortification of untreated samples with the test items and were stored for 0, 1, 2, 4, 8, 12 and 23 months. Two soils were used from different trials of a parallel field study (BASF DocID2013/1343460, soils named 1795 and 1796). The method L0109/02 (BASF DocID 2013/1110235) was used for determination of the test items. The limit of quantitation (LOQ) was 0.005 mg/kg for all analytes. After 23 months of storage at least 98 % of the initial amount of dimethenamid-P, 97 % of the initial amount of M23, 92 % of the initial amount of metabolite M27 and 86 % of the initial amount of metabolite M31 were determined, indicating that dimethenamid-P and its metabolites are stable in soil for at least 23 months of storage under deep-freeze conditions.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	Dimethenamid-P	M23	M27 (Sodium salt)	M31
Purity:	96.4%	98.8%	97.1% / 97.4% / 97.1% (three different batches were used)	98.7%
CAS #:	163515-14-8	--	--	--

B. STUDY DESIGN

3. Experimental Conditions

Two soil samples from a parallel field study were used. The homogenized samples were stored deep-frozen. 5 g of matrix sample was fortified at 0.1 mg/kg (20 x LOQ) of each analyte (separate systems). The test items were distributed to the entire sample with a solvent volume of maximum 500 µL per sample. For each storage interval and matrix a set of at least 4 samples (2 samples for analysis plus 2 backup samples) was prepared.

The temperatures in the freezer were recorded during the entire storage period; temperatures ranged from a minimum of -25°C to a maximum of -14°C with a mean temperature of -22°C. The samples were analysed at least in duplicate, after fortification and storage for 0, 1, 2, 4, 8, 12 and 23 months. In addition, blank matrix samples and freshly fortified recovery samples were analysed at the analysis dates.

4. Procedure for Determination of the Test Item

For the determination of dimethenamid-P and its metabolites, soil samples were analysed by method L0109/02. The samples were extracted twice with 20 mL of methanol/water (60:40, v/v).
The final determination was performed by HPLC-MS/MS detection using at least two characteristic fragment ions.

II. RESULTS AND DISCUSSION

Residues of dimethenamid-P and its metabolites M23, M27 and M31 were determined after deep-frozen storage for 0, 1, 2, 4, 8, 12 and 23 months. The results are given in **Table 7.1.2.2.1-35** to **Table 7.1.2.2.1-38**. No significant decline was observed for dimethenamid-P and its metabolites. They were stable for up to 29 months.

Table 7.1.2.2.1-35: Results of the storage stability of dimethenamid-P in soil

Test item	Storage period	Recovery [% of initial amount]	
		Soil 1795	Soil 1796
Dimethenamid-P	0 months	100	100
	1 months	115	109
	2 months	106	100
	4 months	127	119
	8 months	127	121
	12 months	101	101
	23 months	99	98

Table 7.1.2.2.1-36: Results of the storage stability of M23 in soil

Test item	Storage period	Recovery [% of initial amount]	
		Soil 1795	Soil 1796
M23	0 months	100	100
	1 months	102	102
	2 months	95	91
	4 months	112	114
	8 months	111	102
	12 months	105	105
	23 months	101	97

Table 7.1.2.2.1-37: Results of the storage stability of M27 in soil

Test item	Storage period	Recovery [% of initial amount]	
		Soil 1795	Soil 1796
M27	0 months	100	100
	1 months	111	109
	2 months	102	97
	4 months	120	121
	8 months	106	109
	12 months	113	114
	23 months	92	92

Table 7.1.2.2.1-38: Results of the storage stability of M31 in soil

Test item	Storage period	Recovery [% of initial amount]	
		Soil 1795	Soil 1796
M31	0 months	100	100
	1 months	107	103
	2 months	98	100
	4 months	115	115
	8 months	115	98
	12 months	90	88
	23 months	86	86

III. CONCLUSION

The objective of the current study was to examine the deep-frozen storage stability of dimethenamid-P and its metabolites M23, M27 and M31 in soil over a storage period of up to 23 months. The recovery values in this study did not fall below 70 % of the initial value at each time point and for each analyte. For this reason, residues of dimethenamid-P and its metabolites M23, M27 and M31 can be regarded as stable in the matrix soil for at least 23 months of storage under deep-freeze conditions.

Report: CA 7.1.2.2.1/6
Wiedemann G., 2014b
Calculation of normalised modelling half-lives from terrestrial field dissipation studies with dimethenamid p and its metabolite M27 according to FOCUS kinetics
2014/1031648
Guidelines: FOCUS Degradation Kinetics (2011) Sanco/10058/2005 version 2.0
GLP: no

Report: CA 7.1.2.2.1/7
Wiedemann G., 2014a
Calculation of persistence half-lives from terrestrial field dissipation studies with dimethenamid p and its metabolite M27 according to FOCUS kinetics
2014/1031649
Guidelines: FOCUS Degradation Kinetics (2011) Sanco/10058/2005 version 2.0
GLP: no

Executive Summary

Two field dissipation studies (six trials) with the active substance BAS 656 H – Dimethenamid-P and one study (four trials) with the substance M656H027 (in this study called M27) (see reference CA 7.1.2.2.1/1 – 7.1.2.2.1/3), a metabolite of Dimethenamid-P, were evaluated to derive persistence and normalised modelling half-lives following the recommendations of the guidelines by the FOCUS work group on degradation kinetics [*FOCUS (2011): Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics. Version 1.0, 436pp.*]. The normalised modelling half-lives were calculated on the basis of time-step normalisation.

A summary of fits for persistence and modelling endpoints is presented in the tables below. Only trials with acceptable results are presented in this summary.

Table 7.1.2.2.1-39: Summary of $DegT_{50}$ – persistence endpoints for Dimethenamid-P and M27

Substance	Field trial	Kinetic model	χ^2-error [%]	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Reference
Dimethenamid-P	L110061	SFO	10.5	20.4	67.7	Bayer & Marwitz (2014a), CA 7.1.2.2.1/1
	L110062	SFO	17.3	17.6	58.6	
	L110063	SFO	13.8	14.5	48.1	
	L110064	SFO	9.24	16.5	54.7	
	L110481	FOMC	9.6	17.6	167	Bayer & Marwitz (2014b), CA 7.1.2.2.1/2
	L110482	FOMC	8.3	10.2	68.2	
M27 (applied as parent substance)	L110330	SFO	11.2	31.4	104	Bayer & Marwitz (2014c), CA 7.1.2.2.1/3
	L110331	SFO	3.7	12.0	40.0	
	L110332	SFO	14.6	19.4	64.3	
	L110333	SFO	9.6	23.7	78.6	

Table 7.1.2.2.1-40: Summary of $DegT_{50}$ – modelling endpoints for Dimethenamid-P and M27 (normalised to reference conditions: 20°C, pF2)

Substance	Field trial	Kinetic model	χ^2-error [%]	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Reference
Dimethenamid-P	L110061	SFO	10.1	12.6	42.0	Bayer & Marwitz (2014a), CA 7.1.2.2.1/1
	L110062	SFO	16.4	10.4	34.4	
	L110063	SFO	13.9	10.9	36.1	
	L110064	SFO	8.0	9.7	32.2	
	L110481	SFO	10.4	13.8	45.9	Bayer & Marwitz (2014b), CA 7.1.2.2.1/2
	L110482	SFO	8.2	6.9	22.8	
M27 (applied as parent substance)	L110330	SFO	10.3	14.6	48.6	Bayer & Marwitz (2014c), CA 7.1.2.2.1/3
	L110331	SFO	4.4	8.8	29.3	
	L110332	SFO	12.9	12.7	42.2	
	L110333	SFO	9.2	25.9	86.0	

I. MATERIAL AND METHODS

Study design

Three field dissipation studies with a total of ten trials were conducted with Dimethenamid-P or its metabolite M27. Each trial consisted of three replicates. The substances were applied onto bare soil; no crop was grown during the study period. After application, the soil surface in all trials was covered with a sand layer to prevent loss processes at the soil surface. Details on the locations, soil properties, meteorological data and sampling methods are given in the study summaries above or in the original reports.

Residue data

All replicate values were used in the optimization without averaging. The main part of the residues was found in 0 - 20 cm depth; below 40 cm, no residues > LOQ were detected in any of the samplings. For modelling, the observed residues in the different layers were added.

In the parent study, the main metabolites M23, M27 and M31 were analysed. However, in many trials not enough data points were available to perform a kinetic evaluation: Degradation of M23 was assessed in three out of six trial with Dimethenamid-P, degradation of M27 as metabolite in five trials. Degradation of M31 could not be assessed in any of the trials.

LOQ and LOD (limit of quantification / detection) were 0.005 mg kg^{-1} and 0.001 mg kg^{-1} in all trials. Concentrations given as "< LOQ" were treated according to the guidance of FOCUS (2011): The first sample < LOQ after or before a sample with detection of M27 was set to 0.5 x (LOQ + LOD). All following samples < LOQ were ignored. This rule was applied temporally and also spatially; i.e. if M27 was detected in layer 0 – 10 cm but not in any layer below, the value for layer 10 – 20 cm was set to 0.5 x (LOQ + LOD).

Checklist of the Dutch regulatory authority

The trials were evaluated according to criteria compiled by the Dutch regulatory authority (CTB) in order to ensure that the field study is adequately performed, and that samples are adequately taken and analysed.

Normalisation of residue data

The data of the field trials were normalised to reference conditions (20°C soil temperature, soil moisture at pF 2). The day length normalization was carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors. Actual soil temperature and moisture were derived employing the FOCUS-PEARL 4.4.4 groundwater model which was run with individual climate and soil data corresponding to each trial location.

The soil moisture correction was conducted using the modified Walker equation, as recommended by the FOCUS Kinetics (2011). Day lengths were normalized according to Equation 7.1.2.2.1-1 if the soil moisture calculated with FOCUS-PEARL 4.4.4 was lower than the reference soil moisture at pF 2. If the actual soil moisture was higher than the reference value, the moisture correction factor was set to 1 since wetter conditions do not accelerate the degradation processes. Reference moisture at pF 2 was taken from the soil certificates of the field phase reports converted to volumetric moisture using the measured bulk density.

Equation 7.1.2.2.1-1: Soil moisture correction factor ($\Theta_{ref} \geq \Theta_{act}$) for each day

$$D_{norm_moist} = D * \left(\frac{\theta_{act}}{\theta_{ref}} \right)^B$$

where			
	D_{norm_moist}	day length moisture correction factor	[d]
	D	standard day length (1)	[d]
	B	exponent of the moisture response function (0.7)	[-]
	θ_{act}	actual soil moisture calculated by PEARL	[%]
	θ_{ref}	reference soil moisture at pF 2	[%]

Temperature correction factors were derived using the Arrhenius equation (Q_{10} value = 2.58) as described in the report of the FOCUS Work Group on Degradation Kinetics (2011). The temperature correction factor was calculated according to Equation 7.1.2.2.1-2. If the soil temperature was ≤ 0°C, the correction factor had to be set to 0.

Equation 7.1.2.2.1-2: Temperature correction factor (T > 0°C) for each day

$$D_{norm_temp} = D * Q_{10}^{\frac{T_{act} - T_{ref}}{10}}$$

where			
	D_{norm_temp}	day length temperature correction factor	[d]
	D	standard day length (1)	[d]
	T_{act}	actual temperature calculated by PEARL	[°C]
	T_{ref}	reference temperature (20°C)	[°C]
	Q_{10}	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58)	[-]

The day length normalized for soil temperature and soil moisture was then calculated according to the following equation:

Equation 7.1.2.2.1-3: Day length normalized for temperature and moisture

$$D_{norm} = D * D_{norm_temp} * D_{norm_moist}$$

where			
	D_{norm}	day length normalized for temperature and moisture	[d]
	D	standard day length (1)	[d]
	D_{norm_temp}	day length temperature correction factor	[d]
	D_{norm_moist}	day length moisture correction factor	[d]

Cumulative corrected day lengths values were calculated for each sampling interval (Equation 7.1.2.2.1-4). These "normalized days after applications" were assigned to the residue data of the different field trials and used as input model data for the calculation of modelling endpoints.

Equation 7.1.2.2.1-4: Calculation of cumulative normalized day length

$$t_i = \sum_{j=0}^{i-1} D_{norm,j} \qquad i=1, 2 \ldots t_{max}$$

$$t_0 = 0$$

where	t_i	cumulative normalized day length until day *i*	[d]
	t_{max}	day of last sampling	[d]
	$D_{norm,j}$	normalized day length (20°C, pF2) of day *j*	[d]
	i,j	number of (julian) days after application	

Kinetic evaluation according to FOCUS

The available residue data were evaluated according to recommended procedures as given in the guideline of the FOCUS Work Group on Degradation Kinetics (2011) using the software package KinGUI (version 2).

Persistence endpoints

In trials with Dimethenamid-P and metabolites, the parent parameters were fitted in a first step with SFO and FOMC kinetics. The parameters of this SFO or FOMC model (depending on which model gave a better fit) were considered as relevant for the parent substance. In a second step, all parameters for parent and metabolites were fitted simultaneously using the best-fit model for the parent and SFO kinetics for the metabolites.

Modelling endpoints

In trials with Dimethenamid-P and metabolites, the parent parameters were fitted in a first step. The parameters of this fit were considered as relevant for the parent substance. In a second step, all parameters for parent and metabolites were fitted simultaneously. Only SFO kinetics were applied in the current modelling.

II RESULTS AND DISCUSSION

Persistence endpoints

Estimated degradation parameters of dimethenamid p

The degradation of Dimethenamid-P under field conditions was analysed in six trials. Only SFO and FOMC kinetics were tested since these already provided good fits. No outliers were identified in any of the trials. Details for each location are presented in the following tables. The graphs show the results of the best fit model. The following results are taken from the fits for Dimethenamid-P without metabolites.

Table 7.1.2.2.1-41: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110061 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 726 k: 0.0340 d^{-1}	10.5	k: < 0.001	Good	20.4	67.7
Run FOMC	M_0: 746 Alpha: 523 Beta: 13550	11.5	Alpha: 0.369 Beta: 0.370	Good	18.0	59.8
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

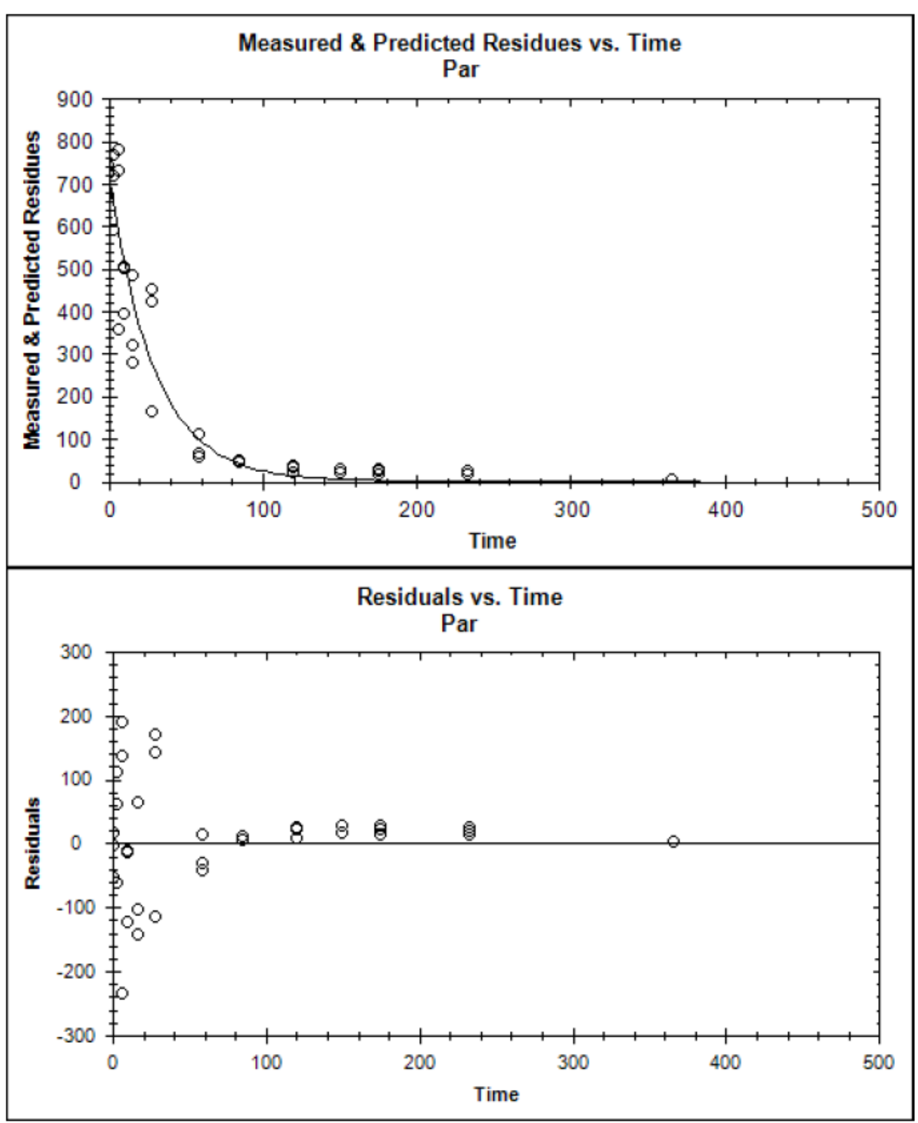


Figure 7.1.2.2.1-1: SFO fit for Dimethenamid-P in trial L110061 – persistence endpoints

Table 7.1.2.2.1-42: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110062 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 858 k: 0.0393 d^{-1}	17.3	k: < 0.001	Good	17.6	58.6
Run FOMC	M_0: 915 Alpha: 1047 Beta: 19956	21.0	Alpha: 0.126 Beta: 0.126	Good	13.2	43.9
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

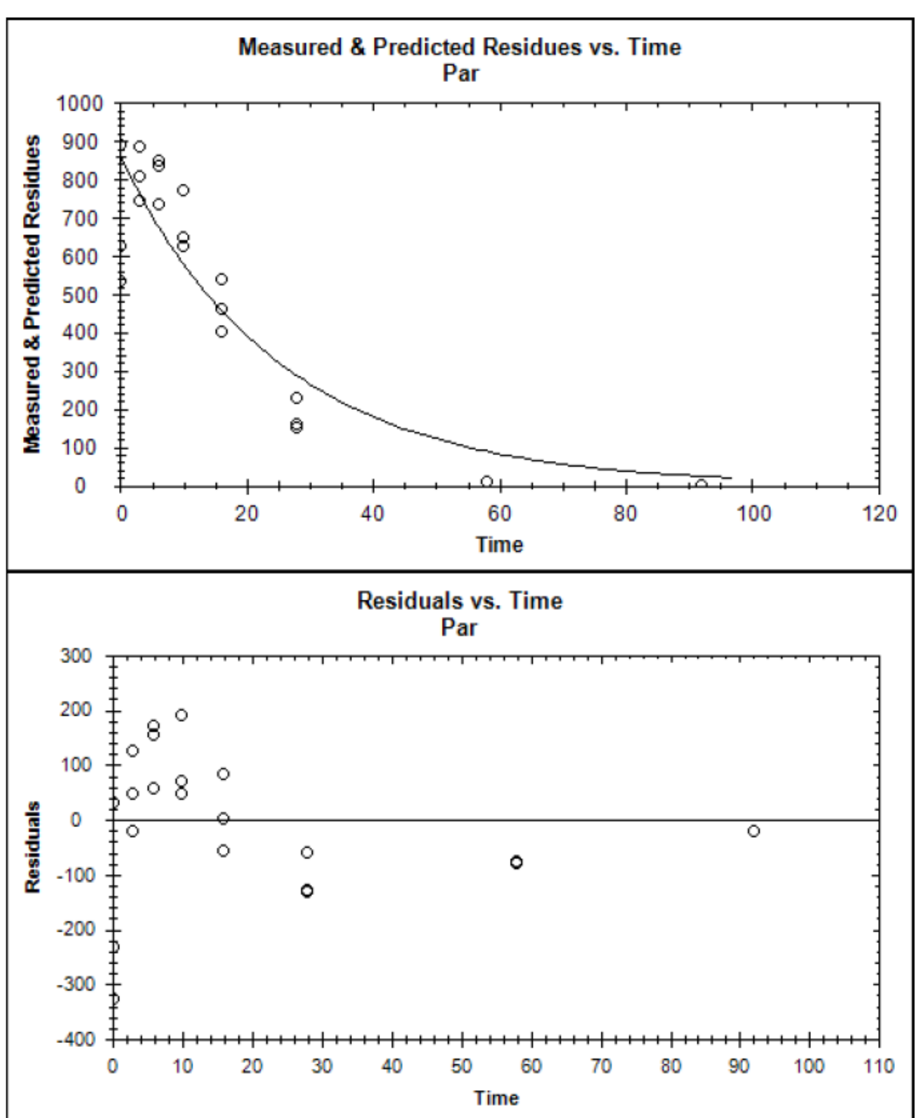


Figure 7.1.2.2.1-2: SFO fit for Dimethenamid-P in trial L110062 – persistence endpoints

Table 7.1.2.2.1-43: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110063 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 880 k: 0.0 479d^{-1}	13.8	k: < 0.001	Good	14.5	48.1
Run FOMC	M_0: 960 Alpha: 861 Beta: 12179	20.7	Alpha: 0.363 Beta: 0.363	Good	9.8	32.6
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

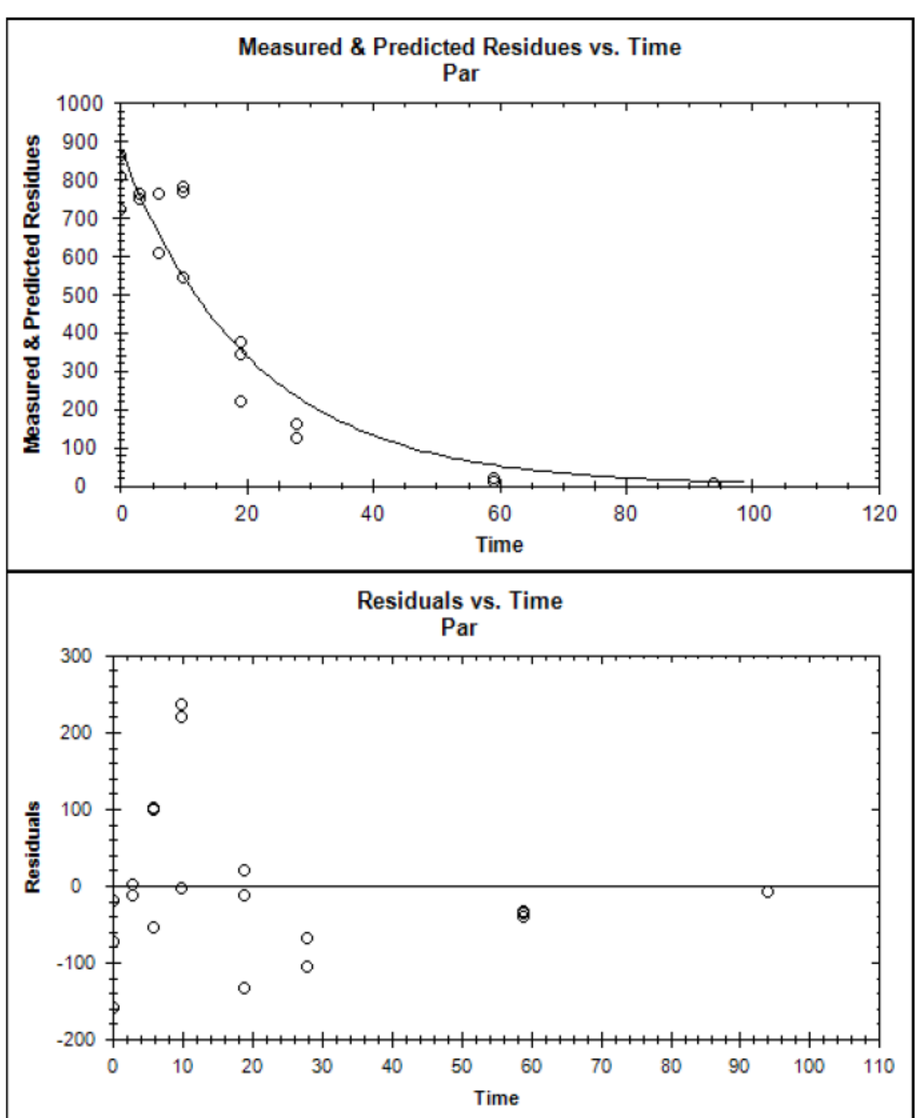


Figure 7.1.2.2.1-3: SFO fit for Dimethenamid-P in trial L110063 – persistence endpoints

Table 7.1.2.2.1-44: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110064 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 913 k: 0.0421 d^{-1}	9.2	k: < 0.001	Good	16.5	54.7
Run FOMC	M_0: 955 Alpha: 1112 Beta: 21617	11.7	Alpha: 0.338 Beta: 0.338	Good	13.5	44.8
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

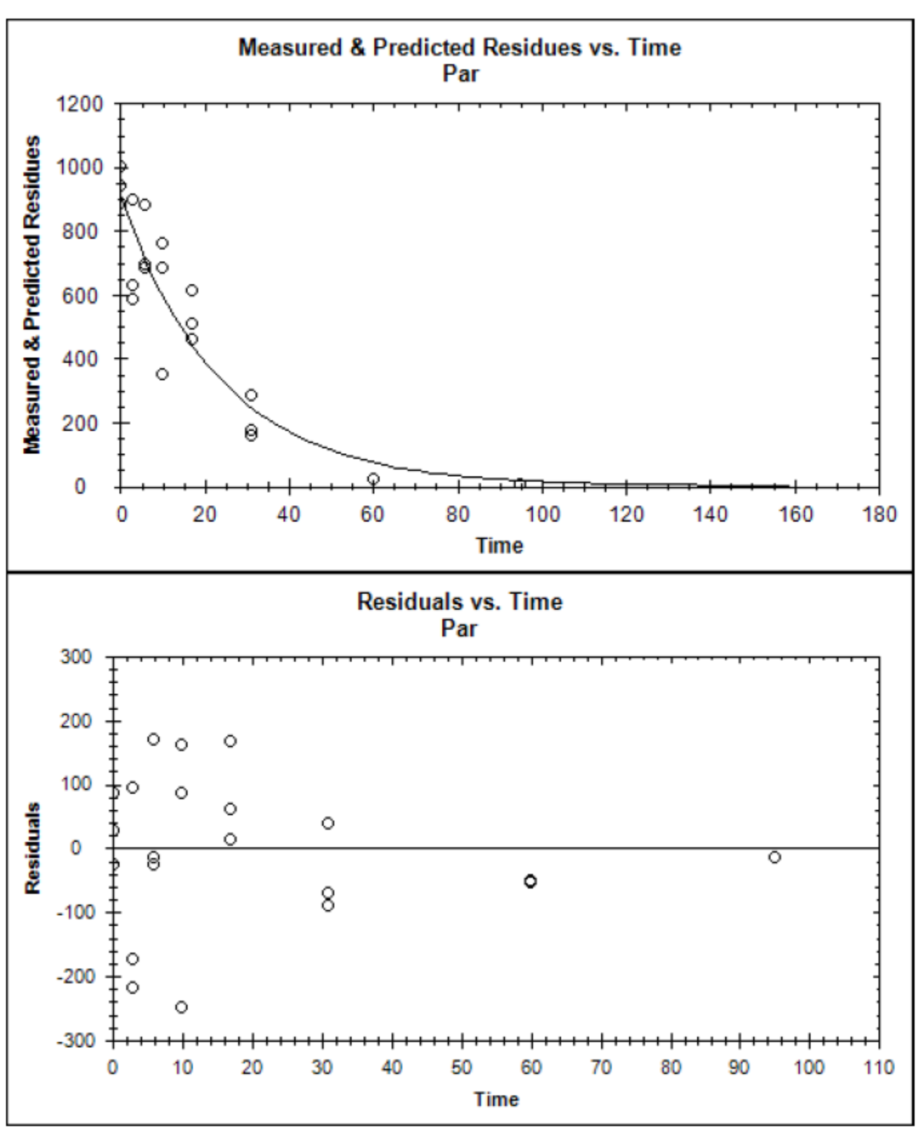


Figure 7.1.2.2.1-4: SFO fit for Dimethenamid-P in trial L110064 – persistence endpoints

Table 7.1.2.2.1-45: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110481 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 490 k: 0.0224 d^{-1}	16.2	k: < 0.001	Good	31.0	103
Run FOMC	M_0: 559 Alpha: 0.955 Beta: 16.5	9.3	Alpha: 0.005 Beta: 0.089	Good	17.6	167
⇨ SFO and FOMC fits both statistically good, FOMC visually better than SFO.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using FOMC kinetics.**						

* Type I error rate

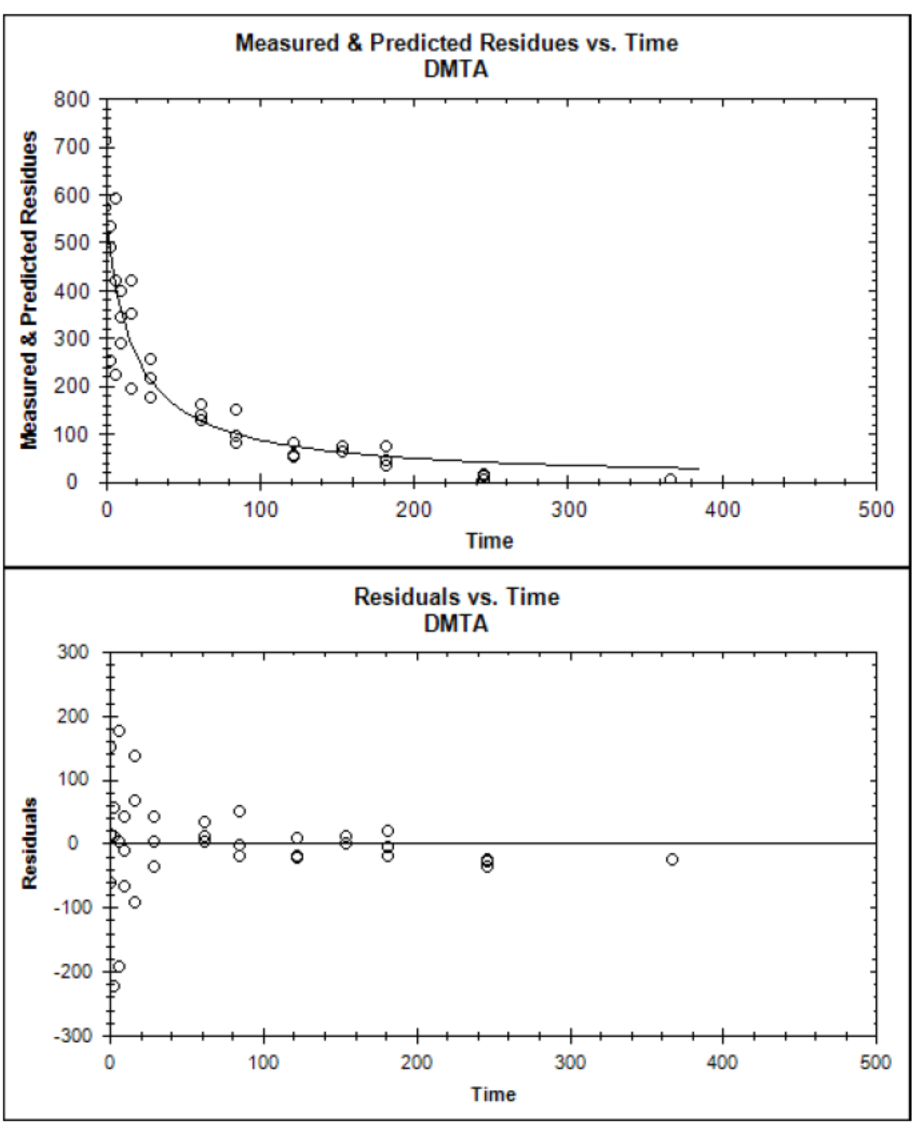


Figure 7.1.2.2.1-5: FOMC fit for Dimethenamid-P in trial L110481 – persistence endpoints

Table 7.1.2.2.1-46: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110482 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 420 k: 0.0565 d^{-1}	12.5	k: < 0.001	Acceptable	12.3	40.8
Run FOMC	M_0: 441 Alpha: 1.36 Beta: 15.4	8.3	Alpha: < 0.001 Beta: 0.003	Good	10.2	68.2
⇨ SFO and FOMC fits both statistically good, FOMC visually better than SFO.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using FOMC kinetics.**						

* Type I error rate

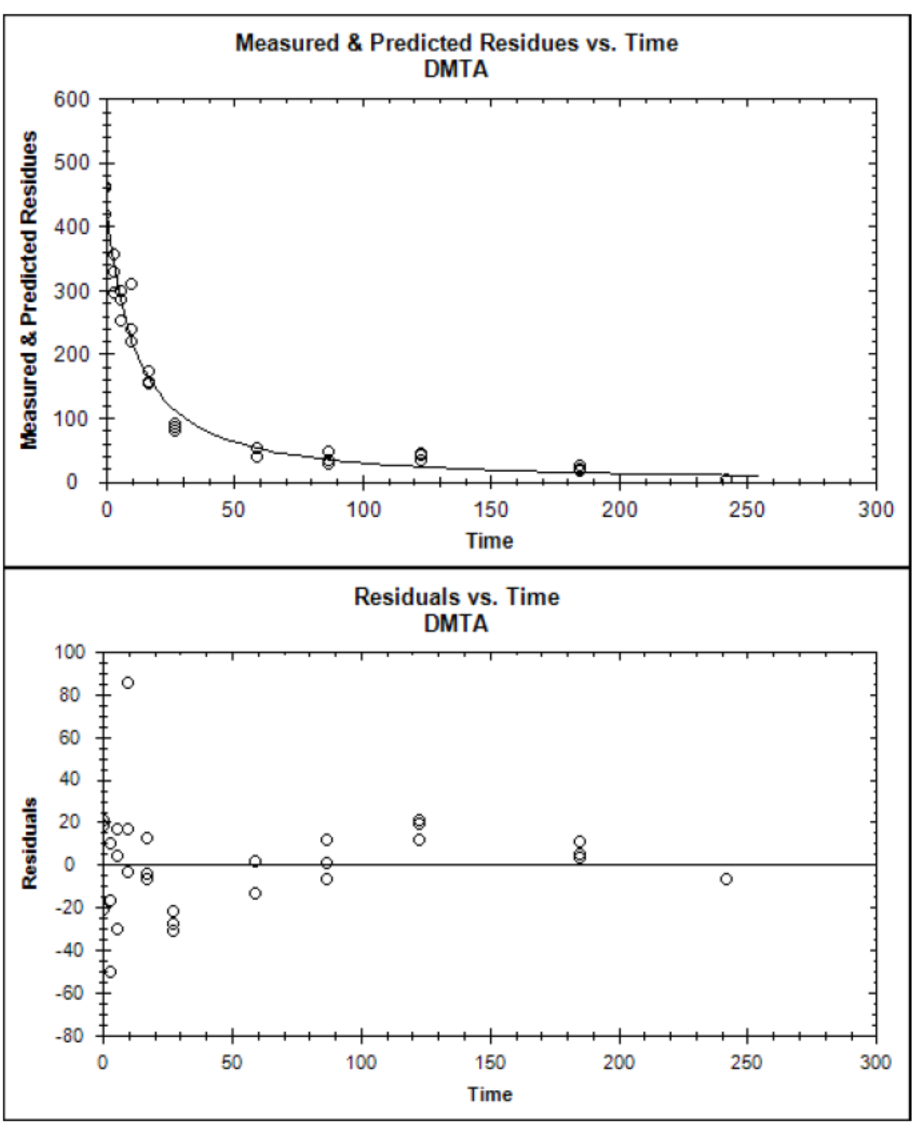


Figure 7.1.2.2.1-6: FOMC fit for Dimethenamid-P in trial L110482 – persistence endpoints

Estimated degradation parameters of M27 and M23 formed as metabolites of Dimethenamid-P

The degradation of M27 as a metabolite of Dimethenamid-P was evaluated for trials L110061, L110062, L110063, L110064 and L110481; for metabolite M23, trials L110062, L110064 and L110481 were considered. The fitted curves for M27 and M23 in all these trials are visually poor, and the χ^2 are very high. Therefore, the resulting parameters are not considered reliable. Please refer to the original report for details on the metabolites.

Estimated degradation parameters of M27 applied as parent substance

The degradation of M27 under field conditions was analysed in four trials, in which M27 was applied as parent substance. Only SFO and FOMC kinetics were tested since these already provided good fits. No outliers were identified in any of the trials. Details for each location are presented in the following tables. The graphs show the results of the best fit model.

Table 7.1.2.2.1-47: Statistical and visual assessment of kinetic models for M27 in trial L110330 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 188 k: 0.0220 d^{-1}	11.2	k: < 0.001	Good	31.4	104
Run FOMC	M_0: 197 Alpha: 953 Beta: 33663	13.8	Alpha: 0.415 Beta: 0.416	Good	24.5	81.4
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

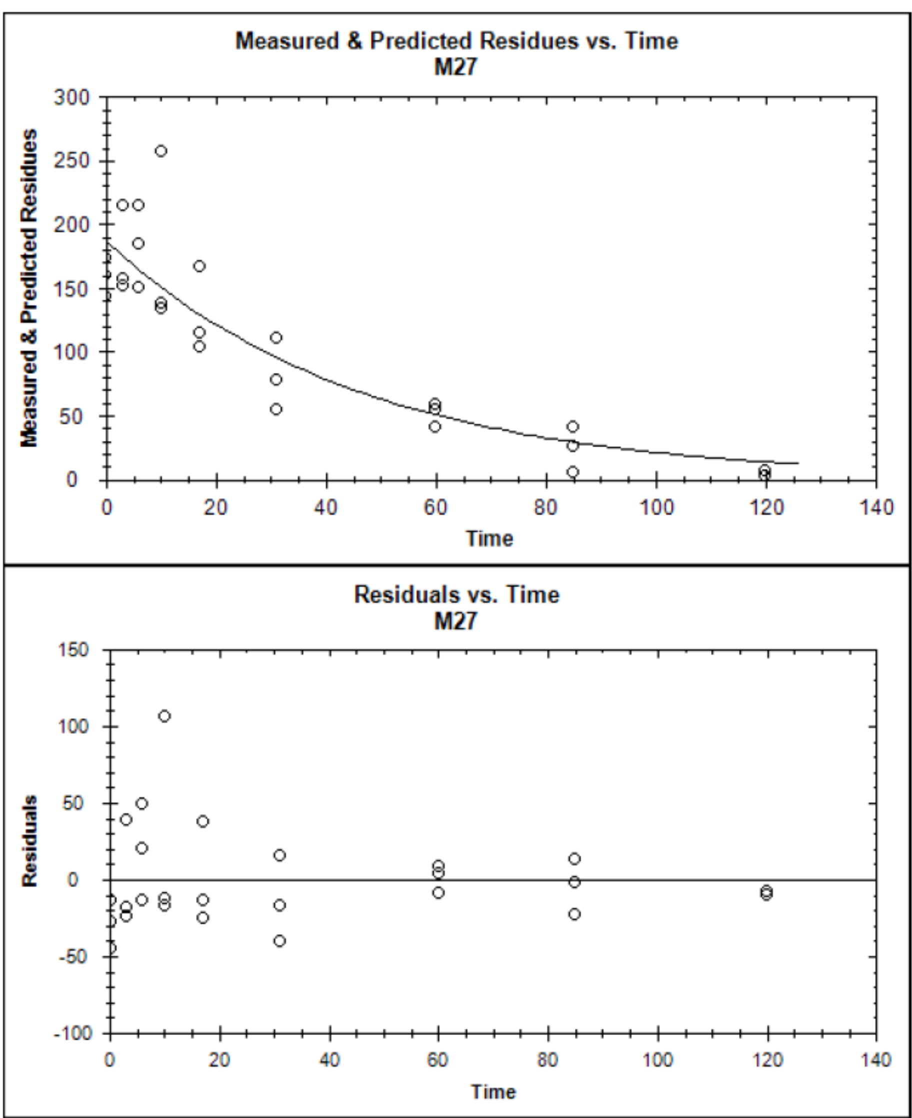


Figure 7.1.2.2.1-7: SFO fit for M27 in trial L110330 – persistence endpoints

Table 7.1.2.2.1-48: Statistical and visual assessment of kinetic models for M27 in trial L110331 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 179 k: 0.0576 d^{-1}	3.7	k: < 0.001	Good	12.0	40.0
Run FOMC	M_0: 182 Alpha: 5.78 Beta: 90.0	3.5	Alpha: 0.232 Beta: 0.255	Good	11.5	44.0
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

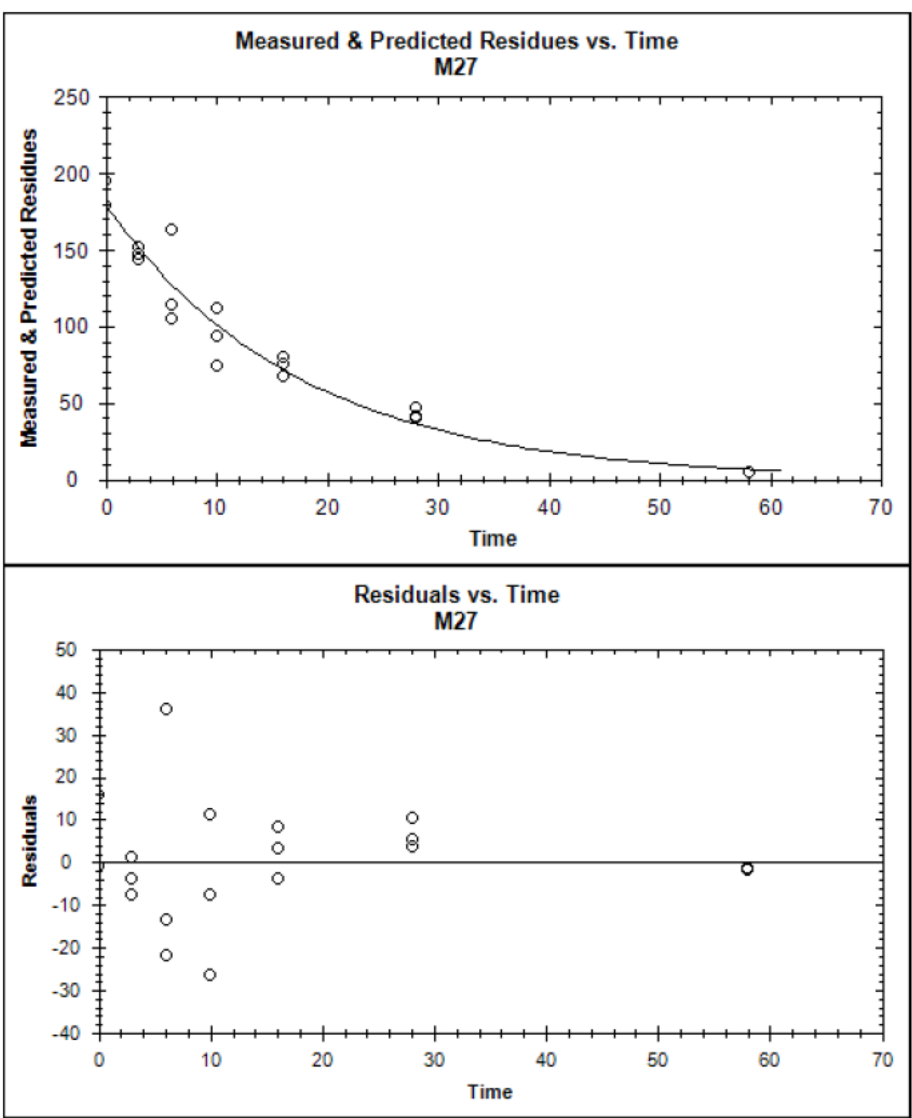


Figure 7.1.2.2.1-8: SFO fit for M27 in trial L110331 – persistence endpoints

Table 7.1.2.2.1-49: Statistical and visual assessment of kinetic models for M27 in trial L110332 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 205 k: 0.0358 d^{-1}	14.6	k: < 0.001	Good	19.4	64.3
Run FOMC	M_0: 218 Alpha: 508 Beta: 10458	18.4	Alpha: 0.393 Beta: 0.393	Good	14.3	47.5
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

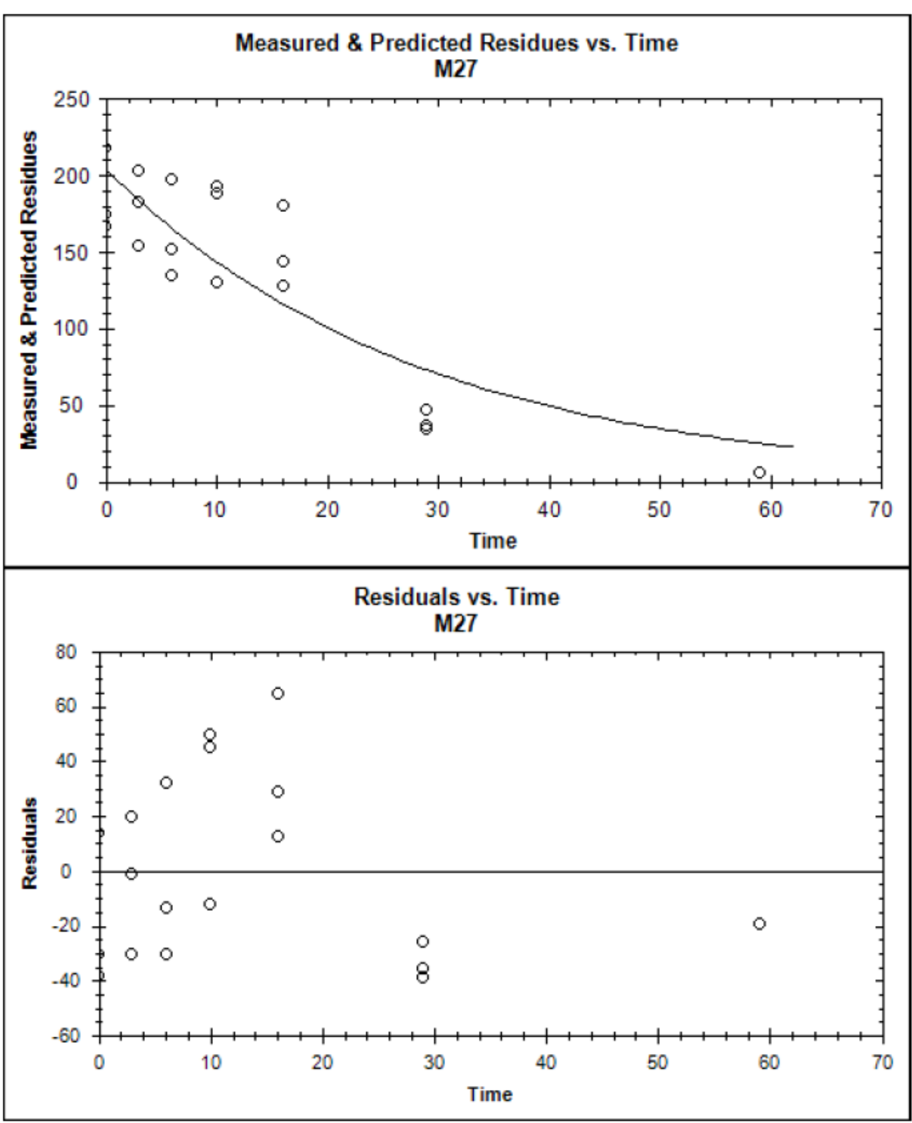


Figure 7.1.2.2.1-9: SFO fit for M27 in trial L110332 – persistence endpoints

Table 7.1.2.2.1-50: Statistical and visual assessment of kinetic models for M27 in trial L110333 – persistence endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 185 k: 0.0293 d^{-1}	9.6	k: < 0.001	Good	23.7	78.6
Run FOMC	M_0: 195 Alpha: 1029 Beta: 26530	13.1	Alpha: 0.409 Beta: 0.409	Good	17.9	59.4
⇨ SFO fit visually and statistically good. FOMC visually good, but t-test fails.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

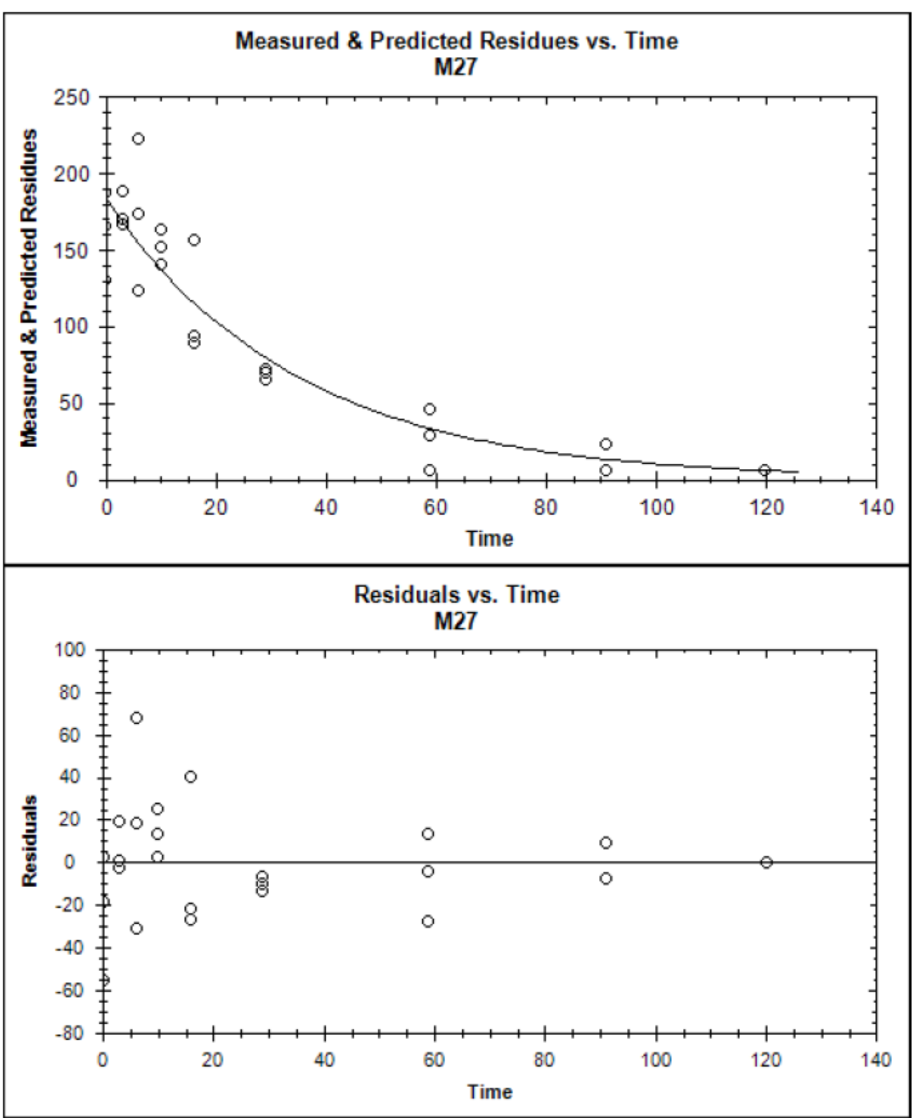


Figure 7.1.2.2.1-10: SFO fit for M27 in trial L110333 – persistence endpoints

Modelling endpoints

Estimated degradation parameters of dimethenamid p

The degradation of dimethenamid p under field conditions was analysed in six trials in order to derive appropriate degradation kinetics and modelling $DegT_{50}$. Only SFO kinetics in combination with time-step normalisation (i.e. with corrected sampling time) were tested since these already provided good fits. No outliers were identified in any of the trials. Details for each location are presented in the following tables. The graphs show the results of the best fit model. The following results are taken from the fits for Dimethenamid-P without metabolites.

Table 7.1.2.2.1-51: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110061 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 712 k: 0.0548 d^{-1}	10.1	k: < 0.001	Good	12.6	42.0
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

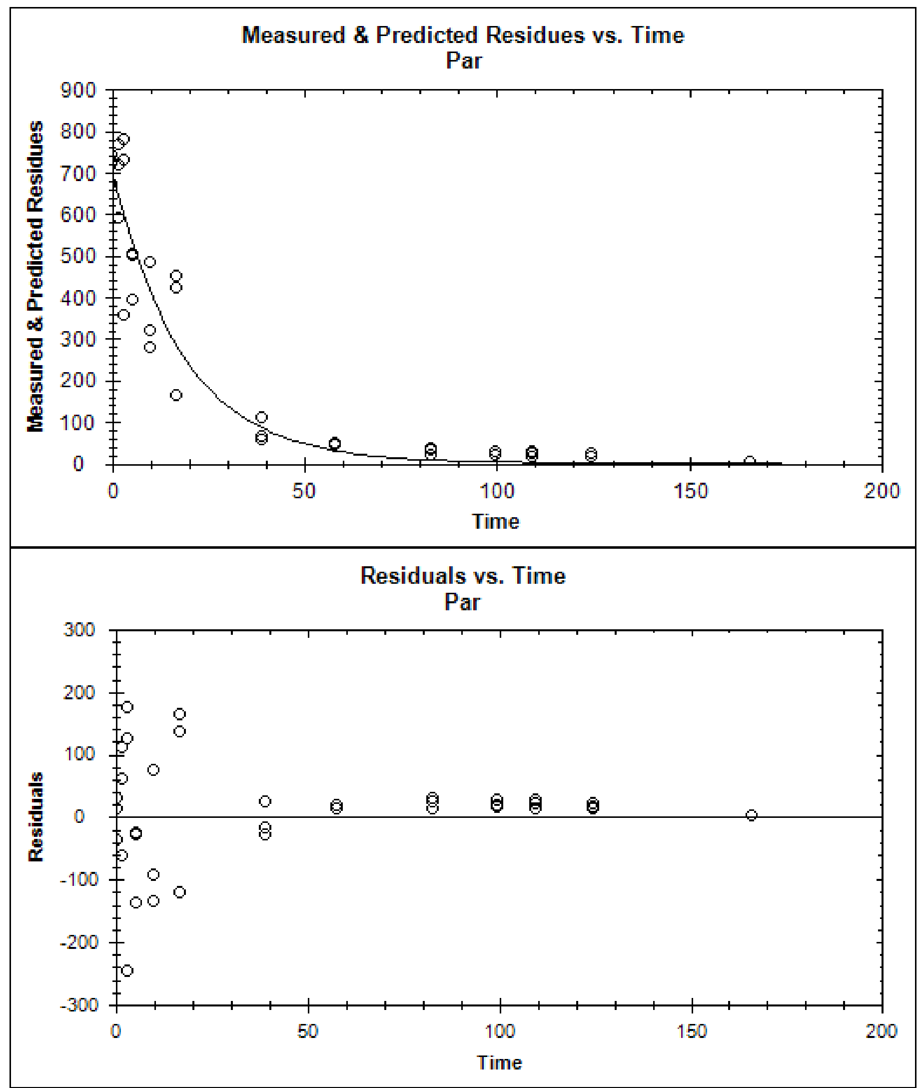


Figure 7.1.2.2.1-11: SFO fit for Dimethenamid-P in trial L110061 – modelling endpoints

Table 7.1.2.2.1-52: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110062 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 855 k: 0.0670 d^{-1}	16.4	k: < 0.001	Good	10.4	34.4
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

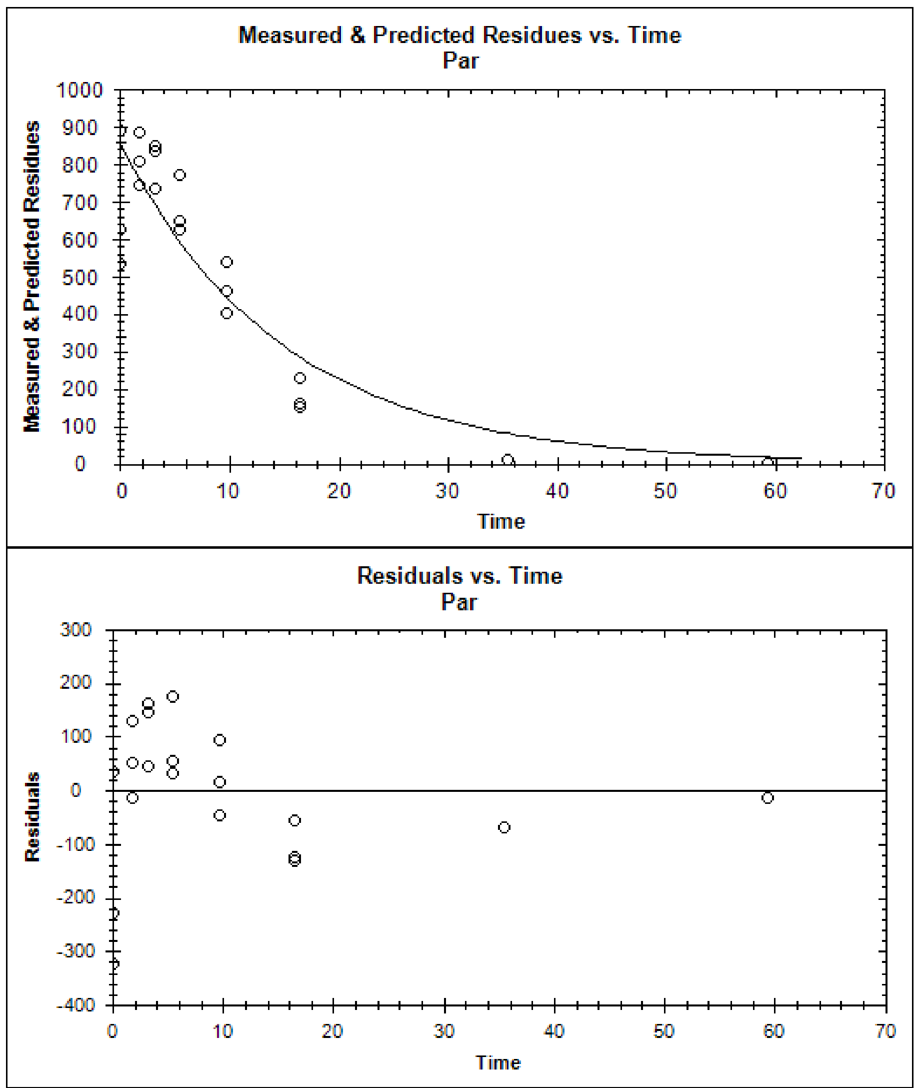


Figure 7.1.2.2.1-12: SFO fit for Dimethenamid-P in trial L110062 – modelling endpoints

Table 7.1.2.2.1-53: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110063 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 883 k: 0.0638 d^{-1}	13.9	k: < 0.001	Good	10.9	36.1
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

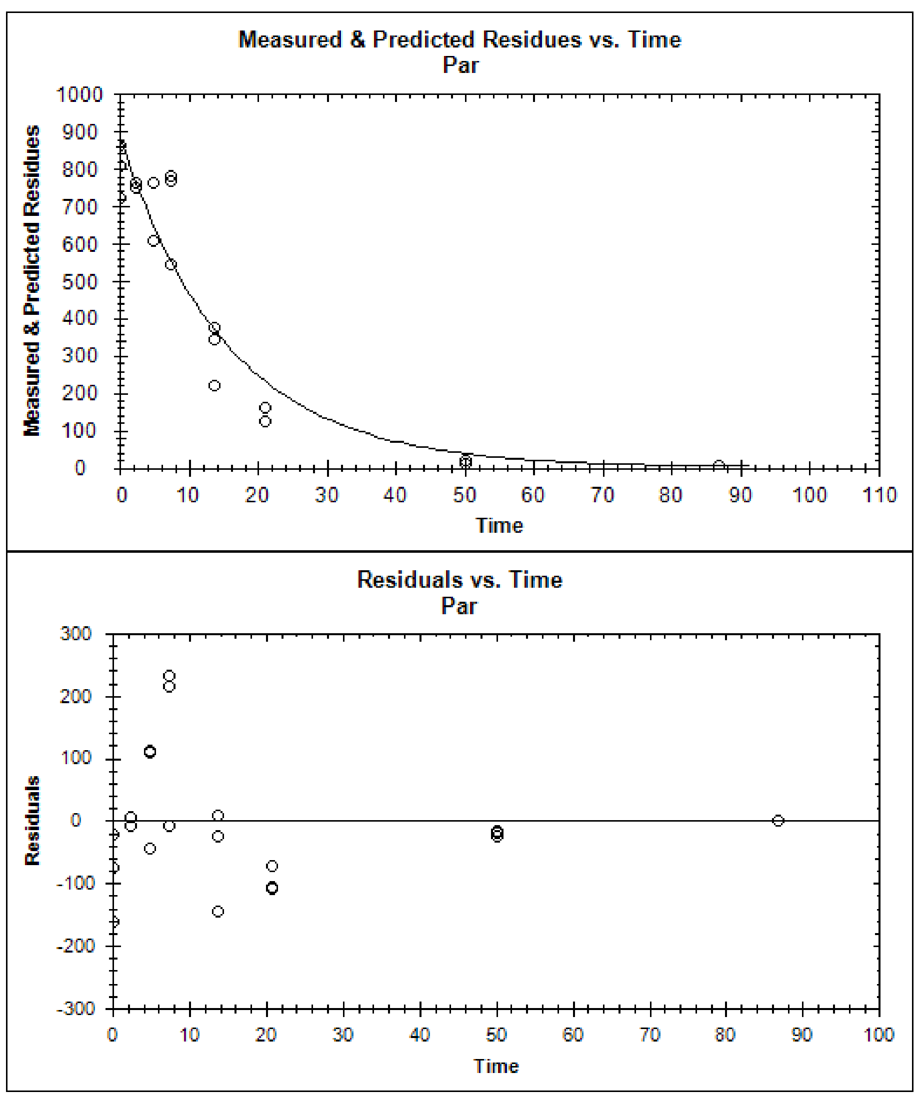


Figure 7.1.2.2.1-13: SFO fit for Dimethenamid-P in trial L110063 – modelling endpoints

Table 7.1.2.2.1-54: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110064 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 899 k: 0.0716 d^{-1}	8.0	k: < 0.001	Good	9.7	32.2
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

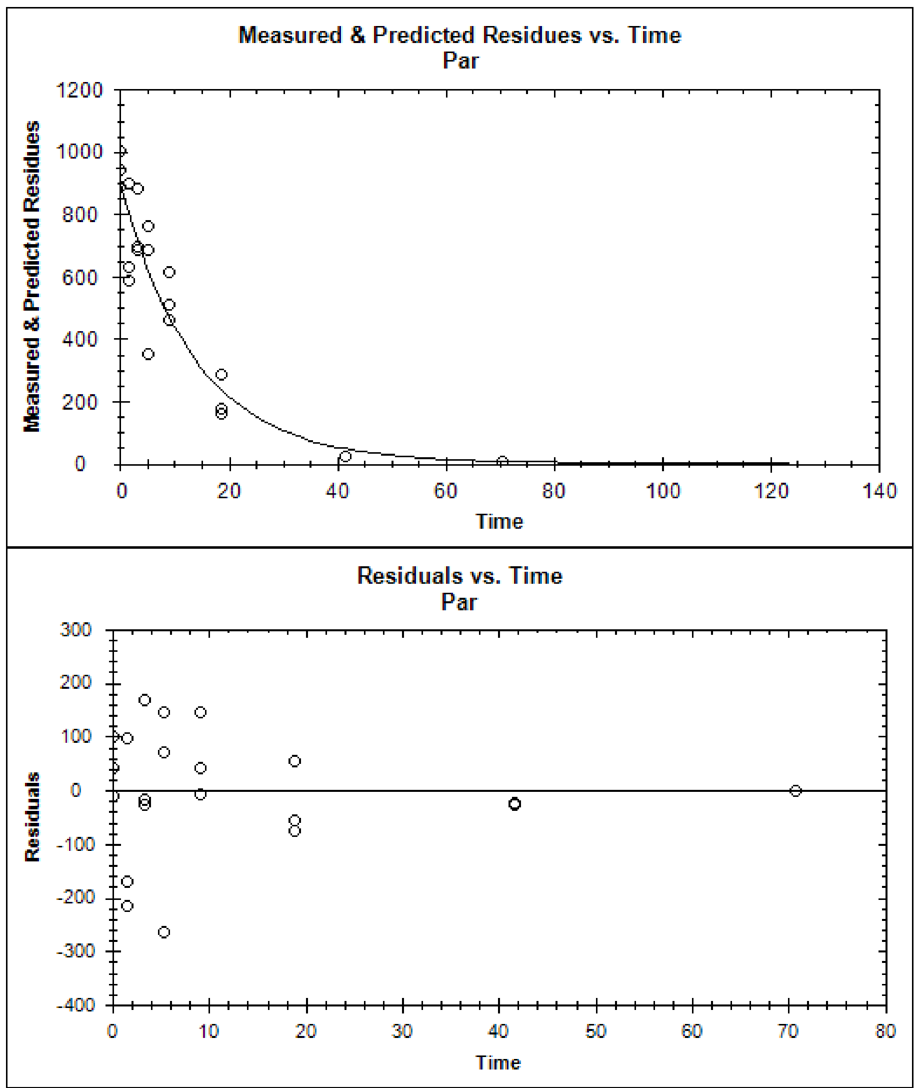


Figure 7.1.2.2.1-14: SFO fit for Dimethenamid-P in trial L110064 – modelling endpoints

Table 7.1.2.2.1-55: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110481 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 530 k: 0.0501 d^{-1}	10.4	k: < 0.001	Good	13.8	45.9
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

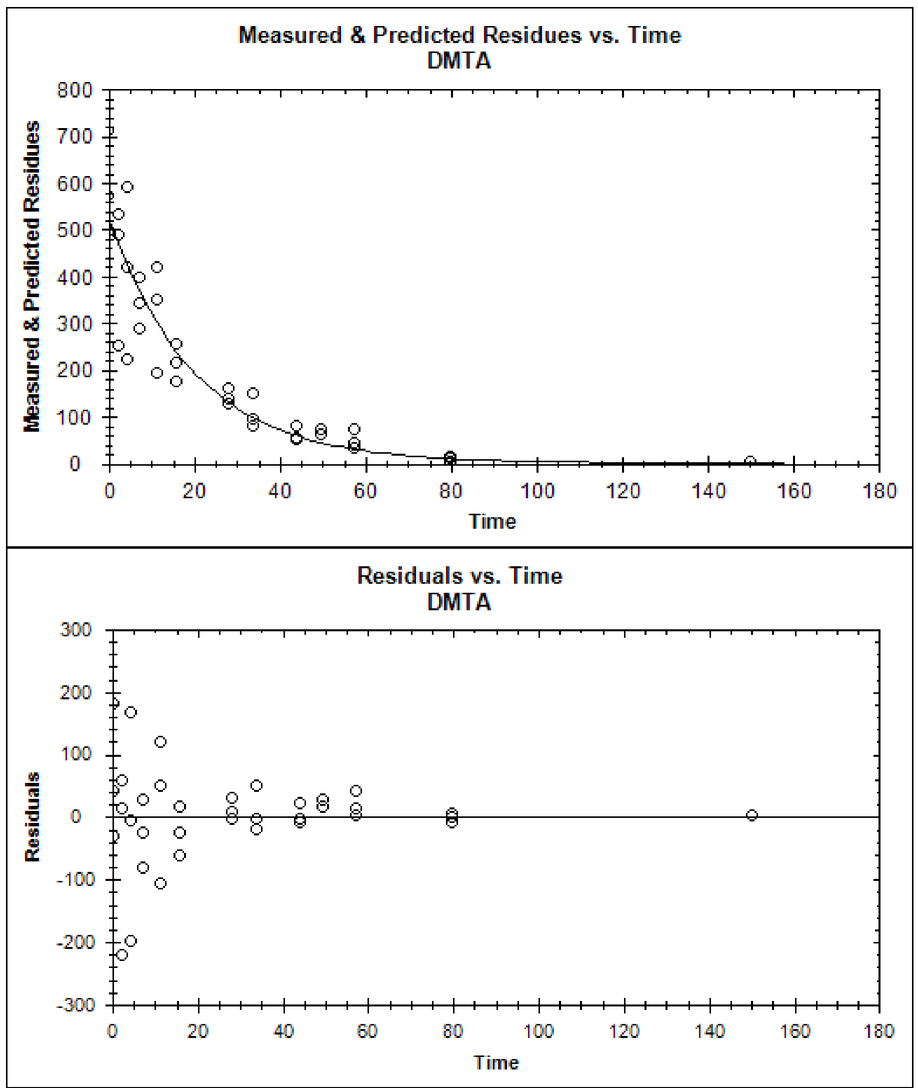


Figure 7.1.2.2.1-15:: SFO fit for Dimethenamid-P in trial L110481 – modelling endpoints

Table 7.1.2.2.1-56: Statistical and visual assessment of kinetic models for Dimethenamid-P in trial L110482 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 429 k: 0.101 d^{-1}	8.2	k: < 0.001	Good	6.9	22.8
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of Dimethenamid-P is well described using SFO kinetics.**						

* Type I error rate

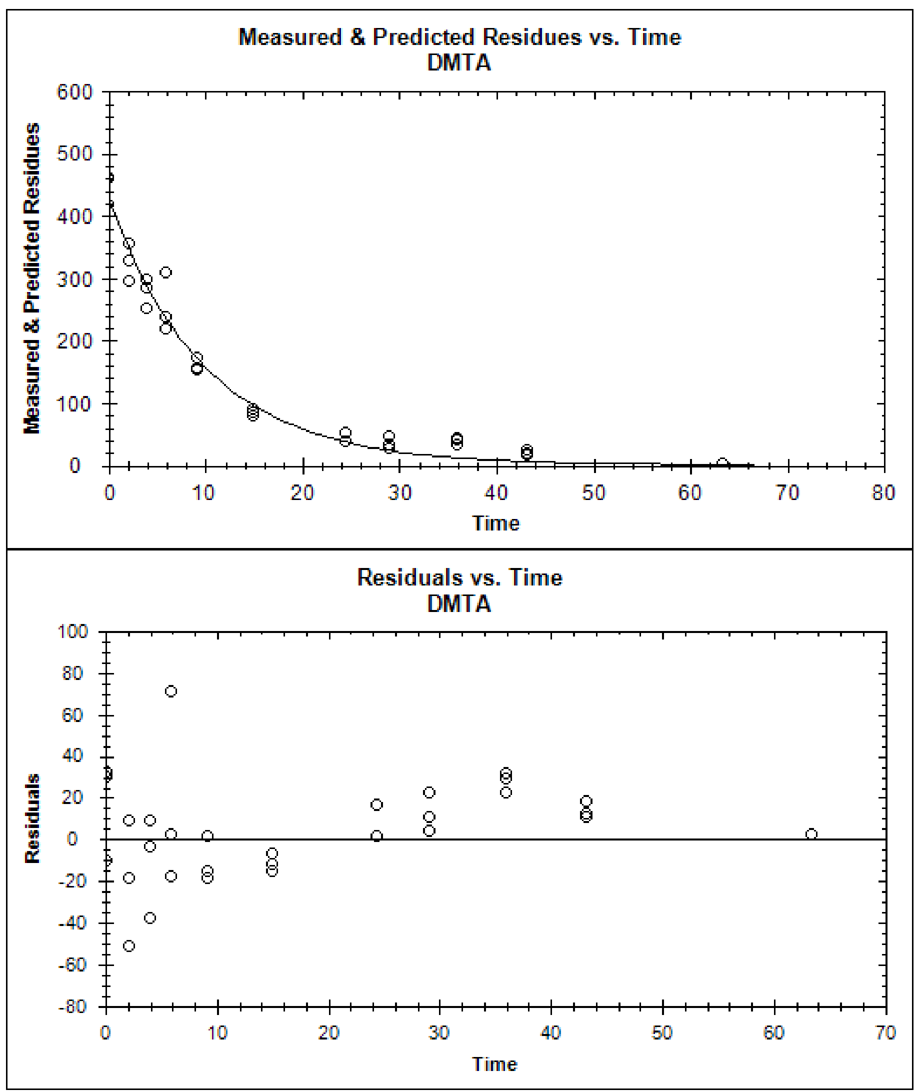


Figure 7.1.2.2.1-16: SFO fit for Dimethenamid-P in trial L110482 – modelling endpoints

Estimated degradation parameters of M27 and M23 formed as metabolites of Dimethenamid-P

The degradation of M27 as a metabolite of Dimethenamid-P was evaluated for trials L110061, L110062, L110063, L110064 and L110481; for metabolite M23, trials L110062, L110064 and L110481 were considered. The fitted curves for M27 and M23 in all these trials are visually poor, and the χ^2 are always > 30 %. Therefore, the resulting parameters are not considered acceptable as input parameters for modelling in environmental risk assessments. Please refer to the original report for details on the metabolites.

Estimated degradation parameters of M27 applied as parent substance

The degradation of M27 under field conditions was analysed in four trials, in which M27 was applied as parent substance. Only SFO kinetics in combination with time-step normalisation (i.e. with corrected sampling time) were tested since these already provided good fits. No outliers were identified in any of the trials. Details for each location are presented in the following tables. The graphs show the results of the best fit model.

Table 7.1.2.2.1-57: Statistical and visual assessment of kinetic models for M27 in trial L110330 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 185 k: 0.0474 d^{-1}	10.3	k: < 0.001	Good	14.6	48.6
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

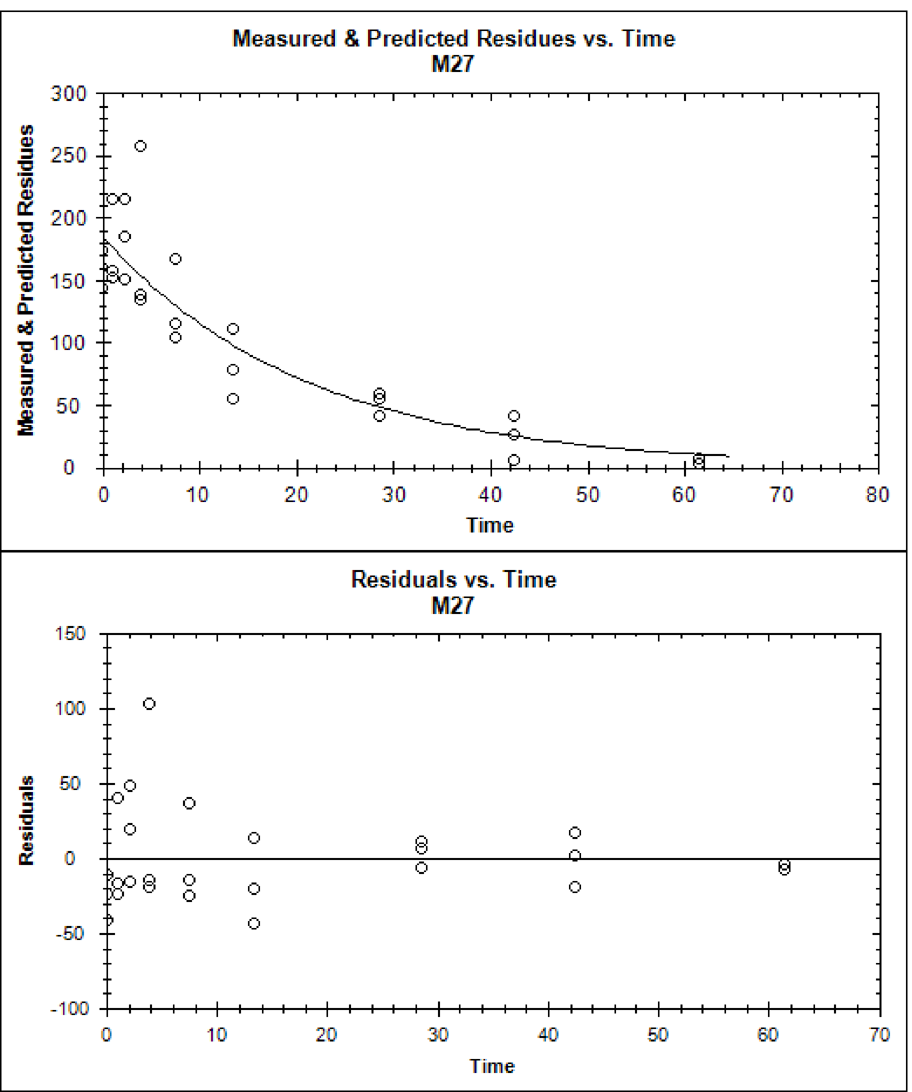


Figure 7.1.2.2.1-17: SFO fit for M27 in trial L110330 – modelling endpoints

Table 7.1.2.2.1-58: Statistical and visual assessment of kinetic models for M27 in trial L110331 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 178 k: 0.0787 d^{-1}	4.4	k: < 0.001	Good	8.8	29.3
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

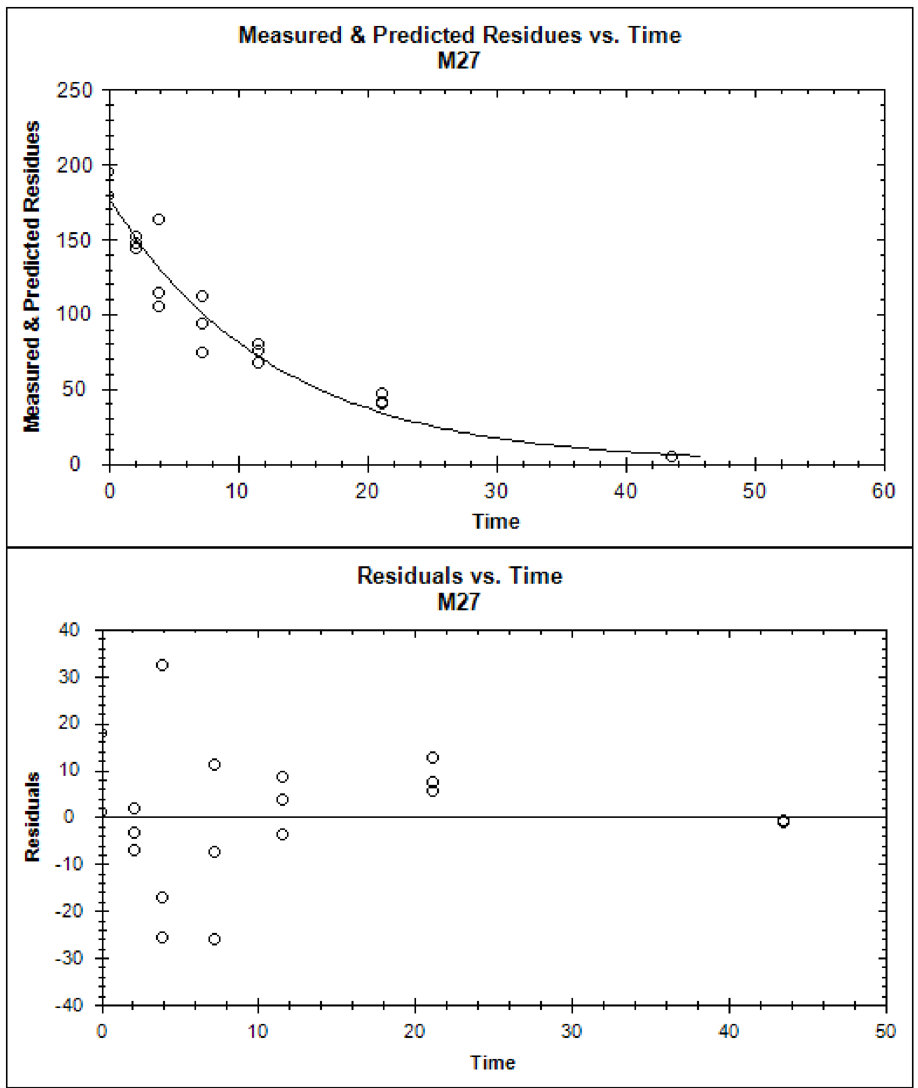


Figure 7.1.2.2.1-18:: SFO fit for M27 in trial L110331 – modelling endpoints

Table 7.1.2.2.1-59: Statistical and visual assessment of kinetic models for M27 in trial L110332 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 203 k: 0.0546 d^{-1}	12.9	k: < 0.001	Acceptable	12.7	42.2
⇨ SFO fit visually and statistically acceptable.						
⇨ **Conclusion: The degradation of M27 is sufficiently well described using SFO kinetics.**						

* Type I error rate

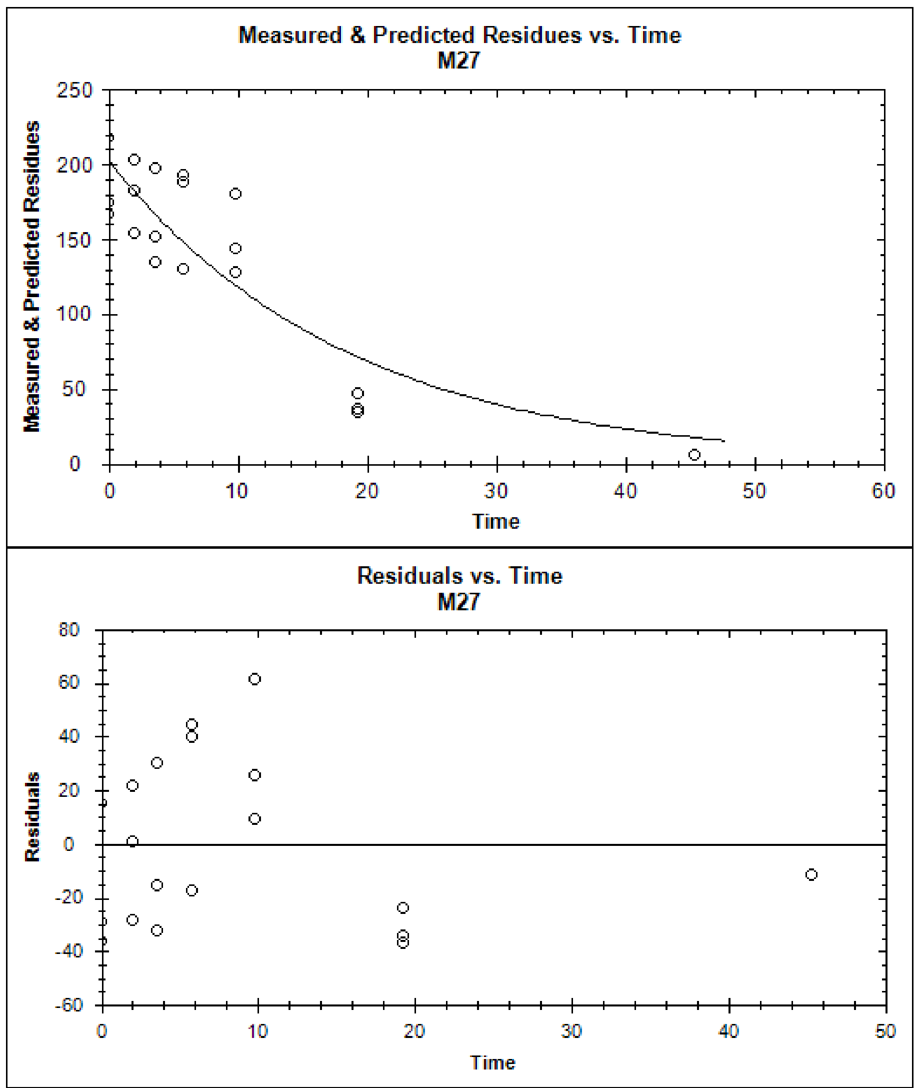


Figure 7.1.2.2.1-19: SFO fit for M27 in trial L110332 – modelling endpoints

Table 7.1.2.2.1-60: Statistical and visual assessment of kinetic models for M27 in trial L110333 – modelling endpoints

Step in FOCUS flowcharts	Fitted parameters	χ^2 error	p (t-test)*	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	M_0: 182 k: 0.0268 d^{-1}	9.2	k: < 0.001	Good	25.9	86.0
⇨ SFO fit visually and statistically good.						
⇨ **Conclusion: The degradation of M27 is well described using SFO kinetics.**						

* Type I error rate

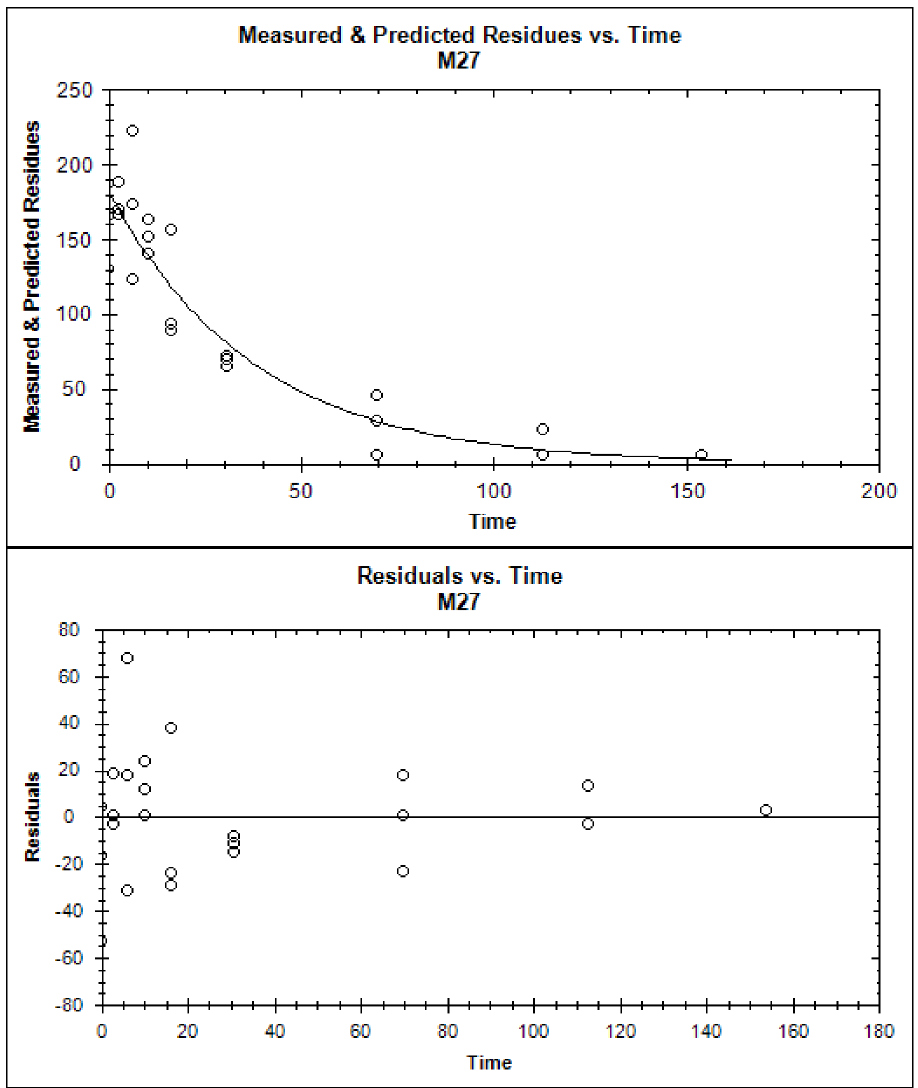


Figure 7.1.2.2.1-20: SFO fit for M27 in trial L110333 – modelling endpoints

III. CONCLUSION

Persistence und normalised modelling endpoints were derived from six field degradation trials with Dimethenamid-P and four field degradation trials with M27. Degradation of Dimethenamid-P and M27 (when applied as parent) was described well by SFO or FOMC kinetics. The degradation of M27 and M23 formed as metabolites of Dimethenamid-P could not be evaluated with sufficient quality. M31 was only found at very few sampling dates; therefore, degradation of M31 was not evaluated.

CA 7.1.2.2.2 Soil accumulation studies

Soil accumulation studies were not submitted for the Annex I inclusion process. Additional studies were not performed nor required.

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

Previously submitted adsorption/desorption studies for Dimethenamid-P are available, however, an amendment is made with respect to evaluation of several experiments, leading to minor changes in the Freundlich sorption coefficient and Freundlich exponent.

Regarding the metabolites M23 and M27 an adsorption/desorption study exists for each compound. However, these studies do not fulfil latest requirements. No significant sorption could be estimated following the criteria outlined in OECD 106, point 71. For metabolite M27 a new adsoption/desorption study was performed in 4 soils. For three out of four soils no significant sorption could be estimated, only for the LUFA 3A soil accurate sorption could be determined. An adsorption/desorption experiment was also performed on metabolite M31. This study with 5 soils did not meet the significant sorption threshold value as well. As a consequence new studies were performed on M23, M27 and M31 with specific modern experimental techniques. These provide reliable adsorption/desorption values according to the latest requirements. The experimental details of these studies are described in the relevant study summaries.

The new ads/des studies have been conducted with a modern technique that allows for more accurate assessment of low adsorbing compounds. The previously collected data did not meet p-criteria standards for reproducibility as outlined in OECD 106. A method, using a syringe, was introduced in order to reduce the remaining volume of test solution in soil after equilibration time, by centrifugation at high speed over a frit. For low sorbing compounds it is important to have full separation of the phases (aqueous and soil). This method allows for more adequate separation of the phases and therefore this enables a more precise determination of the amount of test substance adsorbed on soil. The direct method, which involves the determination of compound concentrations in both phases ($CaCl_2$ solution after adsorption and the soil phase by direct extraction with solvent) performed with this alternative technique provides a more precise determination of adsorption constants of low adsorbing test items. Together with the determined HPLC-MS/MS measuring accuracy (low coefficient of variation combined with double experiments and triplicate aqueous and soil extract injections) information on the significance of adsorption could be shifted to lower limits. This method improves the accuracy for low sorbing compounds and was used in the most recent studies. The p-criteria applied to the statdard methodlogy for determining reliability of data is not applicable to the data generated with this more precise methodology. The standard p-criteria (<0.1) is used to determine the reliability of the macro method. The syringe method, by separating fully the phases by centrifugation, is inherently more accurate and is a physically separate method in which the standard p-criteria is not relevant.

Three newly identified metabolites (M43, M47 and M54) from the re-evaluation of the lysimeter leachates were selected for adsorption/desorption studies using conventional methods. These metabolites were selected for further testing based on the criteria previously described in the grouping strategy discussion.

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Report: CA 7.1.3.1.1/1
Paulick R., 2007a
Amendment 1: Soil adsorption and desorption of SAN-1289H, unaged, by the batch equilibrium method
2007/7003537
Guidelines: EPA 163-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The purpose of the amendment was to correct some minor typographical errors and errors in the calculation of adsorption and desorption parameters, which were reported in the original study [*old EU Annex II 7.1/15, TONG, T.-M.R., SU, L.Y. (1997) - BASF DocID 1997/5180* for the US soils. Some errors were transcription errors from calculation sheets and did not affect the final values. Some were minor transcription errors to the calculation sheets, but did affect the final values. In general the errors were not significant to change the overall conclusion of the study. The errors were corrected and reported in revised tables presented in the amendment.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	SAN-1289 H (Dimethenamid-P)
Lot No.:	RS-1289-010496
Purity:	94.0%
Molecular weight:	275 g/mol

2. Soils

Five European and five US soils were used in the study. Soil characteristic are provided in the original study [TONG, T.-M.R., SU, L.Y. (1997) - BASF DocID 1997/5180]

5180

B. STUDY DESIGN

1. Experimental conditions

Experimental conditions are described in details in the original study [TONG, T.-M.R., SU, L.Y. (1997) - BASF DocID 1997/5180].

2. Description of analytical procedures

Analytical procedures and measurements are given in the original study [TONG, T.-M.R., SU, L.Y. (1997) - BASF DocID 1997/5180].

II. RESULTS AND DISCUSSION

A. FINDINGS

The corrected adsorption and desorption parameters are presented in bold type in **Table 7.1.3.1.1-1** for the US soils. For the loam sediment, a single data entry was wrong in the data set which is used to calculate the exponent 1/n. This error did not change the results significantly; the 1/n changed from 10.04 to 10.03 and the K_d values were the same. For the sandy loam soil, the K_d was calculated incorrectly from the intercept. The corrected value (0.72) replaced the wrong value (1.38) in the amendment; along with the revised K_{oc} value (206) replaced the old value (396). Desorption values for 1/n, K_d and K_{oc} were calculated incorrectly for the sandy loam soil. The correct values (1.4, 1.25 and 357, respectively) were replaced the old values (1.26, 1.4, and 401, respectively).

Table 7.1.3.1.1-1: Freundlich Adsorption and Desorption Coefficients for Dimethenamid-P

US Soils						
Soil	Adsorption			Desorption		
	K_d	1/n	K_{oc}	K_d	1/n	K_{oc}
Clay	2.09	1.05	211	3.24	1.18	327
Clay loam	2.51	0.97	105	3.31	0.90	139
Loam	3.02	**1.03**	247	3.89	0.98	319
Sandy loam	**0.72**	1.04	**206**	**1.25**	**1.4**	**357**
Silt loam	1.95	0.96	129	2.09	0.87	138

III. CONCLUSION

The Freundlich adsorption and desorption coefficients (K_d values) for the five US soils were in the range from 0.72 to 3.02 and from 1.25 to 3.89, respectively. The values for the adsorption and desorption constants (K_{oc} values) ranged from 105 to 247 and from 138 to 357, respectively.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Report: CA 7.1.3.1.2/1
Class T.,Dorn U., 2004a
Dimethenamid metabolite M27 (Sulfanate): Adsorption - desorption on different soils (OECD guidelines 106)
2004/1015224
Guidelines: EEC 91/414, OECD 106
GLP: yes
(certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

The adsorption behaviour of M27, metabolite of Dimethenamid-P was investigated in four different soils. The soils covered a range of pH from 6.1 to 7.3, a range of organic carbon content from 0.8% to 2.72% and different textural classes.

For determination of the adsorption kinetics a concentration of 5 µg/mL of the test item in 0.01 M $CaCl_2$ solution was used. The ratio of soil versus test solution was 1/1. The specimens were shaken at 22-24°C for 2, 4, 6, 8, 24 and 48 h. After each time interval measurements were performed in both aqueous phase (indirect method) and in soil extracts (direct method) by LC-MS/MS.

For the determination of the adsorption isotherm, five different concentrations between 0.05 to 5.0 µg/mL of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 1/1, and the measurements were performed at the appropriate adsorption equilibrium time of 22 h for Sora and LUFA 3A and 2 h for Birnbaum and Bruch West determined within the study. Concentrations of M27 were measured in both the aqueous phase and in extracts of the soil by LC-MS/MS.

Adsorption at equilibrium of M27, metabolite of Dimethenamid-P on soils was generally low (<15%) for all four soils. Only the soil LUFA 3A shows significant sorption. K_d values ranged between 0.087 to 0.16 cm^3/g and resulted in K_{OC} values between 3.4 and 10.9 cm^3/g. The adsorption coefficients K_F derived from Freundlich adsorption isotherms ranged from 0.030 to 0.12 with 1/n ranging from 0.910 to 0.992.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item M27
Reg. No. 360714
Batch No.: 1213-32
Purity: 97.4%
Molecular weight: 343.40 g/mol

2. Soils

The study was conducted with four different soils. The physico-chemical characterisation of the soils is provided in Table 7.1.3.1.2-1

Table 7.1.3.1.2-1: Characterisation of soils used to investigate the adsorption and desorption of M27

Soil designation Origin	Sora (Field preparation August 2000)	LUFA 3A	Birnbaum (Henninger)	Bruch West
Textural class (USDA scheme)	Silt loam	Loam	Loamy sand	Sandy loam
Soil texture [%], (USDA scheme)				
Sand	10.8	48.8	83	60.3
Silt	77.8	35.7	6	26.6
Clay	11.2	15.5	11	13.1
Organic carbon [%]	1.91	2.44	0.8	2.72
Organic matter[1] [%]	3.29	4.20	1.38	4.68
CEC [meq/100g]	16.6	18.8	13	14.0
pH ($CaCl_2$)	6.4	7.2	6.1	7.3
pH (water)	-	7.7	-	8.2

[1] organic matter =organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

To determine adsorption kinetics 10 g of soil were equilibrated with 9.5 mL of 0.01 M $CaCl_2$ overnight by shaking. Then M27 was added to the soil water to obtain a nominal concentration of 5 µg/mL in the aqueous phases. The soil/solution ratio chosen was ≈1/1, as the adsorption of the test item was assumed to be low. Duplicate specimens were dosed per soil type. Two blank controls per soil type were prepared without M27 and two specimens with no soil were dosed with M27 at a nominal concentration of 5 µg/mL. The specimens were shaken at 22-24°C for 2, 4, 6, 8, 24 and 48 h. After each interval an aliquot of the water phase was taken for analysis of M27 by LC/MS/MS. After final sampling the soil pellets were washed with 0.01 M $CaCl_2$ and adsorbed M27 was extracted with methanol/water (6/4, v/v). Combined washes and soil extracts were analysed by LC/MS/MS.

To determine adsorption isotherms, standard solutions of the test item in 0.01 M $CaCl_2$ were prepared with five concentrations ranging from 0.05 to 5.0 µg/mL.
For all experiments, the soil was pre-equilibrated with aqueous 0.01 M $CaCl_2$ overnight, before addition of the test solution. All experiments were performed in glass centrifuge tubes. Per soil type a total of 10 soil samples were dosed (duplicates per amount/concentration level dosed), plus two blank controls without M27 per soil and two specimens dosed with 0.05 µg/mL M27 without soil (adsorption controls).

Aliquots of 2 mL of the test solution were shaken with 2 g of the test soil at 23-25°C. The appropriate time for reaching equilibrium conditions was 22 h for Sora and LUFA 3A and 2 h for Birnbaum and Bruch West as determined in preliminary adsorption kinetics tests (see above).

The adsorbed test item was determined by analysing the aqueous phase (indirect method) and the solvent phase obtained by soil extraction (direct method).

Control specimens with only the test item in aqueous 0.01 M $CaCl_2$ solution (adsorption controls) were used to show that no significant adsorption on the surface of the test vessels occurred.

2. Description of analytical procedures

Aliquots of the supernatant of soil water obtained after equilibrating soil for several hours in fortified 0.01 M aqueous $CaCl_2$ solution were centrifuged, filtered (0.2 µm pore width), diluted (dilution factors DF: 4, 10, and 25) and finally analysed for M27 by LC-MS/MS.
The analytical procedure was assessed and pre-validated for soil water at M27 concentrations of 5.0, 0.20 and 0.005 µg/mL.

Soil pellets (2 g) were washed with 0.01 M $CaCl_2$ and the soil/water phases were separated by filtration (paper filters using Buchner funnels and vacuum suction) or centrifugation. The washed fractions were combined and the total volume noted. The washed soil pellets were extracted repeatedly with methanol/water (6/4, v/v) and then the extracts were combined. Aliquots of the washes and soil extracts were filtered, diluted and subjected to LC/MS/MS analysis.

The applicability of the soil extraction method was demonstrated by fortifying soil pellets of control specimens at 0.050 µg/g with M27 (10 g soil) and 0.025 µg/g with M27 (2 g soil) prior to extraction with methanol/water.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Mass balances were complete and ranged from 95% to 101%, thus indicating that no degradation of the test substance or adsorption on the test vessels occurred.

B. FINDINGS

M27 showed weak adsorption (A(eq) <15%) on all four soils, resulting in little depletion in the aqueous solutions. Adsorption found after 48 to 50 h of equilibration was 11.1% for Sora soil, 13.3% for LUFA 3A soil, 7.2% for Birnbaum soil and 7.7% for Bruch West soil determined by the indirect method. The direct method obtained 12.1% for Sora soils, 9.3% for LUFA 3A soils, 7.2% for Birnbaum soils and 5.8% for Bruch West soils (see **Table 7.1.3.1.2-2**).

The results for adsorption at equilibrium A(eq) obtained from adsorption kinetics tests by direct and indirect method were averaged and used to calculate the distribution coefficients K_d. The K_d values were further used to obtain the organic carbon normalized adsorption coefficients K_{OC}.
K_d values ranged between 0.087 to 0.16 cm^3/g and resulted in K_{OC} values between 3.4 and 10.9 cm^3/g.

The adsorption coefficients K_F derived from Freundlich adsorption isotherms ranged from 0.030 to 0.12 with 1/n ranging from 0.910 to 0.992. The significance of these results is limited as an accurate determination of K_d is problematic if the K_d x m_{soil} /$V_{solution}$ ratio is not >0.1 (for direct determination of sorption, for an indirect measurement the ratio must be above 0.3). Based on the methods of determination only the soil LUFA 3A with a $K_F > 0.1$ shows reliable sorption.

Table 7.1.3.1.2-2: Summary of Adsorption Kinetics and Isotherms Tests

Soil	A(eq) indirect	A(eq) direct	A(eq) Average Indirect/direct	K_d [cm³/g]	K_{OC} [cm³/g]	K_F [$\mu g^{1-1/n} (cm^3)^{1/n} g^{-1}$]	1/n
Sora	11.1	12.1	11.6	0.15	7.8	0.076	0.992
LUFA 3A	13.3	9.3	11.3	0.16	6.5	0.120	0.940
Birnbaum	7.2	8.3	7.7	0.087	10.9	0.036	0.937
Bruch West	7.7	5.8	6.8	0.092	3.4	0.030	0.910

III. CONCLUSION

Adsorption of M27 (metabolite of Dimethenamid-P) at equilibrium was generally low (<15%) for all four soils. Only the soil LUFA 3A shows significant sorption.
K_d values ranged between 0.087 to 0.16 cm^3/g and resulted in K_{OC} values between 3.4 and 10.9 cm^3/g. The adsorption coefficients K_F derived from Freundlich adsorption isotherms ranged from 0.030 to 0.12 with 1/n ranging from 0.910 to 0.992.

Report: CA 7.1.3.1.2/2
Class T., 2011a
Determination of adsorption/desorption behaviour of Reg.No. 360712 (metabolite M31 of BAS 656 H, Dimethenamid-P) on soils (OECD guideline 106)
2011/1277426

Guidelines: EEC 91/414 Annex II (Part A Section 7.1.2), EEC 95/36, OECD 106 (2000)

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

Executive Summary

The aim of this study was to determine the adsorption and desorption behaviour of Reg. No. 260712 (metabolite M31 of BAS 656H, Dimethenamid-P) on five soils with different chemical and physical properties.

The soils covered a range of pH from 5.2 to 7.5, and a range of organic carbon content from 0.52% to 3.84%. The soils were classified as sand (LUFA 2.1), silt loam (Nierswalde Wildacker), loamy sand (Li 10), sandy loam (LUFA 2.3) and a silty clay loam (La Gironda Arahal)

Adsorption kinetics of M31 was determined at one concentration (5 µg/mL) on two soils, LUFA 2.1 and La Gironda Arahal by equilibration in a soil/solution mixture (1:1) for 48 hours. The adsorption isotherms of M31 were established with all five soils. Equilibrium was established by shaking on a horizontal shaker for 48 hours. The test substance was measured using LC-MS/MS in the solution phase after equilibrating the soil sample with 0.01 M $CaCl_2$. Soil pellets were extracted three times, the extracts were combined and adjusted to a final volume, and then diluted for analysis by LC-MS/MS. Control samples were also used and subjected to same procedure as the test systems.

The adsorption of M31 on five soils was found to be variable depending on soil type and ranged from 1% for loamy sand Li 10 soil to 9% for silt loam Nierswalde soil at a nominal concentration level of 5 µg/mL. Kd values ranged from from 0.01 mL/g to 0.087 mL/g, and Koc from 0.9 to 5.3 at the nominal dose of 5 µg/mL. KF values were found between 0.016 µL1-1/n mL1/n g-1 and 0.078 µL 1-1/n mL1/n g-1 depending on concentration level with 1/n exponent ranging from to 0.69 to 1.02. The KF_{oc} was in the range from 1.0 to 6.0 µL1-1/n mL1/n g-1. However, sorption could not reliably be estimated since all soils fail the significance criteria.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	M31
Reg. No.	360712
Batch No.:	L81-46
Chemical name (IUPAC):	[[(2,4-Dimethyl-thiophen-3-yl)-(2-methoxy-1-methy-ethyl)-carbamoyl]-methanesulfinyl]-acetic acid
Purity:	98.7%
Molecular weight:	347.5 g/mol

2. Soils

The study was conducted with five different soils originating from Germany. Soils physico-chemical properties are provided in **Table 7.1.3.1.2-3**.

Table 7.1.3.1.2-3: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 360712 (Dimethenamid-P metabolite M31)

Soil designation Origin	LUFA 2.1	Nierswalde (Wildacker)	Li 10	LUFA 2.3	La Gironda (Arahal)
Textural class (DIN 4220)	Sand	Clay silt	Loamy sand	Loamy sand	Silty clay
Soil texture [%], (ISO 11277) Sand Silt Clay	 88.2 8.9 2.9	 17.6 73.1 9.3	 80.3 13.8 6.0	 54.8 34.0 11.2	 10.2 50.8 39.0
Textural class (USDA)	Sand	Silt loam	Loamy sand	Sandy loam	Silty clay loam
Soil texture [%], (USDA) Sand Silt Clay	 89.1 8.0 2.9	 24.1 66.6 9.3	 81.1 13.0 6.0	 56.8 32.0 11.2	 12.7 48.3 39.0
Organic carbon [%] (ISO 10694)	0.52	1.63	0.88	1.09	3.84
Effective CEC [$cmol^+/kg$]	2.0	7.4	5.4	10.4	29.0
pH ($CaCl_2$) pH (H_2O)	5.2 6.3	6.5 7.1	5.9 6.8	6.9 7.9	7.5 8.1
MWHC [g/100g dry soil]	24.5	39.8	24.2	27.7	36.6
Bulk density [g/L]	1354	1230	1406	1278	1342

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Kinetics Preliminary Test

The test was performed with two soils, LUFA 2.1 with the lowest pH value and organic carbon content, and La Gironda with the highest pH and organic carbon content. Duplicate portions of 5 g dry soil were equilibrated on a horizontal shaker with slightly less than 5 mL $CaCl_2$ solution (1:1 soil/solution ratio, based on actual soil water content determined in the lab) in a centrifuge tube overnight. A 0.25 mL of the 100 µg/mL application solution was added to the mixture to obtain a nominal initial concentration of 25 µg in a total volume of 4.8 mL for LUFA 2.1 and 5 mL for La Gironda Arahal. Test systems were equilibrated on a horizontal shaker for various equilibration times (0.5, 2, 4, 6, and 24 hours) at room temperature (23 – 25 °C). Samples were pre-centrifuged at 4000 rpm for 5 minutes, and the aqueous phases were removed. The supernatants were subsequently centrifuged at 15000 rpm for 30 minutes. The liquid phases obtained after the second centrifugation step were diluted in 1:1 (v/v) methanol/water solution containing 0.1% formic acid by a factor of 1000. Soil pellets were extracted and diluted to a factor of 10 (controls) or 100 (all dosed samples). All aqueous phases and extracts were analysed for M31 by LC-MS/MS

Tier 3: Adsorption Isotherms

The test was performed with all soils using an equilibration time of 48 hours and 1:1 soil/solution ratio. Soil samples (5 g) were equilibrated overnight with < 5.0 mL 0.01 M $CaCl_2$ solution. The test substance was added to the mixture to achieve the following amounts: 0.05, 0.25, 1.25, 5 and 25 µg in 5 mL total aqueous volume. Duplicate samples were used for each concentration and for each soil. The test systems were equilibrated by shaking for 48 hours on a horizontal shaker in the dark at room temperature (21 – 25 °C). Samples were pre-centrifuged for 5 minutes at 4000 rpm. An aliquot of the supernatant was transferred into a 1.8 mL centrifuge vial and centrifuged at 15000 rpm for 30 minutes. A portion of the supernatant was diluted with 1:1 methanol/water solution containing 0.1% formic acid. The total volume of the complete aqueous phase was determined gravimetrically. Soil pellets were extracted and diluted. The amount of M31 was determined in all aqueous phases and extracts by LC-MS/MS

2. Description of analytical procedures

Aqueous phase and solid phases (soil pellets) were analysed separately by means of LC-MS/MS.
Determination of M31 in 0.01 M $CaCl_2$ aqueous solution ("soil water") involved volumetric dilution (dilution factors DF of 10, 100, or 1000) with subsequent LC/MS/MS analyses

Determination of M31 in the soil involved three times extraction of the soil pellets with 5 mL of with acetone/water (1/1 v/v).
First the soil pellet was loosened by brief, intensive shaking by hand and sonication (10 min) then by horizontal shaking (30 min). The soil/solvent phases were separated by centrifugation (5 min at 4000 rpm), then the extracts were combined, filled up to volume (V Ex= 20 mL) with the extraction mixture and diluted (dilution factors DF of 5, 10, 50, 100, 200, or 500) for LC/MS/MS determination of M31.
The applicability of the soil extraction method was investigated by fortifying soil pellets of control specimens with M31 at different concentration levels, extraction and analysis during Tier 1 and Tier 3 testing performance

II. RESULTS AND DISCUSSION

A. MASS BALANCE

LC/MS/MS analysis of diluted soil water resulted in excellent average recoveries (Tier 1: 93 % and 99 %, Tier 3: 95 % to 101 %) and standard deviations (≤ 9 %) for the dose levels (0.01,0.050, 0.25, 1.0, and 5.0 µg/rnL) used during Tier 1 and Tier 3 tests

LC/MS/MS analysis of soil extracts resulted in excellent average recoveries (Tier 1: 100 % and
93 %, Tier 3: 94 % to 98 %) and standard deviations (≤ 6 %) for the dose levels (0.01, 0.05, 0.25, 1.0, and 5.0 µg/g) used during Tier 3 tests

B. FINDINGS

The adsorption kinetics on two soils revealed that reaching adsorption equilibrium requires two days (48 h). Using control specimens with only M31 in 0.01 $CaCL_2$ without soil demonstrated the absence of M31 adsorption on the surface of the test vessels.

The results showed that adsorption of M31 variable depending on the soil type. The adsorption on soil at nominal concentration 5 µg/mL ranged from 1% to 9%. Control specimens demonstrated stability of the test item in $CaCl_2$ solution and absence of adsorption on the surface of test vessels.

The distribution coefficient K_d and K_{oc} values were calculated at nominal concentration of 5 µg/mL K_d values ranged from 0.010 to 0.087 mL/g, K_{oc} values from 0.9 to 5.3 mL/g.

The Freundlich adsorption coefficients K_F ranged from 0.016 to 0.078 $\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1} with the Freundlich exponent 1/n ranging from 0.69 to 1.02. The organic carbon normalised Freundlich coefficient K_{Foc} ranged from 1.0 to 6.0 $\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1}. A summary of the experimental results is provided in Table 7.1.3.1.2-4: . Evaluation of the significance of sorption (K_d x m_{soil} /$V_{solution}$ should be larger than 0.1 due to direct determination of sorption) shows no reliable sorption since all K_d are < 0.1 (using a soil/solution ratio of 5:5).

Table 7.1.3.1.2-4: Summary of adsorption isotherms tests of M31 on five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption at equilibrium [%]	K_d [mL/g]	K_{OC} [mL/g]	K_F [$\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1}]	K_{FOC} [$\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1}]	1/n
LUFA 2.1 (2nd trial)	Sand	0.52	5.2	2	0.027	5.1	0.031	6.0	0.69
Li 10	Loamy sand	0.88	5.9	1	0.010	1.1	0.016	1.8	0.73
Nierswalde Wildacker	Silt loam	1.63	6.5	9	0.087	5.3	0.078	4.8	1.02
LUFA 2.3	Sandy loam	1.09	6.9	5	0.047	4.3	0.046	4.2	0.96
La Gironda Arahal	Silty clay loam	3.84	7.5	3	0.036	0.9	0.037	1.0	0.92
LUFA 2.1 (1st trial)	Sand	0.52	5.2	2	0.023	4.3	Not evaluated-		

Values for adsorption at equilibrium, K_d and K_{oc} are obtained from nominal dose level 5 µg/mL

III. CONCLUSION

The adsorption of M31 on five soils was found to vary depending on soil type and ranged from 1% for loamy sand Li 10 soil to 9% for silt loam Nierswalde soil at a nominal concentration level of 5 µg/mL. K_d values ranged from 0.01 mL/g to 0.087 mL/g, and K_{oc} from 0.9 to 5.3 at the nominal dose of 5 µg/mL. K_F values were found between 0.016 $\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1} and 0.078 $\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1} depending on concentration level with 1/n exponent ranging from to 0.69 to 1.02. The K_{Foc} was in the range from 1.0 to 6.0 $\mu L^{1-1/n}$ $mL^{1/n}$ g^{-1}. However, sorption could not reliably be estimated since all soils fail the significance criteria.

Report: CA 7.1.3.1.2/3
Sacchi R.R., 2013a
Adsorption behavior of M23, M27 and M31 (metabolites of Dimethenamid-P) on different European soils
2013/3012762
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The adsorption behavior of the M23, M27 and M31 (metabolites of Dimethenamid-P) were investigated on five different European soils. The five soils covered a range of pH (in $CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60% to 1.85% and different textural classes.

For low sorbing compounds it is important to have full separation of the phases (water and soil) to utilize the direct method. When a small amount of water remains after centrifugation and subsequent partitioning, results for low sorbing compounds may be inaccurate. To circumvent this problem, a “syringe” method was developed in which after shaking the soil and water mixture inside the syringe, the syringe is then centrifuged and the centripetal force is used to fully separate the phases through a frit. The water is collected for analysis. This method improves the accuracy for low sorbing compounds and was used in these studies.

For the determination of the adsorption isotherm, five different concentrations (nominal 1.0, 0.5, 0.1, 0.05 and 0.01 µg/mL) of the test item in 0.01 mol/L $CaCl_2$ solutions were used. The ratio of mass of soil per test solution volume was 1/1, and the adsorption tests were performed at the adsorption equilibrium time of 48 h for M23 and M27 and 24 hours for M31 for all soils. Concentrations of M23, M27 and M31 were measured in both the aqueous phase and in extracts of the soil by LC-MS/MS.

The following adsorption parameters were measured for the test items in each soil: the Freundlich adsorption coefficient K_F, the Freundlich exponent 1/n, and the corresponding K_{FOC} values. The Freundlich adsorption coefficient K_F covered a range from 0.07 mL/g to 0.14 mL/g for M23, 0.05 mL/g to 0.16 mL/g for M27 and 0.02 mL/g to 0.06 mL/g for M31 for the five soils. The corresponding Freundlich exponents (1/n) covered a range from 0.60 to 0.87 for M23, 0.94 to 1.14 for M27 and 0.77 to 1.01 for M31. The K_{FOC} values ranged from 6.29 mL/g to 22.39 mL/g for M23, 7.73 mL/g to 13.54 mL/g for M27 and 1.72 mL/g to 9.20 mL/g for M31. The p-criteria applied to the statdard methodlogy for determining reliability of data is not applicable to the data generated with this more precise methodology. The standard p-criteria (<0.1) is used to determine the reliability of the macro method. The syringe method, by separating fully the phases by centrifugation, is inherently more accurate and is a physically separate method in which the standard p-criteria is not relevant..

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item M23
Reg. No. 360715
Purity: 100 %
Molar mass: 271.3 g/mol

Test item M27
Reg. No. 360714
Purity: 97.4 %
Molar mass: 343.4 g/mol

Test item M31
Reg. No. 360712
Purity: 98.7 %
Molar mass: 347.5 g/mol

2. Soils

The study was conducted with five different soils originating from Germany. The physico-chemical characterisation of the soils is provided in **Table 7.1.3.1.2-5Table 7.1.3.1.2-5:** .

Table 7.1.3.1.2-5: Characterisation of soils used to investigate the adsorption and desorption of M23, M27 and M31

Soil designation Origin	LUFA 2.1	Nierswalder Wildacker	Li 10	LUFA 2.3	LUFA 5M
Textural class (USDA scheme)	Sand	Silt loam	Loamy sand	Sandy loam	Sandy loam
Soil texture [%], (USDA scheme)					
Sand	90.8	17.7	84.6	68.6	58.1
Silt	6.9	73.5	11.3	23.1	29.7
Clay	2.3	8.8	4.1	8.3	12.1
Organic carbon [%]	0.60	1.85	0.93	0.99	1.07
Organic matter[1] [%]	1.03	3.19	1.60	1.71	1.84
CEC [cmol+/kg]	-0.7	3.1	5.3	7.5	10.1
pH ($CaCl_2$)	5.6	5.7	6.0	6.7	7.4

[1] organic matter =organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

In order to determine the adsorption behaviour of test items in test soils, air-dried and sieved samples were used. Both the indirect and the direct and the parallel method procedures were used.

1.2 Adsorption Equilibrium Test of the Test Items M23, M27 and M31 (Tier 1):

The adsorption equilibrium was determined in Tier 1 testing using standard solutions of the test items with a concentration of 1.0 µg/mL in 0.01 M $CaCl_2$ A soil/solution ratio of 1:1 was chosen. Series of duplicate samples (one duplicate for each sampling) of each soil were prepared. For each sample 3 g of soil were weighed in glass tubes and 3 mL of standard solution were added. The samples were agitated in a temperature controlled dark room at 20 ± 2°C for 4, 8, 24, 32 and 48 h and then the suspensions were separated by centrifugation. The aqueous phase was analysed (after appropriate dilution) by LC-MS/MS and the amount of analyte adsorbed on the soil was calculated as difference between the amount initially dosed and the amount remaining at the end of the experiment in the aqueous phase (indirect method). Subsequently the soil was extracted to analyse the test item adsorbed to the soil (direct method).

1.3 Tier 3 - Adsorption and desorption isotherms testing

The adsorption isotherm determination was performed with all five concentration levels (nominal concentrations: 1.0, 0.5, 0.1, 0.05 and 0.01 µg/mL) and the five soils. All Tier 3 experiments were performed using a constant soil/solution ratio of 1/1. For all samples 1 g of soil was weighed into syringes, and then 1 mL solution of each concentration level was added. Each experiment (one soil and one solution) was done in duplicate. All samples were shaken on a horizontal shaker at 20 ± 2°C until adsorption equilibrium was reached. The appropriate time for adsorption equilibrium was 48 hours for the test item M23 and M27 and 24 hours for test item M31. The soil / solution suspension was then centrifuged and the filtrate was collected for analysis. Syringes were used for the experiment as this allowed the reduction of the remaining volume of test solution in soil after equilibration time by centrifugation at high speed over a frit.
Aliquots of both suspensions and initial solution applied (after dilution) were analysed in order to determine the initial concentration as well as the concentration of the test item in the aqueous solution after adsorption. This information provides data for indirect determination of adsorption.

Since adsorption of the test item was very low, adsorption was also determined by the direct method. The remaining soil in the syringe was extracted with methanol / water (60/40, v/v) solution for M23 and M27 and with acetone/water (1/1, v/v) for M31.

2. Description of analytical procedures

The test item concentrations in aqueous phase and soil extracts were determined by HPLC-MS/MS. The analytical methods for the determination of M23, M27 and M31 were developed and validated within this study. For HPLC-MS/MS quantitation purposes, calibration standard solutions from 0.01 ng/mL up to 1.0 ng/mL in methanol (M23 and M27) and methanol/water (50/50, v/v) (M31), including at least six levels, were used for all the samples.

For validation of analysis of the test substances M23, M27 and M31 in aqueous solution, aqueous solutions were fortified with the test item at the LOQ (0.5 ng/mL) and at a level of 1000 ng/mL, the highest nominal test concentration. In addition, an aliquot of the aqueous solution (untreated) was analyzed to demonstrate that matrix components do not interfere with the detection and quantification of M23, M27 or M31. The fortified aqueous solutions were diluted with methanol / water (20 / 80, v/v) solution, when appropriate, and analyzed by HPLC-MS/MS.

For validation of analysis of the test substance M23 and M27 in soil, soil samples were fortified with the test item at the LOQ (0.0005 mg/kg) and at a level of 1.0 mg/kg, the highest nominal test concentration. In addition, a soil sample (untreated) was analyzed to demonstrate that matrix components do not interfere with the detection and quantification of M23 and M27. The soil samples fortified were extracted twice with methanol / water (60/40, v/v) solution. Samples were diluted with methanol / water (60/40, v/v) solution, when appropriate, and then analyzed by HPLC-MS/MS. The LUFA 2.1 soil was used for validation. The analytical method for M31 was the same, however the soil samples were extracted three times with acetone / water (1/1, v/v) solution instead of methanol / water (60/40, v/v) solution.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The sum of the amounts of test items M23, M27 and M31 in the aqueous phase and in the soil related to the amount initially applied was calculated for all samplings of equilibrium tests and all concentrations from the Freundlich adsorption isotherm determination. For the equilibrium test, the values for the mass balance ranged from 78.4 % to 102.6 % for M23, 75.4 % to 101.9 % for M27 and 90.0 % to 110.0 % for M31. For the isotherm determinations, the values for the mass balance ranged from 90.4 % to 119.8 % for M23, 71.1 % to 116.2 % for M27 and 92.6 % to 133.4 % for M31.

B. FINDINGS

Adsorption Equilibrium Test

For M23, the soils Nierswalder Wildacker and LUFA 5M showed constant adsorption throughout the test period. For the other soils the adsorption values fluctuated throughout the test period, but the percentage of active ingredient adsorbed at 48 h did not have significant increase from sampling 8 h. The time chosen for conduction of isotherms test was 48 hours.
For M27, the soil Li10 showed constant adsorption throughout the test period. In the other soils the adsorption increased throughout the test period. The time chosen for conduction of isotherms test was 48 hours.
For the M31, all soils showed constant adsorption throughout the test period. The time chosen for conduction of isotherms test was 24 hours.

Method Validation

The analytical methods for the determination of M23, M27 and M31 in $CaCl_2$ aqueous phase and soil were developed within this study. To demonstrate the efficiency of the methods, recovery experiments with untreated $CaCl_2$ aqueous phase and soil of LUFA 2.1 soil were performed. Mean recoveries are given in **Table 7.1.3.1.2-6**.

Table 7.1.3.1.2-6: Summary of validation results – mean recoveries obtained for fortified CaCl2 solution and soil LUFA 2.1

		$CaCl_2$ solution		**Soil**	
		Fortification level:		**Fortification level:**	
		0.5 ng/mL	**1000.0 ng/mL**	**0.5 ng/mL**	**1000.0 ng/mL**
M23		97.3 (n = 7)	87.4 (n = 6)	84.4 (n = 6)	72.5 (n = 5)
M27	Quantitation transition	80.7 (n = 6)	100.7 (n = 5)	115.4 (n = 7)	85.7 (n = 7)
	Confirmation transition	80.5 (n = 6)	103.0 (n = 5)	114.4 (n = 7)	82.8 (n = 7)
M31	Quantitation transition	96.3 (n = 7)	96.8 (n = 7	97.8 (n = 7)	95.3 (n = 7)
	Confirmation transition	94.4 (n = 7)	93.8 (n = 7)	92.5 (n = 7)	100.9 (n = 7)

Freundlich Adsorption Isotherm Determination

The Freundlich adsorption coefficient K_F covered a range from 0.07 mL/g to 0.14 mL/g for M23, 0.05 mL/g to 0.16 mL/g for M27 and 0.02 mL/g to 0.06 mL/g for M31. The K_{FOC} values ranged from 6.29 mL/g to 22.39 mL/g for M23, 7.73 mL/g to 13.54 mL/g for M27 and 1.72 mL/g to 9.20 mL/g for M31. The 1/n values ranged from 0.60 to 0.87 for M23, 0.94 to 1.14 for M27 and 0.77 to 1.01 for M31. The values obtained are summarized in the tables below.

Table 7.1.3.1.2-7: Adsorption of M23 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt Loam	1.85	5.7	0.14	0.68	7.62
Li10	Loamy Sand	0.93	6.0	0.10	0.76	10.53
LUFA 2.1	Sand	0.60	5.6	0.13	0.87	22.39
LUFA 2.3	Sandy Loam	0.99	6.7	0.12	0.70	12.46
LUFA 5M	Sandy Loam	1.07	7.4	0.07	0.60	6.29

Table 7.1.3.1.2-8: Adsorption of M27 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt Loam	1.85	5.7	0.16	1.14	8.55
Li10	Loamy Sand	0.93	6.0	0.09	0.97	9.89
LUFA 2.1	Sand	0.60	5.6	0.05	1.00	7.73
LUFA 2.3	Sandy Loam	0.99	6.7	0.11	0.98	10.96
LUFA 5M	Sandy Loam	1.07	7.4	0.14	0.94	13.54

Table 7.1.3.1.2-9: Adsorption of M31 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt Loam	1.85	5.7	0.04	0.98	2.11
Li10	Loamy Sand	0.93	6.0	0.04	1.01	4.58
LUFA 2.1	Sand	0.60	5.6	0.06	0.77	9.20
LUFA 2.3	Sandy Loam	0.99	6.7	0.04	0.96	4.28
LUFA 5M	Sandy Loam	1.07	7.4	0.02	0.93	1.72

III. CONCLUSION

In traditional adsorption/desorption experiments, residual aqueous phase can remain with the sediment after decanting and/or pipetting off of the aqueous phase. For compounds that have low sorption this low level of error can significantly impact the calculated values for K_D and $K_{OC.}$ The syringe method used for these experiments was introduced in order to reduce the remaining volume of test solution in soil after equilibration time, by centrifugation at high speed over a frit. This method allows for more adequate separation of the phases and therefore this enables a more precise determination of the amount of test substance adsorbed on soil. The direct method, which involves the determination of compound concentrations in both phases ($CaCl_2$ solution after adsorption and the soil phase by direct extraction with solvent) performed with this alternative technique provides a more precise determination of adsorption constants of low adsorbing test items. Together with the determined HPLC-MS/MS measuring accuracy (low coefficient of variation combined with double experiments and triplicate aqueous and soil extract injections) information on the significance of adsorption could be shifted to lower limits. The adsorption behaviour of M23, M27 and M31 was determined on five European soils, which covered a range of pH (in $CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 % to 1.85 % and four different USDA textural classes: loamy sand, sandy loam, silt loam and sand. Freundlich isotherms could be determined for all substances in all soils. The p-criteria applied to the statdard methodlgy for determining reliability of data is not applicable to the data generated with this more precise methodology. The standard p-criteria (<0.1) is used to determine the reliability of the macro method. The syringe method, by separating fully the phases by centrifugation, is inherently more accurate and is a physically separate method in which the standard p-criteria is not relevant..

Report: CA 7.1.3.1.2/4
Class T.,Walter W., 2014b
Determination of adsorption and desorption behavior of M656PH043 (Reg.No. 5917262, metabolite of Dimethenamid-P) in 5 soils (OECD Guideline 106)
2013/1348092

Guidelines: OECD 106 (2000)

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

Executive Summary

The adsorption behaviour of M656PH043 (Reg No. 5917262), metabolite of Dimethenamid-P, was investigated in five different soils. The soils covered a range of pH from 4.1 to 7.4 and a range of organic carbon content from 0.75% to 2.03%. The soils were classified as sand (Schifferstadt), sandy loam (LUFA 2.2), loamy sand (LUFA 5M and Li 10) and sandy clay loam (La Gironda (Arahal)).

Adsorption kinetics of M656PH043 were determined at one concentration (50 ng/mL in the aqueous solution) on two soils, Schifferstadt and LUFA 5M, by equilibration in a soil/solution mixture (1/1) for 2, 4, 6, 24 and 48 hours at 20-21°C. For the Schifferstadt soil, the percentage of adsorption obtained a plateau (Adsorption measured directly ≈ 15 % to ≈ 17 %) after 6 to 48 hours of equilibration time. For the LUFA 5M soil, adsorption plateaued (Adsorption measured directly ≈ 4%) after 2 hours of equilibration time.

For the three other soils, adsorption tests (parallel, direct method) with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time. Adsorption after 48 hours equilibration (Adsorption measured directly) was always < 10 %.

The K_d values obtained with the direct method were (except for soil Schifferstadt) all < 0.1 mL/g, thus indicating that Tier 3 experiments for the other four soils would lead to inaccurate results. Therefore, an adsorption isotherm of M656PH043 was established only for soil Schifferstadt and an equilibration time of 24 hours. Tier 3 adsorption experiments resulted in a Freundlich adsorption coefficient (K_{Fads}, 24 hours) of 0.702 and an organic carbon normalized value ($K_{F,ocads}$) of 94 (results given in $ng^{1-1/n}mL^{1/n}g^{-1}$). The obtained Freundlich exponent was 0.51.
For determination of the adsorption isotherm soil pellets were extracted as only for the direct method accurate results were expected, thus no desorption experiments were performed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	M656PH043
Reg. No.	5917262
Batch No.:	L82-113
Chemical name (IUPAC):	3-{(hydroxyacetyl)[(2S)-1-methoxypropan-2-yl]amino}-4-methylthiophene-2-carboxylic acid
Purity:	94.6 % (tolerance ±1.0 %)
Molecular weight:	287.3 g/mol

2. Soils

The study was conducted with five different soils. The physico-chemical properties of the soils are provided in **Table 7.1.3.1.2-10**.

Table 7.1.3.1.2-10: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 5917262 (Dimethenamid-P metabolite M656PH043)

Soil designation Origin	LUFA 2.2	LUFA 5M	Li 10	La Gironda (Arahal)	Schifferstadt
Textural class (DIN 4220)	Loamy sand (SI4)	Loamy sand (SI2)	Loamy sand (SI2)	Sandy clay loam	Sand
Soil texture [%], (ISO 11277) Sand Silt Clay	 55.9 32.1 12.0	 80.0 13.9 6.1	 81.2 13.2 5.6	 48.0 24.3 27.7	 88.1 7.7 4.3
Textural class (USDA)	Sandy loam	Loamy sand	Loamy sand	Sandy clay loam	Sand
Soil texture [%], (USDA) Sand Silt Clay	 60.3 27.7 12.0	 82.8 11.1 6.1	 82.8 11.6 5.6	 49.2 23.0 27.7	 88.5 7.2 4.3
Organic carbon [%] (ISO 10694)	1.47	2.03	0.84	1.22	0.75
Effective CEC [$cmol^+/kg$]	7.6	11.4	5.3	26.3	0.3
pH ($CaCl_2$) pH (H_2O)	5.4 5.9	7.2 7.9	6.4 6.9	7.4 8.3	4.1 5.0
MWHC [g/100g dry soil]	29.6	25.2	25.1	39.2	27.0
Bulk density [g/L]	1227	1367	1369	1308	1342

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Kinetics Preliminary Test

Adsorption kinetics testing experiments were performed exemplarily for two soils, one with low pH and low organic carbon content (sand Schifferstadt: pH 4.1, 0.75 % organic carbon) and one with higher, neutral pH (loamy sand LUFA 5M: pH 7.2, 2.03 % organic carbon). Duplicate portions of 2 g dry soil were equilibrated on a horizontal shaker with slightly less than 2 mL $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. Then the analyte dose solution was added to the soil water to obtain a nominal amount of 50 ng/mL in the aqueous phases. Test systems were then equilibrated on a horizontal shaker for various equilibration times (2, 4, 6, 24 and 48 hours) at 20-21°C. After shaking/equilibration and subsequent centrifugation, the supernatant aqueous phase was weighed to obtain the volume of the supernatant and to calculate the portion/volume of the aqueous phase remaining in the soil pellet. The liquid phases obtained after centrifugation were diluted and analysed by LC-MS/MS. Soil pellets were extracted and the extracts diluted and analysed by LC-MS/MS.

Adsorption at one Concentration after 48 h for Additional Soils

For the soils LUFA 2.2, Li 10 and La Gironda (Arahal), adsorption tests with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time using the same experimental conditions as described above for the adsorption kinetics preliminary test.

Tier 3: Adsorption Isotherm

The Tier 1 tests showed that the test item is expected to be stable in soils LUFA 5M and Li 10 but possibly disintegrates in the other three soils. However, the K_d values obtained by the direct method and the soil/solution ratio of 1/1 were rather low and thus the test item has to be considered “to be qualitatively mobile” (OECD Guideline 106 Section 69) or not significantly adsorbed. Therefore, Tier 3 testing was only considered to lead to accurate Freundlich adsorption isotherms for acidic sand Schifferstadt.
The test was performed using an equilibration time of 24 hours and 1/1 soil/solution ratio. Soil samples (2 g) were equilibrated overnight with < 2.0 mL of 0.01 M $CaCl_2$ solution. The test substance was added to the mixture to achieve the following amounts: 5, 25, 50, 250 and 500 ng/mL in the aqueous solution. Duplicate samples were used for each concentration. The test systems were equilibrated by shaking on a horizontal shaker at 20-21°C. After equilibration the samples were analysed as described for the adsorption kinetics preliminary test.

2. Description of analytical procedures

Aqueous phase and solid phases (soil pellets) were analysed separately. After equilibration the samples were centrifuged for 5 min at 4000 rpm to separate the soil and water phases. An aliquot of the water phase was centrifuged for 30 min at 15,000 rpm to remove soil particles with a diameter of >0.2 µm. Aliquots of supernatant were diluted volumetrically by a dilution factor into methanol / water (1/1, v/v) for LC/MS/MS analysis.

Determination of M656PH043 in the soil involved one extraction of soil pellets with 5 mL of methanol and two extractions with 5 mL of methanol/water (1/1, v/v). For separation of soil and solvent the samples were centrifuged for 5 minutes at 4000 rpm. Subsequently, the extracts were combined and diluted. An aliquot of the final extract was diluted with methanol/water (1/1) and subjected to LC-MS/MS analysis.
The soil extraction method was concurrently validated for all five soils at two fortification levels (5 and 50 ng/g).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

For sand Schifferstadt (pH 4.1), average mass balances of 86% were obtained after 2 to 48 hours of equilibration time with soil pellet extraction indicating limited stability or irreversibly bounding on the soil. For sandy loam LUFA 5M a complete mass balances was obtained after 2 to 48 hours of equilibration time with soil pellet extraction.

Mean mass balances after soil extraction were 97 % for soil Li 10, 89 % for soil LUFA 2.2 and 79 % for soil La Gironda (Arahal), indicating the stability of the test item during the experiments for LUFA 2.2 but limited stability on the both other soils.

For the adsorption isotherm testing in soil Schifferstadt the mass balances obtained after 24 hours of equilibration time were 84 – 90 % (25 to 500 ng/mL) with exception of 77% for the 5 ng/mL samples.

B. FINDINGS

<u>Adsorption Kinetics (Tier 1) for Schifferstadt (pH 4.1)</u>

The percentage of adsorption reached a plateau (Adsorption measured directly ≈ 15 % to ≈ 17 %) after 6 to 48 hours of equilibration time.
K_d calculated by the direct method reached about 0.2 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allows considering the test item to be significantly adsorbed and as “to be qualitatively mobile” (Section 69). A K_d of about 0.2 mL/g (direct method) multiplied by the soil/solution ratio of 1/1 (= 1) is > 0.1 (Section 71).
Thus (as recommended in Section 71) for this soil the experiments to study the adsorptive behaviour of the chemical in soil and its potential mobility were continued by determining Freundlich adsorption isotherms in Tier 3.

Adsorption Kinetics (Tier 1) for LUFA 5M (pH 7.2)

The percentage of adsorption was observed to reach a plateau (Adsorption measured directly ≈ 4 %) after 2 hours of equilibration time.
K_d calculated by the direct method reached only about 0.06 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allows considering the test item as "to be qualitatively mobile" (Section 69). A K_d of about 0.06 mL/g (direct method) multiplied by the soil/solution ratio of 1/1 (= 1) obviously is < 0.1 (Section 71). As a result, the compound can be considered to be not significantly adsorbed.
Thus (as recommended in Section 71) for this neutral loamy sand LUFA 5M experiments to determine Freundlich adsorption isotherms in Tier 3 were expected not to yield accurate results and were therefore not performed.

Adsorption at One Concentration after 48 hours for Additional Soils (Tier 1, Parallel Method)

For the three other soils, adsorption tests (parallel, direct method) with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time.
Adsorption after 48 hours equilibration (Adsorption measured directly) was always < 10 %.
Mean K_d calculated by the direct method reached for all three soils 0.055 mL/g to 0.097 mL/g after 48 hours of equilibration time. These results (following OECD Guideline 106 Sections 69 to 71) allow considering the test item as "to be qualitatively mobile" (Section 69) as especially for Li 10 and La Gironda soil test item is not significantly adsorbed.
When the results for K_d of 0.055 mL/g to 0.097 ml/g obtained with the direct method are multiplied by the soil/solution ratio of 1/1 (= 1) results for these two soils are significantly < 0.1. The K_d value for LUFA 2.2 soil is around 0.1 and due to low variability between individual experimental replicates and of still well determinable soil residue levels, K_d for this soil can be considered valid, despite it is formally below 0.1.
Thus (as recommended in Section 71) for these soils experiments to determine Freundlich adsorption isotherms in Tier 3, which include low concentrations either, were however expected not to yield accurate results and were therefore not performed.
A summary of the Tier 1 results is given in **Table 7.1.3.1.2-11**.

Table 7.1.3.1.2-11: Summary of Tier 1 results (48 h equilibration, 1/1 ratio)

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption at equilibrium [%]	K_d [mL/g]	K_{OC} [mL/g]
Schifferstadt	Sand	0.75	4.1	16	0.229	30.5
LUFA 5M	Loamy sand	2.03	7.2	6	0.062	3.06
LUFA 2.2	Sandy Loam	1.47	5.4	8	0.097	6.60
Li 10	Loamy sand	0.84	6.4	5	0.055	6.52
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	5	0.074	6.07

Values for adsorption at equilibrium, K_d and K_{oc} are obtained from nominal 50 ng/mL in the aqueous phase

Freundlich Adsorption Isotherm for Soil Schifferstadt

Tier 3 tests resulted for soil Schifferstadt in Freundlich adsorption isotherms based on the direct method. The experimental results are given in **Table 7.1.3.1.2-12**.

Table 7.1.3.1.2-12: Summary of adsorption isotherm test of M656PH043 on soil Schifferstadt

Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Equilibration time [%]	K_F [$ng^{1-1/n}$ $mL^{1/n}$ g^{-1}]	K_{FOC} [$ng^{1-1/n}$ $mL^{1/n}$ g^{-1}]	1/n
Sand	0.75	4.1	24	0.702	94	0.51

III. CONCLUSION

Adsorption of the Dimethenamid-P metabolite M656PH043 (Reg No. 5917262) at equilibrium was demonstrated (Tier 1 testing) for all five soils to be rather low, thus the test item has to be considered “qualitatively mobile”.
Tier 1 tests gave acceptable complete mass balances (>90 %) for the soils LUFA 5M and Li 10 and slightly below for the soils Schifferstadt and LUFA 2.2 (>86%) after 48 hours of equilibration time. For the soil La Gironda (Arahal) with only 79% complete mass balance a limited stability of the test item was shown.
Adsorption of the test item was 15 % for the soil Schifferstadt and <10 % for the other four soils using a soil/solution ratio of 1/1 and the results obtained from the direct method.
The K_d values obtained with the direct method were (except for Schifferstadt) all below or equal to 0.1 mL/g thus indicating that Tier 3 experiments for these four soils would lead to inaccurate results.
However for LUFA 2.2 soil a K_d of 0.097 was obtained, which corresponds to a K_{oc} of 6.6.
Tier 3 adsorption experiments were only performed for acidic sand Schifferstadt and resulted in a Freundlich adsorption coefficient (K_F^{ads}, 24 hours) of 0.702 and an organic carbon normalised value ($K_{F,oc}^{ads}$) of 94 (results given in $ng^{1-1/n}mL^{1/n}g^{-1}$).

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Determination of adsorption and desorption behavior of M656PH047 (Reg.No. 5917260, metabolite of Dimethenamid-P) in 5 soils
2013/1348093
Guidelines: OECD 106
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The adsorption behaviour of M656PH047 (Reg No. 5917260), metabolite of dimethenamid-P was investigated in five different soils. The soils covered a range of pH from 4.1 to 7.4 and a range of organic carbon content from 0.75% to 2.03%. The soils were classified as sand (Schifferstadt), sandy loam (LUFA 2.2), loamy sand (LUFA 5M and Li 10) and sandy clay loam (La Gironda Arahal).

The adsorption kinetic of M656PH047 was determined at one concentration (50 ng/mL in the aqueous solution) on two soils, Schifferstadt and LUFA 5M, by equilibration in a soil/solution mixture (1/1) for 2, 4, 6, 24 and 48 hours at 20-21°C (using the parallel direct method).

For the three other soils, adsorption tests (parallel, direct method) with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time.
Tier 1 tests gave acceptable mass balances with 92 % for the soil LUFA 5M after 48 hours of equilibration time, but insufficient mass balances for the soils Schifferstadt with 67 %, LUFA 2.2 with 79 %, Li 10 with 88 % and La Gironda with 86 % after 48 hours of equilibration time, indicating degradation or formation of bound residues. This indicates that the Reg. No. 5917260 has a limited stability.

Adsorption after 48 hours equilibration (Adsorption measured directly) was always < 2 %. The K_d values obtained with the direct method were all < 0.1 mL/h, thus indicating that Tier 3 experiments for the other four soils would lead to inaccurate results. Therefore, Tier 3 adsorption and desorption experiments were not performed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item M656PH047
Reg. No. 5917260
Batch No.: L82-137
Chemical name (IUPAC): 3-{[(2S)-1-methoxypropan-2-yl](sulfoacetyl)amino}-4-methylthiophene-2-carboxylic acid
Purity: 90.7 % (tolerance ±1.0 %)
Molecular weight: 351.4 g/mol

2. Soils

The study was conducted with five different soils. The physico-chemical properties of the soils are provided in **Table 7.1.3.1.2-13**.

Table 7.1.3.1.2-13: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 5917260 (Dimethenamid-P metabolite M656PH047)

Soil designation Origin	LUFA 2.2	LUFA 5M	Li 10	La Gironda (Arahal)	Schifferstadt
Textural class (DIN 4220)	Loamy sand (SI4)	Loamy sand (SI2)	Loamy sand (SI2)	Sandy clay loam	Sand
Soil texture [%], (ISO 11277)					
Sand	55.9	80.0	81.2	48.0	88.1
Silt	32.1	13.9	13.2	24.3	7.7
Clay	12.0	6.1	5.6	27.7	4.3
Textural class (USDA)	Sandy loam	Loamy sand	Loamy sand	Sandy clay loam	Sand
Soil texture [%], (USDA)					
Sand	60.3	82.8	82.8	49.2	88.5
Silt	27.7	11.1	11.6	23.0	7.2
Clay	12.0	6.1	5.6	27.7	4.3
Organic carbon [%] (ISO 10694)	1.47	2.03	0.84	1.22	0.75
Effective CEC [$cmol^+/kg$]	7.6	11.4	5.3	26.3	0.3
pH ($CaCl_2$)	5.4	7.2	6.4	7.4	4.1
pH (H_2O)	5.9	7.9	6.9	8.3	5.0
MWHC [g/100g dry soil]	29.6	25.2	25.1	39.2	27.0
Bulk density [g/L]	1227	1367	1369	1308	1342

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Kinetics at One Concentration

Adsorption kinetics testing experiments were performed exemplarily for two soils, one with low pH and low organic carbon content (sand Schifferstadt: pH 4.1, 0.75 % organic carbon) and one with higher, neutral pH (sandy loam LUFA 5M: pH 7.2, 2.03 % organic carbon). Duplicate portions of 2 g dry soil were equilibrated on a horizontal shaker with slightly less than 2 mL $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. Then the analyte dose solution was added to the soil water to obtain a nominal amount of 50 ng/mL in the aqueous phases. Test systems were then equilibrated on a horizontal shaker for various equilibration times (2, 4, 6, 24 and 48 hours) at 20-21°C. After shaking/equilibration and subsequent centrifugation, the supernatant aqueous phase was weighed to obtain the volume of the supernatant and to calculate the portion/volume of the aqueous phase remaining in the soil pellet. The liquid phases obtained after centrifugation were diluted and analysed by LC-MS/MS. Soil pellets were extracted and the extracts diluted and analysed by LC-MS/MS.

Adsorption at one Concentration after 48 h for Additional Soils

For the soils LUFA 2.2, Li 10 and La Gironda, adsorption tests with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time using the same experimental conditions as described above for the adsorption kinetics preliminary test.

2. Description of analytical procedures

Aqueous phase and solid phases (soil pellets) were analysed separately. After equilibration the samples were centrifuged for 5 min at 4000 rpm to separate the soil and water phases. An aliquot of the water phase was centrifuged for 30 min at 15,000 rpm to remove soil particles with a diameter of >0.2 µm. Aliquots of supernatant were diluted volumetrically by a dilution factor into methanol / water (1/1, v/v) for LC/MS/MS analysis.

Determination of M656PH047 in the soil involved one extraction of soil pellets with 5 mL of methanol and two extractions with 5 mL of methanol/water (1/1 v/v). For separation of soil and solvent the samples were centrifuged for 5 minutes at 4000 rpm. Subsequently, the extracts were combined and diluted. An aliquot of the final extract was diluted with methanol/water (1/1) and subjected to LC-MS/MS analysis.
The soil extraction method was concurrently validated for all five soils at two fortification levels (5.0 and 50 ng/g) resulting in an overall average recovery of 94 % (RSD 3 %).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

For acidic sand Schifferstadt (pH 4.1) soil mass balance decreased from 87 % after 2 hours to 67 % for the 48 hours equilibration time, indicating limited stability with prolonged equilibration.

For neutral loamy sand LUFA 5M (pH 7.2), a mass balance 100% was obtained with soil pellet extraction after 2 h decreasing to 92% after 48h .

For the adsorption testing of soils LUFA 2.2, Li 10 and La Gironda mass balances were 79%, 88% and 86% after 48h of equilibration time, indicating degradation or formation of bound residues.

B. FINDINGS

Adsorption Kinetics (Tier 1) for Schifferstadt (pH 4.1)

The percentage of adsorption did not change significantly with prolonged equilibration time, and was about (Adsorption measured directly) 1 % after 48 hours of equilibration time.
K_d calculated by the direct method reached only about 0.02 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allowed considering the test item as “to be qualitatively mobile” (Section 69). A K_d of maximal 0.02 mL/g (direct method) multiplied by the soil/solution ratio of 1/1 (= 1) is < 0.1 (Section 71). As a consequence, following the reported procedures, adsorption has to be considered not to be significant.
Thus (as recommended in Section 71) for this acidic sand Schifferstadt experiments to determine Freundlich adsorption isotherms in Tier 3 were expected not to yield accurate results and were, therefore, not performed.

Adsorption Kinetics (Tier 1) for LUFA 5M (pH 7.2)

The percentage of adsorption did not change significantly with prolonged equilibration time, and was about (Adsorption measured directly) 2 % after 48 hours of equilibration time.
K_d calculated by the direct method reached only about 0.02 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allowed considering the test item as “to be qualitatively mobile” (Section 69). A K_d of about 0.02 mL/g (direct method) multiplied by the soil/solution ratio of 1/1 (= 1) is < 0.1 (Section 71). If K_d is below this value, adsorption is supposed to be not significant according to OECD Guideline 106.
Thus (as recommended in Section 71) for this neutral loamy sand LUFA 5M experiments to determine Freundlich adsorption isotherms in Tier 3 were expected not to yield accurate results and were, therefore, not performed.

Adsorption at One Concentration after 48 hours for Additional Soils (Tier 1, Parallel Method)

Adsorption after 48 hours equilibration (Adsorption measured directly) was always ≤ 1 %. Mean K_d calculated by the direct method were for all three soils 0.01 mL/g. These results (following OECD Guideline 106 Sections 69 to 71) allowed considering the test item as “to be qualitatively mobile” (Section 69.).
When these results for K_d obtained with the direct method are multiplied by the soil/solution ratio of 1/1 (= 1) the results are all < 0.1.
Thus (as recommended in Section 71) for these soils experiments to determine Freundlich adsorption isotherms in Tier 3 were expected not to yield accurate results and were, therefore, not performed.

A summary of the Tier 1 results is given in **Table 7.1.3.1.2-14**.

Table 7.1.3.1.2-14: Summary of Tier 1 results (48 h equilibration, 1/1 ratio)

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption at equilibrium [%]	K_d [mL/g]	K_{OC} [mL/g]
Schifferstadt	Sand	0.75	4.1	1.2	0.019	2.48
LUFA 5M	Loamy sand	2.03	7.2	1.5	0.016	0.79
LUFA 2.2	Sandy Loam	1.47	5.4	0.5	0.006	0.42
Li 10	Loamy sand	0.84	6.4	1.0	0.012	1.37
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	0.7	0.009	0.72

Values for adsorption at equilibrium, K_d and K_{oc} are obtained from nominal 50 ng/mL in the aqueous phase

III. CONCLUSION

Adsorption of the dimethenamid-P metabolite M656PH047 (Reg No. 5917260) at equilibrium was demonstrated (Tier 1 testing) for all five soils to be very low, thus the test item has to be considered “qualitatively mobile”.
Tier 1 tests gave acceptable mass balances with 92 % for the soil LUFA 5M after 48 hours of equilibration time, but insufficient mass balances for the other four soils of < 90 % after 48 hours of equilibration time, indicating degradation or formation of bound residues. This indicates that the Reg. No. 5917260 has a limited stability.
For all five soils, adsorption was always ≤ 2 % using a soil/solution ratio of 1/1 and the results obtained from the direct method.
The K_d values obtained with the direct method were all < 0.1 mL/g, thus indicating that Tier 3 experiments for all five soils would lead to inaccurate results. Therefore Tier 3 adsorption experiments were not performed.

Report: CA 7.1.3.1.2/6
Class T.,Walter W., 2014a
Determination of adsorption and desorption behavior of M656PH054 (Reg.No. 5920718, metabolite of Dimethenamid-P) in 5 soils - (OECD guideline 106)
2013/1348094

Guidelines: OECD 106 (2000)

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

Executive Summary

The adsorption behaviour of M656PH054 (Reg No. 5920718), metabolite of Dimethenamid-P was investigated in five different soils. The soils covered a range of pH from 4.1 to 7.4 and a range of organic carbon content from 0.75% to 2.03%. The soils were classified as sand (Schifferstadt), sandy loam (LUFA 2.2), loamy sand (LUFA 5M and Li 10) and sandy clay loam (La Gironda Arahal).

Adsorption kinetics of M656PH054 were determined at one concentration (50 ng/mL in the aqueous solution) on two soils, Schifferstadt and LUFA 5M, by equilibration in a soil/solution mixture (1/1) for 2, 4, 6, 24 and 48 hours at 20°C. For soil Schifferstadt the percentage of adsorption increased with prolonged equilibration time, reaching a plateau (Adsorption measured directly ≈ 10 % to ≈ 15 %) after 24 to 48 hours of equilibration time. For soil LUFA 5M adsorption increased with prolonged equilibration time, reaching a plateau (Adsorption measured directly ≈ 7 % to 8 %) after 48 hours of equilibration time.

For the three other soils, adsorption tests (parallel, direct method) with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time. Adsorption after 48 hours equilibration (Adsorption measured directly) was always < 10 %.

The K_d values obtained with the direct method were (except for soil Schifferstadt) all < 0.1 mL/h, thus indicating that Tier 3 experiments for the other four soils would lead to inaccurate results. Therefore, an adsorption isotherm of M656PH054 was established only for soil Schifferstadt and an equilibration time of 48 hours. Tier 3 adsorption experiments resulted in a Freundlich adsorption coefficient (K_{Fads}, 48 hours) of 0.196 and an organic carbon normalized value ($K_{F,ocads}$) of 26 (results given in $ng^{1-1/n}mL^{1/n}g^{-1}$). The obtained Freundlich exponent is 0.81.
For determination of the adsorption isotherm soil pellets were extracted as only for the direct method accurate results were expected, thus no desorption experiments were performed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item M656PH054
Reg. No. 5920718
Batch No.: L82-121
Chemical name (IUPAC): N-(2,4-dimethylthiophen-3-yl)-N-(sulfoacetyl)-L-alanine
Purity: 85.1 % (tolerance ±1.0 %)
Molecular weight: 321.4 g/mol

2. Soils

The study was conducted with five different soils. The physico-chemical properties of the soils are provided in **Table 7.1.3.1.2-15**.

Table 7.1.3.1.2-15: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 5920718 (Dimethenamid-P metabolite M656PH054)

Soil designation Origin	LUFA 2.2	LUFA 5M	Li 10	La Gironda (Arahal)	Schifferstadt
Textural class (DIN 4220)	Loamy sand (SI4)	Loamy sand (SI2)	Loamy sand (SI2)	Sandy clay loam	Sand
Soil texture [%], (ISO 11277)					
Sand	55.9	80.0	81.2	48.0	88.1
Silt	32.1	13.9	13.2	24.3	7.7
Clay	12.0	6.1	5.6	27.7	4.3
Textural class (USDA)	Sandy loam	Loamy sand	Loamy sand	Sandy clay loam	Sand
Soil texture [%], (USDA)					
Sand	60.3	82.8	82.8	49.2	88.5
Silt	27.7	11.1	11.6	23.0	7.2
Clay	12.0	6.1	5.6	27.7	4.3
Organic carbon [%] (ISO 10694)	1.47	2.03	0.84	1.22	0.75
Effective CEC [$cmol^+/kg$]	7.6	11.4	5.3	26.3	0.3
pH ($CaCl_2$)	5.4	7.2	6.4	7.4	4.1
pH (H_2O)	5.9	7.9	6.9	8.3	5.0
MWHC [g/100g dry soil]	29.6	25.2	25.1	39.2	27.0
Bulk density [g/L]	1227	1367	1369	1308	1342

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Kinetics Preliminary Test

Adsorption kinetics testing experiments were performed exemplarily for two soils, one with low pH and low organic carbon content (sand Schifferstadt: pH 4.1, 0.75 % organic carbon) and one with higher, neutral pH (loamy sand LUFA 5M: pH 7.2, 2.03 % organic carbon). Duplicate portions of 2 g dry soil were equilibrated on a horizontal shaker with slightly less than 2 mL $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. Then the analyte dose solution was added to the soil water to obtain a nominal amount of 50 ng/mL in the aqueous phases. Test systems were then equilibrated on a horizontal shaker for various equilibration times (2, 4, 6, 24 and 48 hours) at 20-21°C. After shaking/equilibration and subsequent centrifugation, the supernatant aqueous phase was weighed to obtain the volume of the supernatant and to calculate the portion/volume of the aqueous phase remaining in the soil pellet. The liquid phases obtained after centrifugation were diluted and analysed by LC-MS/MS. Soil pellets were extracted and the extracts diluted and analysed by LC-MS/MS.

Adsorption at one Concentration after 48 h for Additional Soils

For the soils LUFA 2.2, Li 10 and La Gironda, adsorption tests with a soil/solution ratio of 1/1 were performed for 48 hours of equilibration time using the same experimental conditions as described above for the adsorption kinetics preliminary test.

Tier 3: Adsorption Isotherm

The Tier 1 tests showed that the test item is expected to be stable in soils. However, the K_d values obtained by the direct method and the soil/solution ratio of 1/1 were rather low and thus the test item has to be considered “to be qualitatively mobile” (OECD Guideline 106 Section 69.). Therefore, based on OECD 106, Tier 3 testing was only considered to lead to accurate Freundlich adsorption isotherms for acidic sand Schifferstadt.
The test was performed using an equilibration time of 24 hours (not reported) and 48 hours and 1/1 soil/solution ratio. Soil samples (2 g) were equilibrated overnight with < 2.0 mL of 0.01 M $CaCl_2$ solution. The test substance was added to the mixture to achieve the following amounts: 5.0, 25, 50, 250 and 500 ng/mL in the aqueous solution. Duplicate samples were used for each concentration. The test systems were equilibrated by shaking on a horizontal shaker at 20-21°C. After equilibration the samples were analysed as described for the adsorption kinetics preliminary test.

2. Description of analytical procedures

Aqueous phase and solid phases (soil pellets) were analysed separately. After equilibration the samples were centrifuged for 5 min at 4000 rpm to separate the soil and water phases. An aliquot of the water phase was centrifuged for 30 min at 15,000 rpm to remove soil particles with a diameter of >0.2 µm. Aliquots of supernatant were diluted volumetrically by a dilution factor into methanol / water (1/1, v/v) for LC/MS/MS analysis.

Determination of M656PH054 in the soil involved one extraction of soil pellets with 5 mL of methanol and two extractions with 5 mL of methanol/water (1/1 v/v). For separation of soil and solvent the samples were centrifuged for 5 minutes at 4000 rpm. Subsequently, the extracts were combined and diluted. An aliquot of the final extract was diluted with methanol/water (1/1) and subjected to LC-MS/MS analysis.
The soil extraction method was concurrently validated for all five soils at two fortification levels (5.0 and 50 ng/g).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

In the adsorption kinetics testing mass balances of ≥ 98 % were obtained for acidic sand Schifferstadt after soil pellet extraction (except for 48 hours equilibration: Mass balance ≥ 85 %).
For sandy loam LUFA 5M mass balances of ≥ 96 % were obtained after soil pellet extraction.

For the adsorption testing of soils LUFA 2.2, Li 10 and La Gironda mass balances were between 93 and 98% considering individual samples.

For the adsorption isotherm testing in soil Schifferstadt the mass balances obtained after 48 hours of equilibration time were 79 – 91 % (25 to 500 ng/mL) with exception of 63% for the 5 ng/mL samples.

B. FINDINGS

Adsorption Kinetics (Tier 1) for Schifferstadt (pH 4.1)

The percentage of adsorption increased with prolonged equilibration time, reaching a plateau (Adsorption measured directly ≈ 10 % to 15 %) after 24 to 48 hours of equilibration time.
K_d calculated by the direct method reached about 0.2 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allows to consider the test item as “to be qualitatively mobile” (Section 69), with the results for K_d of about 0.2 mL/g obtained with the direct method and multiplied by the soil/solution ratio of 1/1 (= 1) obviously being > 0.1 (Section 71).
Thus (as recommended in Section 71) for this soil the experiments to study the adsorptive behaviour of the chemical in soil and its potential mobility were continued by determining Freundlich adsorption isotherms in Tier 3.

Adsorption Kinetics (Tier 1) for LUFA 5M (pH 7.2)

The percentage of adsorption increased with prolonged equilibration time, reaching a plateau (Adsorption measured directly ≈ 6 % to 8 %) after 24 to 48 hours of equilibration time.
K_d calculated by the direct method reached only about 0.08 mL/g after 48 hours of equilibration time. This result (following OECD Guideline 106 Sections 69 to 71) allows to consider the test item as "to be qualitatively mobile" (Section 69), with the results for K_d of about 0.08 mL/g obtained with the direct method and multiplied by the soil/solution ratio of 1/1 (= 1) obviously being < 0.1 (Section 71).
Thus (as recommended in Section 71) for this soil the experiments to study the adsorptive behaviour of the chemical in soil and its potential mobility by determining Freundlich adsorption isotherms in Tier 3 were considered not to result in accurate results and therefore were not performed.

Adsorption at One Concentration after 48 hours for Additional Soils (Tier 1, Parallel Method)

The percentage of adsorption after 48 hours equilibration (Adsorption measured directly) were always < 10 %, indicating that (acc. to OECD 106, Section 70) results obtained by the indirect method are considered inaccurate.
K_d calculated by the direct method reached for all three soil only between 0.05 and 0.09 mL/g after 48 hours of equilibration time. These results (following OECD Guideline 106 Sections 69 to 71) allow considering the test item as "to be qualitatively mobile" (Section 69).
When these results for K_d obtained with the direct method are multiplied by the soil/solution ratio of 1/1 (= 1) the results are still < 0.1.
Thus (as recommended in Section 71) for these soils the experiments to determining Freundlich adsorption isotherms in Tier 3 were considered not to result in accurate results and therefore were not performed. A summary of the Tier 1 results is given in **Table 7.1.3.1.2-16**.

Table 7.1.3.1.2-16: Summary of Tier 1 results (48 h equilibration, 1/1 ratio)

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption at equilibrium [%]	K_d [mL/g]	K_{OC} [mL/g]
Schifferstadt	Sand	0.75	4.1	15	0.217	28.90
LUFA 5M	Loamy sand	2.03	7.2	7	0.078	3.84
LUFA 2.2	Sandy Loam	1.47	5.4	8	0.087	5.93
Li 10	Loamy sand	0.84	6.4	7	0.080	9.53
La Gironda Arahal	Sandy clay loam	1.22	7.4	5	0.052	4.30

Values for adsorption at equilibrium, K_d and K_{oc} are obtained from nominal 50 ng/mL in the aqueous phase

Freundlich Adsorption Isotherm for Soil Schifferstadt

Tier 3 tests resulted for soil Schifferstadt in Freundlich adsorption isotherms based on the direct method. The experimental results are given in **Table 7.1.3.1.2-17**.

Table 7.1.3.1.2-17: Summary of adsorption isotherm test of M656PH054 on soil Schifferstadt

Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Equilibration time [%]	K_F [$ng^{1-1/n}$ $mL^{1/n}$ g^{-1}]	K_{FOC} [$ng^{1-1/n}$ $mL^{1/n}$ g^{-1}]	1/n
Sand	0.75	4.1	48	0.196	26.2	0.81

III. CONCLUSION

Adsorption of the Dimethenamid-P metabolite M656PH054 (Reg No. 5920718) at equilibrium was demonstrated (Tier 1 testing) for all five soils to be rather low, thus the test item has to be considered qualitatively mobile.
Tier 1 tests gave acceptable mass balances (> 90 %) after 24 hours of equilibration time.
Except for sand Schifferstadt, with an OC of 0.75 % and a pH of 4.1 (thus, considered the most acidic soil examined), adsorption was always < 10 % using a soil/solution ratio of 1/1 and the results obtained from the direct method.
The K_d values obtained with the direct method were (except for Schifferstadt) all < 0.1 mL/g, thus, indicating that Tier 3 experiments for the other four soils would lead to inaccurate results. Therefore, Tier 3 adsorption experiments were only performed for acidic sand Schifferstadt and resulted in a Freundlich adsorption coefficient (K_{Fads}) for the Schifferstadt sand of 0.196 and an organic carbon normalized values ($K_{F,ocads}$) of 26 (results given in $ng^{1-1/n}mL^{1/n}$ g^{-1}).

CA 7.1.3.2 Aged sorption

No new data was generated in aged sorption studies.

CA 7.1.4 Mobility in soil

A previous lysimeter study conducted with racemic Dimethenamid had been submitted for use in the risk assessment. This study was found to be valid from a study conduct perspective but the HPLC data were re-evaluated upon consultation with the designated RMS, Germany. The reassessment of the original lysimeter study involved both re-evaluation of the HPLC peak assignments as well as further structure elucidation of metabolites. The chromatograms were reflective of the degradation profile of the active substance; complex and variable. Great efforts were taken to provide a comprehensive and conservative approach to this data.

A bulk soil incubation and a mini-lysimeter study were performed on Dimethenamid-P with soil from the same location as the original study (“Borstel”) to confirm the degradation pattern. Dimethenamid-P was applied to larger amounts of soil or at an exaggerated rate to generated larger quantities of degradation products for structure elucidation. This led to numerous newly proposed structures in the metabolic scheme. Careful quantitation of peaks led to new estimated concentrations of several metabolites in the lysimeter leachate. The conservative approach ensured reasonable worst case assumptions for ground water PEC calculations. In addition, a relevance assessment for the outdoor lysimeter for Europe was performed.
Considering the tremendous complexity of the metabolite situation in the lysimeter leachate, these metabolites will be grouped according to chemical similarity and metabolic relationship. Representative metabolites of each group are selected for further investigation and assessment.

Besides the studies above a non-guideline mini-lysimeter study was performed for metabolites M656H23 and M656H27 together with a conservative tracer, in order to obtain degradation and sorption values for both metabolites under more realistic conditions. This study was performed due to the uncertainty of obtaining reliable sorption rates for the conventional adsorption/desorption studies of these two metabolites. The study was performed in parallel to the adsorption/desorption studies with the syringe method, and could potentially serve as an alternative method to obtain sorption values. As it turned out, the new syringe method to determine adsorption/desorption could provide reliable sorption values. Therefore the non-guideline mini-lysimeter study serves only as indicative information.

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

The mobility in soil of Dimethenamid-P and its metabolites were evaluated during the Annex I inclusion. No additional column leaching studies have been performed.

The leaching behavior of ^{14}C-Dimethenamid was investigated in three German standard soils and two Swiss agricultural soils.

Under the worst case conditions of laboratory leaching experiments with unaged soil residues, one light sandy soil with very low organic matter (BBA 2.1) allowed mobility of Dimethenamid (40.2% of TAR found in leachate; 33.4% TAR = Dimethenamid in leachate). Other more typical soils allowed less than 10% of the TAR to move to the leachate. Metabolites M23, M27 and M31 were also found in the leachates, but none at levels exceeding 2.8% TAR, which means that limited degradation of the parent molecule occurred during the leaching experiment.

In two studies the leaching behavior of ^{14}C-Dimethenamid residues, which had been aged for 31 days in standard soil BBA 2.1 or for 22 days in standard soil BBA 2.2, was investigated. The leachates contained 23.8% TAR and 22.7% TAR, respectively.
Only one of the two leachates contained minor traces of parent compound (0.09 % TAR). The transformation products of Dimethenamid showed a tendency to move through the soil. Among the metabolites, only the oxalic acid metabolite (M23) was found in amounts significantly exceeding 2% TAR. However, this metabolite, along with M27 and M31 undergoes degradation in soil, as previously summarized in the degradation studies.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

See point CA 7.1.4.1.1 above.

CA 7.1.4.2 Lysimeter studies

The mobility in soil of Dimethenamid-P and its metabolites was evaluated during the Annex I inclusion. The fate and mobility of Dimethenamid was studied in two outdoor lysimeters with undisturbed soil monoliths taken from agricultural land with a sandy soil and low organic carbon content (Borstel near Neustadt a. R., Lower Saxony/Germany). The radiolabeled test substance, was applied to the bare soil of both lysimeters in the first year at an application rate of 1.44 kg a.s./ha. One year later the second lysimeter was treated again with the same rate. The study was run for three years. The amount of precipitation was between 910 and 1159 mm. The leachate volumes were between 318 and 533 L.
The results of the study clearly demonstrated that after treatment with ^{14}C-Dimethenamid, there is no substantial displacement of active substance into deeper soil layers and subsequently into groundwater. The concentrations of the active substance found in the leachates were far below the value of 0.1 µg/L. Metabolites M23 and M27 were detected in amounts >0.1 µg/L and <4 µg/L in the leachate. Additional studies revealed that these metabolites are of no concern with regard to toxicity, ecotoxicity and biological activity. M31 was not detected.

While the original (Burgener, 1996) lysimeter study was sound from a study conduct perspective, interpretation of the HPLC chromatograms left room for improvement. As stated before, the degradation of Dimethenamid-P is extensive and complex due to comprehensive metabolism. This degradation pattern (combined with the multiple potential isomeric forms) directly corresponds to the complexity of the HPLC chromatograms. This, in part, led to the inefficiencies in HLPC interpretation from the original study. The chromatograms were reviewed for greater accuracy in peak assignment and delineation. These efforts have led an overall more accurate (albeit conservative) interpretation of the data.

Due to the conservative evaluation mentioned above, several metabolites that were previously uncharacterized were selected for further structural evaluation and elucidation. To this end a “microlysimeter” [see CA 7.1.4.2/1] study was run to generate samples for structural elucidation. It must be pointed out that the chromatographic profiles between the original lysimeter study and the “microlysimeter” study were similar. This both validates the use of the microlysimeter samples for structural assignment but also demonstrates the reproducibility of the results.

The above mentioned investigations (see CA 7.1.4.2/1, CA 7.1.4.2/2 and CA 7.1.4.2/5) were presented as an Addendum 3 to the previous Annex-I review. These studies are included in the current updated submission for completeness, but should be supplanted with the most recent evaluations as well as the structure elucidation efforts in this submission (CA 7.1.4.2/3, CA 7.1.4.2/4 and CA 7.1.4.2/6).

The vulnerability or relevance of the outdoor lysimeter study was evaluated for applicability to agricultural practices in Europe (CA 7.1.4.2/7). The high vulnerability of the lysimeter study can clearly be shown regarding pesticide leaching to groundwater for the entire intended use area of Dimethenamid-P in Europe. A good representativeness and a high relevance of the higher tier lysimeter study could be concluded.

Column leaching (or “mini-lysimeter") studies were additionally performed on metabolites M656H023 and M656H027 (CA 7.1.4.2/8). This study was used to illustrate the breakthrough behavior of the metabolites relative to a conservative bromide tracer. These results were then further analyzed (CA 7.1.4.2/9) to derive sorption and degradation parameters with mathematical models.

Report:	CA 7.1.4.2/1 Fent G., 2008a Microlysimeterstudy (soil Borstel) with 14C-Dimethenamid-P for characterisation of the metabolite pattern in leachates 2008/1051489
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The purpose of this study was the characterisation of the leachate metabolite pattern of ^{14}C-Dimethenamid-P in undisturbed soil columns of an agricultural soil using a discontinuous irrigation system. The soil used in this study (”Borstel”) was the same as used in a previous outdoor lysimeter leaching study with ^{14}C-dimethenamid. The nature and amount of metabolites in the leachates of the mini-lysimeters of this study may be compared with the results of the outdoor lysimeter study in order to obtain more information about metabolites in the leachate water.

In a non-GLP pre-test in total five test variants were established. Two different application amounts (corresponding to 1 and 3 kg a.s./ha), one outdoor variant with 3 days sunlight exposure after application and three different irrigation patterns were tested. The different variants in the pre-test were selected in order to investigate the leachate metabolite pattern affected by:

- application rate
- sunlight after application
- irrigation scheme (resulting in different infiltration depths and incubation conditions like aerobic vs. anaerobic)

In the pre-tests the main known metabolite in the leachate was M27 (in average 24.5 % of total leachate radioactivity) followed by the metabolite M23 (in average 15.6 % of total leachate radioactivity). Up to 9 unknown metabolites with distinct peaks were detectable. With the exception of the metabolite E (average 6.2% of the total leachate radioactivity) all other unknown metabolites represented < 5% of the total leachate radioactivity. Most of the radioactivity (about one third) represented unknown radioactivity showing an increased baseline without a specific retention time. With the exception of the metabolite M23 (decreasing proportions in the course of the study) the leachate metabolite pattern did not change significantly during the course of the study.

In the pre-tests the metabolite pattern was not affected significantly by the application rate, sunlight after application and/or irrigation scheme.

Since there were no important differences between the variants compared in the pre-test, the following boundary conditions were selected for the definitive test under GLP:
- application rate corresponding to 2.5 kg a.s./ha
- no sunlight after application
- irrigation scheme corresponding to 3 mm/day (Monday to Friday) from day 3 after application until day 30 after application and 6 mm/day (Monday to Friday) from day 31 after application until day 140 after application

Because the two replicate soil columns (Borstel-A and Borstel B) showed no different results, mean values are given in the following:
The soil columns were irrigated with a total amount of 18.93 L water corresponding to 540.8 mm of irrigation. 95.3 % of the irrigation amount was sampled as leachate (18.04 L corresponding to 515.5 mm). 39.2 % of the radioactivity applied was found in the leachate. The radioactivity in the leachate was subjected to radio-HPLC analysis. Like in the pre-tests the main known metabolite in the leachate was M27 (in average 24.3 % of total leachate radioactivity) followed by the metabolite M23 (in average 16.7 % of total leachate radioactivity). Again up to 9 unknown metabolites with distinct peaks were detectable. With the exception of the metabolite E (average 5.4% of the total leachate radioactivity) all other unknown metabolites represented < 5% of the total leachate radioactivity. With the exception of the metabolite M23 (decreasing proportions in the course of the study) the leachate metabolite pattern (number and amounts of metabolites) did not change significantly in the course of the study.

Neither in the pre-tests nor in the definitive GLP-study, the test item Dimethenamid-P was detectable in the leachate samples.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BAS 656 H
Reg.No.:	363851
CAS-No.:	16315-14-8
Chemical name (IUPAC):	(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Common name:	Dimethenamid-P

Radiolabeled test item

Position of radiolabel:	thienyl-5-^{14}C
Batch-No.:	824-3102
Specific radioactivity:	6.53 MBq/mg
Radiochemical purity:	99.4%

Non-radiolabeled test item:
Batch-No. BEAU201204

Prior to the applications, radiolabeled and non-radiolabeled test items were mixed at about the following ratios:
- pre-test: 2+1
- definitive test: 1+1

2. Soil

The soil chosen for the study, originated from Borstel, Lower Saxony, Germany. This is the same soil that was used earlier for the lysimeter study with Dimethenamid.
The characteristics of the soil is given in **Table 7.1.4.2-1**.

Table 7.1.4.2-1: Properties of soil Bruch West used to in the mini-lysimeter study with ^{14}C-Dimethenamid-P

Soil designation	**Borstel (17/03/07)**
Origin	Borstel, Germany
DIN Particle size distribution [%]	
sand 0.063 – 2 mm	90.0
silt 0.002 – 0.063 mm	7.3
clay < 0.002 mm	2.7
textural class	sand
USDA Particle size distribution [%]	
sand 0.050 – 2 mm	91.1
silt 0.002 – 0.050 mm	6.3
clay < 0.002 mm	2.7
textural class	sand
Organic C [%]	0.75
Organic matter [%] **	1.29*
pH [H_2O]	6.5
pH [$CaCl_2$]	5.9
Cation exchange capacity [$cmol^+$ / kg]	2.7
Maximum water holding capacity [g/100g dry soil]	23.0
Microbial biomass [mg C/100g dry soil]	14.5

* organic matter = organic carbon x 1.724

For the study twelve undisturbed soil columns were sampled. At the same time, a disturbed soil sample was taken for the determination of soil characteristics and potential further studies.
The soil cores were sampled with a special device. For the sampling of the soil columns an area of about 100 m² without crop cover was used. Stainless steel tubes (211 mm in diameter and 300 mm length) were connected with the sampling device and placed on the sampling site. The device was adjusted horizontally using a water level. The stainless steel tube was forced into the ground with a hammer. When the sampling tube had lowered to 300 mm the sampling device was removed and the tube was dug out of the ground. At the lower end the soil was cut using a knife.

For the determination of the leachate metabolite pattern in the pre-test five soil columns out of the twelve were used. The soil columns that were not used were stored in closed containers at temperatures between +1-+10°C in the dark. For the actual determination of the leachate metabolite pattern in the definitive test two soil columns (replicates Borstel-A and Borstel-B) out of the twelve were used.

B. STUDY DESIGN

1. Experimental conditions

A ceramic plate inserted in a stainless steel lid was mounted at the lower base of the soil columns. An outlet tube with an internal diameter of 3 mm was connected to the steel lid and was filled with a hanging water column of 300 mm to apply a constant tension to the lower end of the soil column to prevent the formation of a capillary fringe.
On top of the column about 30 mm of the soil were replaced by 50 g of quartz sand ensuring a plain surface. Afterwards an irrigation head was placed on top of the column which is connected over a peristaltic pump with the water reservoir. Prior the application the soil columns were equilibrated under the actual irrigation conditions used for the test. This equilibration was conducted for at least 6 days. During this period the actual amount of leachate was determined and recorded. At the end of each irrigation cycle the actual volume of water applied to each column was measured and recorded.

Table 7.1.4.2-2: Test variants and corresponding irrigation and sampling conditions in the pre-test (nominal amounts)

	Day after tretatment	Mini-lysimeter I	Mini-lysimeter II	Mini-lysimeter III	Mini-lysimeter IV	Mini-lysimeter V
Amount applied	0	3 kg/ha	1 kg/ha	1 kg/ha	1 kg/ha	1 kg/ha
Solar radiation	0-2	No (laboratory)	Yes (outdoor)	No (laboratory)	No (laboratory)	No (laboratory)
Irrigation immediately after application	0	No	No	No	No	Yes corresponding to 5 mm
Irrigation Monday to Friday	3-30	Corresponding to 3 mm/day	Corresponding to 3 mm/day	Corresponding to 3 mm/day	Corresponding to 3 mm/day	None
Irrigation Day 31 after treatment	31	None	None	None	Corresponding to 12 mm followed by closing leachate valve	None
Irrigation after Day 32	32	Corresponding to 6 mm/day	Corresponding to 6 mm/day	Corresponding to 6 mm/day	None	Corresponding to 6 mm/day
Irrigation after Day 63	63	Corresponding to 6 mm/day	Corresponding to 6 mm/day	Corresponding to 6 mm/day	Corresponding to 6 mm/day	Corresponding to 6 mm/day
Sampling and analytical work						
Leachate volume and ^{14}C	0-31	Monday to Friday daily fractions and Saturday to Sunday one fraction				None
Leachate	32-58	Weekly fractions			None	Weekly

volume and ^{14}C				fractions
Leachate volume and ^{14}C	59-100	Weekly fractions		
Radio HPLC	> 28-93	Depending on ^{14}C-activity in the leachate		

The different variants were selected in order to investigate the leachate metabolite pattern affected by:
- application rate - mini-lysimeter I vs. mini-lysimeter III
- sunlight after application - mini-lysimeter II vs. mini-lysimeter III
- irrigation scheme- mini-lysimeter III vs. mini-lysimeter IV and mini-lysimeter IV (resulting in different infiltration depths and incubation conditions like aerobic vs. anaerobic)

In the pre-test all variants were treated at a nominal rate of 1 kg a.s./ha except for mini-lysimeter 1 which was treated at a rate 3 kg a.s./ha. The test conditions in the definitive test corresponded largely to those in mini-lysimeter 1 except for the nominal application rate which was slightly lower with 2.5 kg a.s./ha.

For the applications a mask with 20 holes was placed on top of the column. Through each hole equal aliquots of the application solution were applied on the column surface using a pipette.
Upon application, the soil cores were kept at room temperature (at about 20 ^{o}C) in darkness except for variant II which was exposed to sunlight for 3 days.

2. Sampling
Upon the start of the irrigation, the leachate was collected according to the scheme described in **Table 7.1.4.2-2** and the amount of leachate was determined by weighing. The test duration was 100 days in the pre-test and 142 days in the definitive test.

3. Analytical procedures
The radioactivity in the liquid specimens was measured by liquid scintillation counting.
Leachate samples with sufficient amounts of radioactivity were subjected to radio HPLC investigation. Leachate was concentrated by means of a rotary evaporator. Depending on the radioactivity in the leachate samples were concentrated in order to achieve about 50,000 dpm in 500 µl injection volume for the HPLC analysis.
The identity of the ^{14}C-Dimethenamid-P and of the metabolites M23 and M27 was confirmed by means of UV/VIS-radio HPLC co-chromatography with the non-radiolabeled reference items. Peaks were set manually and unknown peaks were named and characterised with their retention time.

II. RESULTS AND DISCUSSION
1. Pre-test

Water Balance
Depending on the irrigation scheme of test variants, the soil columns were irrigated with a total amount between 214 and to 341 mm. With exception of the test variant II (3 days outdoor exposure) more than 95% of the irrigation volume was sampled as leachate. The difference to the total applied amount of water can be explained by evaporation losses and by increase of the soil moisture close to saturation of the soil. The summarised results of the water balance for each soil column are given in **Table 7.1.4.2-3**.

Table 7.1.4.2-3: Water balance of the individual soil columns

Mini-lysimeter	Total irrigation [mL/mm]	Total leachate [mL/mm]	Total leachate [% of irrigation]
I	11,938/341	11,379/325	95.3
II	11,927/341	10,870/311	91.1
III	11,935/341	11,491/328	95.8
IV	7,471/214	7,469/213	99.9
V	10,111/289	9,743/278	96.4

Radioactivity in the leachate
Between 21.6 % and 35.7 % of the applied radioactivity was found in the leachate. Summarized results for each mini-lysimeter are given in **Table 7.1.4.2-4.**

Table 7.1.4.2-4: Radioactivity in leachate of the individual mini-lysimeter

Mini-lysimeter	Radioactivity applied [kBq]	Radioactivity in leachate [kBq]	Radioactivity in leachate [% applied]
I	35179	11799	33.5
II	11726	2538	21.6
III	11726	3823	32.6
IV	11726	2753	23.5
V	11726	4188	35.7

About 18 days after application the radioactivity increased until reaching a maximum about 44 days after treatment. In the following the radioactivity in leachate decreased to a level < 50 µg/L a.s. equivalents indicating that most of the mobile radioactivity had leached through the soil cores. With the exception of the test variant V (irrigation and leachate formation started 32 days after application) all other test variants showed a similar breakthrough behavior.

The radioactivity in the leachate was characterized by means of radio-HPLC. Independent on the test variant no test item was detectable in the leachate. The main known metabolite in the leachate was M27 (in average 24.5 % of total leachate radioactivity) followed by metabolite M23 (in average 15.6 % of total leachate radioactivity).
In total up to 9 unknown metabolites with distinct peaks were detectable. Independent on the test variant the metabolite pattern was almost identical. Most of the unknown metabolites represented < 5% of the total leachate radioactivity. Only the Metabolite "E" was detected in amounts slightly greater than 5% of the total leachate radioactivity. Most of the radioactivity (about 32%) represented unknown radioactivity showing an increased baseline without a specific retention time. With the exception of the metabolite M23 (decreasing proportions in the course of the study) the leachate metabolite pattern did not change significantly in the course of the study.
Detailed results of all test variants are shown in **Table 7.1.4.2-5 to Table 7.1.4.2-9**.

Table 7.1.4.2-5: Metabolite pattern of leachate radioactivity in test variant I

	Metabolite fraction (Rt in min + 0.5 min) in % of total radioactivity in the leachate										
Rt	2.8	9.4	11.7	13.1	19.2	21.2	22.3	23.3	25.6	28.5	30.7	
DAT[1)]	**Start**	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714 (M27)**	**360715 (M23)**	**F**	**n.c.[2)]**
31-38		3.3	5.8	5.7			2.9	3.4	24.3	29.9		24.7
39-44		2.0	4.1	4.7			2.8	4.1	25.9	29.3		27.0
45-51		2.8	3.4	3.7			4.9	6.3	26.4	22.6		30.0
52-58		2.4	3.5	3.4	3.5	2.4	5.6	7.7	27.5	16.8		27.1
59-65		2.3	4.1	3.5	3.7	2.7	6.0	7.0	26.9	14.5	1.1	28.3
66-72		2.6	3.2	3.7	3.9	3.3	5.7	6.8	24.9	13.8	1.8	30.2
73-79		4.3	2.7	3.9	3.9	4.1	5.2	6.2	22.9	12.4	2.9	31.7
80-86		3.2	3.2	4.7	3.4	3.2	5.2	6.6	22.3	12.3	2.7	33.3
87-93		3.2	2.8	3.8	4.8	3.2	4.7	5.6	22.0	13.9	3.7	32.2
Mean		**2.9**	**3.7**	**4.1**	**3.9**	**3.1**	**4.8**	**6.0**	**24.8**	**18.4**	**2.4**	**29.4**

1) Days after treatment
2) n.c.= not characterized by a retention time

Table 7.1.4.2-6: Metabolite pattern of leachate radioactivity in test variant II

	Metabolite fraction (Rt in min + 0.5 min) in % of total radioactivity in the leachate										
Rt	2.8	9.4	11.7	13.1	19.2	21.2	22.3	23.3	25.6	28.5	30.7	
DAT[1)]	**Start**	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714 (M27)**	**360715 (M23)**	**F**	**n.c.[2)]**
31-38		7.4	6.7	8.1			2.3	2.4	16.5	11.6		45.0
39-44		3.0	3.5	4.9			3.8	5.2	26.8	14.6		38.1
45-51		3.6	2.9	4.1			6.8	8.9	26.5	11.7		35.5
52-58		3.7	3.2	3.1	3.2	3.0	6.9	9.4	26.5	9.5		31.4
59-65		2.6	3.8	4.4	3.5	3.7	5.7	7.2	27.5	8.6		32.9
66-72		4.0	3.7	4.3	3.5	3.3	5.1	6.1	22.8	8.8	1.4	36.9
73-79	6.9	4.3	3.0	4.4	3.3	3.5	4.9	6.2	23.1	7.3	2.6	37.5
80-86	6.0	4.2	3.6	5.7	2.3	2.6	4.4	6.2	22.5	8.8	3.4	36.2
87-93	5.5	3.5	4.0	4.2	3.9	3.0	4.8	6.6	21.0	9.4	4.0	35.6
Mean	**6.1**	**4.0**	**3.8**	**4.8**	**3.3**	**3.2**	**5.0**	**6.5**	**23.7**	**10.1**	**2.9**	**36.6**

Table 7.1.4.2-7: Metabolite pattern of leachate radioactivity in test variant III

	Metabolite fraction (Rt in min + 0.5 min) in % of total radioactivity in the leachate											
Rt	2.8	9.4	11.7	13.1	19.2	21.2	22.3	23.3	25.6	28.5	30.7	
DAT[1)]	**Start**	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714 (M27)**	**360715 (M23)**	**F**	**n.c.[2)]**
31-38		3.1	4.2	5.7			2.1	3.8	29.0	24.0		28.1
39-44		3.2	4.3	4.1			3.2	5.1	32.4	19.2		28.6
45-51		2.4	3.9	3.3			4.4	5.9	31.5	17.4		31.2
52-58		3.0	3.6	3.3	2.8	2.2	5.4	7.1	31.5	12.9		28.3
59-65		2.8	3.6	3.5	3.2	3.5	5.9	8.3	28.2	11.3	1.0	28.7
66-72		3.2	3.1	3.6	3.2	3.0	5.1	7.1	27.2	10.2	2.6	31.9
73-79		3.2	3.4	4.9	2.6	2.9	5.7	6.3	25.6	8.8	2.7	33.8
80-86		4.8	4.0	4.0	3.2	3.0	5.3	6.6	23.2	9.6	3.6	32.8
87-93		4.2	2.9	3.8	3.6	2.3	5.8	6.4	24.6	9.2	4.4	33.0
Mean		**3.3**	**3.7**	**4.0**	**3.1**	**2.8**	**4.8**	**6.3**	**28.1**	**13.6**	**2.9**	**30.7**

Table 7.1.4.2-8: Metabolite pattern of leachate radioactivity in test variant IV

	Metabolite fraction (Rt in min + 0.5 min) in % of total radioactivity in the leachate											
Rt	2.8	9.4	11.7	13.1	19.2	21.2	22.3	23.3	25.6	28.5	30.7	
DAT[1)]	**Start**	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714 (M27)**	**360715 (M23)**	**F**	**n.c.[2)]**
28-30		4.6	4.7	4.3			2.9	4.4	25.2	28.9		24.9
31-38												
39-44												
45-51												
52-58												
59-65		4.1	5.0	7.0			2.9	4.8	24.3	19.8		32.1
66-72		5.1	5.3	8.0			3.0	4.3	22.9	13.6		37.9
73-79		3.8	3.9	4.8	3.2	2.3	4.6	6.6	24.1	15.7		31.1
80-86		3.7	3.0	2.9	2.9	4.1	7.5	9.5	19.0	13.6		33.8
87-93		3.0	3.1	3.8	6.5	4.0	8.5	10.6	17.8	10.1	2.2	30.2
Mean		**4.0**	**4.2**	**5.1**	**4.2**	**3.5**	**4.9**	**6.7**	**22.2**	**17.0**	**2.2**	**31.7**

Table 7.1.4.2-9: Metabolite pattern of leachate radioactivity in test variant V

	Metabolite fraction (Rt in min + 0.5 min) in % of total radioactivity in the leachate											
Rt	2.8	9.4	11.7	13.1	19.2	21.2	22.3	23.3	25.6	28.5	30.7	
DAT[1)]	**Start**	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714**	**360715**	**F**	**n.c.[2)]**
39-44		3.7	4.0	6.9			0.0	0.0	19.2	34.9		31.3
45-51		1.3	3.1	3.8			4.0	6.1	20.6	32.0		29.1
52-58		2.2	3.1	3.7	3.7	3.3	6.3	8.5	24.3	20.5		24.5
59-65		2.1	3.4	3.2	3.4	3.1	6.1	6.7	27.3	15.3		29.5
66-72		2.6	3.3	3.9	4.0	3.6	5.1	6.3	25.9	12.7	2.3	30.3
73-79		2.5	2.3	4.0	3.6	3.3	5.6	5.9	24.3	11.7	3.7	33.1
80-86		3.1	2.4	4.5	2.7	2.5	4.3	6.0	22.7	11.8	4.7	35.5
87-93	4.9	3.6	3.2	5.0	2.3	2.8	5.4	6.6	23.6	12.6	5.8	24.2
Mean	**4.9**	**2.6**	**3.1**	**4.4**	**3.3**	**3.1**	**4.6**	**5.8**	**23.5**	**18.9**	**4.1**	**29.7**

It was concluded from the pre-test that the pattern of metabolites in the leachate is largely independent of the different conditions in the individual variants.
For the definitive test, conditions similar to test variant I were chosen.

2. Definitive test

Water Balance
The soil columns were irrigated with a total amount of 18.9 L corresponding to 541 mm. In average 95.3% of the irrigation volume was sampled as leachate. The summarised results of the water balance for both soil columns are given in Table 7.1.4.2-10: .

Table 7.1.4.2-10: Water balance of the individual soil columns

Mini-lysimeter	Total irrigation [mL/mm]	Total leachate [mL/mm]	Total leachate [% of irrigation]
Borstel-A	18,933/541	18,103/517	95.6
Borstel-B	18,920/541	17,983/514	95.0
Mean	18,927/541	18,043/516	95.3

Radioactivity in the Leachate
39.2 % of the applied radioactivity was found in the leachate. Summarised results for each mini-lysimeter are given in Table 7.1.4.2-11:.

Table 7.1.4.2-11: Radioactivity in leachate of the individual mini-lysimeter

Mini-lysimeter	Radioactivity applied	Radioactivity in leachate	
	[kBq]	[kBq]	[% applied]
Borstel-A	37521	14696	39.2
Borstel-B	33769	13237	39.2
Mean	35645	13967	39.2

About 18 days after application the radioactivity increased by reaching a maximum about 44 days after treatment. In the following the radioactivity in leachate decreased to a level < 20 µg/L a.s. equivalents indicating that most of the mobile radioactivity had leached through the soil cores. Both replicates showed a similar breakthrough behavior.

The metabolite pattern in the leachate as determined by radio HPLC was very similar to that observed in the pre-test.
No test item was detectable in the leachate. The main known metabolite in the leachate was M27 (in average 24.5 % of total leachate radioactivity) followed by the metabolite M23 (in average 15.6 % of total leachate radioactivity).

In total up to 9 unknown metabolites with distinct peaks were detectable. Most of the unknown metabolites represented <5% of the total leachate radioactivity. Only metabolite “E” was detected in amounts slightly greater than 5% of the total leachate radioactivity. Most of the radioactivity (about 32%) represented unknown radioactivity showing an increased baseline without a specific retention time. With exception of metabolite M23 (decreasing proportions in the course of the study) the leachate metabolite pattern did not change significantly in the course of the study.
Detailed results of soil column "Borstel A" are shown exemplarily in Table 7.1.4.2-12: .
The results for soil column "Borstel B" were similar.

Table 7.1.4.2-12: Metabolite pattern of leachate radioactivity mini-lysimeter Borstel-A

	Metabolite fractions (Rt in min + 0.5 min) in % of total radioactivity in the leachate											
Rt[min]	11.2	13.5	16.3	23.5	25.4	26.2	27.5	29.7	32.4	35.8	41.0	
DAT[1)]	**A**	**B**	**C**	**C1**	**C2**	**D**	**E**	**360714 (M27)**	**360715 (M23)**	**F**	**G**	**n.c.[2)]**
31-37	1.91	3.86	4.62	1.88	0.94	3.77	5.67	26.50	29.53			21.32
38-44	1.77	2.31	3.08	3.00	2.27	5.15	5.73	25.74	27.90			23.05
45-51	1.99	2.64	2.90	3.47	2.49	5.41	5.47	26.34	22.11	0.82	8.26	18.10
52-58	2.17	2.16	2.44	2.55	2.47	5.10	7.41	25.19	18.70	1.34	0.55	29.92
59-65	1.45	2.93	3.40	4.40	2.77	4.91	6.10	25.75	18.18	2.27	0.54	27.30
66-72	3.60	2.55	4.36	3.59	1.55	3.27	4.43	29.27	15.01	3.98	0.77	27.62
73-79	2.20	2.08	2.51	2.83	2.40	5.50	5.36	27.53	14.18	2.96		32.45
80-86	1.34	2.41	3.03	5.21	2.13	5.36	5.31	24.02	14.25	3.31		33.63
87-93	2.03	2.11	2.94	2.62	2.64	4.45	5.53	26.20	13.70	4.09		33.69
94-100	2.40	2.34	4.38	4.58	2.54	4.03	5.79	22.73	10.93	5.07		35.21
101-107	2.77	2.47	4.13	4.06	2.55	5.18	3.97	22.46	11.82	4.16		36.43
108-114	3.32	2.91	4.43	4.14	1.54	4.14	5.21	24.78	12.24	3.94		33.35
115-121	3.19	3.42	4.69	3.01	2.55	5.44	5.85	21.70	13.67	3.44		33.04
122-128	2.95	3.63	4.91	4.12	1.84	4.85	4.94	22.16	13.14	3.51		33.95
129-135	3.45	2.24	4.12	3.53	2.44	4.91	4.80	20.24	13.59	3.56	2.17	34.95
136-142	2.69	3.00	3.59	3.68	2.70	4.70	4.44	21.84	12.44	3.20		37.72
Mean	**2.45**	**2.69**	**3.72**	**3.54**	**2.24**	**4.76**	**5.38**	**24.53**	**16.34**	**3.26**	**2.46**	**30.73**

1) Days after treatment
2) n.c.= not characterized by a retention time

III. CONCLUSION

In this mini-lysimeter study with ^{14}C-Dimethenamid-P a characteristic pattern of metabolites was observed in the leachate consisting of the known metabolites M23 and M27 and further up to 9 unknown metabolites. Additionally, unknown radioactivity showing an increased baseline without a specific retention time was observed. This metabolite pattern was largely independent of a variety of different conditions that were tested in a pre-test.

Report: CA 7.1.4.2/2
Staudenmaier H., 2009a
Structure elucidation of metabolites of Dimethenamid in lysimeter leachate
2009/1011362
Guidelines: OECD 307, BBA IV 4-1, EPA 162-1
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 7.1.4.2/3
Staudenmaier H., 2014b
Amendment No. 1 -Structure elucidation of metabolites of Dimethenamid in lysimeter leachate
2014/1031599
Guidelines: OECD 307, BBA IV 4-1, EPA 162-1
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

In the report amendment to the above report, two metabolite structures were revised because of new insights gained during a follow-up study [see 2013/1246087 Staudenmaier H.,Kuhnke G. 2014 a]. Additionally the new metabolite codes were introduced. Furthermore, one chapter of the original study report was revised because of new information on the identity of metabolite isomers. All changes introduced with the report amendment are included in the following summary.

Executive Summary

In the leachate of a lysimeter study with dimethenamid (BASF DocID 1996/10707), numerous fractions of unidentified radioactivity were observed. Since no lysimeter leachate was available any more, the material required for structure elucidation had to be newly generated. This was attempted by both, a soil study that used incubations of the a.s. with soil under different conditions and a mini-lysimeter study.
The present soil study was set up as a soil metabolism study with the purpose to generate information about the formation of degradation products under aerobic, anaerobic and photolytic conditions and to elucidate their structures as far as possible. During the conduct of the study, it turned out that the underlying task - the structure elucidation of metabolites in lysimeter leachate - may be supported by the analysis of the radioactive material detected in the leachate of the mini-lysimeter study. Therefore, it was decided to additionally use samples of leachate water of the latter study for structure elucidation.
Dimethenamid-P, the S-enantiomer of dimethenamid was used as test item.

Soil incubations

Soil from Borstel, Lower Saxony (the same soil that had been used in the lysimeter study) was treated with a mixture of thienyl-5-^{14}C-labeled and non-radiolabeled Dimethenamid-P.

Aerobic soil incubation:

Soil was treated at a nominal rate of 2.7 mg per kg of dry soil which corresponds to a field application rate of 1 kg test item per hectare. Soil aliquots of 100 g dry soil equivalents were weighed into test vessels and were incubated at 20 ^{o}C in the dark. Samples were taken at 0, 2, 7, 14, 28, 58, 89 and 119 days after treatment. At each sampling time, two vessels were taken for sampling.

Additional soil was treated at an exaggerated rate of 8 mg Dimethenamid-P per kg of dry soil corresponding to a field application rate of 3 kg a.s. per hectare. Aliquots of 500 g were incubated under aerobic conditions as described above and sampled 58, 89 and 119 days after treatment.

Anaerobic soil incubation:

Soil aliquots of 100 g dry soil equivalents, treated at the exaggerated rate were filled into test vessels and were incubated as described above. After 30 days, the soil in the test vessels was flooded with water in order to establish anaerobic conditions. From day 48 after treatment on, the test vessels were additionally purged with nitrogen. At day 119, one test vessels was sampled.

Soil photolysis:

Untreated soil aliquots of about 45 g each were filled into metal dishes of a soil photolysis apparatus. The soil was treated by pipetting a solution of the test item to the soil surface at a nominal rate corresponding to 3 kg Dimethenamid-P per hectare. The photolysis was performed with an intensity of radiation of 3 mW/cm^2 in a Suntest apparatus. After 15 days one test vessel was sampled and worked up and at day 16 the soil of the remaining test vessels was pooled and worked up.

The soil samples were extracted 3 times with MeOH for about 20 min each by shaking on a laboratory shaker and the phases were separated by centrifugation. The supernatant was decanted, filtered and made up to volume. The extraction procedure was repeated three times with MeOH/H_2O (8:2). For some of the samples a further extraction step with MeOH/H_2O (2:8) was added for the extraction of possible polar metabolites. Aliquots of the pooled extracts were subjected to radio HPLC analysis. Various samples of the soil incubations were investigated by LC-MS/MS analysis, either directly or upon fractionation by HPLC.

Mini-lysimeter

Leachate water from a laboratory mini-lysimeter study with Dimethenamid-P in Borstel soil conducted under a separate GLP study protocol [see 2008/1051489 Fent G. 2008 a] became available and was known to contain substantial amounts of metabolites. Selected leachate water samples were further analyzed in the present study by radio HPLC analysis. Suitable leachate samples were worked up by solid phase extraction and were fractioned by HPLC and the fractions were subjected to LC-MS/MS analysis.

Substantial degradation of the test item was observed in the aerobic incubation. Significant amounts of the known metabolites M656PH023, M656PH027 and M656PH031 were formed, but all others remained rather low.
Under anaerobic conditions, a similar pattern of degradates as under aerobic conditions was observed. However, some additional or more prominent metabolite peaks were detected under anaerobic conditions compared to aerobic conditions.
Under the influence of light, the pattern of degradates was quite dissimilar to that obtained in the incubations in darkness. At least 12 degradation products were formed, however all in rather low amounts.

A complex pattern of metabolites was observed in the mini-lysimeter leachate.

Comparison of the HPLC chromatograms of the aerobic and anaerobic soil incubations revealed good matches with peaks in the chromatograms of the mini-lysimeter leachate. In contrast many of the peaks of the soil photolysis experiment did not match exactly with peaks of the mini-lysimeter leachate.

In the course of the LC-MS/MS investigations, the identity of the main peaks in the aerobic soil incubation was confirmed to be M656PH023, M656PH027 and M656PH31. Structure elucidation of further metabolites was mainly based on compounds isolated from the mini-lysimeter leachate and in some cases of peaks observed in the anaerobic soil incubation. Finally, structures could be proposed for numerous metabolite peaks. Many of these structures had previously not been observed in other studies with Dimethenamid or Dimethenamid-P.

Additional HPLC analyses were performed with isolated fractions in order to allow for the assignment of identified structures to the peaks in the mini-lysimeter leachate.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No.: 363851
BAS Code: BAS 656 H
Chemical name (IUPAC): (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Common name: Dimethenamid-P

Radiolabeled test item
Batch No.: 824-3102
Position of radiolabel: thienyl-5-^{14}C
Specific radioactivity: 6.53 MBq/mg (~391800 dpm/µg)
Radiochemical purity 99.4 %

<u>Non-radiolabeled test item</u>
Batch No.: L74_120
Chemical purity: 96.5 %

For the test, a mixture of radiolabeled and non-radiolabeled test item was used.

2. Soil

Soil from Borstel, Lower Saxony, Germany was used for this study. This is the same soil that was used earlier for the lysimeter study with dimethenamid.
The soil was taken from the field, passed through a 2 mm sieve and then characterized (see Table 7.1.4.2-13:).

Table 7.1.4.2-13: Soil Characteristics

Origin	**Borstel**
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 90.0 7.3 2.7 sand
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 91.1 6.3 2.7 sand
Organic C [%] Organic matter [%] **	0.75 1.29
pH [H_2O] pH [$CaCl_2$]	6.5 5.9
cation exchange capacity [cmol / kg]	2.7
water holding capacity pF0 [g/100g dry weight]	23.0
microbial biomass * (end of study) [mg C/100g dry soil]	14.5

* determined at the test site according to Anderson & Domsch using a BSB digi
** organic matter = organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

Aerobic soil metabolism (low application rate)
The soil was treated at a nominal rate of 2.7 mg Dimethenamid-P per kg dry soil, which corresponds to a field application rate of 1 kg test item per hectare, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm^3. Prior to the application, the soil was adjusted to 50 % of the maximum water holding capacity (MWHC), homogenized and pre-incubated at 20 °C for eight days. Soil portions of 100 g dry soil equivalents were filled into test vessels. The test vessels were incubated in the dark at 20 ± 2 °C in an incubator with an open gas flow system.

Sampling intervals were 0, 2, 7, 14, 28, 58, 89 and 119 days after treatment. At each sampling time two vessels were removed from the incubator and were either worked up directly or stored in the freezer.
Volatiles were trapped in a trapping system of gas washing flasks containing ethylene glycole and 0.5 M NaOH.

Aerobic soil incubation (high application rate)
The soil was treated at a nominal rate of 8.0 mg Dimethenamid-P per kg dry soil, which corresponds to a field application rate of 3 kg test item per hectare. The soil that was treated as described here was used for the aerobic incubation experiment with high application rate as well as for the incubation under anaerobic conditions (see below).

3 soil portions of 500 g dry soil equivalents were filled into test vessels. The test vessels were incubated in the dark at 20 ± 2 °C in an incubator. with an open gas flow system. Sampling intervals were 58, 89 and 119 days after treatment. At each sampling time an aliquot of 50 g soil was removed from a test vessel and was worked up directly.

Anaerobic soil incubation
5 soil portions of 100 g dry soil equivalents were filled into the test vessels. The test vessels were incubated in the dark at 20 ± 2 °C in an incubator with an open gas flow system. After 30 days of incubation the soil in the test vessels was flooded with water and the incubation was continued. Because the redox potential decreased only very slowly, the test vessels were purged with nitrogen gas from day 48 after treatment on. At day 119 one test vessel was sampled and worked up to check the formation of potential metabolites.
Volatiles were trapped in a trapping system of gas washing flasks containing ethylene glycole and 0.5 M NaOH.

Soil photolysis
Untreated soil was filled into 11 small metal dishes of the soil photolysis apparatus (about 45 g soil, approximately 12 mm soil height). With a pipette 0.1 mL of the treatment solution (9.06 mg Dimethenamid-P / mL) were distributed equally on the soil surface in very small droplets. This corresponds to a field application rate of 3 kg test item per hectare. The photolysis was started with an intensity of radiation of 3 mW/cm^2 (λ> 290 nm). After 15 days one test vessel was sampled and worked up. One day later (day 16) the soil of the 9 remaining test vessels was pooled and extracted to get a larger amount of metabolites.

2. Analytical procedures

Workup of soil samples

The soil samples were extracted once with MeOH for about 20 min each by shaking on a laboratory shaker and the phases were separated by 10 min centrifugation. The supernatant was decanted, filtered, made up to volume and aliquots were measured by LSC. The extraction procedure was repeated three times with MeOH/H_2O (8:2). For selected samples, a further extraction step with MeOH/H_2O (2:8) was added for the extraction of possible polar metabolites.
The MeOH extract and the corresponding MeOH/H_2O extracts were pooled and concentrated using a rotary evaporator. The dry precipitates were redissolved and subjected to radio HPLC analysis.

Mini-lysimeter leachate

For the purpose of comparison of HPLC patterns and structure elucidation of metabolites, leachate water from a mini-lysimeter study performed at RLP Agroscience was used [see 2008/1051489 Fent G. 2008 a, 7.1/24). The purpose of the mini-lysimeter study was the regeneration of lysimeter leachate metabolites of dimethenamid(-P) using the same soil (Borstel) as in the present study.
A total of 32 leachate samples of the replicate mini-lysimeters "Borstel-A" and "Borstel-B" were received in frozen state. Additionally, samples of a non-GLP pre-test to the mini-lysimeter study were obtained.
Selected samples of the mini-lysimeter leachate were analyzed by radio-HPLC directly or after concentration.

Structure elucidation

Selected extracts of the aerobic soil metabolism, the anaerobic incubation and the soil photolysis were used for structure elucidation either directly or after fractionation.
Additionally, selected mini-lysimeter leachate water samples with a high concentration of radioactivity were worked up:
(1) 1st work-up: Water sample "Borstel A, fraction 31-37":
The leachate water was purified by NH_2-SPE (solid phase extraction) with SPE cartridges. The radioactivity in the percolate was concentrated by SPE using strata-X-CW cartridges. The adsorbed radioactivity was eluted with MeOH and MeOH / 5% NH_4OH. The MeOH and MeOH / NH_4OH eluates were combined, reduced to dryness and redissolved. The sample fractioned by semi-preparative HPLC. Selected fractions were analyzed by LC-MS/MS
(2) 2nd work-up: Water samples "Borstel A, fraction 66-72" and "Borstel B, fraction 52-58":
The two samples were concentrated and were separately worked up in a similar way as described above:
The NH_2-SPE columns were loaded with leachate and the purified percolates were recovered for further work-up. The columns were eluted with 20 ml each of MeOH and MeOH / 2.5% HCl. The MeOH eluates were reduced to dryness, redissolved and fractioned by semi-preparative HPLC. Selected fractions of both water samples were combined and analyzed by LC-MS/MS.

The radioactivity in the NH_2-SPE percolate was concentrated by SPE using strata-X-CW cartridges. The column was loaded with the purified percolate. The percolate was collected for further work-up. The strata-X-CW percolates of both water samples were combined. The concentrated sample was fractioned by semi-preparative HPLC. A selected fraction (11.5 - 12.5 min) was reduced to dryness and redissolved. The identity of the isolated main peak in this fraction was checked by HPLC and finally analyzed by LC-MS/MS.

II. RESULTS AND DISCUSSION

A: MASS BALANCE

The main focus of this study was the generation of suitable amounts of metabolites for structure elucidation rather than quantitative aspects like a complete mass balance. Nevertheless a mass balance of ≥97% was achieved in the aerobic degradation (low rate) and in the anaerobic degradation experiments. The setup of the aerobic degradation at the high rate and the photolysis experiment was not suitable for exact quantification of volatiles and no complete mass balance was established.

B. EXTRACTABLE AND BOUND RESIDUES

The percentage of extractable radioactivity decreased from about 100% at day 0 to about 38 - 46% of applied after 119 days in the aerobic incubations. In the anaerobic degradation, it decreased less resulting in 69% extractable after 119 days. In contrast, in the photolysis experiments low extractability of only 23 - 24 % of applied was observed already after 15/16 days.

Bound residues were formed in the aerobic experiments up to 42% of applied whereas a slightly lower percentage of 27% was observed in the anaerobic incubation after 119 days. Similar values (25 - 29%) were also observed in the photolysis experiment - in the latter however already after 15/16 days.

C. VOLATILIZATION

Meaningful values were established only for the aerobic degradation at the low rate, where mineralisation of about 18% of applied was determined after 119 days. Besides $^{14}CO_2$, no other volatile compounds were detected.

D. TRANSFORMATION OF PARENT COMPOUND

<u>*Aerobic soil metabolism (low application rate)*</u>
The results of the aerobic incubation at the low application rate are shown in Table 7.1.4.2-14: . The active ingredient BAS 656 H decreased from 100 %TAR at day 0 to 8 %TAR at day 119. Three metabolites exceeded 5 %TAR at later sampling times: M656PH027 with 5.4 %TAR at day 119, M656PH031 with the maximum of 5.0 %TAR after 89 days and M656PH023 with the maximum of 12.2 %TAR after 58 days. Six other metabolites reached amounts between 1.1 and 3.1 %TAR. Further degradation products in low amounts (<1 % TAR each) were additionally detected.

Table 7.1.4.2-14: Radio-HPLC analysis of the soil extracts of the incubation with 14C-BAS 656 H under aerobic conditions

DAT	% TAR											
		uk	uk	**M27**	uk	**M31**	**M23**	uk	uk	uk	**BAS 656 H**	others [b]
	t_R [a]	23.5	24.1	25.6	27.9	29.0	29.6	30.8	31.7	34.9	37.7	
	^{14}C total											
0	101.2	0.7									100.5	
	100.0	0.4									99.6	
0 mean	100.6	0.5									100.0	
2	94.0		0.3		0.3		0.4			0.2	91.9	0.9
	94.5	0.2	0.3	0.3	0.3	0.1	0.5		0.2	0.1	91.3	0.9
2 mean	94.3	0.1	0.3	0.2	0.3	0.1	0.5		0.1	0.2	91.6	0.9
7	88.3		0.3	0.8		1.0	1.2	0.4	0.4	0.4	81.8	2.0
	89.3		0.5	0.9	1.1	0.9	1.3	0.5	0.4	0.3	82.1	1.3
7 mean	88.8		0.4	0.8	0.5	0.9	1.2	0.5	0.4	0.4	81.9	1.7
14	80.9		0.5	1.4	1.9	2.0	2.8	1.0	0.4		69.1	1.8
	79.7		0.5	1.4	1.8	2.5	2.0	1.0	0.6	0.7	68.0	1.3
14 mean	80.3		0.5	1.4	1.8	2.3	2.4	1.0	0.5	0.3	68.6	1.5
28	70.9	0.5	0.9	2.7	3.1	4.3	2.9	1.6	0.8	0.8	51.4	1.9
	69.7		0.7	2.6	3.1	3.2	4.9	0.6	1.5		51.4	1.7
28 mean	70.3	0.3	0.8	2.6	3.1	3.7	3.9	1.1	1.2	0.4	51.4	1.8
58	54.3	0.7	0.6	4.4		4.3	12.2		0.7	1.0	27.6	2.8
	54.3	0.8	0.7	4.7		4.8	12.2		0.9	1.1	26.8	2.3
58 mean	54.3	0.8	0.7	4.6		4.6	12.2		0.8	1.0	27.2	2.5
89	45.4	1.6		5.4		5.0	12.2		1.3	1.0	15.7	3.2
	44.8	0.8	1.2	5.2		5.1	12.0		1.1	1.0	15.3	3.1
89 mean	45.1	1.2	0.6	5.3		5.0	12.1		1.2	1.0	15.5	3.2
119	37.0	1.1	1.0	5.4		4.3	10.4		0.6	1.0	7.9	5.4
	39.0	0.9	1.2	5.4		4.4	11.6		0.4	1.1	8.1	5.8
119 mean	38.0	1.0	1.1	5.4		4.3	11.0		0.5	1.1	8,0	5.6

[a] t_R: approximate retention time in min
[b] each ≤ 1% TAR

Aerobic soil incubation (high application rate)
The soil extract from the incubation under aerobic conditions with the high application rate showed the same degradation products as that with the low application rate.

Anaerobic soil incubation
Under anaerobic conditions, a similar pattern of degradates as under aerobic conditions was observed. However, some additional or at least more prominent metabolite peaks were observed under anaerobic conditions compared to aerobic conditions.

Photolysis on soil
Under the influence of light, the pattern of degradates was quite dissimilar to that obtained in the incubations in darkness. At least 12 degradation products were formed, however all in rather low amounts of no more than 2.3 %TAR each.

Mini-lysimeter leachate
The HPLC pattern as determined in this study was in good agreement to that reported in the mini-lysimeter study. Furthermore, the retention times of selected reference compounds (e.g. M656PH023, M66PH027) were comparable to those in the lysimeter study.

Comparison of radio-HPLC patterns
Soil extracts were reanalyzed on the HPLC system used in the mini-lysimeter study in order to exactly compare their HPLC patterns with those of the mini-lysimeter leachate.
The major peaks in the sample of the aerobic incubation, metabolites M656PH023, M656PH027 and M656PH031 corresponded well with prominent peaks in the mini-lysimeter leachate.
The sample of the anaerobic incubation showed also some major peaks that corresponded to major peaks in the mini-lysimeter leachate. These were presumably M656PH023 and M656PH027, but not M656PH031. Additional peaks were observed that either matched minor peaks in the mini-lysimeter leachate or were observed at retention times corresponding to unknown fractions of the lysimeter leachate (BASF DocID 1996/10707).
The complex pattern of HPLC peaks of the extract of the photolytic soil incubation showed only few matches to the peaks in the mini-lysimeter leachate. It was concluded that the metabolites that are formed under the conditions of photolysis on soil are mostly different from those observed in the mini-lysimeter leachate.

Samples for structure elucidation

Structure elucidation of metabolites was attempted by using samples or isolated peaks from both, the extracts of the different soil incubations and the mini-lysimeter leachate:

(1) Aerobic soil incubation

The identity of the prominent soil metabolites M656PH023, M656PH027 and M656PH031 was confirmed by LC-MS/MS investigation. This is in line with the previous knowledge and is not described in further detail here. Metabolites M656PH023, M656PH027 and M656PH031were also positively identified in the mini-lysimeter leachate by LC-MS/MS.

(2) Anaerobic soil incubation

The anaerobic incubation led to some additional peaks that matched peaks in the mini-lysimeter leachate or were observed at retention times corresponding to unknown fractions of the lysimeter leachate. HPLC fractions of the anaerobic incubation were used for structure elucidation of few peaks/fractions that were not represented by samples of the mini-lysimeter leachate.

(3) Photolytic soil incubation

The photolytic incubation led to a very complex pattern of HPLC peaks. Major efforts were undertaken to isolate individual peaks and to investigate these peaks by LC-MS/MS. However, it turned out later on that many of these peaks did not match exactly with the peaks in the mini-lysimeter leachate. Since there were doubts if the photolytical metabolites are identical to those in the leachate, it was decided not to rely on these for the assignment of structures to metabolites in the leachate.

(4) Mini-lysimeter leachate

The leachate fractions of the mini-lysimeter contained substantial amounts of radioactivity and were therefore considered to be a suitable direct source for the investigation of leachate metabolites. Selected leachate samples were concentrated and split into suitable fractions by semi-preparative HPLC.

E. IDENTIFICATION OF METABOLITES

General Remarks

Metabolites of Dimethenamid-P were identified by electrospray time-of-flight mass spectrometry (ESI-TOF MS) in positive- and negative-ion mode.
Several metabolites appeared in isomeric forms for which no clear assignment to the type of isomerism can be given based on the spectral data. Depending on the overall structure of a metabolite, the following isomeric variations – listed in order of falling chemical similarity - are possible:

a) regioisomerism, in molecules where a methyl group on the thiophene ring has been oxidized (e.g., metabolite M656PH047)
b) Z/E isomerism, in molecules where the thiophene ring has been oxidatively opened (e.g., M656PH052)
c) centrochiral diastereoisomerism with two or more chiral centers being present (e.g., M656PH059)
d) axial-chiral diastereoisomerism, possible in all metabolites with an intact thiophene ring and two further substituents on the nitrogen atom (e.g. M656PH027)
e) rotamerism about the amide N-CO bond, possible in all metabolites with carbonyl substituent on the nitrogen.

In cases d) and e), the isomeric forms are able to isomerize over a more or less high rotational barrier. Depending on the moieties in the vicinity, the barrier may easily be high enough to produce chromatographically separable entities. With these possibilities in mind, the forms with maximum chemical difference were assigned to double peaks in order to cover the whole chemical variety of possible isomers by the interpretation. If two or more indistinguishable isomers of a metabolite exist, they were assigned different metabolite codes – usually in line with increasing retention time.

Overview on metabolite structure identification

An overview on the results of the structure identification is presented in Table 7.1.4.2-15: . Numerous chemical structures were proposed upon LC-MS/MS investigation covering most of the peaks in the HPLC chromatograms of the mini-lysimeter leachate and additionally some peaks in the extracts of the anaerobic soil incubation. Many of these metabolite structures (M656PH043 - M656PH062) had not been observed previously in other studies.

For the following metabolites, unique structures could be proposed and assigned to specific peaks:

M656PH003	M656PH045,
M656PH010	M656PH047
M656PH023	M656PH050 (rota)
M656PH027	M656PH051
M656PH031	M656PH054
M656PH032	M656H055
M656PH043	M656PH062

There were several pairs and one triplet of isomers of metabolites which are distinguishable by their retention time. Most of the metabolite pairs were shown to be rotamers which are in equilibrium:

M656PH027	two rotamers detected
M656PH043	two rotamers detected
M656PH045	two rotamers detected
M656PH047	two rotamers detected
M656PH050 (rota)	one rotamer detected
M656PH054	two rotamers detected

However, there are other metabolites which are marked with the suffix "(iso)" e.g. M656PH053 (iso) that have further stereochemical features (additional to rotamerism or without it) that give rise to multiple peaks for which no formation of an equilibrium was observed. Since it could not finally be decided from the MS results which of these isomeric forms is actually present in the respective HPLC peak, in these cases the same structures were given for each of the isomer peaks. This applies for the following isomeric pairs/triplet of metabolites:

M656PH049 (iso)	one isomer detected
M656PH052 (iso)	two isomers detected
M656PH053 (iso)	two isomers detected
M656PH059 (iso)	three isomers detected

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
1	mini-lysimeter leachate	8.5	10.3	or isomer	337	M656PH052 (iso)	---
2	mini-lysimeter leachate	10.2 9.0	12.5 10.9	or isomer	337	M656PH052 (iso)	**13.2**/14.5
3	mini-lysimeter leachate	14.0	16.4		249	M656H055	**15.6**/16.7
4	mini-lysimeter leachate	14.4	16.4	or isomer	287	M656PH049	16.7

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
5	mini-lysimeter leachate	19.0	20.4		301	M656PH045 (one of two rotamers)	---
6	mini-lysimeter leachate	19.6	21.6	or possibly	335	M656PH059 (one of three isomers)	**21.3**/22.7/ 23.6
7	mini-lysimeter leachate	19.6	21.6		351	M656PH047 (one of two rotamers)	**21.3**/22.7/ 23.6

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
8	mini-lysimeter leachate	20.8	22.8		351	M656PH047 (one of two rotamers)	21.3/**22.7/ 23.6/24.5**
9	mini-lysimeter leachate	21.4	23.3	[or poss bly]	335	M656PH059 (one of three isomers)	21.3/**22.6/ 24.5**
10	mini-lysimeter leachate	21.4	23.3		301	M656PH045 (one of two rotamers)	21.3/**22.6/ 24.5**

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
11	mini-lysimeter leachate	22.2	23.9	H, S, O, N, O, S, O, OH, HO, O	301	M656PH045 (one of two rotamers)	---
12	mini-lysimeter leachate	23 - 28	25.7	H, S, O, N, O, S, O, OH, HO, O	301	M656PH045 (one of two rotamers)	**24.5**/25.3/ 26.5/27.2
13	mini-lysimeter leachate	23.3	25.7	H, S, O, N, O, O, S, SO_3H [or poss bly H, S, O, N, O, O, S, SO_3H]	335	M656PH059 (one of three isomers)	**24.5**/25.3/ 26.5/27.2

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
14	mini-lysimeter leachate	24.2	25.7	or isomer	351	M656PH053 (one of two isomers)	**24.5**/25.3/ 26.5/27.2
15	mini-lysimeter leachate	24.3	25.7		321	M656PH054 (one of two rotamers)	**24.5**/25.3/ 26.5/27.2
16	mini-lysimeter leachate	24.9	26.9	or isomer	351	M656PH053 (one of two isomers)	25.3/26.5/ **27.2**

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
17	mini-lysimeter leachate	25.1	26.9	H, O, OH, S, N, OH, S, O, O, O, S	321	M656PH054 (one of two rotamers)	25.3/26.5/ **27.2**
18	mini-lysimeter leachate	25.6	26.9	H, S, O, NH, S, OH, O	229	M656PH062	25.3/26.5/ **27.2**
19	anaerobic soil incubation	26.1	29.1	H, S, O, N, S, O, OH, O, HO	287	M656PH043 (one of two rotamers)	28.3

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
20	anaerobic soil incubation	27.4	30.2		287	M656PH043 (one of two rotamers)	28.9
	mini-lysimeter leachate	27.7	30.1				
21	mini-lysimeter leachate	29.8	30.9		321	M656PH027 (two rotamers)	30.3
22		29.6	30.9				
23	anaerobic soil incubation	31.5	34.5		257	M656PH050 (rota)	31.8

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
24	anaerobic soil incubation	34.4	37.3		347	M656PH031	31.1
	mini-lysimeter leachate	34.7	37.0				
25	mini-lysimeter leachate	35.2	37.9		271	M656PH023	32.5
	anaerobic soil incubation	35.2	38.4				
	mini-lysimeter leachate	35.6	38.4				
26	mini-lysimeter leachate	37.3	40.3		363	M656PH051	34.3

Table 7.1.4.2-15: Overview on identified metabolites and their assignment to peaks in different HPLC systems (continued)

No.	Source	t_R in LC/MS [min]	t_R in Acn/H_2O HPLC system [min]	Structure proposal	Molecular mass	Metabolite Code	t_R in phosphate buffer HPLC system [min]*
27	mini-lysimeter leachate	38.1	41.0		319	M656PH010	34.3
	anaerobic soil incubation	38.0	41.2				
28	anaerobic soil incubation	38.0	41.2		331	M656PH032	39.0
29	anaerobic soil incubation	38.9	42.0		241	M656PH003	39.4

Assignment of identified metabolites to HPLC peaks
Due to the technical requirements of LC/MS, the isolation of HPLC fractions and LC-MS/MS investigation was done using a phosphate buffer free HPLC system.
Further HPLC analyses were performed in order to clearly assign the identified compounds to HPLC peaks in the original buffer-containing HPLC system used in the lysimeter and mini-lysimeter studies. Furthermore, some of the HPLC fractions were further fractioned by HPLC into subfractions containing single peaks in order to assist the assignment of individual peaks.
It turned out that reanalysis of the isolated single peaks with the phosphate buffer HPLC system resulted frequently in the separation into several peaks. This is in line with the observation during the LC-MS/MS investigation that often more than one compound is observed within one HPLC peaks. That means that the number of metabolites is even greater than expected from visual inspection of the HPLC chromatograms. Nevertheless, it was possible in most cases to assign the structure of the main peaks of the fractions to corresponding HPLC peaks in the total mini-lysimeter leachate. The results of this HPLC exercise are also shown in Table 7.1.4.2-15: (last column).

III. CONCLUSION

Structure elucidation of metabolites of dimethenamid was performed using extracts of soil treated with Dimethenamid-P and incubated under different conditions. Furthermore, leachate of a mini-lysimeter study with Dimethenamid-P served as a source of metabolites for structure elucidation. Numerous metabolite structures were proposed upon LC-MS/MS investigation. The majority of these structures had not been observed so far in other studies.

Report: CA 7.1.4.2/4
Staudenmaier H.,Kuhnke G., 2014a
Further investigations on structural identity of metabolites of Dimethenamid-P in lysimeter leachate
2013/1246087
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The aim of this study was to investigate mini-lysimeter leachate samples and leachate extracts originating from preceding studies in order to generate further information on the structure of metabolites of Dimethenamid-P.
In study BASF DocID 2008/1051489 [see 2008/1051489 Fent G. 2008 a] a mixture of radiolabeled and non-radiolabeled Dimethenamid-P was applied to two 300 mm long mini-lysimeter columns filled with undisturbed soil from Borstel (Lower Saxony, Germany). Leachate originating from this mini-lysimeter trial and extracts thereof generated in study BASF DocID 2009/1011362 [see 2009/1011362 Staudenmaier H. 2009 a] were further processed and investigated within the current study.

Two work-ups of mini-lysimeter leachate were performed to generate samples for MS analysis, one for the eluate and another for the percolate of a solid phase extraction (SPE) step.
The work-up of the SPE eluate (work-up I) yielded a fraction that contained a number of peaks of medium retention time.
The work-up of the concentrated percolate (work-up II) yielded numerous polar compounds of short retention time as well as a number of compounds of medium retention time with part of them being also present in the eluate fraction.
Compounds in the concentrated percolate were strongly enriched. Consequently the concentration of individual polar compounds that are found only in the percolate fraction is very low when related to the original total leachate. Due to their continuous sequence in HPLC chromatograms they give rise to the appearance of an elevated baseline in addition to small peaks.
Further work-up of mini-lysimeter leachate was performed for NMR analyses. However, the NMR analyses did not significantly contribute to the overall structure assignments and are presented further here.
Finally 13 subfractions were obtained from of the first work-up and 15 fractions were generated in the second work-up of which 6 and 13 fractions, respectively were selected for LC/MS-MS analysis.

The work-ups aimed at the generation of samples containing single HPLC peaks for LC/MS-MS analysis. Unexpectedly, most of the isolated fractions contained more than one peak upon re-analysis on HPLC. Further investigation revealed that there were several pairs of peaks that were in equilibrium to each other. In such cases none of the two peaks could be isolated without formation of the second peak. Upon mass spectrometric investigation, the same structure was assigned to each peak of those pairs. The two peaks are considered to be rotamers, i.e. structures with hindered intramolecular rotation around a single bond that are separated into two peaks in HPLC.

Structure elucidation by mass spectrometry resulted in structure proposals for several metabolite peaks:
In the SPE eluates: M656PH027 (two rotamers), M656PH045 (two rotamers) and an isomer thereof (two rotamers), M656PH047, M656PH054 and M656PH059 (iso) [three isomers].
In the SPE percolates partly the same structures were identified: M656PH045 (two rotamers) and an isomer thereof (two rotamers), M656PH047 (two rotamers), M656PH054 (two rotamers) and M656PH059 (iso) [two isomers].
The following metabolites were found in the SPE percolate only: M656PH049 (iso), M656H055, M656PH109 (rota), M656PH110 (rota) [two rotamers] and an isomer thereof. Additionally, numerous further compounds were observed in mass spectrometry for which only the molecular mass could be determined.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No.: 363851
BAS Code: BAS 656 H
Chemical name (IUPAC): (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Common name: Dimethenamid-P

Radiolabeled test item
Batch No.: 824-3102
Position of radiolabel: thienyl-5-^{14}C
Specific radioactivity: 6.53 MBq/mg (~391800 dpm/µg)
Radiochemical purity 99.4 %

Non-radiolabeled test item
BAS code: BAS 656 H
Common name: Dimethenamid-P
Batch-No.: BEAU201204
Chemical structure:

B. STUDY DESIGN

Test samples

In this study no application was performed. Analyses were performed with leachates and leachate extracts from preceding studies: The original leachates were generated in a mini-lysimeter study (BASF DocID 2008/1051489, [see 2008/1051489 Fent G. 2008 a]and then further investigated in study BASF DocID 2009/1011362,[see 2009/1011362 Staudenmaier H. 2009 a].
In the mini lysimeter study the test item ^{14}C-Dimethenamid-P, blended with non-radiolabeled Dimethenamid-P was applied to two mini-lysimeters with soil from Borstel, Lower Saxony (mini-lysimeters designated “Borstel A” and “Borstel B”).

Detailed data on the history of soil, collection, handling, storage of soil and soil characterization as well as on the generation of leachate samples can be found in the studies BASF DocID 2008/1051489, [see 2008/1051489 Fent G. 2008 a]and BASF DocID 2009/1011362, [see 2009/1011362 Staudenmaier H. 2009 a], respectively.

Selected samples of the mini-lysimeter leachate were analyzed by radio-HPLC directly or after concentration. For structure elucidation samples were further worked up.

Workup of leachate samples

<u>Preparation of samples for mass spectroscopy</u>
Workup I: Water sample “Borstel A” (days 38-44):
The sample was subjected to clean-up on an NH_2-SPE (solid phase extraction) column. The column was loaded with leachate and the purified percolate was recovered for further workup. The percolate was further processed on a strata-X-CW-SPE column. The column was once eluted with methanol and once with methanol / 5% ammonium hydroxide. The combined eluates were evaporated to dryness and dissolved in a small amount of methanol.
The concentrated eluate was subjected to HPLC fractionation. Four fractions were collected.
Fraction 2 (17-32 min) was concentrated, dissolved in acetonitrile / water (1/1, v/v) and further fractionated using HPLC. Thirteen fractions were sampled and aliquots of selected fractions were analysed by HPLC and LC-MS/MS

Workup II: water sample “Borstel A” (days 45-51):
The sample was filtered followed by a cleanup on NH2-SPE columns. Three columns were loaded with aliquots of the filtered sample (985 mL). The percolate was further processed on strata-X-CW-SPE columns. The percolate of the strata-X-CW-SPE columns was concentrated and taken up in a small amount of methanol. The concentrated percolate was subjected to HPLC fractionation. Fifteen fractions were collected and aliquots of selected fractions were concentrated to dryness, taken up in water / acetonitrile (4/1, v/v) and analysed by HPLC and LC-MS/MS.

Further work-up of mini-lysimeter leachate was performed for NMR analyses. However, the NMR analyses did not significantly contribute to the overall structure assignments and are not presented here.

Mass Spectroscopy
Samples were analysed with a Thermo Finnigan Linear-Ion-Trap (LTQ) Fourier-Transform (FT) Ultra mass spectrometer in ESI mode, which allows a very high resolution and precision of the accurate mass measurement.

II. RESULTS AND DISCUSSION

In this study existing samples from preceding studies were further worked up and analysed for metabolites of Dimethenamid-P. The samples originated from leachate of a mini-lysimeter study and were used for structure elucidation of metabolites in a preceding study. Samples of the latter study were transferred to the present study and subjected to additional work on characterization and elucidation of metabolites.

A. WORK-UP OF MINI-LYSIMETER LEACHATE

Samples of mini-lysimeter leachate were worked up in order to generate material for subsequent analyses. For the generation of samples for LC-MS/MS analyses, two different work-ups were performed which aimed at different fractions of the original leachate. In work-up I the eluates of a solid phase extraction (SPE) step were used whereas in work-up II the percolate of the SPE (containing highly polar components) was further processed and investigated.

Work-up I
Work up I included two subsequent HPLC fractionations on different HPLC systems. Fraction 2 out of 4 fractions of the first step contained the peaks of interest of the SPE eluate. About 5 larger and a number of small peaks of medium retention time (i.e. ~20 - 35 min) were observed in this fraction. Fraction 2 was further fractioned in the second step into thirteen subfractions of which six were analysed by LC-MS/MS.
All subfractions subjected to LC-MS/MS analysis contained more than one peak - even those that were isolated clearly as a single HPLC peak.

Work-up II
In workup II concentrated SPE percolate was further worked up. At least 20 peaks were observed in the concentrated percolate. Numerous polar compounds of short retention time (~3 - 20 min) but also a number of compounds of medium retention time were present in the eluate fraction.
Upon further analysis (including mass spectrometry) it became clear that some compounds of medium retention time were present in both, the eluate and the percolate whereas others were observed only in the percolate.
Compounds in the concentrated percolate were strongly enriched during the work-up. Consequently the concentration of individual (mostly very polar) compounds that are found only in the percolate fraction is very low when related to the original total leachate. Due to their continuous sequence in HPLC chromatograms they give rise to the appearance of an elevated baseline in addition to small peaks.

The concentrated percolate was fractioned by HPLC into fifteen fractions of which thirteen were analysed by LC-MS/MS.
All fractions contained more than one peak although in most cases a single HPLC peak was isolated.

All extracts or fractions were re-chromatographed on an additional HPLC system for comparison and confirmation of results.

B. RADIO-HPLC ANALYSES
More peaks than expected were observed in isolated metabolite fractions. In several cases additional peaks were obtained upon re-analysis of isolated peaks that clearly deviated from the original retention time. A more detailed investigation of this phenomenon revealed that there are several pairs of peaks that are in equilibrium to each other. This resulted in the effect that the same pair of HPLC peaks was observed if the one or the other of these peaks was isolated and then re-chromatographed. In such cases none of the two peaks could be isolated without formation the second peak.

Upon structure elucidation, the same structure and consequently the same metabolite code was assigned to each peak of the pairs. The two peaks are considered as rotamers, i.e. structures with hindered intramolecular rotation around a single bond that result in two peaks which however convert into each other

C. STRUCTURE ELUCIDATION
Subfractions of mini-lysimeter leachate were investigated by LC-MS/MS and numerous metabolite structures were proposed. The new coding system for metabolites of Dimethenamid-P was used to assign metabolite codes to the identified structures. Additionally, the extensions "(rota)" and "(iso)" for the metabolite codes were introduced in order to account for the complex situation with different types of isomerism. Briefly, the suffix "(rota)" is added if rotamers but no other chirality elements appear besides those given by the parent compound. The suffix "(iso)" is added if additionally E/Z isomerism or additional chirality elements are present and the occurrence of the respective isomer species is possible. Further details may be taken from the original final report.

The following compounds were identified for which structures were proposed.
In the SPE eluates: M656PH027 (two rotamers), M656PH045 (two rotamers) and an isomer thereof (two rotamers), M656PH047, M656PH054 and M656PH059 (iso) [three isomers].
In the SPE percolates partly the same structures were identified: M656PH045 (two rotamers) and an isomer thereof (two rotamers), M656PH047 (two rotamers), M656PH054 (two rotamers) and M656PH059 (iso) [two isomers].
The following metabolites were found in the SPE percolate only: M656PH049 (iso), M656H055, M656PH109 (rota), M656PH110 (rota) [two rotamers] and an isomer thereof. Additionally, numerous further compounds were observed in mass spectrometry for which only the molecular mass could be determined.
Metabolites M656PH109 (rota), M656PH110 (rota) were identified for the first time in this study.

The identified metabolite structures and their attribution to HPLC peaks is shown in **Table 7.1.4.2-16** below.

Table 7.1.4.2-16: Overview on identified metabolites and their assignment to peaks

No.	t_R in LC/MS [min]	t_R in minilysimeter leachate [min]	Structure proposal	Molecular mass	Metabolite Code
1	8.5*	16.5	or isomer	287	M656PH049 (iso)
2	8.5*	16.8		249	M656H055
3	11.8*	20.4, 22.0	and isomer	301	M656PH110 (rota) (2 rotamers) and isomer
	13.7*, 15.0*	21.8, 22.4			
4	14.1*	21.8		351	M656PH109 (rota)
5	15.2* 18.4* 27.8** 31.0** 33.0**	21.8 23.6 24.3		335	M656PH059 (iso) (rota- and/or isomers)
6	16.4*, 19.2* 28.9**	22.4, 23.6		351	M656PH047 (2 rotamers)

Table 7.1.4.2-16 Overview on identified metabolites and their assignment to peaks (continued)

No.	t_R in LC/MS [min]	t_R in minilysimeter leachate [min]	Structure proposal	Molecular mass	Metabolite Code
7	14.7*, 19.3* 25.0**, 29.9**	21.8, 24.3	and isomer	301	M656PH045 (2 rotamers) and isomer (2 rotamers)
	20.9*, 25.2* 32.3**, 35.8**	25.9, 27.4			
8	26.5*, 27.8* 37.8**	27.4, 28.4		321	M656PH054 (2 rotamers)
9	43.9**, 45.0**	32.6, 33.0		321	M656PH027 (2 rotamers)

* retention time in HPLC system used for SPE percolates
** retention time in HPLC system used for SPE eluates

Report: CA 7.1.4.2/5
Staudenmaier H., 2014a
Investigation of metabolites in the leachate of a lysimeter study with Dimethenamid - Updated version January 2014
2013/1334938
Guidelines: <none>
GLP: no

Executive Summary
The above report is replaced by the following report which is an updated version of it. It contains the information of the above document updated with new information and new insights attained in the course of the more recent investigations
In this summarizing document the HPLC results of the lysimeter study are aligned to those of the new mini-lysimeter study showing that overall the HPLC pattern is comparable. Numerous metabolite structures that were proposed upon LC-MS/MS investigation are assigned to the unknown lysimeter leachate fractions. The limitations of the structure elucidation due to potential multiple isomerism of several metabolites are discussed. A conservative estimation is presented for concentrations of individual compounds in the leachate.

II. RESULTS AND DISCUSSION
A. REVIEW OF THE LYSIMETER STUDY

The lysimeter study (BASF DocID 1996/10707) has been found to be in line with current study guidelines with respect to important parameters. Since the experimental conditions of the study were selected appropriately and the study was conducted in a scientifically sound way, it can be considered still valid as a whole according to current guidance.

However, looking more specifically at the HPLC analyses of the leachate water that led to the definition of the unidentified fractions in question, there are concerns about the evaluation of HPLC chromatograms with regard to metabolites. The main concerns with regard to HPLC analysis are

- the extreme complexity and variability of HPLC patterns
- the limitations to identify clear peaks (peak shape)
- the possibility that not only true substance peaks but also regions of background radioactivity were considered during the HPLC evaluation
- the low extent of structure identification by co-chromatography with known reference compounds

It was concluded that the interpretation of HPLC results with regard to the metabolites may be a subject for improvement.
As a first step it was found useful to perform a detailed investigation of the HPLC chromatograms. The various fractions of identified and unidentified radioactivity in the leachate of the lysimeter study were described in detail and their potential for structure elucidation was discussed. An overview and a short summary of these descriptions is given in Table 7.1.4.2-17: (left part).

In sum, definitive and reproducible peaks were observed in fractions U7, U8, U9, U10, U11, U12, U14 and U16. The same applies for the fractions containing metabolites M23 and M27. Percentages as given in the report are expected to roughly represent the size of these actual peaks. In some cases, peaks may be suspected to be caused by more than one compound or additional radioactivity may be present.
Fractions U3, U4, U5, U6, U13, U15, U17 exhibit only partly reproducible or small peaks but consist mainly of unresolved radioactivity or fragmented peaks. Percentages of the peaks in these fractions - and the compounds potentially identified behind these peaks - will only represent a small fraction of the respective entire "unidentified fraction".
Fraction U1 is an artifact peak and fraction U2 does not contain significant peaks.

B. MINI-LYSIMETER STUDY

In order to reconstitute the metabolites in the lysimeter leachate, a laboratory mini-lysimeter study was conducted with dimethenamid-P (BASF DocID 2008/1051489) In this study the same soil as in the original lysimeter study was used ("Borstel", Lower Saxony, Germany) in order to reproduce as far as possible the original situation.

Several variants of incubation conditions were tested in a non-GLP pre-test which differed with regard to the influence of
- application rate
- outdoor sunlight after application
- low redox potential or slightly anaerobic conditions
- prolonged metabolism in the soil

Despite these variations of the experimental conditions, the metabolite pattern was very similar in all cases.

The definitive mini-lysimeter study was conducted under GLP with two soil columns over 142 days. The leachate metabolite pattern observed in the pre-test was almost exactly reproduced in the main study. Two major peaks were identified as the metabolites M656PH023 and M656PH027 by co-chromatography with non-radiolabeled reference compounds. There were up to 9 minor peaks which were reproducibly detected in most of the leachate fractions. In between these peaks further small peaks and unresolved radioactivity were detected.

C. COMPARISON OF HPLC PATTERNS OF LYSIMETER AND MINI-LYSIMETER STUDY

Since the same HPLC system as in the original lysimeter study was used, the chromatograms of the mini-lysimeter study can be directly compared to those of the original lysimeter study within the range of the usual variability.

The fractions defined in the HPLC chromatograms of the lysimeter study were aligned with the respective sections of chromatograms of the mini-lysimeter leachate. The result of this alignment is presented in Table 7.1.4.2-17: .
Despite differences of their relative percentages, it turned out that the principle distribution of definite peaks and sections of unresolved radioactivity or fragmented peaks is quite similar in both studies.

Overall it can be stated that HPLC peaks in the lysimeter leachate correlate with peaks of the mini-lysimeter leachate. The metabolite pattern of the lysimeter leachate could largely be reproduced in the mini-lysimeter leachate. It is concluded that the metabolism is similar in both systems. This is further supported by the observation that the variation of experimental conditions in the mini-lysimeter study did not significantly affect the metabolite pattern in the leachate. It can be reasonably assumed that the HPLC peaks are caused by the same metabolites in both studies. This would mean that structure elucidation of lysimeter metabolites can be performed using mini-lysimeter leachate or soil extracts bearing the same HPLC peaks as the mini-lysimeter leachate.

Table 7.1.4.2-17: Comparison of HPLC results of the guideline lysimeter study and the mini-lysimeter study

Guideline lysimeter			**Mini-lysimeter**		
t_R [min]	**Designation**	**Description/comment**	**t_R [min]**	**Designation**	**Description/comment**
3	**U1**	**artifact peak**			
4-11	**U2**	**unresolved radioactivity, no significant peaks**	**3-11**		**unresolved radioactivity/ minor peaks**
11-13.5	**U3**	**unresolved radioactivity, minor peaks**	**11-13.5**	**n.i.A**	**peak at t_R~11 min, additionally some unresolved radioactivity/minor peaks**
13.5-16	**U4**	**unresolved radioactivity, minor peaks**	**13.5-16**	**n i.B**	**peak at t_R~13.5 min, additionally some unresolved radioactivity/minor peaks**
16-18.5	**U5**	**unresolved radioactivity, poorly reproducible peaks**	**16-18.5**	**n.i.C**	**peak at t_R~16.5 min, additionally unresolved radioactivity/minor peaks**
18.5-24	**U6**	**unresolved radioactivity, poorly reproducible peaks**	**18.5-23**		**unresolved radioactivity / minor peaks**
23-24.5	**U7**	**peak, partly split into insignificant peaks**	**23-24.5**	**n i.C1**	**small peak at t_R~23.5 min, minor peaks**
24-25.5	**U8**	**peak, sometimes split into fragmented peaks**	**24-25.5**	**n i.C2**	**small peak at t_R~25.5 min, additional minor peak**
26	**U9**	**large peak**	**26**	**n.i.D**	**peak**
27.5	**U10**	**large peak**	**27.5**	**n i.E**	**peak**
28	**U11**	**small peak**	**~28**		**minor peak**
29	**U12**	**peak, frequently split**	**~29**		**minor peak**
31	**M27**	**peak, sometimes double peak**	**30**	**M27**	**double peak**
33	**U13**	**unresolved radioactivity/ minor peaks**	**31-32**		**unresolved radioactivity/minor peaks**
33.5	**M23**	**peak, may include other, minor peaks**	**32.5**	**M23**	**broad peak**
35.5	**U14**	**peak, may include other, minor peaks**	**33-35**		**radioactivity poorly separated from M23**
36-37	**U15**	**no reproducible peaks**	**36-40**	**n.i.F**	**peak at t_R~36 min, additionally minor peaks**
39	**U16**	**peak**	**41**	**n.i.G**	**small peak**
40-42	**U17**	**small peak, unresolved radioactivity**	**>41**		**low radioactivity**
40.5		**active substance (not detected)**	**42**		**active substance (not detected)**

D. STRUCTURE ELUCIDATION

In order to elucidate the structures of the metabolites in the leachate, different approaches were combined. Details are described in [see 2009/1011362 Staudenmaier H. 2009 a] and its Report Amendment [see 2014/1031599 Staudenmaier H. 2014 b]. Further structure elucidation work is reported in [see 2013/1246087 Staudenmaier H.,Kuhnke G. 2014 a].

In one approach, leachate of the mini-lysimeter study was worked up in order to isolate peaks directly out of the leachate.
In a second approach, soil was treated with ^{14}C-dimethenamid-P and incubated in order to produce sufficient amounts of metabolites. In addition to the aerobic incubation, an anaerobic and a photolytic incubation was set up in order to generate different sets of metabolites depending on the incubation conditions.
The different metabolite patterns in the soil extracts were compared to that of the lysimeter / mini-lysimeter leachate in order to select peaks of corresponding retention time. Isolated peaks or fractions were subjected to LC-MS/MS investigation.

Further HPLC analysis and the LC-MS/MS investigation revealed that the number of metabolites and the complexity of the HPLC pattern is even higher than expected from the initial HPLC runs in the mini-lysimeter study. It is concluded that the complex HPLC results obtained in the original lysimeter study are indeed due to an extremely complex metabolism of (racemic) dimethenamid in the lysimeter leading to numerous metabolites which are further split into various isomers. Findings like the atypical peak shape and numerous fragmented peaks can be explained by this behavior.

Taking together the results of the HPLC analyses and the LC-MS/MS investigations, metabolite structures could finally be proposed for most of the unidentified fractions. Most of the proposed structures were directly deduced from leachate peaks of the mini-lysimeter. Only few peaks from soil extracts of the anaerobic incubation had to be taken into account since corresponding peaks were not available from the leachate in sufficient amounts.

An overview on the results of the structure elucidation is given in Table 7.1.4.2-18: .
For a better orientation, the content of Table 7.1.4.2-17: is repeated in this table. Some of the structures shown in Table 7.1.4.2-15: and Table 7.1.4.2-16 do not appear in this table since there was no clear correlation to peaks in the original HPLC system or they were only observed in strongly enriched subfractions.
There were several pairs and one triplet of isomers of metabolites which are distinguishable by their retention time. Most of the metabolite pairs were shown to be rotamers which are in equilibrium. Additionally, there are other metabolites which are marked with the suffix "(iso)" e.g. M656PH053 (iso) that have further stereochemical features (additional to rotamerism or without it) that give rise to multiple peaks for which no formation of an equilibrium was observed. Since it could not finally be decided from the MS results which of these isomeric forms is actually present in the respective HPLC peak, in these cases the same structures were proposed for each of the isomer peaks.

Many of the proposed metabolite structures had not been observed so far. This supports the finding in the lysimeter study that only few matches with known references were observed. Most of the new metabolites can however be regarded as derivatives of known metabolites like e.g. M656PH011, M656PH023 and M656PH27 which have undergone further metabolic reactions (e.g. further oxidation or opening of the thiophene ring). The new metabolites are therefore considered to represent steps further downstream in the degradation process which

are not observed under other experimental conditions. In Figure 7.1.4.2-1: the metabolites in the leachate are shown in their putative metabolic order.

Upon further discussion, a structural ambiguity resp. variability of certain metabolites became apparent. Some metabolites may exist as an alternative structure to the original structure proposal or may be in equilibrium with isomeric structures. An example is given in [see 2013/1334938 Staudenmaier H. 2014 a].

Table 7.1.4.2-18: Structure identification of metabolites corresponding to HPLC peaks of the lysimeter leachate

Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Designation	Description/ comment	t_R [min]	Designation	Description/ comment		
3	**U1**	artifact peak					
4-11	**U2**	unresolved radioactivity, no significant peaks	3-11		unresolved radioactivity/ minor peaks		
11-13.5	**U3**	unresolved radioactivity, minor peaks	11-13.5	**n i.A**	peak at 11 min, additionally some unresolved radioactivity/ minor peaks	or isomer	M656PH052 (iso)
13.5-16	**U4**	unresolved radioactivity, minor peaks	13.5-16	**n.i.B**	peak at 13.5 min, additionally some unresolved radioactivity/ minor peaks		M656H055
16-18.5	**U5**	unresolved radioactivity, poorly reproducible peaks	16-18.5	**n.i.C**	peak at 16.5 min, additionally unresolved radioactivity/ minor peaks	or isomer	M656PH049(1)
18.5-24	**U6**	unresolved radioactivity, poorly reproducible peaks	18.5-23		unresolved radioactivity/ minor peaks		M656PH059 (iso)(2) one of three isomers detected

Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Design ation	Description/ comment	t_R [min]	Design ation	Description/ comment		
						and	M656PH047 one of two rotamers
23-24.5	U7	peak, partly split into insignificant peaks	23-24.5	**n.i.C1**	small peak at 23.5 min, minor peaks	and	M656PH047 one of two rotamers
						and	M656PH059 (iso)(2) one of three isomers detected
							M656PH045 one of two rotamers

Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Designation	Description/ comment	t_R [min]	Designation	Description/ comment		
24-25.5	**U8**	peak, sometimes split into fragmented peaks	24-25.5	**n.i.C2**	small peak at 25.5 min, additional minor peak	and and or isomer	M656PH045 one of two rotamers M656PH059 (iso)[(2)] one of three isomers detected M656PH053 (iso)[(4)] one of two isomers detected
26	**U9**	large peak	26	**n i.D**	peak		M656PH054 one of two rotamers

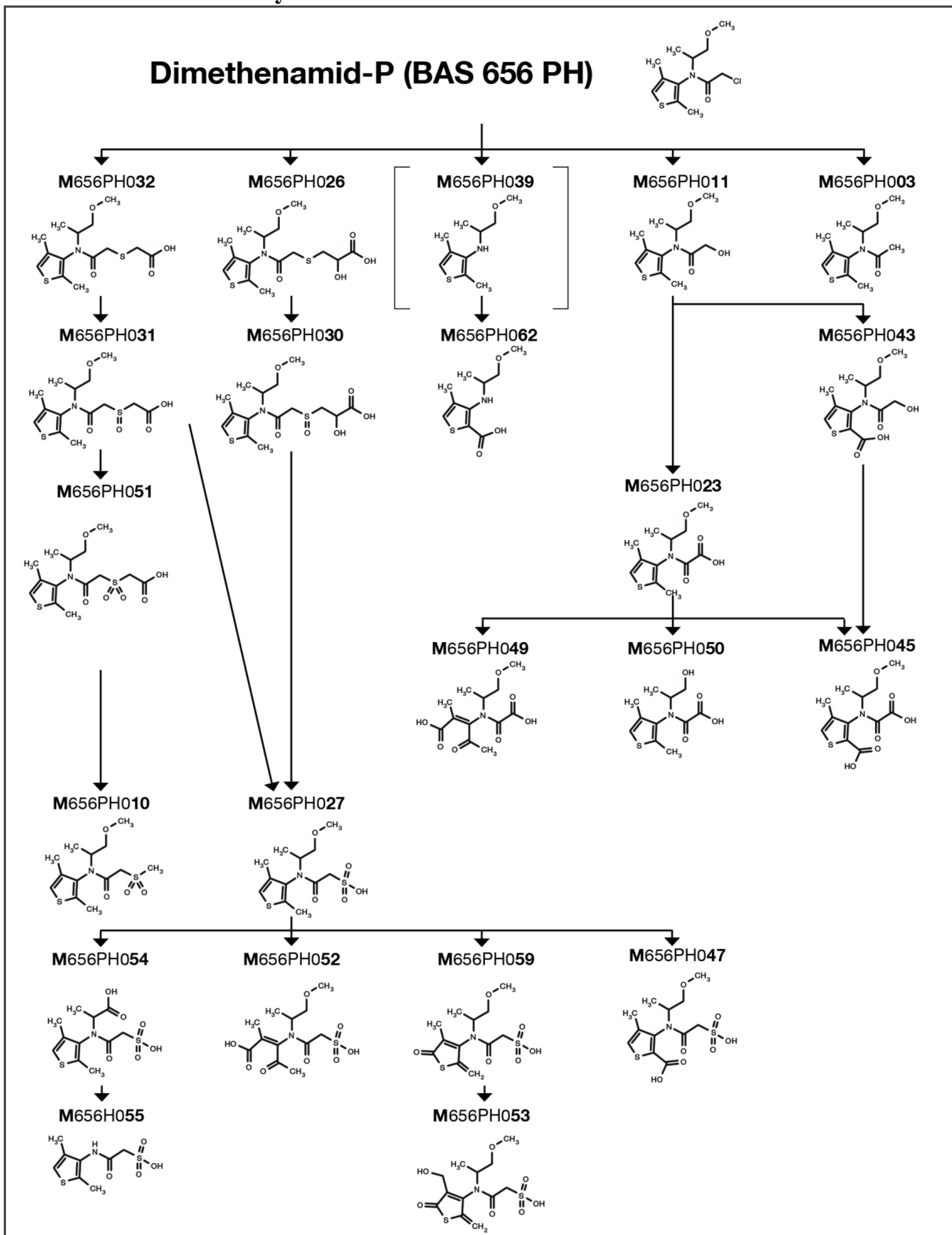
Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Designation	Description/ comment	t_R [min]	Designation	Description/ comment		
27.5	**U10**	large peak	27.5	**n.i.E**	peak	or isomer and and	M656PH053 (iso) one of two isomers detected M656PH054 one of two rotamers M656PH062
28	**U11**	small peak	~28		minor peak		M656PH043 one of two rotamers, isolated from t_R~31-32 min, isolated peaks showed different retention time

Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Design ation	Description/ comment	t_R [min]	Design ation	Description/ comment		
29	**U12**	peak, frequently split	~29		minor peak		M656PH043 one of two rotamers, isolated from t_R~31-32 min, isolated peaks showed different retention time
31	**M27**	peak, sometimes double peak	30	**M27**	double peak		M656PH027 double peak = two rotamers
33	**U13**	unresolved radioactivity/ minor peaks	31-32		unresolved radioactivity/ minor peaks		M656PH050 (rota) two rotamers expected but only this one detected structure derived from anaerobic sample
33.5	**M23**	peak, may include other, minor peaks	32.5	**M23**	broad peak	and	M656PH031

Guideline lysimeter			Mini-lysimeter			Proposed metabolite structure	Designation/ Remarks
t_R [min]	Designation	Description/ comment	t_R [min]	Designation	Description/ comment		
							M656PH023
35.5	**U14**	peak, may include other, minor peaks	33-35		radioactivity poorly separated from M23		M656PH051
36-37	**U15**	no reproducible peaks	36-40	**n.i.F**	peak at 36 min, additionally minor peaks		M656PH010
39	**U16**	peak	41	**n i.G**	small peak		M656PH032 isolated form anaerobic incubation, peak at this t_R in the leachate disappeared after storage

40-42	**U17**	small peak, unresolved radioactivity	>41		low radioactivity		M656PH003 isolated from anaerobic incubation

Figure 7.1.4.2-1: Dimethenamid-P: Metabolic order of metabolites in the lysimeter / mini-lysimeter leachate



E. ESTIMATION OF METABOLITE CONCENTRATIONS

Due to the limitations of the available HPLC analyses in the lysimeter study, distinct concentrations of individual compounds can hardly be derived directly from the study report. In order to provide a reasoned estimate of the concentration of the individual metabolites in the leachate, the compounds identified in fractions U3 - U17 were subjected to a calculation exercise as described below. Fractions U1 and U2 were already previously excluded (see Section II, A. above).

The calculation was based on the maximum annual average concentrations of the unidentified fractions from both lysimeters and all 3 experimental years, i.e. the derived concentrations for the identified metabolites are maximum annual average concentrations.

The calculation of concentrations of individual metabolites involves three steps:

Step 1: Conservative estimation of individual annual average concentrations

The calculation starts from the maximum annual average concentrations of the unidentified fractions U3 - U17 in the lysimeter study.

In order to account for the uncertainties in the quantification in the lysimeter study, a conservative approach is proposed. For each identified peak a factor is attributed in order to account for its percentage within the respective HPLC fractions. These factors are selected in a conservative way, i.e. they are considered to result in an overestimation of the individual metabolites.

The assignment of factors (percentages) was performed according to the following rules:

- if there are no clear peaks within a HPLC fraction of the lysimeter study (like e.g. U3), one third (= factor 0.33) of the radioactivity of the fraction was attributed to the identified peak(s)
- if there are clear peaks within a HPLC fraction in only part of the analyses (like e.g. U7), two thirds (= factor 0.66) of the radioactivity of the fraction was attributed to the identified peak(s)
- if there are clear peaks within a HPLC fraction in the majority of the analyses (like e.g. U9), the entire radioactivity of the fraction (= factor 1) was attributed to the identified peak(s)
- if there is more than one identified compound per fraction, 3/4 (= factor 0.75) of the entire radioactivity as calculated in the previous steps is attributed to each of the compounds

In this way a substantial concentration is calculated even for those compounds that were identified within HPLC fractions that bear mainly an elevated HPLC baseline or scattered peaks rather than clear metabolite peaks. Furthermore, if there was more than one compound within one HPLC fraction, a major proportion was assumed for each of these compounds resulting overall in a significant overestimation of metabolite concentrations.

The results of this calculation exercise is shown in **Table 7.1.4.2-19**.

Table 7.1.4.2-19: Estimation of the concentration of individual metabolites in the lysimeter leachate, refinement step 1 for individual peaks

R_t [min]	Unidenti-fied fraction	Max. annual average conc. of HPLC fraction [µg/L]	Compound			Clear peak	Estimation of peaks in fraction		Estimation of individual peaks	
			old code	new code	comment		factor A(1) (percentage of entire fraction)	all peaks in fraction [µg/L]	factor B(2)	individual peak [µg/L]
3	U1	12.9				no				
4-11	U2	3.9				no				
11-13.5	U3	2.6	M52	M656PH052		no	0.33	0.9	1	0.9
13.5-16	U4	2.0	M55	M656H055		no	0.33	0.7	1	0.7
16-18.5	U5	3.0	M49	M656PH049		no	0.33	1.0	1	1.0
18.5-24	U6	3.4	M59	M656PH059	isomer 1	no	0.33	1.1	0.75	0.8
			M47	M656PH047	rotamer 1				0.75	0.8
23-24.5	U7	0.8	M48	M656PH047	rotamer 2	partly	0.66	0.5	0.75	0.4
			M60	M656PH059	isomer 2				0.75	0.4
			M45	M656PH045	rotamer 1				0.75	0.4
24-25.5	U8	3.2	M46	M656PH045	rotamer 2	partly	0.66	2.1	0.75	1.6
			M61	M656PH059	isomer 3				0.75	1.6
			M53	M656PH053	isomer 1				0.75	1.6
26	U9	1.3	M54	M656PH054	rotamer 1	yes	1	1.3	1	1.3
27.5	U10	2.6	M57	M656PH053	isomer 2	yes	1	2.6	0.75	2.0
			M58	M656PH054	rotamer 2				0.75	2.0
			M62	M656PH062					0.75	2.0
28	U11	0.4	M43	M656PH043	rotamer 1	yes	1	0.4	1	0.4
29	U12	1.2	M44	M656PH043	rotamer 2	partly	0.66	0.8	1	0.8
31	M27	4.0	M27 (2 isomers)	M656PH027	rotamer 1+2	yes	1	4.0	1	4.0
33	U13	0.5	M50	M656PH050		yes	1	0.5	1	0.5
33.5	M23	1.0	M31	M656PH031		yes	1	1	0.1(3)	0.1
			M23	M656PH023					1(3)	1.0
35.5	U14	1.1	M51	M656PH051		yes	1	1.1	1	1.1
36-37	U15	0.2	M10	M656PH010		no	0.33	0.07	1	0.07
39	U16	1.5	M32	M656PH032		yes	1	1.5	1	1.5
40-42	U17	0.3	M3	M656PH003		no	0.33	0.1	1	0.1

(1) factor A is defined depending on the occurrence of clear peaks in the respective HPLC fraction; further explanation see text
(2) factor B is defined depending on the occurrence of one or more compounds in the respective HPLC fraction, further explanation see text
(3) individually set; further explanation see text

Step 2: Adjustment for application rate

Since the concentrations of the metabolite fractions depend on the application rate, an adjustment is made for the application rate:
The application rate in the lysimeter study was 1440 g a.s./ha, but the maximum application rate in the GAP for the Annex I renewal is only 864 g a.s./ha.
864 g/ha / 1440 g/ha corresponds to a factor of 0.6. This factor is applied to the concentrations determined in the previous step.
The result of this calculation is presented in Table 7.1.4.2-20.

Step 3: Accounting for rotamers in equilibrium

As described above, several metabolites do appear in HPLC analyses as two rotamer peaks that are in equilibrium. The concentrations of the individual rotamers are summed up in order to end up with the overall concentration of the respective metabolite.
The result of this calculation is given in Table 7.1.4.2-27**:** .

Additional considerations:

Metabolite M656PH031 was present in the mini-lysimeter leachate in considerable amounts leading to a broad peak due to a partial overlap with M656PH023.
In the guideline lysimeter, where M656PH031 would be expected in the fraction called "M23", a similar broad peak shape is not observed leading to the conclusion that M656PH031 was not present in high amounts. This is in line with the finding that this metabolite was not identified by co-chromatography with its reference compound in the lysimeter study. It is concluded that under the conditions of the guideline lysimeter, the majority of the respective fraction "M23" indeed represents metabolite M656PH023 whereas metabolite M656PH031 is present only in small amounts (if at all).
Applying the precautionary principle, the entire fraction is attributed to metabolite M656PH023 in order not to underestimate it. M656PH031 is additionally kept in the table in order not to disregard it but set to 0.1 µg/L since it was not detected under the conditions of the lysimeter study.

The above concentration estimates refer to the compounds that were identified as most prominent constituents of the fractions U3 - U17. It may be recalled that additional radioactivity was present in the samples that was not separated into distinct peaks. This will - at least partly - be due to further (minor) metabolites or a further splitting of metabolites into various types of isomers - each of them with a very low concentration. This is further substantiated by the identification of additional metabolites or isomers in strongly enriched sample fractions by LC-MS/MS (e.g. an isomer of M656PH045, M656PH109, M656PH110 and an isomer thereof).
As far as the not separated radioactivity corresponds to the identified metabolites, it may contribute to some extent to their overall concentration if e.g. the proportion of individual rotamers is summed up. However, it is considered that this potential contribution is already addressed by the conservative estimation of metabolite concentrations.

Table 7.1.4.2-20: Estimation of the concentration of individual metabolites in the lysimeter leachate, refinement step 2: adjustment to current application rate

R_t [min]	Unidentified fraction	Max. annual average conc. of HPLC fraction		Compound			Max. annual average conc. of individual compound	
		in original lysimeter [µg/L]	adjusted to current GAP [µg/L]	old code	new code	comment	in original lysimeter [µg/L]	adjusted to 864 g/ha application rate [µg/L]
3	U1	12.9	7.7					
4-11	U2	3.9	2.3					
11-13.5	U3	2.6	1.6	M52	M656PH052		0.9	0.5
13.5-16	U4	2.0	1.2	M55	M656H055		0.7	0.4
16-18.5	U5	3.0	1.8	M49	M656PH049		1.0	0.6
18.5-24	U6	3.4	2.0	M59	M656PH059	isomer 1	0.8	0.5
				M47	M656PH047	rotamer 1	0.8	0.5
23-24.5	U7	0.8	0.5	M48	M656PH047	rotamer 2	0.4	0.2
				M60	M656PH059	isomer 2	0.4	0.2
				M45	M656PH045	rotamer 1	0.4	0.2
24-25.5	U8	3.2	1.9	M46	M656PH045	rotamer 2	1.6	1.0
				M61	M656PH059	isomer 3	1.6	1.0
				M53	M656PH053	isomer 1	1.6	1.0
26	U9	1.3	0.8	M54	M656PH054	rotamer 1	1.3	0.8
27.5	U10	2.6	1.6	M57	M656PH053	isomer 2	2.0	1.2
				M58	M656PH054	rotamer 2	2.0	1.2
				M62	M656PH062		2.0	1.2
28	U11	0.4	0.2	M43	M656PH043	rotamer 1	0.4	0.2
29	U12	1.2	0.7	M44	M656PH043	rotamer 2	0.8	0.5
31	M27	4.0	2.4	M27 (2 isomers)	M656PH027	rotamer 1+2	4.0	2.4
33	U13	0.5	0.3	M50	M656PH050		0.5	0.4
33.5	M23	1.0	0.6	M31	M656PH031		0.1	0.06
				M23	M656PH023		1.0	0.6
35.5	U14	1.1	0.7	M51	M656PH051		1.1	0.7
36-37	U15	0.2	0.1	M10	M656PH010		0.07	0.04
39	U16	1.5	0.9	M32	M656PH032		1.5	0.9
40-42	U17	0.3	0.2	M3	M656PH003		0.1	0.06

Table 7.1.4.2-21: Estimation of the concentration of individual metabolites in the lysimeter leachate, refinement step 3: accounting for rotamers in equilibrium

Compound			Observed in fraction	Individual amounts	Sum
old code	new code	comment		[µg/L]	[µg/L]
M52	M656PH052		U3	0.5	0.5
M55	M656H055		U4	0.4	0.4
M49	M656PH049		U5	0.6	0.6
M59	M656PH059	isomer 1	U6	0.5	0.5
M47/M48	M656PH047	rotamer 1+2	U6 + U7	0.5 + 0.2	0.7
M60	M656PH059	isomer 2	U7	0.2	0.2
M45/M46	M656PH045	rotamer 1+2	U7 + U8	0.2 + 1.0	1.2
M61	M656PH059	isomer 3	U8	1.0	1.0
M53	M656PH053	isomer 1	U8	1.0	1.0
M54/M58	M656PH054	rotamer 1+2	U9 + U10	0.8 + 1.2	2.0
M57	M656PH053	isomer 2	U10	1.2	1.2
M62	M656PH062		U10	1.2	1.2
M43/M44	M656PH043	rotamer 1+2	U11 + U12	0.2 + 0.5	0.7
M27	M656PH027	rotamer 1+2	M27	2.4	2.4
M50	M656PH050		U13	0.3	0.3
M31	M656PH031		M23	0.06	0.06
M23	M656PH023		M23	0.6	0.6
M51	M656PH051		U14	0.7	0.7
M10	M656PH010		U15	0.04	0.04
M32	M656PH032		U16	0.9	0.9
M3	M656PH003		U17	0.06	0.06

III. CONCLUSION

A conservative estimation is given for the maximum annual average concentrations of identified metabolites of Dimethenamid-P in lysimeter leachate based on the maximum application rate of 864 g a.s./ha. The estimated concentrations range from 0.04 µg/L for M656PH010 to 2.4 µg/L for M656PH027. The metabolites can be grouped in the following concentration ranges:

(1) Below 0.1 µg/L:
M656PH003 M656PH031
M656PH010

(2) Between 0.1 µg/L and 0.75 µg/L:
M656PH023 M656PH051
M656PH043 M656PH052
M656PH047 M656H055
M656PH049 M656PH059 (2 isomers)
M656PH050

(3) Between 0.75 µg/L and 2.4 µg/L:
M656PH027 M656PH054
M656PH032 M656PH059 (1 isomer)
M656PH045 M656PH062
M656PH053 (2 isomers)

Report: CA 7.1.4.2/6
Haering T., 2013a
Relevance assessment of lysimeter study for BAS 656 H - Dimethenamid-P and its metabolites for agricultural areas of Europe as well as Germany, France, and UK
2012/1262498
Guidelines: <none>
GLP: no

Executive Summary

The leaching behavior of the active substance BAS 656 H – Dimethenamid-P and its soil metabolites M23, M27 and M31 has been analyzed in a higher tier lysimeter study (Burgener, 1996) [*old EU Annex II, chapter 7.1.3.3, reference 7.1/23: Burgener, A., [3-14C-thienyl]dimethenamid: mobility and degradation in soil in outdoor lysimeters, BASF DocID 1996/10707*]. The lysimeter study has been conducted with a coarse textured soil (Borstel soil) at a study site with high annual rainfall amounts (Itzingen, Switzerland, 1046.5mm/a), thus ensure vulnerable conditions regarding groundwater leaching.
In this study, the relevance of the lysimeter study for the agricultural area of Europe has been analyzed. Therefore, the site characteristics of the lysimeter has been set in context to the European conditions in different ways using spatial analysis and spatially distributed modeling in a Geographic Information System (GIS).
Both approaches show clearly the high vulnerability of the lysimeter study regarding pesticide leaching to groundwater for the entire intended use area of Dimethenamid-P in Europe. A good representativeness and a high relevance of the higher tier lysimeter study could be concluded.

I. MATERIAL AND METHODS

MetaPEARL

As an alternative and advanced (higher-tier) procedure to point-scale leaching models, spatially distributed leaching models have been developed and are proposed by the Ground Water Work Group of FOCUS e.g. for higher-tier leaching assessments. Such models may provide maps of the predicted leaching concentrations and allow for identification of high and low risk areas in terms of spatially varying environmental and land use properties [*TIKTAK, A., BOESTEN, J.J.T.I., VAN DEN LINDEN, A.M.A. and VANCLOOSTER, M. (2006): Mapping ground water vulnerability to pesticide leaching with a process-based metamodel of EuroPEARL, J. Environ. Qual. 35: 1231-12266].*

However, the drawback of spatially distributed, process based numerical models is their complexity and the need for a high number of scenarios (often more than 1000), which have to be parameterized with overlaying environmental maps in a Geographic Information System (GIS). The delineation of a large number of parameters can be difficult, and some of the required environmental parameters are even unavailable at larger scales.

To overcome this problems MetaPEARL has been developed as a Metamodel of the process-based EuroPEARL model. The complex nature of EuroPEARL has been simplified by considering only those processes and parameters for which the considered simulation output is sensitive or for which input data are available. As such, a simpler model can be obtained which encompasses the behaviour of the complex model and which is more compatible with available geodatabases. The model was developed within the EU project “Harmonized environmental Indicators for pesticide Risk” (HAIR). Detailed description of the derivation of the model can be found in *Tiktak et al.* (2006) and FOCUS Generic Guidance for Groundwater.

Basically, MetaPEARL is a regression model which predicts the 80th percentile of the leaching concentration at 1 m depth. The regression function is a process-based Metamodel of EuroPEARL. To account for climatic differences in Europe, e.g. seasonal dynamics in weather, model calibration was conducted with four subsets of the leaching sets, namely one for each climate zone. The definition of climate zones was adopted from FOCUS (2000), see **Table 7.1.4.2-22**.

Table 7.1.4.2-22: Major climate zones of the European Union

Zone	Mean annual rainfall m yr^{-1}	Mean annual temperature °C
Temperate, dry (TD)	<0.8	<12.5
Temperate, wet (TW)	>0.8	<12.5
Warm, dry (WD)	<0.8	>12.5
Warm, wet (WW)	>0.8	>12.5

MetaPEARL was calibrated with a leaching dataset for spring application as well as with a leaching dataset for autumn applications.

Relevance assessment

In order to estimate the relevance of the Dimethenamid-P lysimeter study for the European agricultural area, the “conservativeness” of the study site has to be set in context. The site characteristics of the study site have to be conservative regarding groundwater leaching in order to be vulnerable. Therefore, two different GIS-based approaches have been applied.

1. Single site characteristics, which have an influence on the leaching behavior of compounds, have been analyzed - namely mean annual precipitation, soil organic matter content, and soil texture. European wide GIS maps of these parameters have been used to plot the cumulative empirical distribution. In addition, the value of the variable at the study location is added to this distribution plot. By doing so, the relative agricultural area of Europe with a higher/lower value compared to the study location can be illustrated.

2. In addition to analyzing single environmental conditions, MetaPEARL has been used to set the vulnerability of the study site in a spatial context. As for the single environmental parameters, empirical cumulative distribution functions for the MetaPEARL maps have been plotted. In addition to the map of MetaPEARL concentrations also the MetaPEARL concentration for the lysimeter study has been calculated (Borstel soil + Itingen climate). This concentration has been added to the cumulative distribution plot to illustrate the relative agricultural area of Europe with a higher/lower MetaPEARL concentration compared to the lysimeter study, i.e. area with higher/lower vulnerability.

II. RESULTS AND DISCUSSION

Annual average precipitation

The empirical cumulative distribution function for the annual average precipitation for the relative agricultural area of Europe as well as for the study site Itingen is illustrated in Figure 7.1.4.2-2**:** :

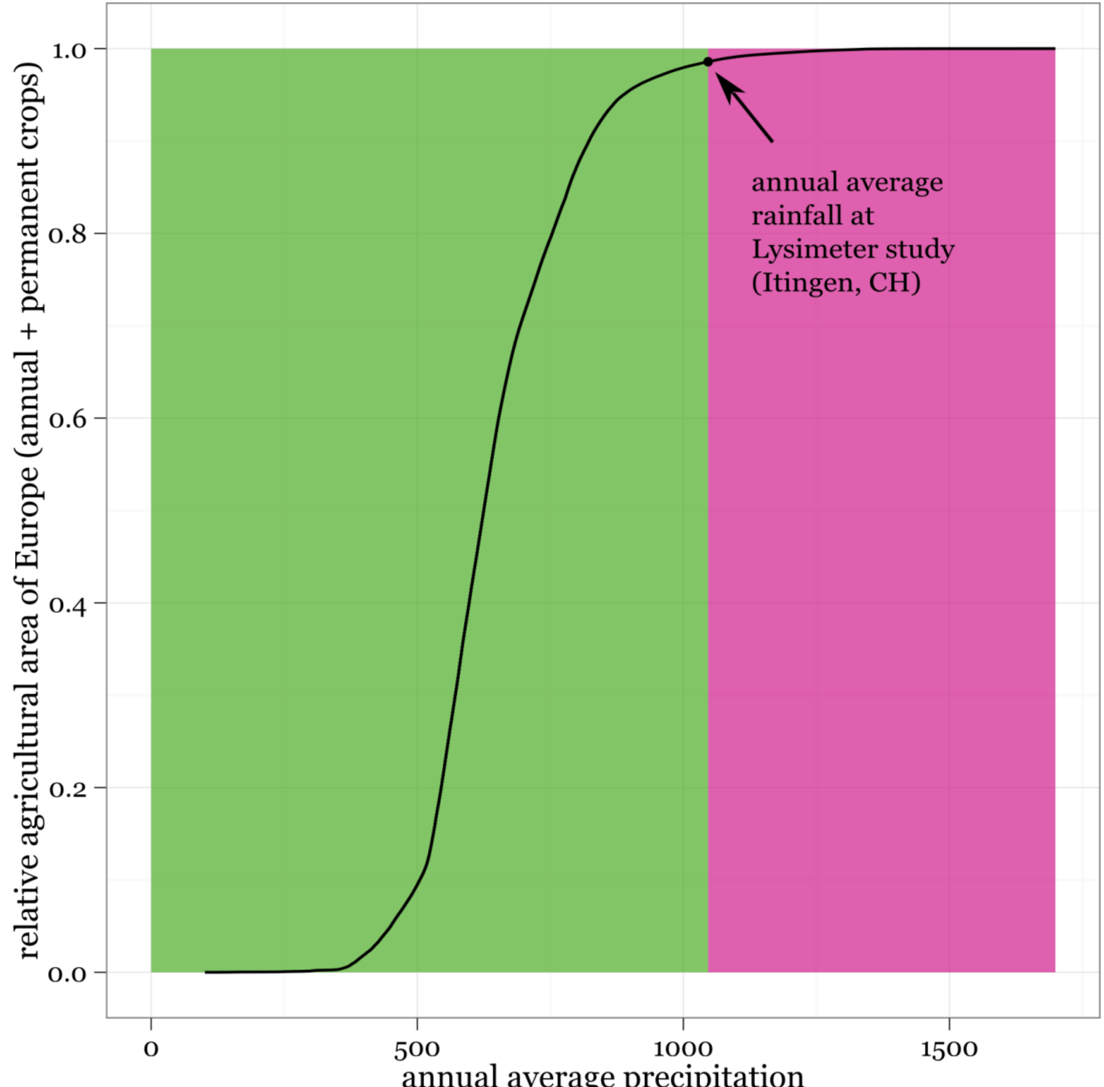


Figure 7.1.4.2-2: Empirical cumulative distribution function of annual average precipitation for the relative agricultural area of Europe and the value for the lysimeter study

The plot indicates that the annual average precipitation at the lysimeter study location (1046.5 mm/a) is higher than 98.1% of the agricultural area of Europe. Since higher rainfall amounts are directly linked to higher leaching to groundwater it can be concluded that the lysimeter study is characterized by vulnerable conditions regarding annual amount of rainfall.

Topsoil organic matter content

The empirical cumulative distribution function for topsoil organic matter content for the relative agricultural area of Europe is illustrated in Figure 7.1.4.2-3:

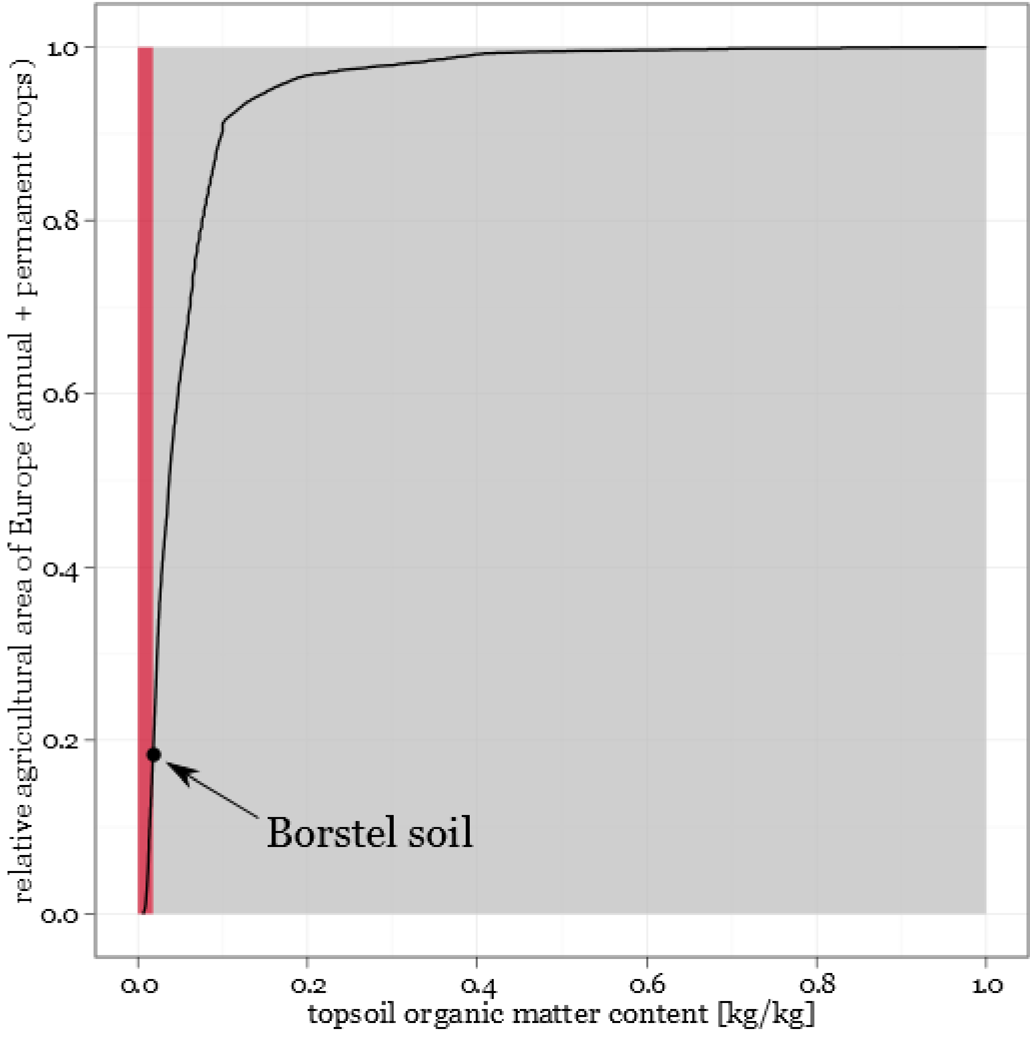


Figure 7.1.4.2-3: **Empirical cumulative distribution function of topsoil organic matter content for the relative agricultural area of Europe and the value for the lysimeter study**

The plot indicates that the organic matter content in the top 30cm at the lysimeter study location (0.0181 kg/kg) is higher than only 18.6% of the agricultural area of Europe, i.e. 81.4% of the agricultural area of Europe has a higher content of soil organic matter. Since lower soil organic matter content means less potential of sorption it can be concluded that the lysimeter study is characterized by vulnerable conditions regarding soil organic carbon content.

Soil texture

Soil texture properties couldn`t be illustrated with cumulative distribution plots because of their level of measurement (nominal data). Typically, soil texture is plotted with a texture triangle, in which the soil texture class is plotted according to their particle size distribution (sand, silt, and clay content). To compare the texture properties of the European agricultural area with the Borstel soil the density over all raster cells of the soil texture map (topsoil, 1 km cell size) has been plotted into a texture triangle, cf. Figure 7.1.4.2-4.

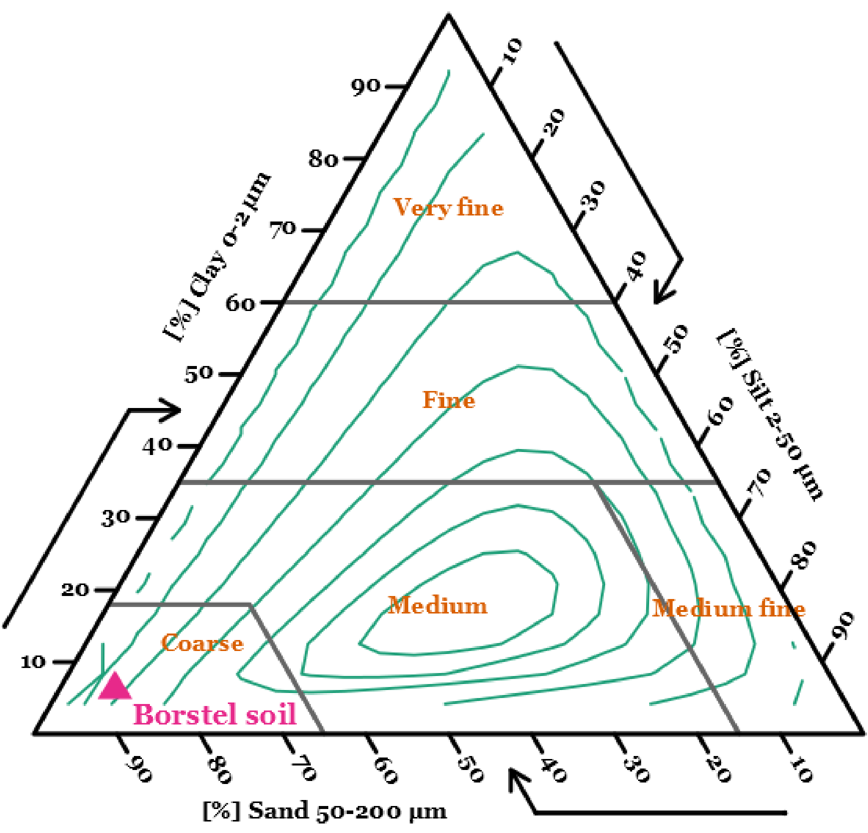


Figure 7.1.4.2-4: **Texture triangle according to the EUSoilDB indicating the distribution of topsoil texture in the agricultural area of Europe (isolines in green) and the texture properties of the Borstel soil**

Figure 7.1.4.2-4 indicates the highest texture density in the agricultural area in Europe in the Medium texture class whereas the texture properties of the Borstel soil show clear coarse texture. Since coarse textured soils have potentially higher infiltration rates and faster movement of water it can be concluded that the lysimeter study is characterized by vulnerable conditions regarding soil texture.

Vulnerability regarding groundwater leaching

For both the spring and autumn application relative MetaPEARL concentrations are depicted below. The lysimeter study shows mostly higher MetaPEARL concentrations than the entire agricultural area of Europe.

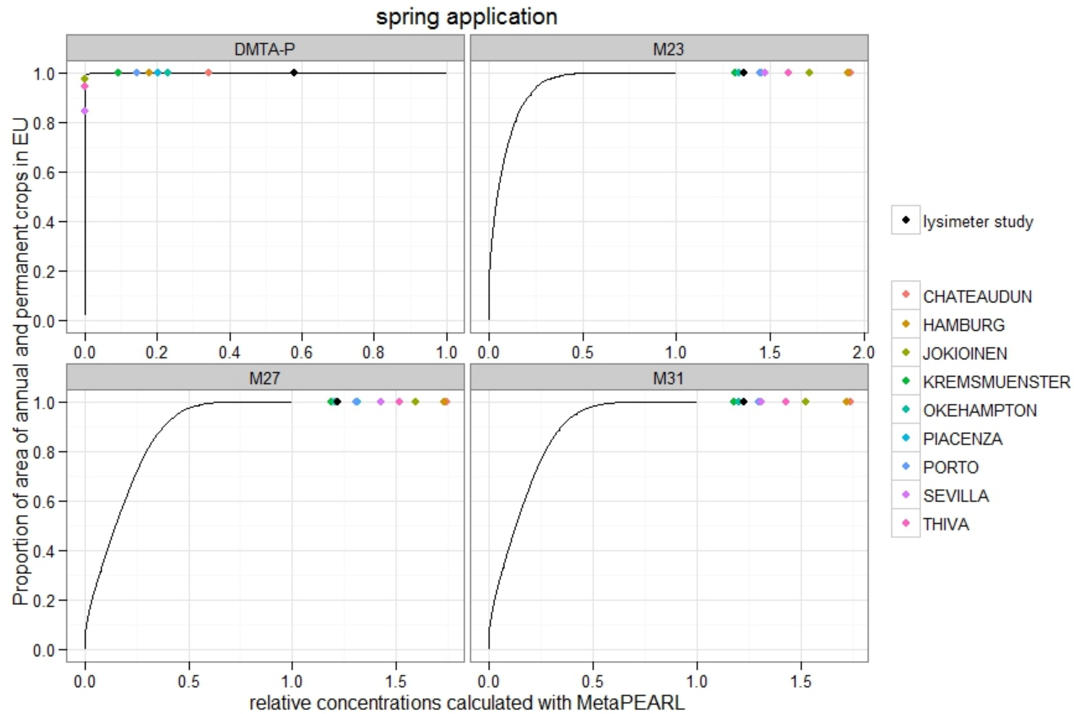


Figure 7.1.4.2-5: Empirical cumulative distribution function of the relative MetaPEARL concentration for the agricultural area of Europe as well as the lysimeter study and the focus scenarios – spring application

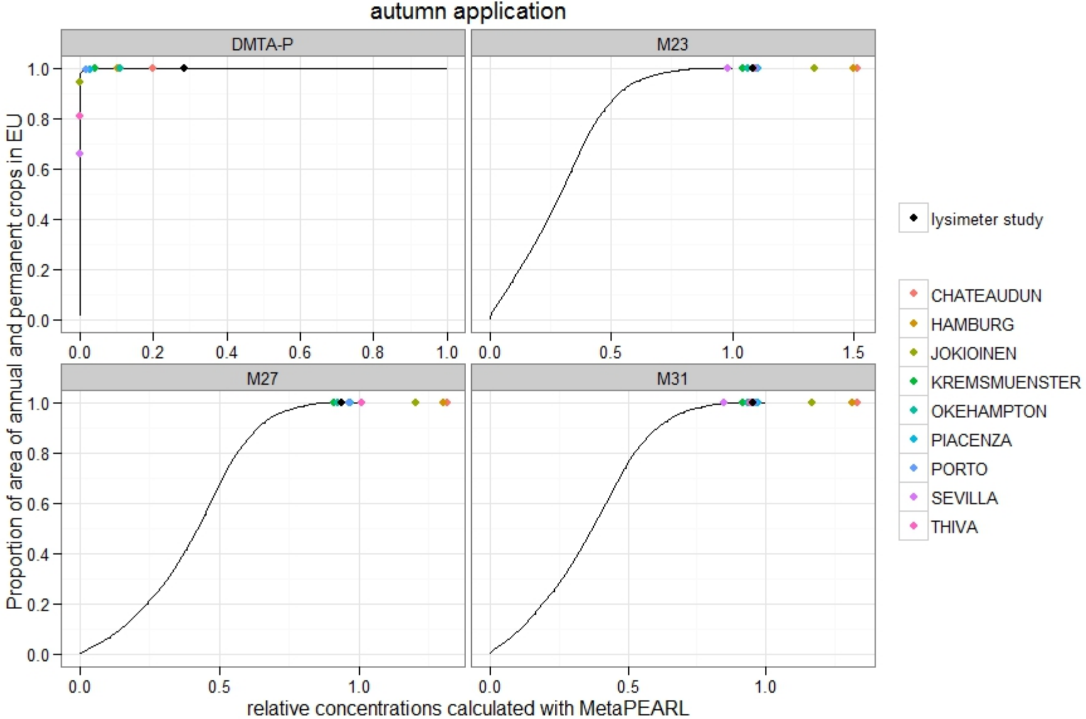


Figure 7.1.4.2-6: Empirical cumulative distribution function of the relative MetaPEARL concentration for the agricultural area of Europe as well as the lysimeter study and the focus scenarios – autumn application

In most plots MetaPEARL concentrations of the lysimeter study is lower than FOCUS Hamburg, even though the conditions at the lysimeter site are more vulnerable than FOCUS Hamburg (same soil but higher rainfall amount). This could be explained by the disaggregation of Europe in four climatic zones for MetaPEARL calculations. With the higher annual rainfall amount (Itingen: 1046.5 mm/yr, FOCUS Hamburg: 786 mm/yr) the lysimeter site has to be modeled with the temperate-wet model whereas FOCUS Hamburg lies within the temperate-dry class.
Bearing in mind that the cumulative distribution plots cover the entire agricultural area it may be confusing that the lysimeter site as well as several FOCUS scenarios have higher concentrations than the maximum concentration of the mapped values (relative MetaPEARL concentrations > 1.0), i.e. are more vulnerable than the entire area which is physically not possible. However, the differences in MetaPEARL concentrations between map and point calculations come from the different scales of measurements as well as the different sources of information, e.g. maps of climatic conditions (worldclim data) vs. local measurements, maps of topsoil organic matter vs. detailed description of soil profiles. In addition, some FOCUS scenarios as well as the lysimeter site are combinations of soil and weather data, which do not occur in reality, e.g. Borstel soil carried to Itingen or the West München weather station for Kremsmünster scenario, which may lead to higher concentrations as for the absolute location of Borstel or Kremsmünster *[FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. - Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000, 202 pp.*].

However, even taking these unavoidable inconsistencies due to the simplified character of MetaPEARL into account, the lysimeter study could be regarded as vulnerable regarding pesticide leaching to groundwater for entire agricultural area of Europe.

III. CONCLUSION

The relevance of a higher-tier lysimeter study for Dimethenamid-P and its metabolites M23, M27 and M31 for the agricultural area of Europe has been analysed using two different approaches. Both approaches show clearly the high vulnerability of the lysimeter study regarding pesticide leaching to groundwater for the entire intended use area of Dimethenamid-P in Europe. A good representativeness and a high relevance of the higher tier lysimeter study could be concluded.

Report: CA 7.1.4.2/7
Hein W.,Baudy M., 2013a
Determination of the breakthrough behaviour of two metabolites (M23 and M27) of Dimethenamid-P and of a conservative tracer using microlysimeters
2013/1294765
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The purpose of the study was the determination of the breakthrough behaviour of two metabolites (M23 and M27) of the herbicide Dimethenamid-P using microlysimeters. The soil used in this study ("Birkenheide") is a typical lysimeter soil with a sand content of >70% and a C_{org} of <1.5%. The test item was applied to three total microlysimeter systems. The irrigation scheme used for the microlysimeters was; 5 days of irrigation two days without irrigation. The irrigation duration was 150 days at a rate of 2 mm/day.

0.35 mg of each test item (M23 and M27) were applied onto each soil column (0.035 m^2 soil column surface) corresponding to an application rate of 200 g/ha. Metabolites M23 and M27 of Dimethenamid-P are formed in different branches of the proposed degradation pathway. The formation of the individual metabolites is therefore considered to be independent from each other and can be investigated together in a single experiment. Additionally, 500 mg of bromide as conservative tracer were applied to the surface of the soil as well. The concentration of the test item and the conservative tracer in the outflow of the soil columns was measured over time. The concentration of test item in soil was also determined.
The concentration of the tracer was determined three times per week up to Day 120 when concentrations reached the LOQ of the analytical method. After breakthrough of bromide the irrigation solution was changed from tap water to a 0.01M solution of $CaCl_2$ at DAT 53. The data may be used to derive specific model transport parameters such as sorption and degradation coefficients by means of inverse modelling.

The maximum concentration observed for the bromide tracer in column 1 was Day 50. The maximum concentrations for the bromide tracer in columns 2 and 3 were observed on Day 43.

Concentrations of M23 and M27 were determined three times per week using LC-MS/MS.
The days of maximum concentrations and the maximum concentrations measured are given in the following table:

Table 7.1.4.2-23: Day of maximum concentration and maximum concentrations of M23 and M27

		Day of maximum concentration	Maximum concentration (µg/L)	Maximum of % applied
M23	1	50	169.3	0.63
	2	41	321.0	2.33
	3	41	363.0	3.19
M27	1	71	161.10	0.2
	2	41	219.0	1.59
	3	50	180.3	0.74

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Test item	sodium[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)carbamoyl]-methanesulfonate (synonym M27)
Reg. No.	360714
Batch No.	01311-28
Chemical purity	97.1%
Test item 2	N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)oxalamic acid (synonym M23)
Reg. No.	360715
Batch No.:	L59-64
Chemical purity:	100.0%

In order to characterise the movement of the soil water and the retardation of the test items in the soil column, bromide was used as a conservative tracer, because it shows no sorption or degradation. For this study, the tracer bromide was applied as potassium bromide.

2. Soils

The soil chosen for the study originates from "Birkenheide" (Rhineland-Palatinate / Germany). The physico-chemical characterisation of the soils is presented in Table 7.1.4.2-24.

Table 7.1.4.2-24: Characterisation of soil used in this study

Textural class (USDA scheme)	Loamy sand
Soil texture [%], (USDA scheme) Sand Silt Clay	 84.7 9.1 6.2
Total organic carbon [%]	0.86
Microbial Biomass [mg C_{mikr}/kg Soil]	22.7
Soil depth (cm)	0-30
pH ($CaCl_2$)/(H_2O)	6.8/7.2

B. STUDY DESIGN

1. Experimental conditions

Stainless steel tubes (211 mm in diameter and 300 mm length) filled with undisturbed soil columns were used. The length of the soil core was approximately 280 mm. In total four soil columns from the sites mentioned were sampled. Three soil columns out of the four were used for the determination of the breakthrough curves. On top of the columns about 30 mm of the soil were replaced by 500 g of quartz sand ensuring a plain surface. A ceramic plate was mounted at the lower base of the soil column. A stainless steel lid, fitted with the outlet tube, was placed on the lower base column and fixed with long screw rods. The outlet tube had an internal diameter of 3 mm and was filled with a hanging water column of about 500 mm to apply a constant tension to the lower end of the soil column to prevent the formation of a capillary fringe.
.
Prior to application, the soil columns were equilibrated for 23 days using an irrigation rate of 105 -210 mL/d. During this period the amount of leachate was measured (weight density = 1 g/mL) and recorded.
An application rate of 200 g/ha was assumed for the test items. Considering the surface of each soil column of 0.035 m² an application rate of 0.7 mg/column was calculated. 35.0 mg of M23 were solved in 100 mL (methanol/water 1:1 (v/v)). 35.0 mg of M27 were also solved in 100 mL (methanol/water 1:1 (v/v)) This solutions served as application solution.

500 mg bromide (as Br-) per column was applied, as that amount was considered to be sufficient to quantify the breakthrough of the bromide tracer. Considering the molecular weight of Br- of 79.90 g/mol and of potassium bromide of 119.01 g/mol a total amount of 744 mg potassium bromide were applied per soil. To each of the three soil columns were applied 350 µg of M23 and M27.

The irrigation was performed according to the following scheme for 150 days.

Table 7.1.4.2-25: Irrigation program

Irrigation		Irrigation within 6 hours [mL]
Day of application Day 0 (Friday)		None
Saturday – Sunday Day 1 – Day 2 after application	24 hours 0.000 mL/min = 0.00 mm/h	None
Monday – Friday Day 3 - Day 150 after application	6 hours 0.194 mL/min = 0.33 mm/h 18 hours 0.000 mL/min = 0.00 mm/h	70
Saturday and Sunday	24 hours 0.000 mL/min = 0.00 mm/h	None

At the end of each irrigation cycle the actual volume of water applied to each column was measured and recorded. Irrigation was with tap water until breakthrough of potassium bromide, afterwards 0.01 M $CaCl_2$ solution was used.

2. Description of analytical procedures

For each leachate specimen the actual volume/weight was determined. From each leachate specimen taken on Monday, Wednesday and Friday an aliquot was directly filtered through a 0.20 µm filter into a HPLC sample vial and injected into the HPLC for test item and bromide analysis. The bromide determinations were carried out in duplicate and the determination limit was 0.1128 mg/l. Based on the results of the analysis additional leachate specimens were analysed by HPLC.
Furthermore, the test item concentrations in the leachates were determined using LC-MS/MS.

The room temperature beside the soil columns was recorded every 20 minutes with a data logger.

II. RESULTS AND DISCUSSION

A. Water balance

Within the test period of 150 days the soil columns were irrigated with a total amount of water of 7.403 L (mean value) corresponding to 212 mm. The irrigation rate throughout the study was 2 mm/day within a five day period. Regardless of the irrigation volume applied, between 82.41% and 83.32% (**Table 7.1.4.2-26:**) of the original irrigation amount was sampled as leachate. The difference between the total applied amount of water and the amount measured as leachate can be explained by evaporative losses and by increasing saturation of the soil. Detailed results for the individual soil columns and each individual sampling date are given in the **Table 7.1.4.2-26:** .

Table 7.1.4.2-26: Water balance of the individual soil columns

Soil	Test duration [days].	Total irrigation	Soil	Test duration [days].
268/1	150	7356	6087	82.75
268/2	150	7426	6187	83.32
268/3	150	7426	6120	82.41

B. Bromide mass balance and bromide breakthrough

The soil columns were treated with potassium bromide (bromide as tracer) equivalent to 500 mg bromide. Bromide concentrations were measured three times per week in order to characterise the tracer breakthrough behaviour. The final measurement was carried out on Day 120, at which time the tracer concentration had reached values lower than the method LOQ. The maximum concentration observed for the bromide tracer in column 1 was Day 50. The maximum concentrations for the bromide tracer in columns 2 and 3 were observed on Day 43.

C. Test item breakthrough behaviour

For column 1 the maximum M23 concentration was determined on Day 50. The maximum M23 concentration for column 2 and 3 was determined on Day 41. The maximum M27 concentration was determined for column 1 on Day 71, for column 2 on Day 41 and for column 3 on Day 50. The maximum concentrations are given in the following table.

Table 7.1.4.2-27: Day of maximum concentration and maximum concentrations of M23 and M27

		Day of maximum concentration	Maximum concentration (µg/L)	Maximum of % applied
M23	1	50	169.3	0.63
	2	41	321.0	2.33
	3	41	363.0	3.19
M27	1	71	161.10	0.2
	2	41	219.0	1.59
	3	50	180.3	0.74

III. CONCLUSION

Three undisturbed soil columns with soil taken from Birkenheide were used for the determination of the breakthrough behaviour of the Dimethenamid-P metabolites M23 und M27. Each column was treated with 0.35 mg of M23 and M27, respectively. Also 500 mg of Bromide tracer were applied in order to characterise the leachate breakthrough behaviour.

The irrigation rate was 2 mm/day within a five day period. Regardless of the irrigation volume applied, between 82.41% and 83.32% (**Table 7.1.4.2-26:**) of the original irrigation amount was sampled as leachate.

The maximum bromide concentration for column 2 and 3 was determined on Day 43 for column 1 the maximum tracer concentration was determined on Day 50.

Concentrations of M23 and M27 were determined three times per week using LC-MS/MS. The maximum M23 concentration for column 1 was determined on day 50, for column 2 and 3 the maximum concentrations were determined on Day 41. For M27 the maximum concentration was determined for column 1 on Day 71, for column 2 on Day 41 and for column 3 on Day 50.

Report: CA 7.1.4.2/8
Schroeder T., 2014a
Estimation of sorption and degradation parameters of metabolite M23 and M27 of BAS 656 H from mini-lysimeter studies by inverse modeling
2013/1348579
Guidelines: <none>
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

An experimental study with three mini-lysimeters was performed with both an inert tracer (bromide) and the metabolites M23 (M656H023) and M27 (M656H027) of BAS 656 H – Dimethenamid. From the breaktrough curves (BTCs) of the inert tracer soil and transport specific parameters for each lysimeter are determined. These parameters are then used for evaluation of the BTCs of the metabolites, with the goal to obtain sorption and degradation parameters of the metabolites. Parameter estimation is acquired by the inverse modeling tool PEST 11.3 in combination with the transport model PEARL 4.4.4.
Regarding metabolite M27 all three mini-lysimeters could be evaluated with high accuracy, with respect to M23 only one mini-lysimeter could be evaluated.

I. MATERIAL AND METHODS

Test items

Test item 1

Reg No. 360715
Batch L59-64
Internal (Metabolite) Code M23
IUPAC-Name Englisch N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)oxalamic acid
Molecular formula $C_{12}H_{17}NO_4S$
Molecular weight [g/mol] 271.3

Test item 2

As provided on the Certificate of Analysis in study 427728

Reg No. 360714
Batch 01311-28
Internal (Metabolite) Code M27
IUPAC-Name sodium[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)carbamoyl]-methanesulfonate
Molecular formula $C_{12}H_{18}NNaO_5S_2$
Molecular weight [g/mol] 343.4

Test item 2 is declared with the Sodium, however, analyzed in the reference study is the molecule without the Sodium (Na^+). In this report we consider as metabolite M27 the molecule below without the sodium (with molecular weight of 312.4 g/mol).

Internal (Metabolite) Code M27
IUPAC-Name 2-[(2,4-dimethylthiophen-3-yl)(1-methoxypropan-2-yl)amino]-2-oxoethanesulfonic acid

Molecular formula: $C_{12}H_{19}NO_5S_2$
Molecular weight [g/mol] 321.4

Mini-lysimeters

Three mini-lysimeters were investigated in the experimental study CA 7.1.4.2/8. The length of a mini-lysimeter column (all three systems are equal) was 28 cm. At the top of the mini-lysimeter irrigation was applied. At the bottom of the mini-lysimeter a constant pressure head of -50 cm was applied which initiates an active flow of water from the top to bottom. Over a period of 150 days measured values of concentrations in the percolate (i.e. the water that leached out of the soil system) of the inert tracer and the metabolites were obtained.

Inverse modelling procedure

The inverse modelling tool PEST is used to fit the following parameters for the tracer BTC: the saturated water content (θ_{sat}), the saturated soil hydraulic conductivity (K_{sat}), the dispersion length (D) and the application rate of bromide. From the BTCs of the test items the degradation rate in soil (DegT50), the sorption coefficient of equilibrium sorption on organic matter (K_{om}) and the Freundlich coefficient (1/n) were derived. PEST calls PEARL with predefined initial conditions for the parameters to be fitted and changes these variables within a pre-defined range until an optimized parameter set is acquired, in comparison to the measured BTC values. PEST stops when no better parameter set can be found statistically.

Statistical evaluation

In order to evaluate the quality of the fit as well as the quality of the parameters in accordance with the recommendations of the FOCUS kinetics workgroup the following procedures were followed.

1. Visual assessment
2. Coefficient of variation
3. Chi square statistics (χ^2)
4. Student's t-test

Strategic procedure

Tracer

The general procedure is to first estimate all parameters of interest at once, thus all parameters are released, and free to be optimized. If it is not possible to acquire statistically sound parameters, one may choose to fix a parameter based on initially estimated or conservative parameters.

Metabolites

In a first run, it was tested whether the resulting parameters would fall within the boundary limits and are statistically sound. If yes, a second run with the First-run-results as initial parameter settings were performed to check whether a proper solution without correlation of coefficients was achieved.

II. RESULTS AND DISCUSSION

A visual assessment of the raw data of bromide over time showed that for all three columns three data points (DAT 43, DAT 50 and DAT 53) had larger discrepancies from the mean (optimal) curve. Therefore, these observations were weighted, with a lesser weight than the other data points (0.5 instead of 1). In a first attempt, all parameters were released. However, neither was the fit of the bromide BTC statistically accurate (too high coefficients of variation), nor resembled the estimated water content a sandy soil physically. The next step was then to fix one of the four parameters. It was chosen to fix θ_{sat} to its initial value, since this parameter has physically the least influence. A K_{sat} belonging to this fixed value can be found to describe the soil hydraulic properties properly.

The BTCs of the metabolites were analyzed for all parameters without any weighting of the experimental data.

Mini-lysimeter: Column 1

Step 1: bromide BTC – Column 1

The fitted parameters with their statistics are given **Table 7.1.4.2-28** and **Table 7.1.4.2-29**. The parameters are fitted with good accuracy (maximum coefficient of variation of <8 %, p-values ≤ 0.001).

Table 7.1.4.2-28: Fitted parameters from bromide BTCs in column 1

Compound	**D [m]**			**Θ_{sat} [m^3 m^{-3}]**		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]
Tracer (Br)	0.011	<0.001	6.4	0.380*	-	-
	K_{sat} [m d^{-1}]			**Application rate [kg ha^{-1}]**		
	mean	st. Dev	CV [%]	mean	st. dev	CV [%]
	0.734	0.052	7.1	113.6	2.29	2.0

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)
* Parameter fixed

Table 7.1.4.2-29: Statistical parameter evaluation of the bromide BTCs in column 1

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*			
		D [m]	Θ_{sat} [m^3 m^{-3}]	K_{sat} [m d^{-1}]	Applica-tion rate [kg ha^{-1}]
Tracer (Br)	16.86	<0.001	-	<0.001	<0.001

* p-values obtained for a 5% significance level

Step 2: Metabolites' BTC – Column 1

The fitted parameters with their statistics are given in **Table 7.1.4.2-30** and **Table 7.1.4.2-31** and are estimated with good accuracy (coefficients of variation <7 %, p-values ≤ 0.001) for M27. Regarding M23, the boundary limits of the Freundlich exponent and the sorption coefficient were reached, which means that no reasonable optimization could be realized.

Table 7.1.4.2-30: Fitted parameters from M23 and M27 BTCs – Column 1

Compound	$DegT_{50}$ [d]			K_{om} [L kg^{-1}]			1/n [-]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]	mean	st. dev	CV [%]
M23 - run 1	22.80	-	-	0.0001	-	-	0.60	-	-
M27 - run 1	39.65	-	-	5.40	-	-	1.11	-	-
M27 - run 2	39.64	0.782	2.0	5.51	0.346	6.3	1.12	0.056	5.0

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

Table 7.1.4.2-31: Statistical parameter evaluation of the metabolites' BTCs in column 1

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*		
		$DegT_{50}$ [d]	K_{om} [L kg^{-1}]	1/n [-]
M27	13.58	<0.001	<0.001	<0.001

* p-values obtained for a 5% significance level

Mini-lysimeter Column 2

Step 1: bromide BTC – Column 2

The fitted parameters with their statistics are given in **Table 7.1.4.2-32** and **Table 7.1.4.2-33**. The parameters are fitted with good accuracy (maximum coefficient of variation of <10%, p-values ≤ 0.001).

Table 7.1.4.2-32: Fitted parameters from bromide BTCs in column 2

Compound	D [m]			Θ_{sat} [m^3 m^{-3}]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]
Tracer (Br)	0.020	0.001	5.4	0.380*	-	-
	K_{sat} [m d^{-1}]			**Application rate [kg ha^{-1}]**		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]
	1.31	0.125	9.5	117.4	2.187	1.9

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)
* Parameter fixed

Table 7.1.4.2-33: Statistical parameter evaluation of the bromide BTCs in column 2

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*			
		D [m]	Θ_{sat} [m^3 m^{-3}]	K_{sat} [m d^{-1}]	Applica-tion rate [kg ha^{-1}]
Tracer (Br)	11.79	<0.001	-	<0.001	<0.001

* p-values obtained for a 5% significance level

Step 2: Metabolites' BTC – Column 2

The fitted parameters with their statistics are given in **Table 7.1.4.2-34** and **Table 7.1.4.2-35**, and are estimated with good accuracy for M27 (maximum coefficient of variation of <4 %, p-values ≤ 0.001). The fit of M23 on the other hand is not as accurate, due to the higher coefficient of variation for the sorption parameters, but still acceptable, as the visual fit is good and the parameters do not deviate in great extent from run 1.

Table 7.1.4.2-34: Fitted parameters from M23 and M27 BTCs – Column 2

Compound	$DegT_{50}$ [d]			K_{om} [L kg^{-1}]			1/n [-]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]	mean	st. dev	CV [%]
M23 - run 1	26.36	-	-	1.02	-	-	0.985	-	-
M23- run 2	26.51	0.695	2.6	1.12	0.286	25.5	0.991	0.220	22.2
M27 - run 1	36.46	-	-	3.40	-	-	0.877	-	-
M27 - run 2	36.46	0.862	2.4	3.40	0.090	2.7	0.877	0.027	3.1

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

Table 7.1.4.2-35: Statistical parameter evaluation of the metabolites' BTCs in column 2

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*		
		$DegT_{50}$ [d]	K_{om} [L kg^{-1}]	1/n [-]
M23	18.66	<0.001	<0.001	<0.001
M27	13.10	<0.001	<0.001	<0.001

* p-values obtained for a 5% significance level

Mini-lysimeter Column 3

Step 1: bromide BTC – Column 3

The fitted parameters with their statistics are given in **Table 7.1.4.2-36** and Table 7.1.4.2-37: . The parameters are fitted with good accuracy (maximum coefficient of variation of <7%, p-values ≤ 0.001).

Table 7.1.4.2-36: Fitted parameters from bromide BTCs in column 3

Compound	D [m]			Θ_{sat} [m^3 m^{-3}]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]
Tracer (Br)	0.009	<0.001	4.2	0.380*	-	-
	K_{sat} [m d^{-1}]			Application rate [kg ha^{-1}]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]
	2.46	0.154	6.3	108.1	1.49	1.4

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)
* Parameter fixed

Table 7.1.4.2-37: Statistical parameter evaluation of the bromide BTCs in column 3

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*			
		D [m]	Θ_{sat} [m^3 m^{-3}]	K_{sat} [m d^{-1}]	Applica-tion rate [kg ha^{-1}]
Tracer (Br)	11.08	<0.001	-	<0.001	<0.001

* p-values obtained for a 5% significance level

Step 2: Metabolites' BTC – Column 3

The fitted parameters with their statistics are given in Table 7.1.4.2-38**:** and Table 7.1.4.2-39**:** . The parameters are fitted with good accuracy (maximum coefficient of variation of <4 %, p-values ≤ 0.001) for M27. Regarding M23, the boundary limits of the Freundlich exponent were reached, which means that no reasonable optimization could be realized.

Table 7.1.4.2-38: Fitted parameters from M23 and M27 BTCs – Column 3

Compound	$DegT_{50}$ [d]			K_{om} [L kg^{-1}]			1/n [-]		
	mean	st. dev	CV [%]	mean	st. dev	CV [%]	mean	st. dev	CV [%]
M23 - run 1	25.21	-	-	0.877	-	-	0.600	-	-
M27 - run 1	33.05	-	-	2.19	-	-	0.662	-	-
M27 - run 2	33.05	0.658	2.0	2.19	0.054	2.5	0.662	0.024	3.6

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

Table 7.1.4.2-39: Statistical parameter evaluation of the metabolites' BTCs in column 3

Compound	Min. error (err) at which χ^2 test is passed (5% sign. level)	t-test: p-values*		
		$DegT_{50}$ [d]	K_{om} [L kg^{-1}]	1/n [-]
M27	15.13	<0.001	<0.001	<0.001

* p-values obtained for a 5% significance level

Overview all columns

Table 7.1.4.2-40: Summary of DegT50 for Test item 1 (M23) and Test item 2 (M27)

Mini-lysimeter study	$DegT_{50}$ [d]	
	M23	**M27**
Column 1	-	39.64
Column 2	26.51	36.46
Column 3	-	33.05
Geometric mean	26.51	36.28

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

Table 7.1.4.2-41: Summary of K_{om} for Test item 1 (M23) and Test item 2 (M27)

Mini-lysimeter study	K_{om} [L kg^{-1}]	
	M23	**M27**
Column 1	-	5.51
Column 2	1.12	3.40
Column 3	-	2.19
Arithmetic mean	1.12	3.70

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

Table 7.1.4.2-42: Summary of Freundlich exponent (1/n) for Test item 1 (M23) and Test item 2 (M27)

Mini-lysimeter study	Freundlich exponent (1/n) [-]	
	M23	**M27**
Column 1	-	1.12
Column 2	0.991	0.877
Column 3	-	0.662
Arithmetic mean	0.991	0.887

st. dev = standard deviation
CV = coefficient of variation (= st. dev / mean *100%)

III. CONCLUSION

Degradation and sorption parameters of the metabolites M23 and M27 could reliably be determined by means of inverse modelling using breakthrough curves of mini-lysimeters including information of a conservative tracer.

CA 7.1.4.3 Field leaching studies

The mobility in soil of Dimethenamid-P was evaluated during the Annex I inclusion. No field leaching studies were performed.

STUDIES RELATED TO PLANTS FOR REFINEMENT OF EXPOSURE SCENARIOS

Three additional studies were performed focusing on the role that the agricultural plant themselves play in the risk assessment. The studies focus on plant intercept (and thus foliar DT50 values) as well as plant uptake of molecules from root proximal soil. This is realistic for Dimethenamid-P and its degradation products in particular considering that field studies show that compounds are not located below 30 cm and thus more likely to be taken up by the plants root system. Since there is no suitable Annex point, it was chosen to summarize these within the soil chapter, since it is the environmental assessment of the soil compartment we wish to refine.

Two dislodgeable foliar residue (DFR) studies were performed with the purpose of obtaining a dissipation rate from the surface of plant leaves (foliar DT50). This data will help to refine the aquatic exposure scenario for post-emergent applications by giving a more realistic picture of soil inputs from leaf surfaces.

In addition, a Plant Uptake Factor (PUF) study is presented. This study was run to provide further higher tiered data for refinements of PECgw calculations. The study was run with a representative metabolite of Dimethenamid-P, M656H027. The similarity of structures (all acidic side chains with minor differences) of M656H023, M656H027 and M656H031 allowed for a single metabolite to represent the three. Fundamental chemical principles (discussed below) demonstrate that M656H027 represents a “worst case” selection.

Plant Uptake Factor

Metabolites M23, M27 and M31 of Dimethenamid-P are considered in the risk assessment for groundwater. One factor which plays a vital role in refining the Predicted Environmental Concentrations (PECs) of metabolites reaching ground water is the plant uptake factor (PUF). A PUF study was performed for metabolite M27 for two major crops to which Dimethenamid-P is applied in the EU (maize and oilseed rape) and for various pHs, under greenhouse conditions.

Because of the structural similarity between the three metabolites we wished to choose a representative molecule to obtain a PUF value to be used in the PEC value refinement of all three metabolites. The only difference in the metabolites is the side chain (R1) and nature of the acidic moiety.

It has been shown that partitioning between solution and the lipophilic root system is an important mechanism for plant uptake. Briggs (1983) [*Briggs, G. G., Bromilow, R. H., Evans, A. A. & Williams, M. 1983. Relationships between lipophilicity and the distribution of non- ionised chemicals in barley shoots following uptakeby the roots. Pesticide Science 14, 492-500*] has shown that, for neutral molecules, a linear relationship exists between the Log K_{ow} and the absorption of chemicals to roots. It was also shown in this same body of work that polar and ionic compounds partitioned more weakly to root systems. When a molecule becomes ionic the K_{ow} partitioning coefficient becomes very small as the charged nature of the ion forces partitioning almost exclusively to water giving a very low K_{ow}.

Keeping this in mind we wished to choose a representative molecule that would give a "worst case" scenario. Following the logic of Briggs, this would be the molecule with the lowest K_{ow} or most ionic character. Again, the structural similarities and distinct differences in our molecules should be observed. The only difference in the molecules is the nature of their acidic side chains. M23 and M31 are carboxylic acids (pKa of acetic acid is 4.76) and a M27 is a sulfonic acid (pKa roughly -1.9). The compound that would be ionized to the greatest extent (and therefore least lipophilic) in all the proposed testing pH's is the sulfonic acid (M27). This molecule would never be protonated to a greater degree than 0.000004%. In contrast the carboxylic acids (M23, M31) would see their neutral (and most lipophilic species) up to 15% in the lowest pH. Therefore M27 is selected as representative to obtain a PUF by means of a PUF study.

Report:	CA 7.1.4.3/1 Gourlay V., 2013b Plant uptake of Dimethenamid-P metabolite M656PH027 (Reg.No. 360714) in corn and oil seed rape under greenhouse conditions 2013/1251908
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The purpose of this study was to determine the plant uptake factor (PUF) of soil metabolite M656PH027 (M27) of the herbicidal active substance Dimethenamid-P for use in the parameterization of leaching models. The study design was chosen in order to mimic the soil pore water that contains the test compound and to focus on the gross uptake of this compound with the soil pore water via the root system into the plant. Other processes influencing the concentration of the compound in soil, such as degradation or adsorption to soil particles were intentionally excluded in this test system. Corn and oilseed rape plants were investigated under three pH levels (5.5, 6.5 and 7.5) to estimate the potential influence of the crop type or the pH level on the uptake of the test item. The plants were kept for eight days in the test solutions with known concentration of the test item and volume of the solution. The variation of these two parameters allowed calculating the PUF.
The radiochemical purity of both test items in solution was stable under the applied test conditions so that the plant uptake factors calculated corresponded solely to the investigated test items. No glass adsorption, volatilisation or direct evaporation was detected during the course of the experiment. Volume and concentration could therefore be used to determine the plant uptake factors. Water consumption after eight days was sufficient, between 118 and 329 mL. Similar volumes were determined with the corresponding plants in the non-treated solutions, indicating no influence of the test item.

The radioactivity measurements of the final compartments (plant material, solution at the end of the test, root washing solution) showed that the test protocol allows the recovery of most of the test item given to the system on application, and that the test item was transferred to the shoots and leaves of the plants.

The pH level had no significant effect on the PUF. Overall, PUF values determined in solution were between 0.7 and 1.1.

I. MATERIAL AND METHODS

Test and reference items

The test item [thienyl-5-^{14}C] M650PH027 (synonym M27) and its non-labelled reference item were provided by the sponsor. The reference item was used to confirm the identity of the test item by means of co-radio-HPLC. Details of the HPLC method are given in in the section on HPLC. The information concerning the test and reference items, such as purity, structure, specific activity, position of radioactive label were given by the sponsor and is documented in the raw data.

Test Material	Dimethenamid-P (BAS 656 H)
Reg.No.:	363851
Chemical name:	(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Molar mass:	275.8 g/mol (non-labelled)
Molecular formula:	$C_{12}H_{18}ClNO_2S$

Labelled test item

Label:	thienyl-5-^{14}C
Specific radioactivity:	8.21MBq/mg
Radiochemical purity:	99.0 %

Test System

Cultivation of the test plants (non-GLP)

Corn *"Ronaldinio"* and oil seed rape "*Primavera*" plants were cultivated under greenhouse conditions for seven weeks (non-GLP). Pots (ø = 13 cm) with removed bottoms, placed on trivets and filled with very silty sand were used to avoid root damage during the later withdrawal. For each plant, eight seeds per pot were sown between 0.5 and 1 cm depth into the soil. The temperature, air humidity and light regime were established as given in Table 7.1.4.3-1.

Table 7.1.4.3-1: Greenhouse conditions during plant cultivation (non-GLP)

Temperature (daily mean value)	22 °C ± 10 °C
Air humidity (daily mean value)	70% ± 25%
Light regime	16 h light / 8 h dark
(additional artificial light when daylight was less than 5 klx: sodium light SON-T Agro 400W)	
Soil medium and fertilization: Very silty sand, regularly irrigated with liquid fertilizer (0.5% Flory 9-Hydro)	

Prior to placing the plants into the test and control plants vessels, the loamy sand was completely removed by gently showering the roots with water.

The plants were kept for about one hour in a non-buffered $CaCl_2$ solution without test item, before inserting them into the corresponding test and plant control solutions, in order to let them slowly adapt to the change of root medium. The number of plants per vessel was chosen in order to have similar fresh biomasses. The exact biomasses were determined gravimetrically by weighting the test systems before and after inserting the plants.

The opening between the plant stems and the glass wall was covered with Parafilm® to prevent direct evaporation from the solution. The whole setup was placed in a greenhouse.

The plant development stages of each test plant, on application, are given in **Table 7.1.4.3-2**.

Table 7.1.4.3-2: BBCH and fresh biomass on application

Crop	BBCH	Fresh biomass [g]
Corn	32	179 – 204
Oilseed rape	32	55 – 85

Test system for PUF Test

The test system consisted of a 1 L brown glass test vessel filled with 1000 mL of 0.01 M $CaCl_2$ buffered solution. Brown glass was used in order to avoid algae growth and to exclude photolytic transformation processes. The diameter of the opening at the top of the glass vessel was sufficient to insert the plant roots without damage. Furthermore, the shape of the vessel ensured that during the experimental period the roots were freely suspended in the $CaCl_2$ solutions to enable continuous root exposure to the test solution.

The opening around the plant stems was covered with Parafilm® in order to prevent photolytic degradation, evaporation and algae growth. The whole setup was placed in a greenhouse at similar temperature and air humidity conditions used for the growth period in order to avoid stress for the test plants.

The mean concentration over all applied vessels were found to be 100.5 $\mu g.L^{-1}$ (CV = 0.9%). Individual values are given in Table 7.1.4.3-5**:** and Table 7.1.4.3-6**:** .

Control solutions

For each crop and pH level an *untreated plant control* (three replicates) was prepared in order to monitor the influence of the test item on the volume uptake of solution and on the variation in biomass during the test period. The vessels were filled with 1000 mL of the corresponding buffered 0.01 M $CaCl_2$ solution and plants were inserted in such numbers that each vessel contained an almost equal amount of fresh plant biomass.

A *stability control* without plants was prepared for each pH level in order to monitor the stability of the test item under actual test conditions (one replicate). For this a vessel was filled with 1000 mL of buffered solution and treated with the test item. The vessel was sealed with Parafilm®, following the same application procedure as described above.

An *evaporation control* was prepared for each pH level in order to monitor the loss of water by direct evaporation in the absence of plants (one replicate). For this a vessel was filled with 1000 mL buffered solution and sealed with Parafilm® containing a hole about the same size as the one in the test vessels with plants.

Overview of the test chamber
The following Table 7.1.4.3-3 gives an overview of the whole testing scheme.

Table 7.1.4.3-3: Overview of the testing scheme for corn and oil seed rape

	pH 5.5			pH 6.5			pH 7.5			Sum of test vessels
	NP	C	OSR	NP	C	OSR	NP	C	OSR	
with test item										
Test solutions (^{14}C)	-	3	3	-	3	3	-	3	3	21
Stability controls (^{14}C)	1	-	-	1	-	-	1	-	-	
without test item										
Untreated plant controls	-	3	3	-	3	3	-	3	3	21
Evaporation controls	1	-	-	1	-	-	1	-	-	
Sum of test vessels	14			14			14			42

NP: no plants, C: Corn, OSR: Oilseed rape

Incubation and Sampling

Incubation conditions
The test was conducted under greenhouse conditions for eight days. The daily cultivation conditions in the greenhouse were set up to achieve a mean daily temperature of 22 °C (recorded hourly in addition to the air humidity with a Tinytag temperature/humidity logger), with a light regime corresponding to 16 h light / 8 h dark (recorded with a Hobo® light logger [λ = 400–700 nm]). Additional artificial light was supplied when natural daylight was less than 5 klux (sodium light SON-T Agro 400W).

Sampling
The plants were kept in their respective test solutions for eight days. Two intermediate samplings were performed after two and five days. The remaining volumes of the solutions were determined by weighting the test system and subtracting the masses of the test vessel, the initial fresh biomass of the plants and the Parafilm® cover. It was assumed that the mass density of the $CaCl_2$ solution was equivalent to water ($\rho = 1000$ g L^{-1}) and that the fresh biomass of the plant remained constant over time. From each test solution two 250 µL aliquots were measured by LSC to determine the concentration of the test item in solution.

After eight days, at the end of the incubation period, the plants were carefully removed from the test vessels. The test solution that retained on the root system was collected by placing the roots in a funnel and letting the excess solution drip into the corresponding test vessel for about ten minutes. Subsequently, the roots were carefully dried with tissue paper.

In order to quantify the amount of test item adsorbed on the root system the roots were shaken in 250 mL of water/acetonitrile (1:1, v/v) solution for three minutes. The washing solution was then filtrated and two 5 mL aliquots were analysed by LSC to determine the amount of radioactivity.

The remaining test solutions were transferred into tarred polyethylene flasks and weighted. Two 250 µL aliquots were measured for radioactivity by LSC to determine the concentration of the test item. For each pH level, a 2 mL aliquot was taken from one plant replicate and from the stability control to determine the radiochemical purity of the test item by means of radio-HPLC.

The plant material was fractionated into root and stem/leaves system. Fresh weights were determined for each fraction and the separated roots and stem/leaves were freeze dried. The plant materials were then homogenized using a ball mill and aliquots of the powders were combusted in a sample oxidizer and analysed for radioactivity via LSC.

A radioactive mass balance was compiled taking into account the test solution, the root washing solution and the plant material. As all recoveries were found to be > 90%, no further analysis of the tissue paper or the filter was pursued.

Accompanying Measurements and Photo Documentation
During the test phase the pH level, redox potential and oxygen saturation of the non-treated $CaCl_2$ solutions were determined every day during the week. Throughout the experimental period, the plant development stage, plant health and the condition of the root system were documented by photos, including the control plants.

Analytical methods
Liquid Scintillation Counting (LSC)
Liquid specimens were measured using a liquid scintillation counter (TRI-CARB 2550 TR/LL or TRI-CARB 2300 TR; Canberra Packard Corp.) employing Ultima Gold™ as scintillator.

High Performance Liquid Chromatography (HPLC)
The following HPLC method was used for the identification of M656PH027 by co-chromatography with the reference item and to determine the radiochemical purity of the test item in the stock solution and in the applied vessels after eight days of incubation.

Mixer	Jasco LG 980-02 S, Jasco Germany GmbH.
Pump	Jasco Gradient Pump PU-980, Jasco Germany GmbH.
Auto Injector	Jasco Autosampler AS-1555, Jasco Germany GmbH.
Detectors	Radio-HPLC-Detector A-525 AX, Canberra Packard Corp.
	UV-Detector Jasco UV-1575
Column	Beckman Coulter Ultrasphere RP18; 250 x 4.6 mm; 5µm
Pre-Column	Phenomenex Octadecyl Security Guard; 4 x 3 mm
Mobil phase A	0.01 M NaH_2PO_4, pH 2.0 – 2.3 (H_3PO_4)
Mobil phase B	Methanol
Flow rate	1.00 mL min^{-1}
UV	230 nm

Gradient:

Time [min]	% A	% B
0	90	10
15	65	35
35	30	70
36	20	80
40	20	80
41	90	10
48	90	10

Data evaluation

PUF values were calculated for each test vessel according to the equation given in Equation 7.1.4.3-1. A statistical evaluation was performed on the PUF values and volume uptake values in order to investigate potential influences of the test item and pH levels. All statistical tests were obtained by using R statistical system and Excel® functions.

Equation 7.1.4.3-1

$$PUF = \frac{\ln\left(\frac{m_{solution-8}}{m_{solution-0}}\right)}{\ln\left(\frac{V_{solution-8}}{V_{solution-0}}\right)} \quad [-]$$

With

PUF	plant uptake factor	[-]
$m_{solution-8}$	mass of test item remaining in test solution after eight days	[µg]
$V_{solution-8}$	remaining volume of test solution after eight days	[L]
$m_{solution-0}$	initial mass of test item	[µg]
$V_{solution-0}$	initial volume of test solution	[L]

II. RESULTS

Characterisation of the test system and test conditions

Test conditions in the chamber over eight days

The study was conducted under greenhouse conditions for eight days. The daily cultivation conditions in the greenhouse are given in **Table 7.1.4.3-4**.

Table 7.1.4.3-4: Test chamber conditions

	Mean	Min	Max
Temperature	20.8 °C	15.8 °C	32.3 °C
Air humidity	59 %	27 %	86 %
Luminance intensity (6:00 am – 9:00 pm)	16.0 klux h^{-1}	13.7 klux h^{-1}	20.6 klux h^{-1}

The greenhouse conditions were kept in a constant range throughout the experimental period.

Plant conditions over the eight days

The fresh biomass of the test plants was determined at the start and at the end of the test period. The fresh biomass was 56.8% to 84.3% of the initial biomass for all crop types and treatments. No substantial differences were observed between treated and control test systems, indicating that there was no significant effect of the test item on plant behaviour.

Independent of crop type, plant tissue of older leaves partly turned yellow by the end of the test period, presumably due to senescence processes and allocation of plant nutrients towards younger plant tissues. The observed decrease in total fresh biomass may have been induced by plant stress due to the transfer of the plants from soil to pseudo-hydroponic nutrient-free medium and the resulting differences in the physical and nutritional environment of the root system. Further, as test vessels were sealed with Parafilm® and the solutions were not continuously aerated, the root system may have been affected by a lack of oxygen towards the end of the test period. Nevertheless, all test plants showed sufficient water uptake during the eight days.

Water consumption over eight days

One important pre-condition for the determination of a reliable PUF is a sufficient volume of water used as transport media for the test item from the artificial soil solution into the plants. A substantial uptake of water is required to ensure that differences in the test concentrations in the solution remaining in the vessel can be determined with accuracy (ideally volume uptake > 15% of the initial volume). The concentration changes are the basis for determining the plant uptake factor.

Time courses of the volume of each solution over the time of experiment are given in Table 7.1.4.3-7**:** and Table 7.1.4.3-8**:** . Data for the cumulative volume uptake for each crop and pH level (see Tables 11 and 12 of the original report) show a volume uptake from 149–315 mL for all treated plants over the eight day period. Thus, the requirement of a minimum volume uptake to obtain a sufficient precision in the calculation of the PUF was met. For all experiments the plants continued to consume water until the last sampling day.

Impact of the test variables on the cumulative volume uptake

An analysis of variance was performed over crop type, pH levels and treatment (treated or control solution).

- pH level

No significant differences in the volume uptake were observed between pH levels (see Tables 11 and 12 of the original report).

- Test item
No significant differences were observed between the volume uptake of treated and control test systems, indicating that there is no effect of the test items on the volume uptake (see Tables 11 and 12 of the original report).

- Crop type
A significantly higher uptake of water per initial fresh biomass was observed for oil seed rape compared to corn (see Tables 13 and 14 of the original report) which presumably resulted from the natural variation in water needs of the different crops and growth stages.

Direct evaporation from solution (evaporation controls)
In order to ensure that the volume loss in the different vessels was only a result of the plant transpiration, the volume of the evaporation control solutions without plants was recorded over time. For all control solutions, the water loss by direct evaporation was ≤ 1.1%, indicating that the variation in volume observed with plants can be attributed primarily to water consumption by the respective plants.

Parameters in the non-treated solutions over eight days (plant and evaporation controls)
- pH level
The pH values of the plant control solutions and of the evaporation control solutions were measured over time to confirm stable pH levels. Overall, the pH levels of the buffered solutions with plants were steady over time. For the incubations with corn, the pH 7.5 level decreased slightly. Nevertheless, the mean pH over eight days was close to pH 7. The pH level of the evaporation controls, without plants, showed low variation over the eight days.

- Internal temperature
As a coupled measurement with the pH and the oxygen saturation, the internal temperature of the solutions was determined and ranged between 22.6 °C and 24.0 °C. These values were in the same range as the air temperature.

- Oxygen saturation
The hydroponic-like test system used in this study may lead to oxygen deficiencies in the root system. However, aeration of the test solutions could not be carried out since this would have caused substantial evaporation losses that would conflict with the determination of the uptake volume. Air exchange of the test solutions was moreover limited due to the Parafilm® cover. Despite lower oxygen levels in the root zone sufficient water uptake was confirmed, indicating adequate plant health during the experimental period.

- Redox potential
The redox potential of a given soil or sediment is a measure of its anaerobiosis. Aerated soils have redox potentials in the range of +400 to +700 mV and anaerobic conditions display redox potentials from +400 to as low as -300 mV (Pt electrode). Independent of crop type and pH level, measured redox potentials during the experiment were always < 250 mV indicating anaerobic conditions in the root zone of the test plants. Analogous to the low oxygen content of the solutions the anaerobic conditions did not limit sufficient water uptake indicating adequate plant health.

Characterization of plant uptake behaviour

Radioactive mass balance

In order to validate the study design, a radioactive mass balance was established at the end of the study. After eight days the sum of all processed fractions and solutions yielded in recoveries between 93.4% and 98.7% of applied radioactivity for both crops. A large proportion of the radioactivity was detected in the test solution and the plant parts. No substantial losses were observed during the experiment (volatilization, glass adsorption) or during the analysis of the plant material (homogenization, combustion). The test protocol is therefore well adapted to retrieve most of the radioactivity applied to the system.

Stability of the test item in the test solutions

The radiochemical purity of the test item was assessed before the application by radio-HPLC analysis of the application solution. To confirm the stability of the test item in the buffered solutions during the experiment the radiochemical purity of the test item in solution was determined at the end of the incubation time for each test solution with and without plants. With an initial purity of the application solution of 100%, the purity of the test item in solution after eight days remained at 100% in the stability control solutions without plants as well as in the test solutions with plants. This confirmed that the test item was stable in the test solutions over time and that the radioactivity measured in the solutions and taken up by the plants corresponded exclusively to the test item.

Glass adsorption of test item (stability controls)

The radioactivity in solution was measured in the stability controls without plants in order to ensure that there was no loss of test item by glass adsorption or volatilization and that the variation was only due to plant uptake and/or root adsorption. The variations observed after eight days for all pH levels indicate that possible losses from glass adsorption or from volatilization were negligible. This confirmed that the variation in concentration measured in the test solutions with plants corresponded predominantly to plant uptake.

PUF

It is important to provide a derivation on the PUF, but since it is very detailed we refer to Appendix 2 of the original study. The most important part is the mathematical description determining the PUF (Equation 7.1.4.3-1).

The concentrations and water volumes in the test solution are provided for both crops in the tables below, the mass can be derived from the concentration in conjunction with the volume of water. Please refer to Appendix 2 of the original study for an example derivation.

Table 7.1.4.3-5: Concentration of M656PH027 in the test solution over eight days (corn)

pH	Replicate	Concentration in solution [µg L^{-1}]			
		Day 0	*Day 2*	*Day 5*	*Day 8*
5.5	a	100.2	95.0	96.9	101.3
	b	101.2	97.6	97.9	100.8
	c	101.3	97.4	100.2	107.4
	Mean	**100.9**	**96.7**	**98.3**	**103.2**
	CV	*0.6%*	*1.5%*	*1.7%*	*3.6%*
6.5	a	100.9	97.3	99.5	106.6
	b	100.6	96.3	95.9	103.1
	c	101.5	97.1	96.0	106.0
	Mean	**101.0**	**96.9**	**97.1**	**105.2**
	CV	*0.5%*	*0.5%*	*2.1%*	*1.8%*
7.5	a	101.3	97.1	99.9	108.9
	b	100.7	96.8	98.2	107.2
	c	100.4	97.4	99.7	108.1
	Mean	**100.8**	**97.1**	**99.3**	**108.1**
	CV	*0.5%*	*0.3%*	*0.9%*	*0.8%*

Table 7.1.4.3-6: Concentration of M656PH027 in the test solution over eight days (oil seed rape)

pH	Replicate	Concentration in solution [µg L^{-1}]			
		Day 0	*Day 2*	*Day 5*	*Day 8*
5.5	a	100.6	98.1	100.5	98.8
	b	97.4	97.7	100.4	99.4
	c	100.7	98.3	99.5	97.8
	Mean	**99.6**	**98.0**	**100.1**	**98.7**
	CV	*1.9%*	*0.3%*	*0.6%*	*0.8%*
6.5	a	101.1	100.6	101.1	98.7
	b	100.7	100.1	101.8	100.5
	c	101.0	100.3	99.0	97.8
	Mean	**100.9**	**100.3**	**100.6**	**99.0**
	CV	*0.2%*	*0.3%*	*1.4%*	*1.4%*
7.5	a	100.2	99.5	100.2	100.3
	b	101.6	100.8	102.1	101.2
	c	101.0	99.8	101.2	99.8
	Mean	**100.9**	**100.0**	**101.2**	**100.4**
	CV	*0.7%*	*0.7%*	*0.9%*	*0.7%*

Table 7.1.4.3-7: Time course of the volume of test solutions over eight days (corn)

pH	Replicate	Volume of test solution [mL]			
		Day 0	*Day 2*	*Day 5*	*Day 8*
5.5	a	999.4	866.1	776.3	737.4
	b	998.7	864.4	775.4	728.9
	c	999.0	884.8	813.8	738.1
	Mean	**999.0**	**871.8**	**788.5**	**734.8**
	CV	*0.0%*	*1.3%*	*2.8%*	*0.7%*
6.5	a	999.2	856.6	758.5	688.1
	b	998.4	868.2	790.3	734.7
	c	998.7	846.3	750.6	690.8
	Mean	**998.8**	**857.0**	**766.5**	**704.5**
	CV	*0.0%*	*1.3%*	*2.7%*	*3.7%*
7.5	a	998.8	863.2	782.9	700.5
	b	1002.0	836.8	745.7	671.4
	c	999.7	861.4	764.7	677.6
	Mean	**1000.2**	**853.8**	**764.4**	**683.1**
	CV	*0.2%*	*1.7%*	*2.4%*	*2.2%*

Table 7.1.4.3-8: Time course of the volume of test solutions over eight days (oilseed rape)

pH	Replicate	Volume of test solution [mL]			
		Day 0	*Day 2*	*Day 5*	*Day 8*
5.5	a	1002.6	912.3	837.0	820.8
	b	999.8	866.3	797.2	790.6
	c	999.2	897.9	824.7	817.4
	Mean	**1000.5**	**892.2**	**819.6**	**809.6**
	CV	*0.2%*	*2.6%*	*2.5%*	*2.0%*
6.5	a	998.7	913.4	867.5	852.5
	b	998.8	902.9	847.0	811.0
	c	998.9	930.0	892.5	879.1
	Mean	**998.8**	**915.4**	**869.0**	**847.6**
	CV	*0.0%*	*1.5%*	*2.6%*	*4.0%*
7.5	a	999.7	887.0	814.1	774.8
	b	999.1	902.3	824.0	776.3
	c	998.9	876.5	804.3	774.3
	Mean	**999.2**	**888.6**	**814.1**	**775.1**
	CV	*0.0%*	*1.5%*	*1.2%*	*0.1%*

Over all pH levels, the mean PUF values for oil seed rape ranged from 0.99 to 1.01, and from 0.72 to 0.81 for corn (Table 7.1.4.3-9**:**).

Table 7.1.4.3-9: Average plant uptake factors after eight days

pH	Corn		Oilseed rape	
	PUF	CV	PUF	CV
5.5	0.81	13.7%	1.00	12.1%
6.5	0.77	0.7%	1.09	9.0%
7.5	0.72	5.3%	0.99	3.0%

The possible impact of test parameters such as crop type and pH level was statistically evaluated. There was no significant effect of the pH level on the plant uptake factor values, but there was a significant difference between crop types.

III. DISCUSSION AND CONCLUSION

A simple standardized test system with intact plants was successfully applied to determine reliable plant uptake factors (PUFs) for metabolite M656PH027 (M27) of Dimethenamid-P for two crops and for three different pH levels in an artificial pore water solution.
For the intended use in corn the mean plant uptake factor over all pH levels and replicates was 0.77 (CV 9.3%). For the intended use in oil seed rape the plant uptake over all pH levels and replicates was 1.03 (CV 9.1%).
The pH level of the buffered solution did not show any direct influence on the PUF. The experimental setup combined with appropriate statistical tools was suitable to identify potential pH effects caused by a pH-dependent behaviour of the test item.

The test system described in this report is suitable for the experimental determination of PUF to replace conservative PUF default values by higher tier compound specific factors. Independent from crop type, the experiments showed that the average PUF for M27 is larger than any default values proposed for simulating plant uptake.

Report:	CA 7.1.4.3/2 Friedemann A.,Teresiak H., 2014b Amended report - Determination of dislodgeable foliar residues of Dimethenamid-P (BAS 656 H) and determination of foliar DT 50 after application of BAS 656 12 H to corn, 2013 2014/1036905
Guidelines:	EPA 875.2100 (1996), Guidance for the determination of dislodgeable foliar residue (California EPA Rev. 1 February 2002)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

The objective of this study was to determine the magnitude of dislodgeable foliar residues of Dimethenamid-P resulting from a single application of BAS 656 12 H to corn. The test item was applied to corn plants (*Zea mays L.*) grown in pots in a greenhouse using spraying equipment that simulated a commercial application to corn. The amount of dislodgeable foliar residues was determined. The data are required for worker re-entry risk assessments and for the determination of residue deposit half-life values on corn leaves.
The maximum recommended application rate is 1.2 L/ha BAS 656 12 H. 88 % were actually applied in a water volume corresponding to about 177 L/ha. The crop growth stage at the application time was BBCH 17-18. Leaf discs were sampled for dislodging approximately 0 - 2, 6, 24 and 48 hours after application and at 4 and 7 days after the application. The amount of dislodgable residues was calculated per leaf surface area as well as per leaf weight.
Dislodging of leaf residues was performed by means of rinsing the leaf discs with 100 mL dislodging solution (Water / Aerosol OT-B (0.01%)) twice for 10 minutes respectively. Each dislodging event was performed on five replicate leaf specimens (using 20 leaf discs randomly sampled from the treated crop).
Analytical determination of Dimethenamid-P (BAS 656 H) in the leaf discs dislodging solutions was performed by HPLC-MS/MS.

The analytical results of field fortification experiments (87 % mean recovery) show that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.
The mean Dimethenamid-P (BAS 656 H) residues dislodgeable from corn leaves are summarized below.

Table 7.1.4.3-10: Summarized results – BAS 656 H in dislodging solutions

Dislodging event	BAS 656 12 H [mg/specimen]*	Relative Standard Deviation
0-2 (+/-30 min) HALA	0.465	12 % (n = 5)
6 (+/-30 min) HALA	0.285	11 % (n = 5)
24 (+/-2 h) HALA	0.059	13 % (n = 5)
48 (+/-4 h) HALA	0.009	25 % (n = 5)
4 DALA	< LOQ	-- (n = 5)
7 DALA	< LOQ	-- (n = 5)

*specimen = 200mL dislodging solution of 20 leaf discs
HALA: Hours after last application
DALA: Days after last application
< LOQ: below limit of quantitation of 0.001 mg/specimen

Foliar DT_{50} and DT_{90} values were estimated from residue measurements of the a.s. Dimethenamid-P (BAS 656 H) on the leaf surfaces of corn. Dimethenamid-P dissipates rapidly from the leaves with a DT_{50} of 0.36 days, in other words, with a DT_{50} < 9 hours.

I. MATERIAL AND METHODS

Test item

Table 7.1.4.3-11: Test item

Name of product:	**BAS 656 12 H**			
Batch No.:	0004701751			
Active Ingredient	Name	BAS Code	nominal	analyzed
	Dimethenamid-P	BAS 656 H	720.0 g/L	707.8 g/L
Formulation Type:	EC			
Density	1.119 g/cm^3			
Storage Advice:	Keep at ambient temperature (+5 to +30°C)			
Certificate of analysis:	346279 35 (February 23, 2012)			
Expiration date:	February 28, 2014			
Chemical stability in water and	sufficient for planned trial conditions			
Safety Precautions	see MSDS			

Test System

The test system consisted of corn plants (*Zea mays L.*) grown and maintained in plastic pots (12 cm in diameter) with commercial potting mixture (Floradur) in the greenhouse. The seeds were sown on 21st August 2013. At application the corn plants were at BBCH 17-18 and approximately 86 cm high on average. The plant density was one plant per pot.
All pots used for the study were identified by labels specifying the trial number and the treatment group (control or treated).

Table 7.1.4.3-12: Treatment regime

Treatment description	Plot	Purpose	Number of pots	Plant pot IDs
Untreated	1	Control specimens	5	Plot 1
Treated	2	Treated specimens	61	Plot 2
Untreated	3	Field fortifications	20	Plot 3

Pesticide History and Crop Maintenance

During the trial no additional pesticides were applied. After application the plants were cultivated in a greenhouse at defined environmental conditions (daily mean air temperature ranged between 17.9 and 20.5°C, extremes 13°C and 25°C , daily mean relative humidity ranged from 68.9 to 79.3 %, extremes 45 % and 91 %) one day before application to last sampling.

The application equipment operated with constant speed and fixed pressure to simulate typical agricultural conditions. The application equipment was calibrated prior to the application and the obtained data were used to calculate the mixing ratio of BAS 656 12 H with water to meet the application parameters specified in Table 7.1.4.3-13: .

Table 7.1.4.3-13: Nominal treatment volume and rate

	Plot 1 (untreated control) Plot 3 (field fortification,	**Plot 2 (treated)**
Application volume	-	approx. 200 L/ha tap water
Application rate BAS 656 12	-	1.2 L/ha
Application rate BAS 656 H	-	864 g a.i./ha

A total of 61 plant pots were treated with BAS 656 12 H. The applied amount of test item BAS 656 12 H was 88 % of the intended amount.

Sampling
For each sampling event 20 leaf discs (200 cm^2; both leaf sides) were collected randomly from the 5th and 6th leaf which were not covered by other leaves at application time using a leaf puncher (1 inch in diameter). The leaf discs were collected directly into a pre-labeled wide mouth jar (Nalgene®, PC, 125 mL) and subsequently transferred at ambient temperature to the *agro-check* laboratory for weighing and dislodging procedure.
Before application the leaf discs of untreated plants (S1, control and field fortification specimens) were sampled.
Within two hours after application once the spray deposit had fully dried sampling S2 was performed for plot 2. At sampling events S2 to S7 the leaf discs were randomly sampled from 10 treated plants each as described above.

Table 7.1.4.3-14: Target sampling parameters

Sampling No.*	Plot	Timing	Description
S1	1	BA	Control specimens to be collected before the application 1 x 20 leaf discs
S1	3	BA	Field fortifications to be collected before the application 7 x 20 leaf discs
S2	2	0-2 (+30 min) HALA	Specimens of treated crop approximately 2 hours subsequent to the application (once the spray deposit has fully dried). 5 x 20 leaf discs
S3	2	6 (+/-30 min) HALA	Specimens of the treated crop approximately 6 hours subsequent to the application 5 x 20 leaf discs
S4	2	24 (+/-2 hours) HALA	Specimens of the treated crop approximately 24 hours subsequent to the application 5 x 20 leaf discs
S5	2	48 (+/-4 hours) HALA	Specimens of the treated crop approximately 48 hours subsequent to the application 5 x 20 leaf discs
S6	2	4 DALA	Specimens of the treated crop approximately 4 days subsequent to the application 5 x 20 leaf discs
S7	2	7 DALA	Specimens of the treated crop approximately 7 days subsequent to the application 5 x 20 leaf discs

* sampling no. not identical with FREDDY sampling no.
BA Before application;
HALA Hours after last application;
DALA Day after last application

Leaf dislodging procedure

Control specimen and specimen of treated plants

Exactly 20 leaf discs were used for the dislodging procedure of one specimen. 100 mL of the dislodging solution (Water / Aerosol OT-B (0.01%)) was added and the pre-labeled wide mouth jar containing the leaf discs was subsequently transferred to a reciprocating shaker. The jars were shaken at shaking level 2 for a period of 10 minutes. The dislodging solution was then decanted into a beaker and the jar with the leaf discs was subjected to a second dislodging process with another 100 mL of fresh dislodging solution (level 2, 10 minutes). The two dislodging solutions were pooled in the beaker and thoroughly mixed.

Fortification samples

Two fortification solutions at different concentrations containing Dimethenamid-P (BAS 656 H) as outlined in Table 7.1.4.3-15 were prepared at the analytical test site and subsequently sent to *agro - check* on dry ice. The fortification experiments with Dimethenamid-P (BAS 656 H) were performed at 0 DALA before application of test item. To prepare the fortification samples the same routine in washing corn plant discs was used as described.

Table 7.1.4.3-15: Fortification samples

Matrix	DFR Dislodging Solution [mL]	No. of Repl.	LOQ (proposed) [µg/L]	Fortification Level	Fortification solution [mL]	Conc. of solution [µg/mL]
Fortification Control	20	1 [1)]	5	control	-	-
Fortification Low	20	3 [2)]	5	10 x LOQ	0.5	2
Fortification High	20	3 [3)]	5	1000 x LOQ	0.5	200

1) L1307280032
2) marked with A, B and C – L1307280033, L1307280034, L1307280035
3) marked with A, B and C - L1307280036, L1307280037, L1307280038

II. RESULTS AND DISCUSSION

An overview over the results are given in Table 7.1.4.3-16**:** .

Table 7.1.4.3-16: Summarized results – BAS 656 H in dislodging solutions

Matrix	Timing	Mean concentration of analyte BAS 656 H		
		[mg/specimen]*	**[µg/cm^2]**	**[mg/g leaf]**
Mean	Control (BA)	0.000	0.000	0.00
Mean	0 - 2 HALA	0.465	2.323	0.212
RSD		11.8 % (n=5)	11.8 % (n=5)	14.1 % (n=5)
Mean	6 HALA	0.285	1.427	0.136
RSD		11.1 % (n=5)	11.1 % (n=5)	11.3 % (n=5)
Mean	24 HALA	0.059	0.296	0.028
RSD		12.5 % (n=5)	12.5 % (n=5)	14.5 % (n=5)
Mean	48 HALA	0.009	0.046	0.004
RSD		24.7 % (n=5)	24.7 % (n=5)	23.4 % (n=5)
Mean	4 DALA	< LOQ	--	--
RSD		--	--	--
Mean	7 DALA	< LOQ	--	--
RSD		--	--	--

*specimen = 200mL dislodging solution of 20 leaf discs
BA: Before application
HALA: Hours after last application
DALA: Days after last application
< LOQ :below limit of quantitation of 0.001 mg/specimen

The analytical results of the fortifications presented in Table 7.1.4.3-17**:** show that frozen storage and transport did not influence the analytical results of this study.

Table 7.1.4.3-17: Summarized results of the field fortification experiments with BAS 656 H

Fortification levels	Recovery range (%)	Mean recovery ± RSD (%)
10 x LOQ 1000 x LOQ	70.1 – 95.5	86.6 ± 13 (n=6)

Foliar DT50 Results

Foliar DT50 and DT90 values were estimated from residue measurements of the a.s. Dimethenamid-P on the leaf surfaces of corn. Dimethenamid-P dissipates rapidly from the leaves with a DT50 of 0.36 days, in other words, with a DT50 < 9 hours.

Table 7.1.4.3-18: Statistical and visual assessment of the SFO kinetic model and the DT50 and DT90 values for Dimethenamid-P

Leaf	^{2} error [%]	type I error rate (Prob. > t)	Visual fit	$DisT_{50}$ [d]	$DisT_{90}$ [d]
Corn	2.86	k: < 0.001	excellent	0.36	1.21

Kinetic evaluation was performed in order to derive dissipation rates of Dimethenamid-P on the leaf surfaces of corn (Foliar DisT50/DisT90). Despite the fact that no guidance on how to derive dissipation rates from such studies is available, the kinetic analysis and calculations of DisT50 and DisT90 values were performed following the recommendations of the FOCUS Kinetics workgroup. 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 were set to 0.00001 and 100, respectively.

III. CONCLUSION

The objective of this study was to determine the magnitude of dislodgeable foliar residues of Dimethenamid-P (BAS 656 H) resulting from a single application of BAS 656 12 H to corn. The test item was applied to corn plants (*Zea mays L.*) grown in pots in a greenhouse using spraying equipment that simulated a commercial application to corn. The amount of dislodgeable foliar residues was determined. The data are required for worker re-entry risk assessments and for the determination of residue deposit half-life values on corn leaves.

The analytical results of field fortification experiments (87 % mean recovery) show that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.
The mean Dimethenamid-P (BAS 656 H) residues dislodgeable from corn leaves at 0 - 2 hours after the application were 0.465 mg/specimen, 6 hours after the application 0.285 mg/specimen were determined, 24 hours after the application the residues were 0.059 mg/specimen, 48 hours after the application 0.009 mg/specimen were determined and at 4 and 7 DALA the residues were < LOQ.
Foliar DT50 and DT90 values were estimated from residue measurements of the a.s. Dimethenamid-P (BAS 656 H) on the leaf surfaces of corn. Dimethenamid-P (BAS 656 H) dissipates rapidly from the leaves with a DT50 of 0.36 days, in other words, with a DT50 < 9 hours.

Report:	CA 7.1.4.3/3 Friedmann A.,Teresiak H., 2014c Amended report - Determination of dislogdeable foliar residues of Dimethenamid-P (BAS 656 H) and determination of foliar DT 50 after application of BAS 830 01 H to oilseed rape, 2013 2014/1036906
Guidelines:	EPA 875.2100 (1996), Guidance for the determination of dislodgeable foliar residue (California EPA Rev. 1 February 2002)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

The objective of this study was to determine the magnitude of dislodgeable foliar residues of Dimethenamid-P resulting from a single application of BAS 830 01 H to oilseed rape. The test item was applied in a spray chamber to oilseed rape plants (*Brassica napus L.*) grown in pots in a greenhouse. The using spraying equipment simulated a commercial application to oilseed rape. The amount of dislodgeable foliar residues was determined. The data are required for worker re-entry risk assessments and for the determination of residue deposit half-life values on oilseed rape leaves.
Oilseed rape plants were treated with BAS 830 01 H. The maximum recommended application rate is 1.5 L/ha BAS 830 01 H in a water volume corresponding to about 200 L/ha. The crop growth stage at the application time was BBCH growth stage 16. Leaf discs were sampled for dislodging approximately 0 - 2, 6, 24 and 48 hours after application and at 4 and 7 days after the application. The amount of dislodgable residues was calculated per leaf surface area as well as per leaf weight.
Dislodging of leaf residues was performed by means of rinsing the leaf discs with 100 mL dislodging solution (Water / Aerosol OT-B (0.01%)) twice for 10 minutes respectively. Each dislodging event was performed on five replicate leaf specimens (using 20 leaf discs randomly sampled from the treated crop). Analytical determination of Dimethenamid-P in the leaf discs dislodging solutions was performed by HPLC-MS/MS.
The analytical results of field fortification experiments (106 % mean recovery) show that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.
The mean Dimethenamid-P residues dislodgeable from oilseed rape leaves are summarized below.

Table 7.1.4.3-19: Summarized results – BAS 656 H in dislodging solutions

Dislodging event	BAS 830 01 H [mg/specimen]*	Relative Standard Deviation
0-2 (+/-30 min) HALA	0.289	5 % (n = 5)
6 (+/-30 min) HALA	0.086	16 % (n = 5)
24 (+/-2 h) HALA	0.002	38 % (n = 5)
48 (+/-4 h) HALA	0.001	12 % (n = 5)
4 DALA	< LOQ	-- (n = 5)
7 DALA	< LOQ	-- (n = 5)

*specimen = 200mL dislodging solution of 20 leaf discs HALA: Hours after last application
DALA: Days after last application
< LOQ: below limit of quantitation of 0.001 mg/specimen

Foliar DT_{50} and DT_{90} values were estimated from residue measurements of the a.s. Dimethenamid-P on the leaf surfaces of oilseed rape. Dimethenamid-P dissipates rapidly from the leaves with a DT_{50} of 0.14 days, in other words, with a DT_{50} < 4 hours.

I. MATERIAL AND METHODS

Test item

Table 7.1.4.3-20: Test item

Name of product:	**BAS 830 01 H**			
Batch No.:	451008			
Active Ingredient	Name	BAS Code	nominal	analyzed
	Dimethenamid-P	BAS 656 H	333.0 g/L	347.7 g/L
	Quinmerac	BAS 518 H	167.0 g/L	173.0 g/L
Formulation Type:	SE			
Density	1.135 g/cm3			
Storage Advice:	Keep at room temperature (typically +25 °C) or cooler			
Certificate of analysis:	401052_6 (February15, 2013)			
Expiration date:	January16, 2014			
Chemical stability in water and light:	sufficient for planned trial conditions			
Safety Precautions	see MSDS			

Test System
The test system consisted of oilseed rape plants (*Brassica napus L.*) grown and maintained in plastic pots (10.5 cm in diameter) with commercial potting mixture (Floradur) in the greenhouse. The seeds were sown on 21st August 2013. At application the oilseed rape plants were at BBCH 16 and approximately 23-32 cm high on average. The plant density was two plants per pot. All plant pots used for the study were identified by labels specifying the trial number and the treatment group (control or treated). At least 141 pots are assigned for this study and were allocated according to the treatment plan outlined in Table 7.1.4.3-21**:**

Table 7.1.4.3-21: Treatment Regime

Treatment description	Plot	Purpose	Number of pots	Plant pot IDs
Untreated	1	Control specimens	20	Plot 1
Treated	2	Treated specimens	101	Plot 2
Untreated	3	Field fortifications	20	Plot 3

Pesticide History and Crop Maintenance
Irrigation was added to the bottom of the pots when necessary using tap water. Plants were fertilized Hakaphos blau in accordance to good agricultural praxis for the time of cultivation.
Two days before application Folicur (1 l/ha) was applied. After application the plants were cultivated in a greenhouse at defined environmental conditions (daily mean air temperature ranged between 16.0 and 19.1°C, extremes 12°C and 25°C , daily mean relative humidity ranged from 63.9 to 76.8 %, extremes 46 % and 92 %) one day before application to last sampling.

Table 7.1.4.3-22: Nominal Treatment Volume and Rate

	Plot 1 (untreated control) Plot 3 (field fortification, untreated)	Plot 2 (treated)
Application volume	-	approx. 200 L/ha tap water
Application rate BAS 830 01 H	-	1.5 L/ha
Application rate BAS 656 H	-	500 g a.i./ha
Application rate BAS 518 H	-	250 g a.i./ha

Sampling
For each sampling event 20 leaf discs (200 cm^2; both leaf sides) were collected randomly from the upper at application complete developed leaves using a leaf puncher (1 inch in diameter). Covered leaves were avoided. Vertical standing leaves at application were marked and not used for sampling due to the possibility of extensive runoff. The leaf discs were collected directly into a pre-labeled wide mouth jar (Nalgene®, PC, 125 mL) and subsequently transferred at ambient temperature to the *agro- check* laboratory for weighing and dislodging procedure. Before application the leaf discs of untreated plants (S1, control and field fortification specimens) were sampled. Within two hours after application once the spray deposit had fully dried sampling S2 was performed for plot 2.

Table 7.1.4.3-23: Target sampling parameters

Sampling No.*	Plot	Timing	Description
S1	1	BA	Control specimens to be collected before the application 1 x 20 leaf discs
S1	3	BA	Field fortifications to be collected before the application 7 x 20 leaf discs
S2	2	0-2 (+30 min) HALA	Specimens of treated crop approximately 2 hours subsequent to the application (once the spray deposit has fully dried). 5 x 20 leaf discs
S3	2	6 (+/-30 min) HALA	Specimens of the treated crop approximately 6 hours subsequent to the application 5 x 20 leaf discs
S4	2	24 (+/-2 hours) HALA	Specimens of the treated crop approximately 24 hours subsequent to the application 5 x 20 leaf discs
S5	2	48 (+/-4 hours) HALA	Specimens of the treated crop approximately 48 hours subsequent to the application 5 x 20 leaf discs
S6	2	4 DALA	Specimens of the treated crop approximately 4 days subsequent to the application 5 x 20 leaf discs
S7	2	7 DALA	Specimens of the treated crop approximately 7 days subsequent to the application 5 x 20 leaf discs

* sampling no. not identical with FREDDY sampling no.
BA Before application;
HALA Hours after last application;
DALA Day after last application

Leaf dislodging procedure

Control specimen and specimen of treated plants

Exactly 20 leaf discs were used for the dislodging procedure of one specimen. 100 mL of the dislodging solution (Water / Aerosol OT-B (0.01%)) was added and the pre-labeled wide mouth jar containing the leaf discs was subsequently transferred to a reciprocating shaker. The jars were shaken at shaking level 2 for a period of 10 minutes. The dislodging solution was then decanted into a beaker and the jar with the leaf discs was subjected to a second dislodging process with another 100 mL of fresh dislodging solution (level 2, 10 minutes). The two dislodging solutions were pooled in the beaker and thoroughly mixed.

Fortification samples

Two fortification solutions at different concentrations containing Dimethenamid-P (BAS 656 H) as outlined in Table 7.1.4.3-24: were prepared at the analytical test site and subsequently sent to *agro - check* on dry ice. The fortification experiments with Dimethenamid-P (BAS 656 H) were performed at 0 DALA before application of test item. To prepare the fortification samples the same routine in washing oilseed rape plant discs was used as described.

Table 7.1.4.3-24: Fortification samples

Matrix	DFR Dislodging Solution [mL]	No. of Repl.	LOQ[1] (proposed) [µg/L]	Fortificatio n Level	Fortification solution [mL]	Conc. of solution [µg/mL]
Fortification Control	20	1 [2]	5	control	-	-
Fortification Low	20	3 [3]	5	10 x LOQ	0.5	2
Fortification High	20	3 [4]	5	1000 x LOQ	0.5	200

1) applicable for both analytes (Dimethenamind-P)
2) L1307270032
3) marked with A, B and C – L1307270033, L1307270034, L1307270035
4) marked with A, B and C - L1307270036, L1307270037, L1307270038

The fortified specimens were subjected to the same analytical procedure as applied to the specimens obtained from the treated plants.

II. RESULTS AND DISCUSSION

An overview is given in Table 7.1.4.3-25**:**

Table 7.1.4.3-25: Summarized results – BAS 656 H in dislodging solutions

	Timing	Mean conctration of analyte BAS 656 H		
		[mg/specimen]*	[µg/cm^2]	[mg/g leaf]
Mean	Control (BA)	0.000	0.000	0.00
Mean	0 - 2 HALA	0.289	1.446	0.137
RSD		4.7 % (n=5)	4.7 % (n=5)	7.6 % (n=5)
Mean	6 HALA	0.086	0.432	0.040
RSD		15.7 % (n=5)	15.7 % (n=5)	14.6 % (n=5)
Mean	24 HALA	0.002	0.012	0.001
RSD		38.4 % (n=5)	38.4 % (n=5)	34.3 % (n=5)
Mean	48 HALA	0.001	0.005	0.000
RSD		11.8 % (n=5)	11.8 % (n=5)	12.0 % (n=5)
Mean	4 DALA	< LOQ	--	--
RSD		--	--	--
Mean	7 DALA	< LOQ	--	--
RSD		--	--	--

*specimen = 200mL dislodging solution of 20 leaf discs
BA: Before application
HALA: Hours after last application
DALA: Days after last application
< LOQ: below limit of quantitation of 0.001 mg/specimen

The analytical results of the fortifications presented in **Table** 7.1.4.3-26**:** show that frozen storage and transport did not influence the analytical results of this study.

Table 7.1.4.3-26: Summarized results of the field fortification experiments with BAS 656 H

Fortification levels	Recovery range (%)	Mean recovery ± RSD (%)
10 x LOQ 1000 x LOQ	94.6 – 116.4	105.8 ± 7 (n=6)

Foliar DT50 Results

Foliar DT50 and DT90 values were estimated from residue measurements of the a.s. Dimethenamid-P on the leaf surfaces of oilseed rape. Dimethenamid-P dissipates rapidly from the leaves with a DT50 of 0.14 days, in other words, with a DT50 < 4 hours.

Table 7.1.4.3-27: Statistical and visual assessment of the SFO kinetic model and the DT50 and DT90 values for Dimethenamid-P

Leaf	^{2} error%	type I error rate (Prob. > t)	Visual fit	DisT50 [d]	DisT90 [d]
Oilseed rape	0.30	k: < 0.001	excellent	0.14	0.47

Kinetic evaluation was performed in order to derive dissipation rates of Dimethenamid-P on the leaf surfaces of oilseed rape (Foliar DisT50/DisT90). Despite the fact that no guidance on how to derive dissipation rates from such studies is available, the kinetic analysis and calculations of DisT50 and DisT90 values were performed following the recommendations of the FOCUS Kinetics workgroup. 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 were set to 0.00001 and 100, respectively.

III. CONCLUSION

The objective of this study was to determine the magnitude of dislodgeable foliar residues of Dimethenamid-P (BAS 656 H) resulting from a single application of BAS 830 01 H to oilseed rape. The test item was applied in a spray chamber to oilseed rape plants (*Brassica napus L.*) grown in pots in a greenhouse. The using spraying equipment simulated a commercial application to oilseed rape. The amount of dislodgeable foliar residues was determined. The data are required for worker re-entry risk assessments and for the determination of residue deposit half-life values on oilseed rape leaves.

The analytical results of field fortification experiments (106 % mean recovery) show that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.
The mean Dimethenamid-P (BAS 656 H) residues dislodgeable from oilseed rape leaves at 0 - 2 hours after the application were 0.289 mg/specimen, 6 hours after the application 0.086 mg/specimen were determined, 24 hours after the application the residues were 0.002 mg/specimen, 48 hours after the application 0.001 mg/specimen were determined and at 4 and 7 DALA the residues were < LOQ.
Foliar DT50 and DT90 values were estimated from residue measurements of the a.s. Dimethenamid-P on the leaf surfaces of oilseed rape. Dimethenamid-P dissipates rapidly from the leaves with a DT50 of 0.14 days, in other words, with a DT50 < 4 hours.

CA 7.2 Fate and behaviour in water and sediment

Data submitted in the previous Annex-I review gave a clear picture of the fate of Dimethenamid-P in aquatic systems. Dimethenamid-P was relatively stable to hydrolysis at pH's 5, 7 and 9 (DT50 31 days). Dimethenamid-P was readily degraded under aqueous photolysis conditions with a DT50 of 13.7 days. A water/sediment study gave whole system DegT50 values of 23.4 and 33.4 from river and pond systems respectively.

Bridging studies were conducted to demonstrate the similarity of the degradation profiles between racemic Dimethenamid and Dimethenamid-p in water. Hereto, one system was evaluated for aerobic degradation of Dimethenamid-p in water/sediment. Moreover, the already peer reviewed racemic compound study was re-evaluated according to latest kinetic guidance. In order to further our bridging argumentation we used water from the same water sediment (river) system as in the original study.

The route of degradation of Dimethenamid-P was investigated in an aquatic (river) sediment system. This was to serve as bridging data for the previously submitted studies and done with chirality and fate of the isomers as a focal point. The observed degradation was extensive, yielding as many as 15 metabolites from the overall system.

The active substance was present mainly in the water phase and this therefore is where the majority of the degradation took place. Metabolites M656PH023, M656PH027 and M656PH003 were observed in 9.6, 6.3 and 5.7 % TAR respectively. No other metabolite was observed in >4.1% TAR in water.
The degradation profiles observed in these studies were, for the most part very similar. No metabolites were observed that had not already been accounted for in our risk assessments.
The ratio of enantiomers of Dimethenamid was observed throughout the experiment to remain constant illustrating that no enantio-selective degradation was taking place. The whole system DegT50 was estimated at 28 days.

The previously submitted water/sediment study was re-evaluated under new kinetic guidance. This gave DegT50 values for the whole system of 35.1 and 19.8 days for the pond and river systems, respectively.

An aerobic mineralisation in surface water study, which is a new data requirement under 1107/2009, was also conducted. Natural pond water was utilized to investigate the biodegradability of Dimethenamid-P. No significant degradation was observed, therefore no kinetic evaluation of the active could be performed from this study.

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

No new data was generated.

CA 7.2.1.2 Direct photochemical degradation

No new data was generated.

CA 7.2.1.3 Indirect photochemical degradation

No new data was generated. Not triggered as per OECD Draft Guidance Document "Phototransformation of Chemicals in Water".

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

No new data was generated.

CA 7.2.2.2 Aerobic mineralisation in surface water

Report: CA 7.2.2.2/1
Voelkel W., 2013b
Aerobic mineralisation of 14C-Dimethenamid-P in surface water
2013/1125944
Guidelines: OECD 309 (April 2004), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414
GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

The purpose of this study was to determine the aerobic mineralization of ^{14}C-Dimethenamid-P in surface water (pelagic test) under defined laboratory conditions. Additionally, it was investigated if a potential shift between the two enantiomers did occur under the applied test conditions.

The test was performed with two different test item concentrations (high dose: 50 µg/L and low dose: 10 µg/L). Additionally, a test was run at the high concentration under sterile conditions in order to gain information about abiotic degradability of the test item. The test flasks were attached to a flow-through system for continuous aeration and incubated at a temperature of 20.0 ± 2.0°C in the dark for 63 days. After treatment, the flasks (except for day 0 samples) were connected to a volatile trapping system for detection of organic volatiles and $^{14}CO_2$. Duplicate samples (singles for sterile test) were taken for analysis at 0, 1, 3, 7, 14, 28 and 63 days after treatment (DAT). Microbial activity of the surface water was proven by the degradation of [^{14}C(U)]benzoic acid.

The amount of radioactivity in the water samples was determined by LSC. Volatiles trapped in appropriate trapping solutions were also analysed by LSC. Parent substance and metabolite identification and quantification was done by HPLC. Selected samples were analysed by Thin-Layer Chromatography (TLC) to confirm the results. The enantiomer ratio was determined by using a chiral HPLC method.

The obtained results showed that no significant degradation of ^{14}C-Dimethenamid-P was observed in a pure water environment as provided in the pelagic test. Immediately after treatment (day 0), Dimethenamid-P accounted for 100.6% AR (high dose), 99.3% AR (high dose, sterile) and 101.7% AR (low dose). After 63 days of incubation. Dimethenamid-P accounted for 94.8% AR, 100.7% AR and 97.8% AR, respectively. Dimethenamid-P was the only substance observed in the HPLC chromatograms of the aqueous phase. The ratio of the two Dimethenamid-P

isomers was constant in all analysed samples indicating that no enantiomer shift took place during the incubation period.

Overall, the degradation of ^{14}C-Dimethenamid-P was characterized by a low mineralisation rate irrespective of test item concentration. The amount of $^{14}CO_2$ and other organic products never exceeded 2.0% AR within 63 days.

No kinetic evaluation of Dimethenamid-P degradation rates was performed since no significant degradation was observed in the pelagic test.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Dimethenamid-P (BAS 656 H)
Reg.No.: 363851
Chemical name: (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Molar mass: 275.8 g/mol (non-labelled)
Molecular formula: $C_{12}H_{18}ClNO_2S$

Labelled test item
Label: thienyl-5-^{14}C
Specific radioactivity: 8.21MBq/mg
Radiochemical purity: 99.0 %

2. Test system

Water was freshly collected on May 7th, 2013 from Biederthal, a pond located at Biederthal (France).

The water sample was transported to IES Ltd in clean containers, stored for about two weeks at about 4°C in the dark. The water used for the main test was filtered through a 0.2 mm mesh and acclimated at room temperature for five days.

Various hydrological characteristics of the test system, i.e. pH, the O_2 concentration, redox potential and temperature of the water were determined directly at the site of sampling. The depth of sampling, colour and turbidity of the water were recorded. The characterization of the water is summarised in Table 7.2.2.2-1.

Table 7.2.2.2-1: Water characteristics

Name:		**Biederthal**
Origin:		68480 Biederthal, France 47.4701°N / 7.4342°E
Sampling date:		May 07, 2013
Batch:		Biederthal 05/13
Water parameters measured at field sampling[a]:		
Temperature	[°C]	16.1
pH (water)		7.86
Oxygen concentration	[mg/L]	14.32
Redox potential (Eh)*	[mV]	398
Sampling depth	[cm]	0-20
Colour		Green
Turbidity/Visibility		About 50 cm
Water parameters measured post-handling[b]:		
TOC	[mg/L]	3.35
DOC	[mg/L]	2.43
Nitrate	[mg/L]	0.54
Nitrite	[mg/L]	<0.82
Ammonium	[mg/L]	0.40
N total	[mg/L]	2.70
P total	[mg/L]	0.31

[a] Parameters determined by IES Ltd.
[b] Parameters determined by AgroLab AG, 6037 Root, Switzerland (non-GLP).
*The measured potential was corrected to Eh of a standard hydrogen electrode by adding 214 mV.

B. STUDY DESIGN

1. Experimental conditions

A total number of 52 test vessels was prepared for the incubations. The flasks were filled with about 100 mL of water. Table 7.2.2.2-2 illustrates the set-up of experimental conditions. Seven test vessels (used for high dose samples) were sterilised in an autoclave (10 min, 121°C).

Table 7.2.2.2-2: Set-up of the experimental conditions

System	Sampling Intervals		Reserves	Total Number of Treated Samples
	Number	Single or Duplicate		
High dose	7	Duplicate	6	20
Low dose	7	Duplicate	6	20
High dose, sterilised	7	Single	3	10
Reference test item	2	Single	-	2

Note: Two non-treated samples (blank controls) were used to determine the water parameters during the test

All test item treated and untreated vessels were placed on multiplate magnetic stirrers and incubated at 20.0 ± 2.0°C in a metabolism chamber providing the test vessels with a continuous gas-flow through system.

After application of the test item, each test vessel was connected to the air stream leading to a trapping system equipped with a total of two absorption traps. The first trap containing ethylene glycol and the second 2N NaOH (in this sequence) to trap organic volatiles and $^{14}CO_2$, respectively.

For the [^{14}C(U)]benzoic acid treated vessels, degradation was monitored in two samples using the same experimental set-up in order to test the microbial activity of the surface water. After each sampling, the volatile traps were replaced by new traps containing fresh solutions.

Untreated control samples were used for system characterisation and for microbial plate counts at the end of the experiment.

Application

The nominal application rates of the test item were 50 µg/L and 10 µg/L for the high and the low application rate, respectively. This was achieved by pipetting drop-wise 970 µL (high dose) or 1000 µL (low dose) of the corresponding application solutions into the upper water layer of the test vessels. The nominal application rate for viability control (^{14}C-benzoic acid treated vessels) was 10 µg/L. The rate was attained by treating four test vessels with 1000 µL of the ^{14}C-benzoic acid application solution.

2. Sampling

The test systems (two replicates for the non-sterilised system and a single sample for the sterilised system) were sampled at 0, 1, 3, 7, 14, 28 and 63 days after treatment (DAT). Trapping were taken at the corresponding sampling day and the volume of liquid in the ethylene glycol and sodium hydroxide traps were recorded.

For the^{14}C-benzoic acid investigation, the test vessels were disconnected from the air flow system and single samples were taken for analysis after 7 and 14 days of incubation.

3. Description of analytical procedures

At each sampling date, the respective flasks were removed from the incubator. The temperature, the O_2 concentration, pH and redox potential of the water were recorded in all water samples types. The water phases were treated for about 30 seconds in an ultrasonic bath and thereafter transferred into a measuring cylinder. The volume was recorded and aliquots were submitted to LSC and HPLC analysis.

For the high application rate, aliquots (17-19 mL) of the water samples at 0, 28 and 63 DAT were additionally concentrated to 2 mL under reduced pressure and at approximately 35°C for chiral HPLC analysis.

The entire samples from ^{14}C-benzoic acid treated test vessels were taken for work-up. The volume of the aqueous phase was recorded and aliquots were taken per test vessel for LSC measurements and HPLC analysis.

The volume of liquid in the ethylene glycol and sodium hydroxide trap of the corresponding flasks was recorded. Radioactivity present in the trapping solutions was monitored by LSC.

4. Calculation of the degradation/dissipation rates

No significant degradation of Dimethenamid-P in the test vessels was observed in the pelagic test. Therefore no kinetic evaluation of the recorded data was performed.

II. RESULTS AND DISCUSSION

Material Balance

The total mean recoveries were 99.7 ± 1.9% of applied radioactivity (AR) for the high dose, 101.0 ± 2.3% AR for the sterile high dose and 100.8 ± 3.0% AR for the low dose experiments. The material balance and the distribution of radioactivity in the pelagic test for high and low concentrations are shown in Table 7.2.2.2-3 and Table 7.2.2.2-4, respectively.

Table 7.2.2.2-3: Material balance and radioactivity distribution after application of ^{14}C-Dimethenamid-P to pelagic test (high dose). Mean values in percent of the applied radioactivity [%AR]

days after treatment	^{14}C-Dimethenamid-P (high dose)				^{14}C-Dimethenamid-P (high dose, sterile)			
	Water	Volatiles ethylene glycol traps	Volatiles $^{14}CO_2$	Material balance	Water	Volatiles ethylene glycol traps	Volatiles $^{14}CO_2$	Material balance
0	100.6	n.p.	n.p.	100.6	99.3	n.p.	n.p.	99.3
1	99.8	< 0.2	0.2	99.9	102.6	< 0.2	< 0.2	102.7
3	101.8	< 0.2	0.3	102.1	104.9	< 0.2	0.2	105.1
7	99.2	< 0.2	0.6	99.9	99.7	< 0.2	< 0.2	99.8
14	98.9	< 0.2	0.8	99.8	98.2	< 0.2	0.8	99.0
28	97.8	< 0.2	1.1	98.9	99.4	< 0.2	< 0.2	99.4
63	94.8	< 0.2	1.5	96.4	100.7	< 0.2	0.9	101.6
MEAN ± SD	99.7 ± 1.9				101.0 ± 2.3			

n.p. not performed

Table 7.2.2.2-4: Material balance and radioactivity distribution after application of ^{14}C-Dimethenamid-P to pelagic test (low dose). Mean values in percent of the applied radioactivity [%AR]

days after treatment	^{14}C-Dimethenamid-P (low dose)			
	Water	Volatiles ethylene glycol traps	Volatiles $^{14}CO_2$	Material balance
0	101.7	n.p.	n.p.	101.7
1	99.5	< 1.0	< 1.0	100.0
3	105.4	< 1.0	< 1.0	105.9
7	100.2	< 1.0	< 1.0	100.8
14	99.5	< 1.0	< 1.0	99.9
28	97.4	< 1.0	1.2	98.6
63	97.8	< 1.0	1.2	99.0
MEAN ± SD	100.8 ± 3.0			

n.p. not performed

No significant differences were found in Dimethenamid-P behaviour between the high and the low dose concentration of the test substance applied to the water system. The amount of radioactivity in the water was found to be very stable. At the end of the study (63 DAT) it ranged from 94.8% to 100.7% AR. For all samples and sampling time points the radioactivity in the volatile traps never exceeded 2% AR indicating a low rate of mineralization.

The control vessels treated with [^{14}C(U)]benzoic acid showed that the system was microbially active. After 7 days of incubation, [^{14}C(U)]benzoic acid was not detectable anymore in the aqueous phase.

Physico-chemical parameters of the test systems

During the incubation with Dimethenamid-P, the O_2 concentration in the water of system had an averaged value of 8.38 ± 1.59, 8.37 ± 1.62, 8.43 ± 1.52 for the test vessel dosed at high, high sterile and low concentrations, respectively.

The pH in the water of the viable vessels was slightly basic with most values around 8.0. The lowest pH was measured at 7.63 in the test vessels dosed at high concentration.

Considering the untreated surface water (control), the O_2 concentration in the water had an averaged value of 7.69 ± 1.98 and pH mean value of 8.31 ± 0.17.

Characterisation and identification of residues in water and sterile extracts

No significant degradation of Dimethenamid-P in the pelagic test was observed. At 63 DAT, between 94.8% and 100.7% AR was still recovered as unchanged parent for the different concentrations. The detected amounts of the active substance and its metabolites in the water samples are presented in Table 7.2.2.2-5.

No metabolites were detected the aqueous phase.

Table 7.2.2.2-5: Pattern of degradation and formation of metabolites in system treated with ^{14}C-Dimethenamid-P (high dose). Mean values in percent of the applied radioactivity [%AR]

days after treatment	^{14}C-Dimethenamid-P in surface water (high dose)		
	Parent compound	$^{14}CO_2$	^{14}C total
0	100.6	n.p.	100.7
1	99.8	0.2	100.0
3	101.8	0.3	102.2
7	99.2	0.6	99.9
14	98.9	0.8	99.8
28	97.8	1.1	98.9
63	94.8	1.5	96.4
days after treatment	**^{14}C-Dimethenamid-P in surface water (high dose, sterile)**		
0	99.3	n.p.	99.3
1	102.6	< 0.2	102.7
3	104.9	0.2	105.1
7	99.7	< 0.2	99.8
14	98.2	0.8	99.0
28	99.4	< 0.2	99.4
63	100.7	0.9	101.6
days after treatment	**^{14}C-Dimethenamid-P in surface water (low dose)**		
0	101.7	n.p.	101.7
1	99.5	< 1.0	100.0
3	105.4	< 1.0	105.9
7	100.2	< 1.0	100.8
14	99.5	< 1.0	99.9
28	97.4	1.2	98.7
63	97.8	1.1	98.9

n.p. not performed

Enantiomer-specific analyses

The ratio between both types of isomers remained constant throughout the incubation time. The amount of R-Isomer ranged between 5.0 and 6.7%, while the amount of S-Isomer ranged between 93.3 and 95.0%.

Degradation of the reference test items

No kinetic evaluation of Dimethenamid-P degradation rates was performed since no significant degradation was observed under the applied test conditions.

III. CONCLUSION

From the obtained results it can be concluded that Dimethenamid-P does not significantly degrade in a pure water environment as provided in the pelagic test. After 63 days more than 94.8% AR was recovered as the unchanged active substance.

The enantiomer ratio of Dimethenamid-P remained constant in all analysed samples.

No kinetic evaluation of Dimethenamid-P degradation rates was performed since no significant degradation was observed under the applied test conditions.

CA 7.2.2.3 Water/sediment studies

Report:	CA 7.2.2.3/1 Voelkel W., 2014b Route and rate of degradation of 14C-Dimethenamid-P in one aerobic aquatic sediment system 2013/1125942
Guidelines:	OECD 308, EC 1107/2009 (14 June 2011), EEC 79/117, EEC 91/414
GLP:	yes (certified by Swiss Federal Office of Public Health)

Executive Summary

The degradation of ^{14}C- Dimethenamid-P was investigated in an aerobic water/sediment system under dark conditions. ^{14}C-Dimethenamid-P was applied at a rate of 213 µg test item per flask (106.3% of target), corresponding to an application rate of 354 µg/L water

Samples for the experiment were taken at 0, 3, 8, 14, 28, 56, 77, and 100 days after treatment (DAT). Water and sediment were worked up separately. Water samples and sediment extracts were analysed by liquid scintillation counting (LSC). The amount of non-extractable residues and volatiles was determined by LSC.
Chromatographic profiling was performed using Thin-Layer Chromatography (TLC). High-Performance Liquid Chromatography (HPLC) was used as corroborative method for chromatographic profiling of selected samples. Furthermore, chiral HPLC analysis was performed to investigate a possible shift of the enantiomer ratio over time. The ratio between both types of enantiomers remained constant throughout the incubation time (the amount of R-enantiomer ranged between 5.3 and 6.4%, while the amount of S-enantiomer ranged from 93.6 to 94.7%).

The total recovery of applied radioactivity of all individual samples was ≥90.6% of applied radioactivity (AR).
Immediately after treatment (day 0), 93.9% AR was present in the aqueous phase of the water/sediment system. Thereafter, the amount of radioactivity in the aqueous phase decreased, reaching 34.3% AR at 100 days of incubation.

The amount of radioactivity extracted from sediments at room temperature increased over time from 1.5% AR (day 0) to a maximum of 18.1% AR at 8 days of incubation. Thereafter, the amount decreased and accounted 9.6% AR at the end of the incubation (day 100). The amount of radioactivity recovered by Soxhlet extraction varied between 3.6% and 6.0% AR.
The amount of non-extractable radioactivity increased over time from 0.7% AR (day 0) to 35.6% AR at the end of incubation.
Mineralisation of ^{14}C-Dimethenamid-P in the aquatic sediment system was rather slow. The amount of radioactivity in sodium hydroxide increased from 0.3% AR at sampling day 3 to 6.6% AR at day 100, and could be identified as $^{14}CO_2$. Organic volatile products absorbed in the ethylene glycol traps did not exceed 0.1% AR at any sampling interval.

^{14}C-Dimethenamid-P was mainly present in the aqueous phase. The amount of ^{14}C-Dimethenamid-P in the aqueous phase decreased from 92.8% AR (sampling day 0) to 5.4% AR at the end of incubation (day 100). The amount of test item in the extracts increased from 1.5% AR (day 0) to a maximum of 18.1% AR (day 8), followed by a decrease to 3.0% AR at the end of the experiment. The chiral analysis showed that no enantiomer shift occurred.

^{14}C-Dimethenamid-P degraded into 12 radioactive fractions. Only minor radioactive fractions were detected in the aqueous phase throughout the experiment. Fraction F1.1, containing metabolite M656H027, reached a maximum of 6.3% AR at the end of the experiment. Fraction F4, assigned to M656H023, accounted for a maximum of 9.6% AR at the end of the experiment. Fraction F10 assigned to M656H003 reached a maximum of 5.7% AR (day 56), and accounted for 4.0% AR at the end of the experiment. All other radioactive fractions never exceeded 4.1% AR. Only minor radioactive fractions were detected in the sediment extracts and did not exceed 2.9% AR at any sampling interval.

The calculated DT_{50} and DT_{90} values for ^{14}C-Dimethenamid-P in water and sediments are based on single first-order kinetics as presented below.

Table 7.2.2.3-1: Selected endpoints for Dimethenamid-P

System	^{14}C-Dimethenamid-P				
	$DisT_{50}$ [d]	$DisT_{90}$ [d]	χ^2-error [%]	r^2	Prob > t
Water	19.5	64.9	9.25	0.9742	7.77E-009
Sediment	38	126	4.14	0.9859	1.56E-009
	$DegT_{50}$ [d]	$DegT_{90}$ [d]	χ^2-error [%]	r^2	Prob > t
Whole system	28	93.1	1.95	0.9967	7.13E-016

I. MATERIAL AND METHODS

1. Test Material

Test item:	BAS 656 H, Reg.No. 363851
Chemical name:	(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Molecular mass:	275.8 g/mol (non-labelled)
Molecular formula:	$C_{12}H_{18}ClNO_2S$
Label:	thienyl-5-^{14}C
Specific radioactivity:	8.21 MBq/mg
Radiochemical purity:	99.0% (RHPLC)

Unlabelled test item:
Purity: 96.5%
Unlabelled test item was used for co-chromatography purposes and to dilute the radioactivity of the ^{14}C-labelled test item.

2. Test systems

A natural water/sediment system was collected from the river Rhine near 4322 Mumpf, Switzerland (47.546° N / 7.931° E). The sampling was performed in accordance with ISO Guidance on sampling of bottom sediments.

The physico-chemical parameters of the systems measured at field sampling and prior to acclimatisation are summarised in Table 7.2.2.3-2 and Table 7.2.2.3-3. The sediments were passed through a 2 mm sieve. The water and sediment were filled in test flasks within two days of sampling.

Table 7.2.2.3-2: Parameters measured at field sampling

Batch:	Rhein near Mumpf 04/13
Sampling date:	24.04.2013
Water	
Temperature [°C]	9.3
pH (water)	8.17
Oxygen concentration [mg/L]	16.85
Redox potential (E_h) [mV]	530
Sediment	
Depth [cm]	0-10
Colour	Grey
Smell	none

Note The measured redox potential was corrected to E_h of a standard hydrogen electrode (SHE).

Table 7.2.2.3-3: Parameters for river sediment measured prior to acclimatisation

Water	
TOC [a] [mg/L]	1.09
Sediment	
pH ($CaCl_2$) [a]	7.27
pH (water) [a]	7.70
TOC [a] [mg/kg]	13.9
Microbial biomass [b] [mg/kg]	326
Dry weight [b] [g/g]	0.52
Soil (sediment) type (USDA): [b]	Sandy loam
Particle size analyses:	
< 0.002 mm (clay) [a] [%]	7.48
0.002-0.05 mm (silt) [a] [%]	23.38
> 0.05 mm (sand) [a] [%]	69.14

[a] Parameters determined by AgroLab AG, 6037 Root, Switzerland (non-GLP).
[b] Parameters determined by IES Ltd.

Table 7.2.2.3-4: Parameters for river water/sediment system measured during incubation

Water			
pH (water) [b]		Start of incubation Average during incubation End of incubation	8.14 8.07 7.97
TOC [a]	[mg/L]	Start of incubation End of incubation	1.85 9.39
Oxygen conc. [b]	[mg/L]	Start of incubation Average during incubation End of incubation	8.54 8.89 9.17
Redox potential (E_h) [b]	[mV]	Start of incubation Average during incubation End of incubation	459 417 387
Sediment			
pH ($CaCl_2$) [a]		Start of incubation During incubation [c] End of incubation	7.42 7.43 7.43
TOC [a]	[mg/g]	Start of incubation End of incubation	13.7 13.0
Redox potential (E_h) [b]	[mV]	Start of incubation Average during incubation End of incubation	91 23 81
Microbial biomass [b]	[mg/kg]	Start of incubation End of incubation	249 200

a Parameters determined by AgroLab AG, 6037 Root, Switzerland (non-GLP).
b Parameters determined by IES Ltd.
c Determined for sampling interval 56 days.
n.a. Not available.
Note The measured redox potentials were corrected to Eh of a standard hydrogen electrode (SHE).

B. STUDY DESIGN

1. Experimental conditions

The test systems consisted of 1 L all-glass metabolism flasks (inner diameter: approximately 10.6 cm) filled with wet sediment to a height of approximately 2.3 cm (corresponding to about 200 g river sediment). Thereafter, approximately 6.4 cm river water (corresponding to about 600 mL) was added to reach a sediment/water volume ratio of about 1:3.

Following the start of acclimation the flasks were ventilated with moistened air. The samples were allowed to equilibrate in the dark for four weeks at a target temperature of 20 ± 2°C, in order to achieve stable redox potential and oxygen conditions.

Appropriate amounts (720 µL) of the respective application solution were pipetted to the water surface to achieve a nominal amount of about 200 µg test item per test vessel.

The test item ^{14}C-Dimethenamid-P (batch no. 824-6027) was purified before use. The application solution contained 3.07 mg ^{14}C-labelled test item and 3.97 mg of unlabelled test item resulting in a specific activity of 3.58 MBq/mg.
The final concentration of the organic solvent in the treated samples was <0.1% of the water phase volume.

After application of the test item to the flasks, the flasks were connected to a series of two volatile traps. The first trap contained ethylene glycol, the second trap 2 M NaOH. For sampling on day 0, no absorption traps were set up.

2. Sampling

Two replicates of treated samples were taken for extraction and analyses immediately after treatment (day 0) and after 3, 8, 14, 28, 56, 77 and 100 days of incubation.
The pH, oxygen concentration, and redox potential in water and/or sediment were measured in each sampled test vessel. Additionally, the parameters in control flasks were determined at all sampling intervals.
The volume of the liquid in each sodium hydroxide and ethylene glycol trap was recorded at the corresponding sampling intervals. Thereafter, the radioactivity in the absorption solutions was determined by LSC.
Untreated control samples were taken to determine the microbial biomass at the start and the end of incubation.

3. Description of analytical procedures

For sampling the water was withdrawn from the test vessel using a glass pipette. The volume was recorded and aliquots of 1 mL were analysed by LSC to determine the radioactivity.

The sediment samples were exhaustively extracted using the work-up procedures as described below:

- Acetonitrile/water (4:1, v/v) up to two times (until less than 5% of the radioactivity applied was recovered in a single extraction step).
- Soxhlet extraction with acetonitrile/water (4:1, v/v) for at least four hours (not performed for sampling intervals 0 and 3 days).

The amount of solvent used for each room temperature extraction step was 100 mL, and about 200 mL for Soxhlet extractions. Each room temperature extraction was performed on a shaker at approximately 250 revolutions per minute (rpm) for 30 minutes.

The individual extracts were centrifuged at approximately 1300 G for 10 minutes. The volume of individual extracts was recorded and the radioactivity quantified by LSC. Room temperature and Soxhlet extracts containing more than 2% of the radioactivity applied were combined. The pooled extracts were concentrated under a stream of nitrogen, if needed, and analysed by LSC for recovery before chromatographic analysis.
Chromatographic analysis of water and sediment extracts was performed by TLC. HPLC was used as additional method for chromatographic analysis of selected samples.

The residual radioactivity in sediment after extraction was determined by combustion of aliquots of the air-dried and homogenised sediments in a sample oxidiser with subsequent LSC analysis.

4. Calculation of the degradation/dissipation rates

The rate of degradation of ^{14}C-Dimethenamid-P in the aquatic sediment system incubated under aerobic conditions was calculated according to FOCUS [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.9]* using the CAKE software [*CAKE V2.0 developed by TessellaPlc, Abingdon, Oxfordshire, UK*].
Dissipation and degradation rates to derive modelling endpoints according to level P-I were calculated using SFO kinetics. Calculations were performed for the water compartment ($DisT_{50}$ water), the sediment compartment ($DisT_{50}$ sediment) and the whole system (sum of water and sediment) $DegT_{50}$.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The distribution of radioactivity in the different compartments of the water/sediment system treated with ^{14}C-Dimethenamid-P is presented in Table 7.2.2.3-5. The material balance in the test vessels was between 90.6% and 96.1% applied radioactivity (AR).

B. FINDINGS

Microbial biomass of the sediment

The microbial biomass was determined in triplicate before acclimatisation (post handling), as well as at the beginning and the end of the incubation period. The corresponding values were 326 mg, 249 mg, and 200 mg microbial C/kg dry sediment, demonstrating viability of the aquatic systems during acclimatisation and incubation (Table 7.2.2.3-4).

Radioactivity in the water

Immediately after treatment (day 0), 93.9% of the applied radioactivity was present in the water phase of the river system. Thereafter, the amount of radioactivity in the water decreased, reaching 34.3% AR at 100 days of incubation.

Radioactivity in the sediment

The amount of radioactivity extracted from river sediments at room temperature increased over time from 1.5% AR (day 0) to a maximum of 18.1% AR after 8 days of incubation. Thereafter, the amount slowly decreased and accounted for 9.6% AR at the end of the incubation (day 100). The amount of radioactivity recovered by Soxhlet extraction varied between 3.6% and 6.0% AR.
The amount of non-extractable radioactivity increased over time from 0.7% AR (day 0) to 35.6% AR at the end of incubation.

Volatiles

Mineralisation of ^{14}C-Dimethenamid-P in the aquatic sediment system was rather slow. The amount of radioactivity in sodium hydroxide increased from 0.3% AR at sampling day 3 to 6.6% AR at day 100, and was identified as $^{14}CO_2$.
Organic volatile products absorbed in the ethylene glycol traps did not exceed 0.1% AR at any sampling interval.

Table 7.2.2.3-5: Recovery of the applied radioactivity in aquatic sediment system treated with ^{14}C-Dimethenamid-P. (Values in percent of applied radioactivity, mean of two replicates)

DAT	water	sediment					volatiles		material balance
		extractable			NER	total	ethylene-glycole	NaOH (CO_2)	
	total	Room temp. extracts	Soxhlet extracts	total extractability					
0	93.9	1.5	n.p.	1.5	0.7	2.2	n.p.	n.p.	96.1
3	76.2	14.7	n.p.	14.7	4.9	19.5	<0.1	0.3	96.0
8	64.4	18.1	3.6	21.6	7.6	29.2	<0.1	1.5	95.2
14	58.2	17.0	5.1	22.0	12.4	34.4	<0.1	2.1	94.7
28	47.5	15.3	4.7	20.0	21.7	41.8	0.1	3.5	92.9
56	36.7	12.5	6.0	18.5	34.8	53.3	<0.1	3.6	93.6
77	35.5	11.3	5.3	16.6	36.2	52.8	<0.1	3.8	92.1
100	34.3	9.6	4.5	14.1	35.6	49.6	0.1	6.6	90.6

DAT = days after treatment
NER = non extractable residues
n.p. = not performed

Table 7.2.2.3-6: Amount of ^{14}C-Dimethenamid-P in water, sediment and whole system used for kinetic evaluation. (Values in percent of applied radioactivity, mean of two replicates)

Incubation time (day)	Parent (% AR)		
	Water	Sediment	Whole System
0	96.1*	1.5**	96.1*
3	73.6	13.9**	87.5
8	58.7	18.1	76.8
14	51.1	17.9	69.0
28	32.9	12.7	45.6
56	17.3	8.2	25.5
77	8.1	5.4	13.5
100	5.4	3.0	8.4

* For day 0, the total material balance was considered for kinetic evaluation for water and whole system as recommended in FOCUS

** Not used for kinetic evaluation as recommended in FOCUS (kinetics were fitted to the decline of parent concentrations from the maximum onwards).

Characterisation and identification of residues in water and sediment extracts

The results of the one- and two-dimensional TLC analyses are summarised in Table 7.2.2.3-6. The second dimension TLC was applied to verify a correct quantification and to make sure that no compound is hidden under a certain spot.

^{14}C-Dimethenamid-P was mainly present in the water phase. The amount of ^{14}C-Dimethenamid-P decreased from 92.8% AR (sampling day 0) to 5.4% AR at the end of the incubation period (day 100). The amount of test item in the sediment extracts increased from 1.5% AR (day 0) to a maximum of 18.1% AR (day 8), followed by a decrease to 3.0% AR at the end of the experiment.

^{14}C-Dimethenamid-P degraded overall into 15 radioactive fractions. In case where a radioactive spot detected in the first-dimension-TLC split into two or more spots in the second-dimension-TLC, the new spots were designated by an additional number separated by a point, e.g. fraction 1 splits into three fractions, they will be designated as fraction 1.1, 1.2, and 1.3.

In water, radioactive fraction F1.1 reached a maximum of 6.3% AR at the end of the experiment. It could be assigned by co-chromatography with the reference standard as the known Dimethenamid-P-metabolite M656H027 (former code "M27").
Radioactive fraction F4, which accounted for a maximum of 9.6% AR at the end of the experiment, could be assigned to metabolite M656H023 (former code "M23").
Radioactive fraction F10 reached a maximum of 5.7% AR (day 56), and accounted for 4.0% AR at the end of the experiment. Its R_f value matched the one of reference item M656H003 (former code "M3").

All other radioactive fractions in the water phase never exceeded 4.1% of AR.

None of the radioactive fractions detected in the sediment extracts exceeded 3.8% AR at any sampling interval. A proposed route of degradation of pendimethalin in water/sediment systems is given in Figure 7.2.2.3-1.

Ratio between R- and S-enantiomer of Dimethenamid-P

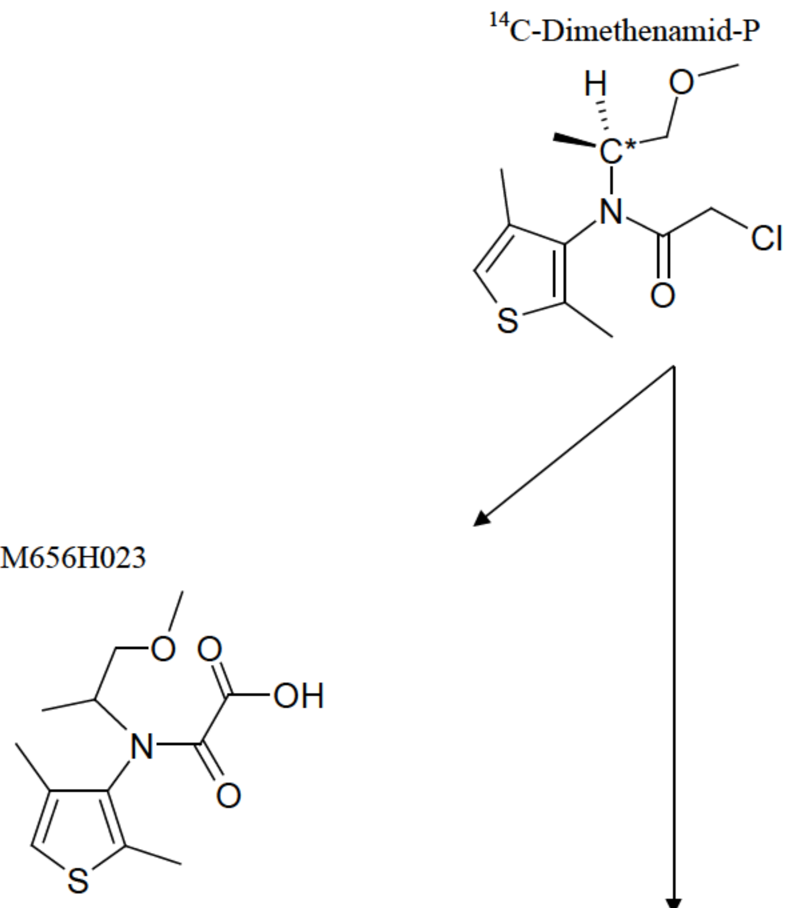
The water samples of intervals 0, 28, and 100 days were analysed by HPLC using a chiral column in order to separate the R- and S-enantiomers. The ratio between both types of enantiomers remained constant throughout the incubation time (the amount of R-enantiomer ranged between 5.3 and 6.4%, while the amount of S-enantiomer ranged from 93.6 to 94.7%).

Table 7.2.2.3-7: Pattern of degradation and formation of metabolites in the water phase and sediment extracts after treatment with ^{14}C-Dimethenamid-P. Values in percent of applied radioactivity, mean of two replicates.

Water		F1				F3	F4	F5	F6				F7	F8	F9	F10	F11	F12	F13
Incubation time	**Total RA**		**F1.1 (M27)**	**F1.2**	**F1.3**	**(M31)**	**(M23)**			**F6.1**	**F6.2**	**F6.3**				**(M3)**		**(Parent)**	
0	93.9	0.3	n.d.	n.d.	n.d.	*	*	*	0.4	n.d.	n.d.	n.d.	*	0.4	*	*	*	92.8	*
3	76.2	0.5	n.d.	n.d.	n.d.	0.4	0.6	*	0.6	n.d.	n.d.	n.d.	*	*	*	0.5	*	73.6	*
8	64.4	2.5	n.d.	n.d.	n.d.	0.7	0.9	*	1.6	n.d.	n.d.	n.d.	*	*	*	*	*	58.7	*
14	58.2	1.5	n.d.	n.d.	n.d.	0.6	1.4	*	1.0	n.d.	n.d.	n.d.	*	0.6	*	2.0	*	51.1	*
28	47.5	4.1	n.d.	n.d.	n.d.	1.0	4.0	*	1.5	n.d.	n.d.	n.d.	*	*	1.1	2.6	0.4	32.9	*
56	36.7	3.3	2.5	0.8	*	1.4	5.4	*	3.6	2.4	0.4	0.8	*	*	*	5.7	*	17.3	*
77	35.5	6.4	4.8	1.1	0.4	1.9	8.5	0.3	4.5	1.6	0.7	2.2	*	*	*	4.6	1.2	8.1	*
100	34.3	7.8	6.3	1.5	*	2.2	9.6	0.8	3.4	n.d.	n.d.	n.d.	*	*	*	4.0	1.2	5.4	*
Sediment		**F1**				**F3**	**F4**	**F5**	**F6**				**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
			F1.1 (M27)	**F1.2**	**F1.3**	**(M31)**	**(M23)**			**F6.1**	**F6.2**	**F6.3**				**(M3)**		**(Parent)**	
0	1.5	*	n.d.	n.d.	n.d.	*	*	*	*	n.d.	n.d.	n.d.	*	*	*	*	*	1.5	*
3	14.7	0.1	n.d.	n.d.	n.d.	*	0.1	*	0.1	n.d.	n.d.	n.d.	*	*	*	0.4	*	13.9	*
8	21.6	1.0	n.d.	n.d.	n.d.	0.1	0.2	*	0.3	n.d.	n.d.	n.d.	*	0.1	0.2	1.0	0.2	18.1	0.3
14	22.0	1.1	n.d.	n.d.	n.d.	0.1	0.4	0.1	0.4	n.d.	n.d.	n.d.	*	0.1	0.3	1.4	0.1	17.9	0.3
28	20.0	1.6	n.d.	n.d.	n.d.	0.2	0.8	0.1	0.4	n.d.	n.d.	n.d.	0.5	0.5	0.4	2.0	0.3	12.7	0.5
56	18.5	2.6	n.d.	n.d.	n.d.	*	1.9	*	2.1	n.d.	n.d.	n.d.	*	*	*	3.8	*	8.2	*
77	16.6	2.5	n.d.	n.d.	n.d.	*	2.2	*	2.0	n.d.	n.d.	n.d.	*	*	*	3.8	0.6	5.4	*
100	14.1	2.9	n.d.	n.d.	n.d.	0.5	1.8	0.1	1.2	n.d.	n.d.	n.d.	*	*	0.4	3.1	0.7	3.0	0.3

* Not detectable.
n.d. Not determined as not separated by TLC.
Parent (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
M31 [[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl]-acetic acid
M3 N-(2,4-dimethyl-3-thiophen-3-yl)-N-(2-methoxy-1-methylethyl)acetamide
M23 N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid
M27 Sodium [(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonate
Note: The second room temperature extract of sampling interval 8, replicate A, was measured by TLC and contained 4.9% AR, but was accidentally spilled prior to pooling of the extracts for chromatographic analysis. The radioactivity was therefore, attributed to the different radioactive fractions based on the results of the pooled extracts (first room temperature extract and Soxhlet extract).

Figure 7.2.2.3-1: Proposed route of degradation of Dimethenamid-P in water/sediment systems



Minor metabolites* bound residues and $^{14}CO_2$
* Including M31, M3 and M27

Degradation rates

The reported values of ^{14}C-Dimethenamid-P in the water, the sediment and the whole system (Table 7.2.2.3-5) were subjected to kinetic evaluation as described in Section I.B4.

The calculated DT_{50} and DT_{90} values for ^{14}C-Dimethenamid-P are based on single first-order kinetics and are presented in Table 7.2.2.3-8.

Table 7.2.2.3-8: Selected endpoints for Dimethenamid-P

System	^{14}C-Dimethenamid-P				
	$DisT_{50}$ [d]	$DisT_{90}$ [d]	χ^2-error [%]	r^2	Prob > t
Water	19.5	64.9	9.25	0.9742	7.77E-009
Sediment	38	126	4.14	0.9859	1.56E-009
	$DegT_{50}$ [d]	$DegT_{90}$ [d]	χ^2-error [%]	r^2	Prob > t
Whole system	28	93.1	1.95	0.9967	7.13E-016

III. CONCLUSION

The rate of degradation of ^{14}C-Dimethenamid-P was investigated in an aerobic aquatic sediment system (river). ^{14}C-Dimethenamid-P degraded with dissipation half-lives of 19.5 days in water and 38 days in sediment. The whole system $DegT_{50}$ was calculated to be 28 days.

The degradation proceeded primarily via formation of the known Dimethenamid-P metabolites M656H003 (former code "M3"), M656H023 (former code "M23"), and M656H027 (former code "M27") in water and bound residues in sediment. The formation of CO_2 was overall rather low.

The ratio between both types of enantiomers remained constant throughout the incubation time (the amount of R-enantiomer ranged between 5.3 and 6.4%, while the amount of S-enantiomer ranged from 93.6 to 94.7%).

Report: CA 7.2.2.3/2
Bastiansen F., 2011a
Kinetic evaluation of BAS 656 H in water/sediment systems under aerobic conditions
DocID 2011/1102522
Guidelines: FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0
GLP: no

Executive Summary

Data on the degradation of BAS 656 H - Dimethenamid and its metabolites M3 and M23 from a water-sediment laboratory study was evaluated according to the FOCUS (2006) recommendations. The study contained data on two systems, "Pond" and "River".

According to FOCUS (2006), data was evaluated on different levels of complexity:
(a) Single compartment approaches for the parent substance for water compartment, sediment compartment and whole system data (level P-I).
(b) Multi-compartment approach for the parent substance including water and sediment compartment and backtransfer (level P-II).
(c) Metabolite degradation/dissipation (level M-I): If data on the decline of the metabolite(s) is available, single compartment approaches for water sediment and whole system data, similar to level P-I, shall be used.

Only level P-I kinetic analyses lead to statistical sound results. The whole system DegT50, for trigger and modeling endpoints, equal 35.1 d (SFO) and 19.8 d (SFO) for the Pond and River test system, respectively. The geometric mean $DegT_{50}$ value is 26.4 d.

I. MATERIAL AND METHODS

Kinetic analysis

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics (*FOCUS, 2006)*. The analysis was done by non-linear regression methods (Marquardt algorithm, ordinary least squares optimization) using the Model Maker software package, version 3.1 [*Anonymous (1997) Model Maker User Manual, Version 3. Cherwell Scientific Publishing Limited.*].

Experimental data

The kinetic evaluation was based on the results of a dark aerobic water/sediment study with two test systems: a "River" sample from the river Rhine and a "Pond" sample from Anwil (Switzerland) [*see old EU dossier, chapter 7.2.1.3.2, reference 7.2/07. Wyss-Benz, M., Völkel, W. (1994): [3-14C-Thienyl]-Dimethenamid: Degradation and metabolism in aerobic aquatic systems. RCC unpublished report 361146. BASF DocID 1994/10641*].

The degradation of Dimethenamid and the formation and degradation of its metabolites M3 and M23 as well as partitioning of the substance between water and sediment were studied under laboratory conditions, over a period of 105 days. Formation and degradation of further metabolites (M13, M31, M11 and PL 36-88) was evaluated as well in the course of the laboratory study, however, their formation and degradation was not considered in the kinetic modelling as the % TAR was < 5%.

Kinetic modeling strategy

For the parent substance and the metabolites M3 and M23 kinetic evaluation was performed considering the different levels proposed by the FOCUS kinetics guidance [*FOCUS (2006)*]. The analysis at P-I level (one-compartmental approach) was done for degradation in the whole system as well as dissipation from the water phase and dissipation in the sediment phase of the test systems for the parent only. Different kinetic models as proposed by the FOCUS Kinetics guidance document were tested in order to identify the best model for the whole system as well as the individual compartments water and sediment to derive appropriate endpoints. It is discerned between persistence and modeling endpoints. The recommended procedures to estimate the persistence endpoints are outlined in [*FOCUS (2006), Figure 10-1, p. 197*]. The recommended procedures to estimate the modelling endpoints are outlined in *[FOCUS (2006), Figure 10-2, p. 198*].
At the P-II level (two-compartmental approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.
For the metabolites M3 and M23 the "Level M-I" approach was followed; since in the water/sediment systems no decline phase occurred, dissipation rates in the water and sediment compartment [*FOCUS (2006), Box 10-3, p.219*] could not be evaluated. An analysis at M-I level for estimation of the degradation rates in the whole water-sediment system [*FOCUS (2006), Box 10-4, p.224*], however, was performed. Whole system data for the parent substance and the metabolites were used to estimate degradation rates of the metabolites M3 and M23. For the parent the best fit kinetic model from P-I analysis was considered, for the metabolites the data was fitted to SFO kinetics. If no acceptable fit to the data could be achieved using SFO kinetics, the default DT_{50} value of 1000 days was used. In addition to the parent substance and the metabolites M3 and M23 two sink compartments are defined: one sink compartment represents the minor metabolites PL36-88, M11 and M31. The second sink compartment represents CO_2 and bound residues, no concentration data is connected to this compartment. Two separate sink compartments are used since for one compartment data is available (minor metabolites) whereas no data is available for the second sink compartment (CO_2 and bound residues).

The kinetic models were implemented in Model Maker by means of a compartment model, considering the underlying equations defined in the FOCUS kinetics guidance document.

II. RESULTS AND DISCUSSION

Pond system

Trigger endpoints

Level P-I: BAS 656 H in the whole system

Table 7.2.2.3-9: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, whole system (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO & FOMC	SFO	2.7	k: <0.001	Good	35.1	116.5
	FOMC	2.8	β: 0.499	Good	35.1	116.5
SFO fit statistically sufficient and visually acceptable; FOMC visually acceptable but statistically not sufficient; moreover χ^2 error larger than SFO, therefore use SFO: **$DegT_{50}$ = 35.1 d**						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-1, along with its statistical evaluation.

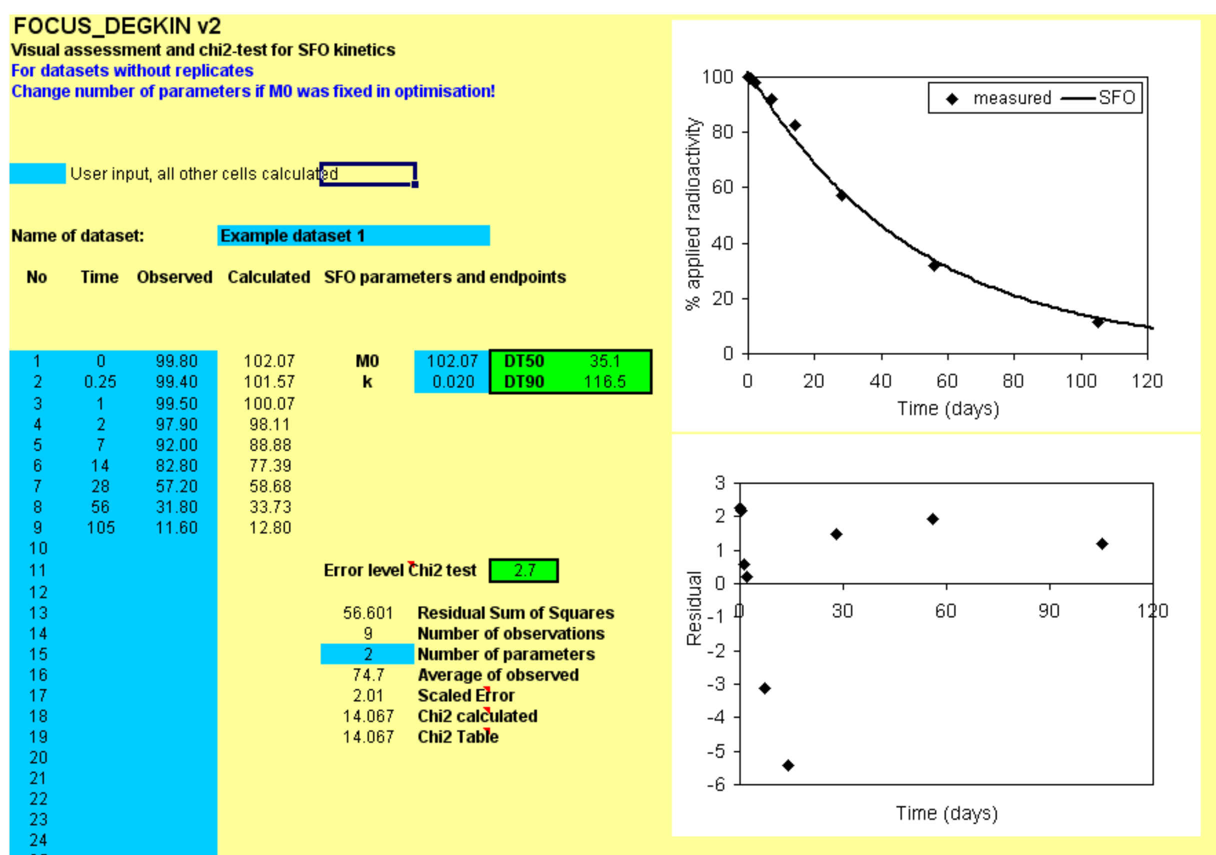


Figure 7.2.2.3-2: Level P-I, Pond, whole system: SFO kinetic evaluation

Level P-I: BAS 656 H in water

Table 7.2.2.3-10: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, water compartment (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO & FOMC	SFO	4.5	k: <0.001	Moderate	23.6	78.3
	FOMC	3.7	β: 0.075	Good	21.1	102.2
SFO results in moderate visual fit and is statistically sufficient, FOMC visually and statistically acceptable and more appropriate than SFO; therefore run modified fitting: Constrain M0.						
Run modified fitting: Constrain M0.	SFO	7.0	k: <0.001	Not acceptable	21.1	102.2
	FOMC	5.1	β: 0.061	moderate	17.8	114.3
Modified SFO fit visually not acceptable. FOMC fit visually moderate, but worse than without M0 constrained, though statistically sufficient. Therefore use FOMC. Modification does not yield significant improvements, therefore use FOMC (unmodified).						
Run DFOP and HS	DFOP	1.1	k1: <0.001 k2: 0.003 g: <0.001	Good	21.4*	86.2*
	HS	2.3	M0: <0.001 hingepoint: 0.059 k1: 0.065 k2: <0.001	Good	21.3	81.2
Conclusion: DFOP fit and HS fit visually acceptable and statistically sufficient. DFOP fit statistically better than HS fit and FOMC fit. Therefore use DFOP fit for parent: **DT_{50} = 21.4 d.**						

* approximated from the calculated values

The measured vs. calculated values and the respective residual values for DFOP kinetics of the parent are depicted in Figure 7.2.2.3-2.

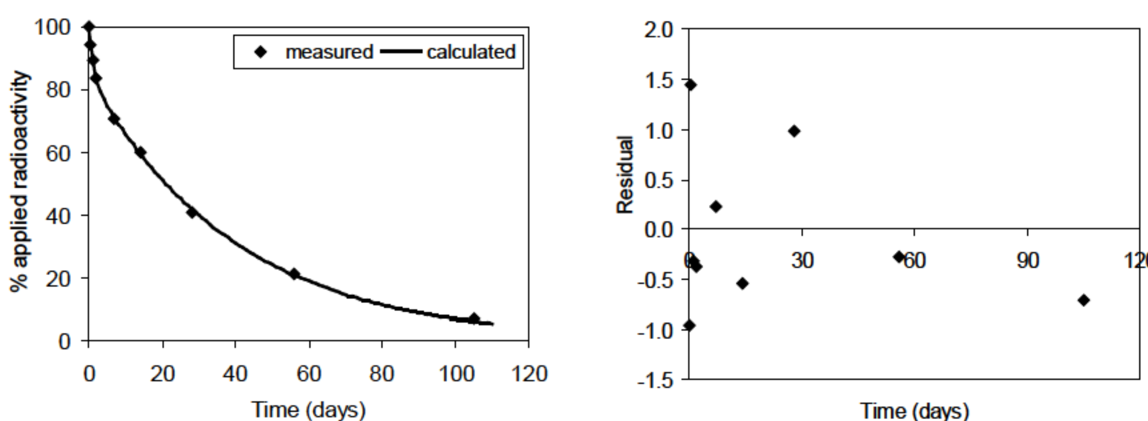


Figure 7.2.2.3-3: Water compartment evaluation (Pond, level P-I): Calculated vs. measured data (left) and residual data (right) of Dimethenamid (DFOP kinetics)

Level P-I: BAS 656 H in sediment

Table 7.2.2.3-3: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, sediment compartment (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO & FOMC	SFO	3.5	k: 0.004	Good	38.2	126.9
	FOMC	3.1	β: 0.500	Good	35.1	159.1
SFO fit is visually acceptable and statistically sufficient, FOMC is visually acceptable but statistically not sufficient; therefore use SFO: DT_{50} = 38.2 d.						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-3.

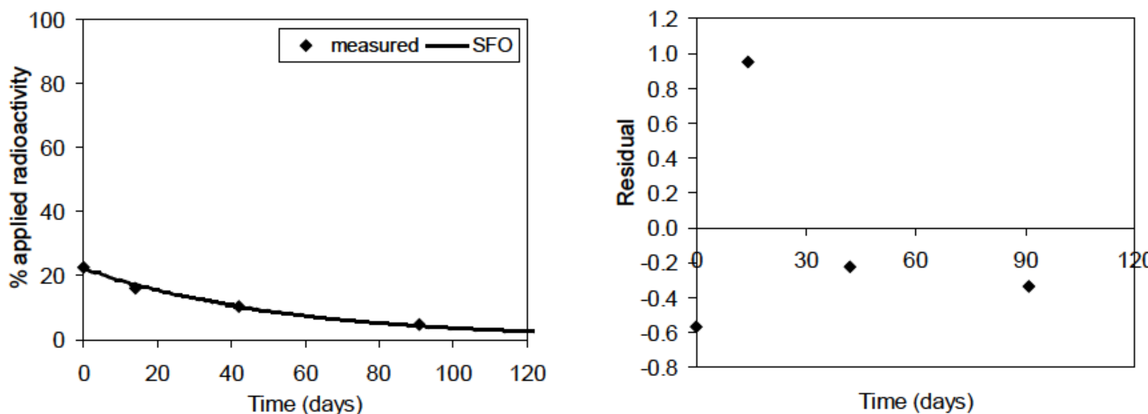


Figure 7.2.2.3-3: Sediment compartment evaluation (Pond, level P-I): Calculated vs. measured data (left) and residual data (right) of Dimethenamid (SFO kinetics)

Modelling endpoints

Level P-I: BAS 656 H in the whole system

Table 7.2.2.3-12: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, whole system (modelling endpoints)

Step in FOCUS flowchart	**Kinetic model**	**χ^2 error**	**p-value**	**Visual assessment**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**
Run SFO	SFO	2.7	k: <0.001	Moderate	35.1	116.5
SFO fit statistically sufficient and visually acceptable. Therefore use SFO fit for parent: **$DegT_{50}$ = 35.1 d.**						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-2 above.

Level P-I: BAS 656 H in water

Table 7.2.2.3-13: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, water compartment (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO & FOMC	SFO	4.5	k: <0.001	Moderate	23.6	78.3
	FOMC	3.7	β: 0.075	Good	21.1	102.2
SFO results in moderate visual fit and is statistically sufficient, FOMC visually and statistically acceptable and more appropriate than SFO; therefore run modified fitting: Constrain M0.						
Run modified fitting: Constrain M0.	SFO	7.0	k: <0.001	Not acceptable	21.1	102.2
	FOMC	5.1	β: 0.061	moderate	17.8	114.3
Modified SFO fit visually not acceptable. FOMC fit visually moderate, but worse than without M0 constrained, though statistically sufficient. Therefore use FOMC. Modification does not yield significant improvements, therefore use FOMC (unmodified).						
Run DFOP and HS	DFOP	1.1	k1: <0.001 k2: 0.003 g: <0.001	Good	21.4*	86.2*
	HS	2.3	M0: <0.001 hingepoint: 0.059 k1: 0.065 k2: <0.001	Good	21.3	81.2
Conclusion: DFOP fit and HS fit visually acceptable and statistically sufficient. DFOP fit statistically better than HS fit and FOMC fit. Therefore use half-life back-calculated from DFOP fit: **DT_{50} = DT_{90} / 3.32 = 26.0 d.**						

* approximated from the calculated values

The measured vs. calculated values and the respective residual values for DFOP kinetics of the parent are depicted in Figure 7.2.2.3-3.

Level P-I: BAS 656 H in sediment

Table 7.2.2.3-14: Statistical and visual assessment of kinetic models for BAS 656 H in Pond, sediment compartment (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO	SFO	3.5	k: 0.004	Good	38.2	126.9
SFO fit is visually acceptable and statistically sufficient; therefore use SFO: **DT_{50} = 38.2 d**.						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-3 above.

River system

Trigger endpoints

Level P-I: BAS 656 H in whole system

Table 7.2.2.3-15: Statistical and visual assessment of kinetic models for BAS 656 H in River, whole system (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO & FOMC	SFO	2.6	k: <0.001	Good	19.8	65.8
	FOMC	2.6	β: 0.230	Good	19.4	69.7
SFO fit visually acceptable and statistically sufficient, FOMC visually acceptable but statistically not sufficient; moreover no improvement against SFO, therefore use SFO: **$DegT_{50}$ = 19.8 d.**						

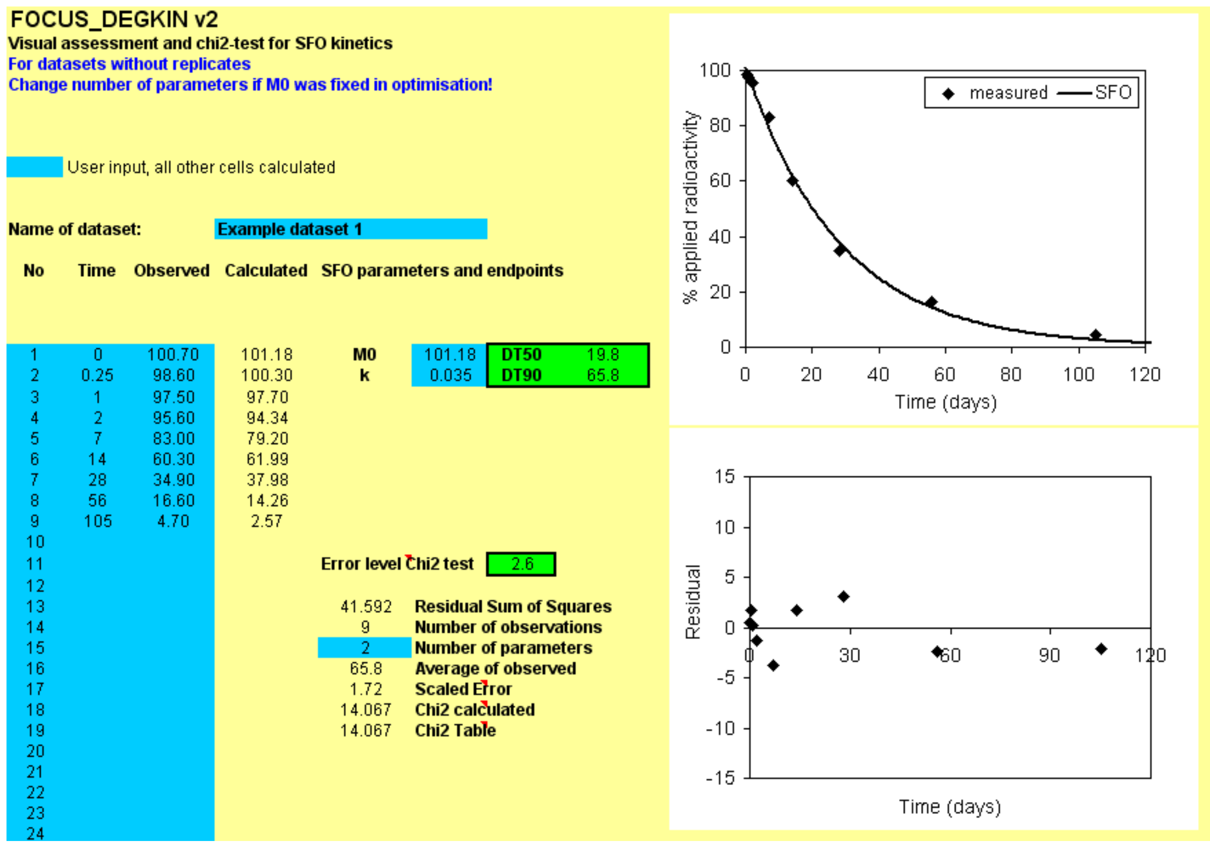


Figure 7.2.2.3-5: Level P-I, river, whole system: SFO kinetic evaluation

Level P-I: BAS 656 H in water

Table 7.2.2.3-16: Statistical and visual assessment of kinetic models for BAS 656 H in River, water compartment (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO & FOMC	SFO	5.4	k: <0.001	Moderate	12.7	42.1
	FOMC	3.6	β: 0.035	Good	11.1	57.7
SFO fit visually not acceptable but statistically sufficient, FOMC visually acceptable and statistically sufficient; therefore run modified fitting: Constrain M0.						
Run modified fitting: Constrain M0.	SFO	7.7	k: <0.001	Not acceptable	11.0	36.6
	FOMC	5.1	β: 0.028	Moderate	9.5	60.9
SFO fit visually still not acceptable, but statistically sufficient. FOMC fit visually acceptable, but worse than without M0 constrained, and statistically sufficient. Therefore use FOMC. Modification does not yield significant improvements, therefore use FOMC (unmodified).						
Run DFOP and HS	DFOP	6.1	k1: 0.452 k2: 0.454 g: 0.499	Moderate	12.8*	42.3*
	HS	3.9	M0: <0.001 hingepoint: 0.066 k1: 0.003 k2: <0.001	Good	11.8	47.5
DFOP fit visually moderate but statistically not sufficient. HS fit visually acceptable and statistically sufficient. FOMC fit visually better than HS fit, and statistically more appropriate (slightly lower χ^2 error). Therefore use FOMC fit: **DT_{50} = 11.1 d.**						

* approximated from the calculated values

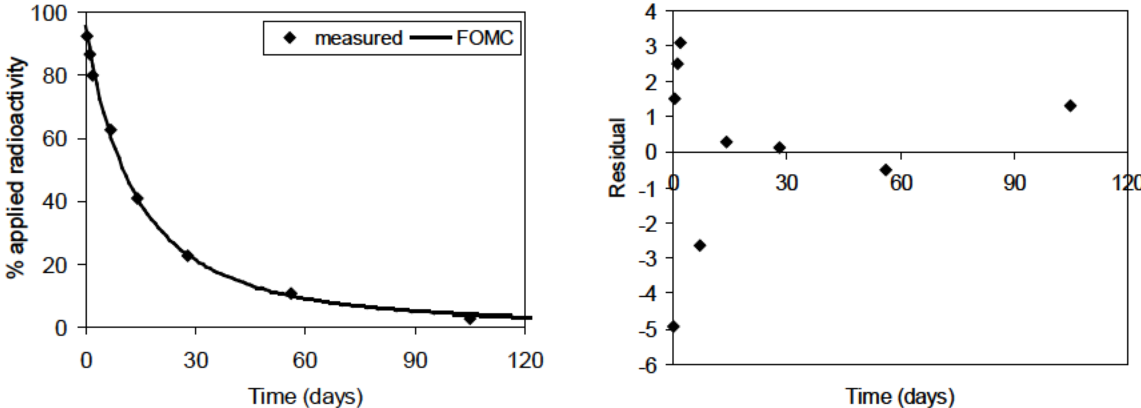


Figure 7.2.2.3-6: Water compartment evaluation (River, level P-I): Calculated vs. measured data (left) and residual data (right) of Dimethenamid (FOMC kinetics)

Level P-I: BAS 656 H in sediment

Table 7.2.2.3-17: Statistical and visual assessment of kinetic models for BAS 656 H in River, sediment compartment (trigger endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO & FOMC	SFO	5.4	k: 0.006	Good	28.5	94.7
	FOMC	6.2	β: 0.500	Good	28.5	94.7
SFO fit visually acceptable and statistically sufficient, FOMC visually acceptable but statistically not sufficient; therefore use SFO: **DT_{50} = 28.5 d.**						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-6.

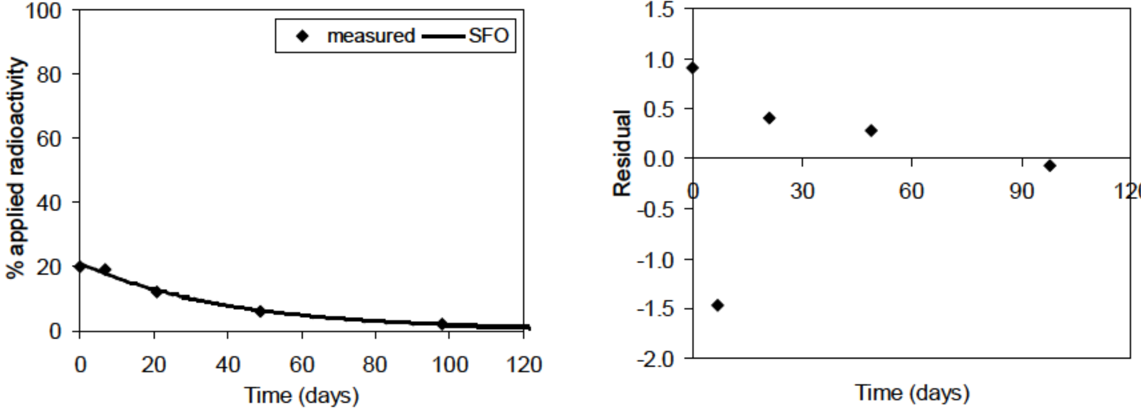


Figure 7.2.2.3-7: Sediment compartment evaluation (River, level P-I): Calculated vs. measured data (left) and residual data (right) of Dimethenamid (SFO kinetics)

Modelling endpoints

Level P-I: BAS 656 H in whole system

Table 7.2.2.3-18: Statistical and visual assessment of kinetic models for BAS 656 H in River, whole system (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Run SFO	SFO	2.6	k: <0.001	Moderate	19.8	65.8
SFO fit visually acceptable and statistically sufficient, therefore use SFO. **$DegT_{50}$ = 19.8 d**.						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-5 above.

Level P-I: BAS 656 H in water

Table 7.2.2.3-19: Statistical and visual assessment of kinetic models for BAS 656 H in River, water compartment (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p-value	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO	SFO	5.4	k: <0.001	Moderate	12.7	42.1
SFO fit visually not acceptable but statistically sufficient; therefore run modified fitting: Constrain M0.						
Run modified fitting Constrain M0.	SFO	7.7	k: <0.001	Not acceptable	11.0	36.6
SFO fit visually still not acceptable, but statistically sufficient. 10% of initially measured concentration reached within experimental period; therefore run DFOP, FOMC and HS.						
Run DFOP, FOMC and HS	DFOP	6.1	k1: 0.452 k2: 0.454 g: 0.499	moderate	12.8*	42.3*
	FOMC	3.6	β: 0.035	Good	11.1	57.7
	HS	3.9	M0: <0.001 hingepoint: 0.066 k1: 0.003 k2: <0.001	Good	11.8	47.5
DFOP fit visually moderate but statistically not sufficient. HS fit visually acceptable and statistically sufficient. FOMC fit visually better than HS fit, and statistically more appropriate (slightly lower χ^2 error). Within experimental period 10% of initially measured concentration was reached. Therefore use half-life back-calculated from FOMC fit: **$DT_{50} = DT_{90}$ / 3.32 = 17.4 d.**						

* approximated from the calculated values

The measured vs. calculated values and the respective residual values for FOMC kinetics of the parent are depicted in Figure 7.2.2.3-6 above.

Level P-I: BAS 656 H in sediment

Table 7.2.2.3-20: Statistical and visual assessment of kinetic models for BAS 656 H in River, sediment compartment (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run SFO	SFO	5.4	k: 0.006	Good	28.5	94.7
SFO fit visually acceptable and statistically sufficient; therefore use SFO: **DT_{50} = 28.5 d**.						

The measured vs. calculated values and the respective residual values for SFO kinetics of the parent are depicted in Figure 7.2.2.3-7 above.

P-I Result overview

The assessment of the kinetic models for the Pond and River test systems are shown in Table 7.2.2.3-21considering the different compartments for trigger and modelling endpoints.

Table 7.2.2.3-21: P-I trigger and modelling endpoints for BAS 656 H for the Pond and River test systems

Test system	Trigger endpoints			Modelling endpoints	
Whole system	**Kinetic model**	**$DegT_{50}$**	**$DegT_{90}$**	**Kinetic model**	**$DegT_{50}$**
Pond	SFO	35.1	116.5	SFO	35.1
River	SFO	19.8	65.8	SFO	19.8
Geometric mean:		**26.4**	**87.6**		**26.4**
Water compartment	**Kinetic model**	**DT_{50}**	**DT_{90}**	**Kinetic model**	**DT_{50}**
Pond	DFOP	21.4	86.2	DFOP	26.0
River	FOMC	11.1	57.7	FOMC	17.4
Sediment compartment	**Kinetic model**	**DT_{50}**	**DT_{90}**	**Kinetic model**	**DT_{50}**
Pond	SFO	38.2	126.9	SFO	38.2
River	SFO	28.5	94.7	SFO	28.5

Level P-II: BAS 656 H in water and sediment

Pond

For the evaluation of the Pond system data on P-II level degradation in water and sediment as well as the partitioning between these compartments is considered. A stepwise approach was used; the estimated parameters are shown in Table 7.2.2.3-22. The degradation in the water phase could not be estimated reliably, therefore no degradation rates in the individual compartments could be determined.

Table 7.2.2.3-22: Level P-II water and sediment: Estimated parameters (Pond system)

Test system	Parameter	estimated value	std error	P-value
Pond	M_0 [%TAR]	99.54	1.48	<0.001
	kwP [d^{-1}]	$5.34*10^{-14}$	0.014	0.500*
	ksP [d^{-1}]	0.073	0.029	0.036
	r_sw [d^{-1}]	0.252	0.056	<0.001
	r_ws [d^{-1}]	0.114	0.016	<0.001

* not significantly different from zero (for a 10% significance level)

River system

For the evaluation of the River system data on P-II level degradation in water and sediment as well as the partitioning between these compartments is considered. The stepwise approach was used; the estimated parameters are shown in Table 7.2.2.3-23. The degradation in the water phase could not be estimated reliably, therefore no degradation rates in the individual compartments could be determined.

Table 7.2.2.3-23: Level P-II water and sediment: Estimated parameters (River system)

Test system	Parameter	estimated value	std error	P-value
River	M_0 [%TAR]	98.18	1.82	<0.001
	kwP [d^{-1}]	$7.2*10^{-14}$	0.022	0.500*
	ksP [d^{-1}]	0.134	0.061	0.023
	r_sw [d^{-1}]	0.216	0.067	0.002
	r_ws [d^{-1}]	0.129	0.022	<0.001

* not significantly different from zero (for a 10% significance level)

Level M-I

For the estimation of modelling endpoints based on metabolite formation and degradation a multi-compartment model is used which incorporates the parent substance BAS 656 H and the metabolites M3 and M23. Since the metabolites M13, M31 and PL 36-88 are not evaluated, the sum of their measured concentrations is used as a sink compartment.

Table 7.2.2.3-24: Statistical and visual assessment of kinetic models at level M-I for BAS 656 H and metabolites M3 and M23 in Pond, whole system (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run Parent best-fit from P-I (SFO) and metabolite 3 SFO	M3: SFO	69.3	k: 0.006	Not acceptable	4.0	13.5
SFO kinetics visually not acceptable and statistically not sufficient. Therefore decide case-by-case: Use default DT_{50} = 1000 days. Set degradation of M3 to the metabolite M23 to the respective degradation rate k=ln(2)/1000 = 6.93E-04.						
Run parent best-fit (SFO), metabolite M3 SFO with fixed degradation rate, metabolite M23 SFO	M23: SFO	78.9	k: 0.500	Not acceptable	-** ($6.93*10^{18}$)	-** ($2.30*10^{19}$)
SFO fit visually not acceptable and statistically not sufficient. Therefore decide case-by-case: Use default DT_{50} of 1000 days for metabolite M23.						

* Half-lives could not be determined from the calculated values since no decline occurs within the study period
** degradation rate ($k=10^{-19}$) was not different from zero according to t-test, therefore no reliable DT_{50} and DT_{90} could be calculated.

Table 7.2.2.3-25: Statistical and visual assessment of kinetic models at level M-I for BAS 656 H and metabolites M3 and M23 in River, whole system (modelling endpoints)

Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	DT_{50} [d]	DT_{90} [d]
Run Parent best-fit from P-I (SFO) and metabolite M3 SFO	M3: SFO	59.4	k: 0.489	Not acceptable	M3: 7.6	M3: 25.3
SFO fit visually not acceptable and statistically not sufficient. Therefore decide case-by-case: Use default DT_{50} = 1000 days. Set degradation of M3 to the metabolite M23 to the respective degradation rate k=ln(2)/1000 = 6.93E-04.						
Run parent best-fit (SFO), metabolite M3 SFO with fixed degradation rate, metabolite M23 SFO	M23: SFO	64.1	k: 0.500	Not acceptable	M23: - ($6.93*10^{18}$)**	M23: - ($2.3*10^{19}$)**
SFO fit visually not acceptable and statistically not sufficient. Therefore decide case-by-case: Set default DT_{50} = 1000 days for metabolite M23.						

* β parameter was not different from zero according to t-test, therefore no reliable DT_{50} and DT_{90} could be calculated.
** degradation rate ($k=10^{-19}$) was not different from zero according to t-test, therefore no reliable DT_{50} and DT_{90} could be calculated.

According to FOCUS recommendations (FOCUS, 2006) as a case by case decision a default DT_{50} of 1000 days is used for the metabolite M3. After adding the metabolite M23 to the model using SFO kinetics for the degradation to sink, no appropriate fit to the data could be obtained. Therefore, for M23 a default DT_{50} of 1000 days was assessed as well.

III. CONCLUSION

Data on the degradation of BAS 656 H - Dimethenamid and its metabolites M3 and M23 from a water-sediment laboratory study was evaluated according to the FOCUS (2006) recommendations. The study contained data on two systems, "Pond" and "River".

Level P-I kinetic analysis of the whole system resulted in trigger and modelling endpoints, specifically $DegT_{50}$ values of 35.1 d (SFO) and 19.8 d (SFO) for the Pond and River test system, respectively. The geometric mean $DegT_{50}$ value is 26.4 d.

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

No study on the degradation in the saturated zone was performed.

CA 7.3 Fate and behaviour in air

Based on its physical-chemical properties, Dimethenamid-p has a potential for volatilisation (vapour pressure 2.5 x 10^{-3} Pa at 25°C). An old volatilisation study exists but does not fulfil the latest requirements. A new volatilisation study on soil and plant surfaces was performed under current guidance.

CA 7.3.1 Route and rate of degradation in air

Report: CA 7.3.1/1
Hassink J., 2013a
Volatilisation of Dimethenamid-P after application of BAS 656 12 H on soil and plant surfaces
2012/1282998
Guidelines: BBA IV 6-1
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The volatilisation behaviour of Dimethenamid-P was investigated after application of the EC formulation BAS 656 12 H. The formulation was spiked with ^{14}C-Dimethenamid-P on soil (LUFA 5M) and plant (bush bean) surfaces in a circulation chamber, based on a nominal field application rate of 864 g active substance/ha. The test duration was 24 h.

The recovery rates for the volatilisation experiments were 103 and 91 % for the soil and the plant experiments, respectively.

The volatilisation rate of BAS 656 H from plants is significant with values from 17.5% to 26.1% after 24 h depending on the applied calculation. From soil, the volatilisation rate of BAS 656 H is significantly reduced to 2.8-5.3 % since adsorption to soil particles can be expected.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal Code: BAS 656 H
Common Name: Dimethenamid-P
Reg. No.: 363851
CAS-No. 163515-14-8
Chem. Name: (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Molar Mass: 275.8 g mol^{-1} (unlabelled)
Emprirical Formula: $C_{12} H_{18} Cl N O_2 S$

Structure:

AND Enantiomer

Labelled test item:

Label: [thienyl-5-^{14}C]
Batch-No.: 824-6027
Specific Activity: 8.21 MBq/mg
Radiochem. Purity: 99 %
Origin: GVC/N

Formulation

Code No.: BAS 656 12 H (EC-formulation)
Batch-No.: 0004701751
Nominal content: 0.72 kg BAS 656 H/l
Analyzed content: 0.71 kg BAS 656 H/l
Density: 1.119 kg/l
Origin: APR/DP

Based on a field application rate of 864 g BAS 656 H/ha and a treated area of 113 cm^2, the plant was treated with about 1212 µg and the soil was treated with about 1261 µg BAS 656 H.
For each volatilisation experiment the application solution was freshly prepared. The formulation BAS 656 12 H was spiked with about 0.8 % labelled BAS 656 H, thus resulting in a total application rate of about 1072 g (plant trial) and 1115 g (soil trial) BAS 656 H / ha.

B. STUDY DESIGN

1. Experimental conditions

The soil and the plant were treated in a closed application chamber made of glass. The formulation was applied via a spray nozzle (1.2 bar) to the plant and to the soil.

For determination of volatilisation from the plant surface, a bush bean planted in a soil-containing glass tray was used for the experiment. The soil was covered during application and application losses were determined by rinsing the glass container and all equipment with acetonitrile and subsequent analysis of the rinsate.

For determination of volatilisation from the soil surface, the soil was adjusted to 60% of the maximum water holding capacity (MWC). The soil characteristics (USDA: sandy loam) were: 58.8% sand, 28.9% silt, 12.2% clay, organic carbon 1.99%, pH 7.2, maximum water holding capacity 28.9 g/100 g dry soil. Moisture losses were compensated for, and evaporation of water from the soil surface led to an average air humidity of 60 %.

After application, the plant and/or the soil were removed from the application chamber and transferred directly to the circulation chamber which allowed the air exchange rate (200 L/h) and the temperature (20 - 21°C) of the air to be controlled. The wind speed was adjusted to 1 m/s. In the plant experiment the timer of the lamp was set to 12 h light, 9 h dark, and 3 h light during the testing period of 72 h. Volatiles were captured in charcoal traps, which were replaced and sampled 1, 3, 6, and 24 h after application. At the same intervals the cryo-traps and the CO_2 traps were exchanged and sampled. At the end of the experiment, the remaining radioactivity in soil and plant (parts of the plant above the soil surface) was determined. Furthermore, the equipment (chamber, tubes, fans) was rinsed and the solutions analysed.

2. Description of analytical procedures

The plant parts above the soil surface were macerated and extracted sequentially with acetonitrile, acetonitrile/water (1/1) and water. The extracts were combined and the radioactivity in the extracts was measured by liquid scintillation counting (LSC). The soil was treated in the same way. The remaining plant or soil material was combusted and also measured by LSC.
All traps were collected and analysed by direct LSC measurement and/or combustion.

II. RESULTS AND DISCUSSION

The volatilisation rate was calculated in three ways:

1. via Traps and Applied Substance (TAS, ‘direct’ measurement):

The sum of the amount of active ingredient detected in the charcoal traps and the equipment wash (and for the plant experiment: the amount of a.s. in the soil) were defined as the volatile part. This value was related to the total amount of a.s. applied to the test system.

2. via Residues and Applied Substance (RAS, ‘indirect’ measurement):

The item in the test system (i.e. either sum of plant extract and plant residues or sum of soil extract and soil residue) plus the a.s. equivalents in the CO_2 traps were the non-volatile part. The difference of this value to the amount applied to the test system was related to the total amount of a.s. applied to the test system.

3. via Residues and the Recovery rate of the Volatilisation experiment (RRV, ‘indirect‘ measurement):

The non-volatile part was calculated as RAS, but corrected with the recovery of the volatilisation experiment.

Recovery rates were calculated both for the complete experiment (all solutions, extracts, combusted samples vs. amount of Dimethenamid-P in application system) and for the respective volatilisation experiment (traps at every sampling time, circulation chamber wash and test system after 24 h vs substance on the test system after deduction of application losses).

The volatilisation rate of BAS 656 H after application of EC formulation BAS 656 12 H to plants is significant with values from 17.5 % to 26.1 % after 24 h depending on the applied calculation. From soil, the volatilisation rate of BAS 656 H is significantly reduced to 2.8-5.3 % since adsorption to soil particles can be expected. The obtained recoveries and volatilisation rates are shown in Table 7.3.1-1.

Table 7.3.1-1: Recovery of radioactivity in % of Dimethenamid-P during plant and soil experiment

Matrix	Recovery Rates [%]		Volatilisation Rate [%]		
	Complete experiment	Volatilisation experiment	TAS	RAS*	RRV*
Plant	97.4	91.4	17.5	26.1	19.1
Soil	100.6	102.5	5.3	2.8	5.2

* Indirect measurement, i.e. including eventual degradation and/or adsorption processes

III. CONCLUSION

Considering the values for both surfaces, a significant difference between volatilisation from plant and soil surfaces was observed. The results demonstrate that the volatilisation potential of EC formulation BAS 656 12 H spiked with ^{14}C-Dimethenamid-P from plants is significant with values from 17.5 % to 26.1 %. From soil, the volatilisation rate of BAS 656 H is significantly reduced to 2.8-5.3 % since adsorption to soil particles can be expected.

CA 7.3.2 Transport via air

Due to the low to moderate volatility observed for Dimethenamid-P from soil and plant surfaces and the significantly low half-life observed in air (DT_{50}=2.45 hrs, as submitted as part of the previous Annex-II review) significant transport via air is not envisioned as a major contributing factor.

CA 7.3.3 Local and global effects

Low to moderate volatility of Dimethenamid-P may be observed. In any case of volatilisation a very low half-life of Dimethenamid-P would prevent any transport of the active ingredient beyond an extremely localized environment. In addition, as evidenced by the small percentage of Dimethenamid-P observed volatilized in a 24 h period, marginal effects, if any, would be expected in an extremely localized area.

The literature paper presented below demonstrates that Dimethenamid-P was observed rarely in air in both rural, agricultural areas as well as in more populated urban areas. Though Dimethenamid-P is thought to be moderately volatile, this shows that in this region it was rarely seen and when it was observed, it was at very low concentrations.

Report: CA 7.3.3/1
Coscolla C. et al., 2010b
Occurrence of currently used pesticides in ambient air of Centre Region (France)
2010/1229759
Guidelines: <none>
GLP: no

Executive Summary

The study aimed to improve the knowledge about the atmospheric behaviour of current-used pesticides in the central region of France. A group of 56 pesticides including dimethenamid was monitored in this region during 2006 to 2008. Weekly, air samples were collected at three rural and two urban sites on quartz fibre filters plus polyurethane foam plugs. Targeted pesticides were extracted from the samples and analysed to determine their concentrations. In 262 samples analysed, dimethenamid was detected at a frequency of 2%. The overall concentration of dimethenamid ranged from 0.16-0.74 ng m^{-3}.

I. MATERIAL AND METHODS

Test material

In the study, three groups of pesticides were tested:

- A group of herbicides including dimethenamid
- A group of insecticides
- A group of fungicides

Air Sampling and analysis

Sampling was performed in three campaigns from 2006 to 2008 [2006: 14 March12 September; 2007: 11 April-11 July, except for Saint Martin d'Auxigny (until 11 September). 2008: 9 April - 2 July, except for Saint Martin d'Auxigny (until 5 November)) using a low-volume sampler (Partisol 2000). Gas samples and particulate matter were collected together on quartz fibre filters (47 mm diameter) followed by polyurethane foam plugs (26 mm diameter x 76 mm length). Sampling was performed on a weekly basis by exposing filters and polyurethane plugs at a flow rate of 1 m^3 h^{-1}. A total volume of 168 m^3 was collected approximately. Samples were analysed after sampling or after a storage period at -18 °C. The studied pesticides were extracted from the samples and the concentrations were determined using LC-MS/MS and GC-MS techniques.

A. MATERIALS

B. STUDY DESIGN

Field study design

The study was carried out within the central region of France on three rural sites (Saint Martin d'Auxigny, Oysonville and Saint Aignan) and two urban sites (Tour and Orléans). The area of the region is about 40000 km^2 and about 57% of the surface is used for agricultural activities. Pesticides are used intensively in the farming activities in this region.

II. RESULTS AND DISCUSSION

Method efficiency
The retention capacity for the polyurethane foam plugs ranged from 60-120% and the obtained recoveries were in the range from 70-110% for PUF plugs plus quartz filters.

Residues in Air
In total, 262 air samples were tested for herbicides along the study period. Dimethenamid was detected at a low frequency of 2%. The overall concentration of dimethenamid ranged from 0.16-0.74 ng m^{-3}.

III. CONCLUSION

In 262 samples analysed, dimethenamid was detected at a frequency of 2%. The overall concentration of dimethenamid ranged from 0.16-0.74 ng m^{-3}.

CA 7.4 Definition of the residue

Soil, water and air

In the previous evaluation only the active substance Dimethenamid-p was given in the residue definition for soil, water and air. Below are updated definitions based on new information and conservative approaches to the exposure estimations.

Soil
For the definition of residue for risk assessment in soil besides Dimethenamid-P, its metabolites M656PH023, M656PH027 and M656PH031 should be considered.

For the definition of residue for monitoring in soil only Dimethenamid-P is relevant.

Groundwater
For the definition of the residue for risk assessment, besides Dimethenamid-P all metabolites above 0.1 µg/L in lysimeter leachate or PEC groundwater should be considered: M656PH003*, M656PHM010*, (*these were found in the original lysimeter study to be > 0.1 µg/L. After re-evaluation these were found below 0.1 µg/L but were also evaluated in the risk assessment.) M656PH023, M656PH027, M656PH031, M656PH032, M656PH043, M656PH045, M656PH047, M656PH049 (iso), M656PH050, M656PH051, M656PH052 (iso), M656PH053 (iso), M656PH054, M656H055, M656PH059 (iso) and M656PH062.

For the definition of the residue for monitoring, besides Dimethenamid-P representative metabolites should be included, following the grouping concept for further investigation and assessment of metabolites. The metabolites for monitoring are as follows: M656PH023, M656PH027, M656PH031, M656PH032, M656PH043, M656PH045, M656PH047 and M656PH054.

Surface water
For the definition of the residue for risk assessment in surface water, Dimethenamid-p is relevant as well as the following metabolites: M656PH003, M656PH023, M656PH027, M656PH031.

For the definition of the residue for monitoring in surface water only Dimethenamid-P is relevant.

Air
For the definition of residue for risk assessment and monitoring in air only Dimethenamid-p is relevant.

CA 7.4.1 Definition of the residue for risk assessment

See point CA 7.4 above.

CA 7.4.2 Definition of the residue for monitoring

See point CA 7.4 above.

CA 7.5 Monitoring data

Several monitoring studies were performed by BASF SE with respect to dimethenamid-p and its metabolites either in surface water (CA 7.5/1) or groundwater (CA 7.5/2 - CA 7.5/8). In addition, summaries of open literature dealing with monitoring in surface water (CA 7.5/9, 7.5/10) and groundwater (CA 7.5/11) were reported.

Surface water

Report: CA 7.5/1
Laabs V., 2010a
Surface water screening for Dicamba, Dimethenamide-P, Bentazone, Tritosulfuron, Topramezone and selected metabolites in three corn growing regions of the EU
2010/1148003
Guidelines: <none>
GLP: no

Executive Summary

The purpose of the study was the monitoring of five corn herbicides and their metabolites in selected surface water bodies in three corn growing regions of Europe. In the following, the results for Dimethenamid-P are presented.

The Rott river (eastern Bavaria, Germany), the Adda and Oglio rivers (northern tributaries of the Po river, ltaly) and the Sió and Danube river (central-western part of Hungary) were chosen, which all drain areas with relatively intensive cultivation of corn.

Surface water samples were taken bi-weekly during the application season and weekly thereafter for five months (April to beginning of September in ltaly; May to beginning of October in Hungary), or weekly from May to November (Germany).

The analysis of samples was done centrally in the laboratory of BASF SE in Limburgerhof (Germany), using a multi-residue analysis method with a quantification limit of 0.01 µg/L for all analysed substances.

Dimethenamid-P was frequently detected in surface water samples in this study. Its maximum concentration reached 0.46 (Germany, Rott) to 0.51 (Hungray, Sió) µg/L at two sampling locations, while much lower peak concentrations were measured at the other sampling locations (<LOQ to 0.02 µg/L). It was mostly present at low concentrations or in traces for medium long periods of time in surface water bodies (>2 months). Dimethenamid-P metabolites were detected in traces at all sampling sites. At the German sampling site, peak concentrations were highest and ranged from 0.02 to 0.13 µg/L (M27 ≈ M31 ≈ M23 > M3). The metabolite maximum peak was recorded roughly one month after the highest observance of the active ingredient.

While in the sampled Italian river bodies only sporadic traces of DMTA-P metabolites were measured, metabolites M23, M27, and M31 were frequently detected at the two Hungarian sites, however at low maximum concentrations ≤0.02 µg/L. M3 was never detected in Italian or Hungarian surface water samples.

In general, the observed concentrations of Dimethenamid-P in surface water fall far below all ecotoxicological protection goals (as defined by environmental quality standards).

The EU limit value for finished drinking water (0.1 µg/L) was only transiently and mostly slightly transgressed in surface water samples in Germany and Hungary.

I. MATERIAL AND METHODS

A. MATERIALS

Reference substance

BAS code:	BAS 656 H
Common name:	Dimethenamide-P
Reg. No.:	363851
CAS-No.:	163515-14-8
Chemical name (IUPAC):	(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamid
Chemical formula:	$C_{12}H_{18}ClNO_2S$
Molar mass:	275.8 g/mol
Purity:	96.4%

B. STUDY DESIGN

Test sites

Sampling locations were chosen within regions of Europe where corn cultivation is one of the major cropping systems. The selection of monitoring points was in accordance with recommendations from national, BASF and local independent scientific experts.

The Rott river (eastern Bavaria, Germany), the Adda and Oglio rivers (northern tributaries of the Po river, ltaly) and the Sió and Danube river (central-western part of Hungary) were chosen, which all drain areas with relatively intensive cultivation of corn.

The surface water sampling spots were chosen to represent regionally independent catchments, providing information about the contamination situation in their basin areas.

An overview of the selected sites is given in Table 7.5-1.

Table 7.5-1: Sampling sites and sampling procedures

River	Rott (Germany)	Adda (Italy)	Oglio (Italy)	Sió (Hungary)	Danube (Hungary)
GPS coordinates	48.4282 N, 13.3341 E	45.1866° N, 9.7782° E	45.0415° N, 10.6499° E	46.3774° N, 18.7266° E	46.3522° N, 18.8945° E
Sample type	72-h time-integrated samples at several dates; grab samples (one sample) at all other sampling dates	Grab composite sample (three subsamples of river transect)			
Surrounding area	A mixture of cropland (corn, cereals) and scattered pastures dominates the land use in this region	Sampling spot located within the town centre of Pizzighettone (town surrounded by agricultural land)	Sampling spot located within agricultural land, dominated by cropped fields	Sampling spot surrounded by agricultural land cropped with corn and sunflowers	The larger area is dominated by agricultural land cropped with corn and sunflowers
Storm flow periods	Unknown	28 April, 01 May	28 April, 05 May	-	30 June, 07 July
Sampling device	Sampling tube of automated sampling device (72-h composite samples) or glass bottles	Horizontal water sampling bottle (PVC), inert silicone layer on inside		Horizontal water sampling bottle (PVC)	
Temperature	Unknown	12.4 to 23.5 °C	13.2 to 26.7 °C	16.4 to 27.6 °C	16.5 to 25.1 °C
pH	Unknown	7.9 to 8.9	8.0 to 8.9	8.2 to 9.1	7.5 to 9.4
Oxygen content	Unknown	7.0 to 8.1 mg/L	6.4 to 9.5 mg/L	62 to 250 % saturation	85 to 132 % saturation
Conductivity	Unknown	250 to 480 µS/cm	430 to 650 µS/cm	712 to 1145 µS/cm	292 to 416 µS/cm
Sample container	Glass bottles with Teflon-lined plastic screw caps	High-density polyethylene bottle (HDPE)			

Sampling and analysis

Surface water samples were taken biweekly during the application season and weekly thereafter for five months (April to beginning of September in Italy, May to end of September in Hungary), or weekly from May to November (Germany) in 2009.

At the sampling site in Rott (Germany), sampling was done at a regular water monitoring site of the Bavarian Environmental Agency (LfU). The samples were either taken within the special pesticide monitoring program PSMRegio (72-h time-integrated samples, using an automated sampling device), or in between the regular intervals as grab samples using 1 L glass bottles. Samples were taken at ca. 30 cm depth, on the right-hand side of the river. Automated samples were pumped over a period of 72 h and cooled (<8°C) within the automated sampling device. During transport to the laboratory and storage samples were kept cool (<8°C).

At the sampling sites in Italy and Hungary, a horizontal water sampling bottle (cleaned, ethanol-rinsed, and once rinsed with river water before use) was submerged in the surface water body to ca. 30 cm depth below the water surface level for sampling. To receive a composite grab sample, three subsamples of ca. 1 L were taken from the cross-section of the rivers; subsamples were evenly spaced out to achieve a representative composite sample for the river at this point. The subsamples were combined and mixed, and a portion of the sample was used to measure basic water parameters on site (temperature, pH, conductivity, and oxygen saturation). The sampling bottles were closed and stored on (dry)ice immediately after sampling and stored deep frozen (<-16°C) until shipment on dry ice for analysis.

The analysis of samples was done centrally in the laboratory of BASF SE in Limburgerhof (Germany). Prior to routine sample analysis, an analytical multi-residue method was developed (BASF No. L149/01) and validated. For analysis, a 50 mL aliquot of the water sample was acidified with 6M HCI to pH 2 and concentrated on a solid phase extraction column; the column was then washed with purified water (pH 2). After drying the column for 1 minute under vacuum the residues were eluted with methanol. The eluate was reduced to dryness by evaporation and the residue was dissolved in methanol/water to prepare the final volume for analysis. The sample was finally analyzed using UPLC-MS/MS. The limit of quantification (LOQ) was determined at 0.010 µg/L, the limit of detection (LOD) at 0.002 µg/L.

Method validation

For quality control, fortified samples (minimum of two samples) were routinely analyzed with each batch of surface water samples. The average recovery rates ranged from 93 to 102% of spiked amount for Dimethenamide-P and its metabolites.

II. RESULTS AND DISCUSSION

Analytical results for Dimethenamid-P (DMTA-P) are presented in Table 7.5-2.

In Germany Dimethenamid-P showed a maximum concentration of 0.46 µg/L in June. Dimethenamid-P could be detected in low amounts during the whole sampling period in samples of the Rott river. The substance concentrations measured in the 72-h time integrated samples fit well into the time course of concentrations from grab samples. Seemingly, pesticide peaks were of mid-term duration (several days) in the Rott river, while short-term peaks (e.g. from drift contamination) were not captured by the chosen sampling regime. The non-relevant metabolites of DMTA-P (M23, M27 and M31) showed maximum concentrations in the range from 0.11 to 0.13 µg/L in surface water at one sampling event in June, and were below 0.1 µg/L or <LOQ/<LOD at all other times All measured concentrations occurred only transiently at >0.05 µg/L.

Samples of the two tributary rivers of the Italian Po river contained only low peak concentrations of Dimethenamid-P (0.02 µg/L in one sample, rest <LOQ/<LOD).

In Hungarian surface water, measured peak concentrations were higher for the Sió river (0.01 to 0.51 µg/L) than for the Danube (<LOQ to 0.01 µg/L), as was to be expected due to dilution of pesticide peak concentrations in the larger river. In both rivers, Dimethenamid-P showed only transient peaks during the sampling period. In the Sió river, a transgression of the EU concentration limit of 0.1 µg/L for finished drinking water occurred only at one sampling event..

Table 7.5-2: Results of Dimethenamid-P analysis at the five surface water sampling sites in three EU countries in 2009

Germany		Italy			Hungary		
Date	**Rott**	**Date**	**Adda**	**Oglio**	**Date**	**Sió**	**Danube**
6.5.09	<LOQ	**14.4.09**	-	-	**5.5.09**	-	<LOQ
***12.05.09**	0.02	**17.4.09**	-	<LOQ	**8.5.09**	-	<LOQ
18.5.09	0.01	**21.4.09**	-	-	**12.5.09**	-	<LOQ
***26.05.09**	0.15	**25.4.09**	-	-	**15.5.09**	-	<LOQ
***03.06.09**	0.46	**28.4.09**	<LOQ	<LOQ	**19.5.09**	-	<LOQ
10.6.09	0.42	**1.5.09**	<LOQ	0.02	**22.5.09**	<LOQ	<LOQ
17.6.09	0.15	**5.5.09**	-	<LOQ	**26.5.09**	-	<LOQ
***23.06.09**	0.05	**8.5.09**	-	-	**29.5.09**	-	<LOQ
30.6.09	0.04	**15.5.09**	-	-	**2.6.09**	0.02	<LOQ
7.7.09	0.02	**21.5.09**	-	<LOQ	**9.6.09**	<LOQ	0.01
***14.07.09**	<LOQ	**28.5.09**	-	<LOQ	**15.6.09**	-	<LOQ
22.7.09	<LOQ	**6.6.09**	-	<LOQ	**23.6.09**	-	<LOQ
28.7.09	<LOQ	**12.6.09**	<LOQ	<LOQ	**30.6.09**	0.51	<LOQ
10.8.09	-	**19.6.09**	<LOQ	<LOQ	**7.7.09**	0.01	<LOQ
19.8.09	-	**26.6.09**	<LOQ	<LOQ	**14.7.09**	<LOQ	<LOQ
26.8.09	-	**3.7.09**	-	<LOQ	**22.7.09**	<LOQ	<LOQ
1.9.09	-	**10.7.09**	-	-	**29.7.09**	<LOQ	<LOQ
8.9.09	0.04	**17.7.09**	-	-	**7.8.09**	<LOQ	0.01
15.9.09	0.02	**24.7.09**	-	-	**10.8.09**	-	<LOQ
***22.09.09**	<LOQ	**31.7.09**	-	-	**19.8.09**	-	<LOQ
5.10.09	<LOQ	**7.8.09**	-	-	**27.8.09**	-	<LOQ
***13.10.09**	-	**17.8.09**	-	-	**1.9.09**	-	<LOQ
21.10.09	<LOQ	**20.8.09**	-	-	**8.9.09**	0.02	<LOQ
29.10.09	-	**28.8.09**	-	-	**15.9.09**	-	<LOQ
***03.11.09**	<LOQ	**4.9.09**	-	-	**21.9.09**	<LOQ	<LOQ
		11.9.09	-	-	**29.9.09**	<LOQ	<LOQ

* Signifies 72-h time integrated sample

\- Signifies measured concentration <LOD

Dimethenamid-P was detected in surface water samples in this study. Its maximum concentration reached 0.46 µg/L in the Rott river and 0.51 µg/L in the Sió river, while only low peak concentrations were measured at the other sampling locations (<LOQ to 0.02 µg/L). It was mostly present at low concentrations or in traces for medium long periods of time in surface water bodies (>2 months). DMTA-P metabolites were often detected at the Rott river sampling site, where peak concentrations ranged from 0.02 to 0.13 µg/L (M27 ≈ M31 ≈ M23 > M3) and the metabolite maximum peak was recorded roughly one month after the one of the active ingredient. In the sampled Italian river bodies only sporadic traces (<LOQ) of Dimethenamid-P metabolites were measured. Metabolites M23, M27, and M31 were detected at the two Hungarian sites, however at low maximum concentrations ≤0.02 µg/L. M3 was never detected in Italian or Hungarian surface water samples.

III. CONCLUSION

In general, the observed concentrations of Dimethenamid-P in surface water are no cause for concern regarding ecotoxicological protection goals (as defined by environmental quality standards). These monitoring results validate the conservative modelling efforts and show that Dimethenamid-P degrades rapidly under field conditions and presents little concern for surface water bodies. These results also validate the conservative approach to metabolite concentrations based on our lysimeter results.

Groundwater

Two groundwater monitoring studies were performed in order to generate information on the metabolites of Dimethenamid-P in groundwater under realistic worst case conditions in North-west Europe (Germany and Netherlands). The field part of the study in Germany consists of three successive studies and is described in CA 7.5/2 - 7.5/4. It was performed in support of another compound (Topramezone) but the samples were then also analysed for metabolites of Dimethenamid-P. The analytical data for the metabolites of Dimethenamid-P for the German monitoring sites were generated in two additional studies reported under CA 7.5/5 and 7.5/6.

The monitoring points were located in regions in Germany, typical for cultivation of maize, with a shallow, vulnerable groundwater table and where maize is cultivated in an upstream area relative to the wells. The selection of the monitoring points was in accordance with recommendations from the water authorities in the federal states. All 20 monitoring wells were from areas potentially vulnerable to leaching. Groundwater from major agricultural areas with intensive maize cultivation flows into the direction of the wells. The groundwater sampling points tap into shallow groundwater or collect the sensitive groundwater respectively. The study comprises analysis of sites with both historical product use as well as investigation of current use (retrospective as well as prospective analysis). All areas represented high water table levels.
On average the products Clio® Top or Clio® Super were applied on more than 10 hectares in the direct upstream areas and with more than 20 hectares in the broader upstream areas, each year and each monitoring well. Due to the fact that the farmers who cultivated maize upgradient to the wells were provided for free with the product, the study simulated a very high market share regarding the areas located upgradient to the monitoring wells. Hence the use scenario represented a worst case situation. Groundwater samples were taken on a bimonthly interval

between May 2007 and March 2010 and on a quarterly interval between June 2010 and March 2013 from all 20 monitoring wells.

For the study in the Netherlands additional samples were taken from selected wells of the official monitoring network in the province North Brabant and analysed for metabolites of Dimethenamid-P. This study is reported under CA 7.5/7.

The groundwater monitoring studies were performed to observe the actual quantities of the metabolites in groundwater and also to demonstrate effectiveness of the conservative methodology employed in the evaluation of the lysimeter study (see CA 7.1.4). The results, in fact, do demonstrate that the metabolites were observed in amounts lower, and in many case much lower, than the estimates using the lysimeter results (note that the application rate used for the monitoring trials ranges fom 807 to 968 g/ha and is on average larger than the corrected use rate of 864 g/ha of the lysimeter).

The German groundwater monitoring study showed that M656PH027 was observed at a maximum level of 1.68 µg/L in comparison to the predicted **2.4** µg/L. M656PH027 was observed most frequently of any metabolite and was observed in approximately 16% of samples. M656PH023 was seen at a maximum level of 0.379 µg/L compared a predicted value of **0.6** µg/L. M656PH023 was observed the second most frequently in approximately 10% of samples. No other metabolite was observed in more than 5% frequency rate. M656PH045 was observed in maximum levels up to 0.045 µg/L compared to a predicted level of **1.2** µg/L. M656PH047 was observed at a maximum level of 0.161 µg/L compared to a predicted value of **0.7** µg/L. Metabolite M656PH054 was seen at 0.049 µg/L in contrast to **2.0** µg/L prediction from the lysimeter studies. Metabolites M656PH031, M656PH010, M656PH043 and M656PH003 were not measureable at all and were obviously well below the predicted values. The results from the entire body of work in the German monitoring study show that the metabolites of Dimethenamid-P were overall rarely observed in groundwater. Of the 20 sampling sites tested in Germany, 14 (70%) showed no detectable levels (and/or < LOQ) of all 10 metabolites of Dimethenamid-P. Six of the sites (Albersloh, Brekendorf, Flechum, Pfarrkirchen, Veltrup and Vinnen-Ahmsen) showed low levels of metabolites. One site, Flechum, showed a higher degree of observances for the metabolites of Dimethenamid-P. This site also gave the highest observed levels for M656PH027 (and highest level of any metabolite, for that matter) in the German groundwater monitoring study, but levels were still well below the predicted values. Other than the Flechum site, the other observances of metabolites of Dimethenamid-P can only be described as sporadic and/or transient. This data strongly supports the conservative estimations for movement of metabolites from Dimethenamid-P to groundwater based on the lysimeter results.

The monitoring study in The Netherlands demonstrated much of the same results. M656PH027 was observed at a maximum level of 1.509 µg/L in comparison to the predicted **2.4** µg/L. Again here in the groundwater study in the Netherlands, M656PH027 was observed most frequently of any metabolite of Dimethenamid-P. The observance rate was higher than in the German study (approximately 35%), but again all values came well below the predicted value based on the lysimeter results. The metabolite M656PH023 was seen at a maximum level of 0.810 µg/L compared a predicted value of **0.6** µg/L. Approximately 77% of analyzed samples contained no observable M656PH023. M656PH031 was below the LOD except for a single sample in which it was observed in 0.042 µg/L. M656PH045 was observed in maximum values up to 0.213 µg/L compared to a predicted level of **1.2** µg/L and at an approximately 16% frequency. M656PH047 was observed at a maximum level of 0.459 µg/L compared to a predicted value of **0.7** µg/L.

M656PH054 was observed at 0.076 µg/L compared to a predicted value of 2.0 µg/L. Metabolites M656PH003, M656PH010 (except for a single sample at 0.033 µg/L), M656PH032 and M656PH043 were all below the limit of detection (0.0075 µg/L). Other than M656PH027, M656PH023 and M656PH047, no other metabolite was observed in a frequency above 6%. For all metabolites observed in all samplings, approximately 90% of data points were either non-detected or < LOD.

To put the monitoring sites into perspective a relevant assessment has been performed (CA 7.5/8). It can be show that the German monitoring sites are representative for most of Europe's agricultural areas and that the data is valid for the metabolites of dimethenamid-p in a retro- and prospective way. With respect to the 80 Dutch monitoring wells and the sampling period, a single analysis for the dimethenamid-P metabolites provides a representative snapshot of the situation in an area with shallow groundwater and high levels of maize production at a time when both groundwater recharge and groundwater levels are expected to be around their annual maxima.

The results presented below demonstrate the conservative nature of the estimations from the lysimeter studies as well as demonstrate under realistic use scenarios the safety of Dimethenamid-P and its metabolites.

Report:	CA 7.5/2 Schmidt B. et al., 2010a Groundwater monitoring for Topramezone (BAS 670 H) in four representative regions in Germany 2010/1069470
Guidelines:	<none>
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report:	CA 7.5/3 Schmidt B.,Schulz H., 2012a Groundwater monitoring for Topramezone (BAS 670 H) in four representative regions in Germany (study period 2010 to 2012) 2012/1159571
Guidelines:	<none>
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 7.5/4 Schmidt B.,Schneider M., 2013a Groundwater sampling in four representative regions in Germany (study period 2012 – 2013) 2013/1338065
Guidelines:	OECD-DOC ENV/MC/CHEM(98)17 Paris 1998
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

The three reports are summarized together since they are part of a single monitoring study. Report CA 7.5/2 summarizes the samplings from 2007 to 2010, report CA 7.5/3 summarizes from 2010 to 2012, and report CA 7.5/4 summarizes from June 2012 to March 2013. Only the field work of the study including sampling is summarized here whereas the analytical results for metabolites of Dimethenamid-P are presented further down.

Executive Summary

The purpose of this study was the monitoring of shallow groundwater in four maize growing regions in Germany. The reports describe the rationale of well selection in addition to the sampling regime during the years 2007 to 2010, from 2010 to 2012 and from 2012 to 2013 (CA 7.5/2, CA 7.5/3 and CA 7.5/4, respectively).

Twenty groundwater wells were chosen with recommendations from the federal and local water authorities of the following federal states: Schleswig-Holstein, Niedersachsen (Lower Saxony), Nordrhein-Westfalen (North-Rhine Westphalia), Mecklenburg-Vorpommern (Mecklenburg-West Pomerania), Bayern (Bavaria) and Baden-Wuerttemberg (Baden-Wuerttemberg). The guidance document from the German regulatory authorities for explanation of findings in groundwater (Aden et al. 2002) was considered when selecting the monitoring well sites.

Groundwater samples were taken on a bimonthly basis between May 2007 and March 2010 and at quarterly intervals between June 2010 and March 2013. All 20 monitoring wells were from areas potentially vulnerable to leaching. Groundwater from major agricultural areas with intensive maize cultivation flows into the direction of the wells. The groundwater sampling points tap into shallow groundwater or collect the sensitive groundwater respectively. The study covers a retrospective monitoring aspect as well as a prospective monitoring purpose. That means that both is checked, a possible impact on the groundwater on a regional scale resulting from the use of products containing Dimethenamid-P in the past in the region, and a possible leaching into the groundwater in the direct upstream area resulting from the use of such products during the study period. In the years 2007 to 2009 applications of the commercial products Clio® Top or Clio® Super directly upgradient to the monitoring wells were performed by local farmers in accordance with normal local agricultural practices. Clio® Top and Clio® Super are combination products containing the active substances Topramezone and Dimethenamid-P.
On average the products Clio® Top or Clio® Super were applied on more than 10 hectares in the direct upstream areas and with more than 20 hectares in the broader upstream areas, each year and each monitoring well. Due to the fact that the farmers who cultivated maize upgradient to the wells were provided for free with the product, the study simulated a very high market share regarding the areas located upgradient to the monitoring wells. Hence the use scenario represented a worst case situation.

During the study period very low distances between the ground surface level and the ground water tables were measured at single monitoring wells. At the wells Pfarrkirchen, Albersloh and Krogaspe distances of the groundwater table to the surface levels fell even below one meter (Postmuenster 0.96 m in July 08 and 0.97 m in November 09; Albersloh 0.89 m in March 08; Krogaspe 0.94 m in January 08, 0.58 m in February 08 and 0.9 m in March 2009), thus representing also extreme worst case conditions.

The study was originally performed for the monitoring of the active substance Topramezone and its metabolite M670H05. In two analytical studies selected samples were additionally analysed for metabolites of Dimethenamid-P. These analytical results are described below in the studies reported under CA 7.5/5 and CA 7.5/6.

I. MATERIAL AND METHODS

Monitoring regions

On the basis of statistical agricultural use data, regions were selected which represent centers of maize cultivation in Germany with regard to their total acreage and the relative percentage of the total agricultural use area. Four regions, the Nordwestdeutsches Tiefland / Geest (Northwest German Lowlands), the Altmark-Prignitz region, the Unterbayerisches Huegelland (Lower Bavarian Hilly Country) and the Suedliches Oberrheintal (Southern Upper Rhine Valley) were chosen.

The following figure gives an overview of the monitoring regions.

Figure 7.5-1: Locations of the groundwater monitoring regions

Selection of monitoring points

The monitoring points are located in regions in Germany, typical for cultivation of maize, with a shallow, vulnerable groundwater table and where maize is cultivated in an upstream area relative to the wells. The selection of the monitoring points was in accordance with recommendations from the water authorities in the federal states. Selection criteria found in the guidance paper from the German regulatory authorities for explanation of findings in groundwater (Aden et al. 2002) were used.
The topmost aquifers (most sensitive and near-surface primary aquifers) which are also used for water management purposes, were monitored at groundwater measurement points which are representative of the regional hydrogeological conditions. The conditions in the selected monitoring regions represent sensitive groundwater situations. These areas are identified as having the potential for infiltration of crop protection products from the proper and intended use. The regions also represent areas of intense maize cultivation.

The following table gives an overview of the selected measuring points and the hydrogeological conditions in the respective regions.

Table 7.5-3: Groundwater monitoring wells (GWM) / hydrogeological situation

No.	Location	Distance of groundwater* [m]	Hydrogeological situation
Suedliches Oberrheintal (Baden-Wuerttemberg)			
1	Rheinau	~ 2.5 - 3	sands and gravels (Quaternary / fluviatile sediments)
2	Ichenheim	~ 2 - 3	sands and gravels (Quaternary / fluviatile sediments)
3	Oberhausen	~ 3 - 4	sands and gravels (Quaternary / fluviatile sediments)
4	Hartheim	~ 6 - 7	sands and gravels (Quaternary / fluviatile sediments)
Unterbayerisches Huegelland (Bavaria)			
5	Glaslern	~ 1.5 - 3	gravel / sand (Quaternary / fluviatile terrace sediments)
6	Osterholzen	~ 9 - 10	gravel / sand (Quaternary / fluviatile terrace sediments)
7	Pfarrkirchen	~ 1 - 2	sand (Quaternary / fluviatile terrace sediments)
8	Rossbach	~ 2.5 – 3.5	gravel / sand / silt (Quaternary - Holocene / fluviatile sediments and slope debris)
9	Asing	~ 8	gravel / sand (Quaternary / fluviatile terrace sediments)
Altmark/Prignitz region (Saxony-Anhalt / Brandenburg)			
10	Gardelegen	~ 8	sand (Pleistocene / glaciofluviatile sediments)
11	Quadendambeck	~ 6 - 7	sand (Pleistocene / glaciofluviatile sediments)
12	Drewen	~ 3 – 4	sand and silt / marl lenses (Pleistocene / glacial moraine sediments)
Nordwestdeutsches Tiefland (Schleswig-Holstein / Lower Saxony / North Rhine-Westphalia)			
13	Albersloh	~ 1 - 2	sand (Pleistocene / glacial moraine sediments)
14	Ostbevern	~ 1.5- 2.5	sand (Pleistocene / glacial moraine sediments)
15	Veltrup	~ 1 - 2	sand (Pleistocene / glaciofluviatile sediments)
16	Flechum	~ 2.5 - 3.5	sand / silt (Pleistocene / glaciofluviatile and moraine sediments)
17	Vinnen-Ahmsen	~ 3.5 - 4	sand / silt (Pleistocene / glaciofluviatile and moraine sediments)
18	Wedel	~ 3 - 4	sand (Pleistocene / moraine sediments)
19	Krogaspe	~ 1 - 2	sand / silt (Pleistocene / glacial sediments)
20	Brekendorf	~ 5 - 6	sand / silt / gravel (Pleistocene / ground moraine sediments)

*distance of groundwater table to soil surface measured at the monitoring well

The study design of the ground water monitoring covers a retrospective aspect as well as a prospective approach:

<u>Retrospective Groundwater Monitoring:</u>

Products containing Dimethenamid/P and formerly Dimethenamid were used since a long time in maize cultivation in Germany and it can be assumed that they were also used in the selected monitoring regions in the past.

Groundwater monitoring points, which penetrate into the upper groundwater layers, were sampled regularly. The groundwater wells were chosen with regard to recommendations of the environmental or water authorities of the federal states. The groundwater units sampled are located in sedimentary rocks of the Quaternary which consists mostly of sands.

The monitoring regions are typical for maize cultivation and represent the prominent maize growing areas in Germany. Groundwater from major agricultural areas with intensive maize cultivation flows in the direction of the wells. The groundwater wells tap into shallow groundwater; the groundwater table is generally at about 2 – 10 m depth. The typical soils in the area of the monitoring points are sands and loams above sandy sediments. The environmental scenario around each well represents a vulnerable situation with regard to pesticide leaching due to the soil conditions or the shallow groundwater table.

Prospective Groundwater Monitoring:

In order to address the aspect of a prospective ground water monitoring, that means to observe possible inputs into the groundwater in the direct upgradient of the monitoring points, products containing Dimethenamid-P were applied on fields in the upstream of the wells.
For this, an area from which groundwater may flow to the monitoring points within three years was defined. The groundwater flow direction may vary and flow velocities of the ground water were conservatively calculated to be at about 0.1 – 2 m per day (40 – 700 m per year). Hence a segment of a circle, which has an apex angle of approx. 45° and a length of approx. 1000 m was defined. Within this segment products containing Dimethenamid-P such as Clio® Super or Clio® Top Pack were applied on areas under maize cultivation.

The applications were done under normal agricultural practice. Applications and gathering of related information were not conducted according to GLP regulations. Applications upgradient from the wells were attempted every year as far as possible. The documentation of the actually treated areas upstream to the monitoring wells was collected and compiled in the report.

Applications

The prospective aspect of the groundwater monitoring was adressed by ensuring treatment of maize fields upgradient from the monitoring points with commercial products containing the active ingredient Dimethenamid-P under normal agricultural conditions. For this farmers, which cultivate maize fields within a distance of approx. 1 km upgradient from the monitoring wells were asked for using products containing Dimethenamid-P (besides Topramezone) on their fields. BASF SE provided the farmers with the commercial products as Clio® Super or Clio® Top Pack.
The exact sizes and locations of the fields treated with products containing Dimethenamid-P in the area directly upgradient from the wells are shown in the report.

Substantial amounts of dimethenamid-P were applied in the course of the documented sponsored applications:
In the years 2007 and 2008 1.8 L/ha Clio® Super, which is equivalent to 968.4 g/ha dimethenamid-P. In the year 2009 1.5 L/ha Clio® Super, which is equivalent to 807 g/ha dimethenamid-P, was applied. In many cases, Clio® Super was provided as the “Clio® Top Pack” which is a package of Clio® Super and terbuthylazine for use as a tank mix. Thus the application rates for DMTA-P documented in the monitoring study are comparable with the current maximum application rate of 864 g/ha DMTA-P.
On average the products Clio® Top or Clio® Super were applied on more than 10 hectares in the direct upstream areas and with more than 20 hectares in the broader upstream areas, each year and each monitoring well.

After 2009 product applications were no longer sponsored by BASF. However further use of Dimethenamid-P and especially of Clio® products containing Dimethenamid-P can be assumed after the successful market introduction and the favorable performance of the Clio® product family.

Monitoring wells

To assess the state of the groundwater, monitoring wells (GWM) were selected that meet the following requirements: 1. The monitoring wells allow the investigation of the topmost vulnerable groundwater; 2. Sampling from the monitoring wells permits evaluation of both local influences (directly upstream areas with documented application of the test item) and regional influences (farther upgrade area) to the groundwater; 3. The monitoring wells are in technically good condition in accordance with existing guidelines (e.g. LAWA 1999) and suitable for taking residue specimens.
Possible influences on natural conditions (such as the direct inflow of surface water into the monitoring well and contributions from farms unconnected to the wastewater system) must be excluded.

Groundwater sampling

Groundwater samples were taken on a bimonthly interval between May 2007 and March 2010 and on a quarterly interval between June 2010 and March 2013 from all 20 monitoring wells. Steps were taken to ensure contamination-free sampling of the shallow groundwater. The well head was cleaned with water/isopropanol before opening the seals. The groundwater-level was measured with an electric level probe. The electric level probe was rinsed with tap water after each measurement.

A submersible pump (Grundfos MP 1) was installed in the well. The pump was lowered to a depth, which represents the middle of the well casing filled with groundwater or at least 3 meters below the groundwater table if the well was deeper than 20 m. The pump was rinsed with tap water after each sampling procedure and all components used were made from inert materials. This ensured that the specimen taken was neither influenced nor altered by the sampling procedure. After obtaining the specimen, the tube material was discarded. For each monitoring well and each sampling new tube material was used.
Before taking the specimen the stagnant water was removed from the well (about three well volumes; at wells deeper than 20 m one well volume was pumped). If during the sampling procedure no sufficient amount of groundwater entered the well tube, it was sufficient to pump until the stability of conductivity was achieved. During pumping the groundwater parameters were monitored on site. pH, conductivity, redox potential and water temperature were determined and recorded.
Specimens were added to four HDPE bottles with a volume of 0.5 L each and put on dry ice immediately after sampling (< -18°C).

II. RESULTS AND DISCUSSION

Groundwater samples were taken on a bimonthly interval between May 2007 and March 2010 and on a quarterly interval between June 2010 and March 2013 from 20 monitoring wells in four maize growing regions in Germany.

The analytical results on concentrations of Dimethenamid-P and its metabolites determined in selected samples in two separate studies regarding the German monitoring sites are presented below in the studies reported under CA 7.5/5 and CA 7.5/6.

Report:	CA 7.5/5 Class T., 2013a Determination of the Dimethenamid-P metabolites M23, M27 and M31 in ground water samples originating from BASF studies 262015 and 392191 2013/1349144
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Report:	CA 7.5/6 Mewis, F., 2013 Determination of Residues of BAS 656 PH and Metabolites in groundwater (monitoring Germany) 2013/1352172
Guidelines:	
GLP:	Yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of these two studies was the determination of residues of metabolites of Dimethenamid-P (M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054) in groundwater samples in Germany.

The groundwater specimens originate from three separate successive groundwater monitoring studies conducted in Germany under GLP. Details on the groundwater monitoring sites and the sampling procedure are described in the respective study and summarised in CA 7.5/2; CA 7.5/3 and CA 7.5/4.

Samples were analysed using two validated methods described in section CA 4.1.2/3 *[Jooß, 2012, Determination of Dimethenamid-P and Its Metabolites M23, M27 and M31 in Water, BASF DocID 2012/1278546]* and CA 4.1.2/4 [*Mewis; A., 2013, Validation of an Analytical Method for Determination of Metabolites of Dimethenamid-P in Water, study S13-0346, BASF DocID 2013/1349800*]

Analysis lead to the following results: Residues of M27 were found up to 1.680 µg/L mainly at the locations Pfarrkirchen, Flechum, Veltrup, Albersloh and Brekendorf.
Residues of M23 were found up to 0.379 µg/L mainly at the locations Flechum, Veltrup and Albersloh.
Residues of M656PH045 were found up to 0.045 µg/L mainly at the locations Veltrup and Albersloh.
Residues of M656PH047 were found up to 0.161 µg/L mainly at the locations Flechum and Albersloh.
Residues of M656PH054 were found up to 0.049 µg/L mainly at the location Flechum.
Residues of M31, M656PH010, M656H032, M656PH043 and M3 were not measurable (<LOQ; <0.025 µg/L) in all samples.

I. MATERIAL AND METHODS

A. MATERIALS

1. Reference items

Reference item 1	Dimethenamid-P Metabolite M23
IUPAC name:	N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid
Reg. No.	360715
Molar Mass:	271.3 g/mol
Chemical purity:	98.8% (98.4% for selected samples)
Reference item 2	Dimethenamid-P Metabolite M27
IUPAC name:	Sodium[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonate
Reg. No.	360714
Molar Mass:	343.4 g/mol
Chemical purity	97.1% (97.4% for selected samples)
Reference item 3	Dimethenamid-P Metabolite M31
IUPAC name:	[[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl]-acetic acid
Reg. No.	360712
Molar Mass:	347.5 g/mol
Chemical purity	98.7%

Reference item 4	Dimethenamid-P Metabolite M656PH032
IUPAC name:	({2-[(2,4-dimethyl-3-thienyl)(2-methoxy-1-methylethyl)amino]-2-oxoethyl}sulfanyl)acetic acid
Reg. No.	395234
Molar Mass:	331.5 g/mol
Chemical purity	92.1%

Reference item 4	Dimethenamid-P Metabolite M3
IUPAC name:	N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)acetamide
Reg. No.	360717
Molar Mass:	241.4 g/mol
Chemical purity	97.0%

Reference item 4	Dimethenamid-P Metabolite M656PH010
IUPAC name:	N-(2,4-dimethylthiophen-3-yl)-N-[(2S)-1-methoxypropan-2-yl]-2-(methylsulfonyl)acetamide
Reg. No.	5931836
Molar Mass:	319.4 g/mol
Chemical purity	94.0%

Reference item 4	Dimethenamid-P Metabolite M656PH047
IUPAC name:	3-{[(2S)-1-methoxypropan-2-yl](sulfoacetyl)amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917260
Molar Mass:	351.4 g/mol
Chemical purity	90.7%

Reference item 4	Dimethenamid-P Metabolite M656PH045
IUPAC name:	3-{(carboxycarbonyl)[(2S)-1-methoxypropan-2-yl]amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917261
Molar Mass:	301.3 g/mol
Chemical purity	99.7%

Reference item 4	Dimethenamid-P Metabolite M656PH054
IUPAC name:	N-(2,4-dimethylthiophen-3-yl)-N-(sulfoacetyl)-L-alanine
Reg. No.	5920718
Molar Mass:	321.4 g/mol
Chemical purity	85.1%

Reference item 4	Dimethenamid-P Metabolite M656PH043
IUPAC name:	3-{(hydroxyacetyl)[(2S)-1-methoxypropan-2-yl]amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917262
Molar Mass:	287.3 g/mol
Chemical purity	94.6%

2. Test sites and monitoring wells

The groundwater specimens originate from three separate successive groundwater monitoring studies conducted in Germany under GLP. Details on the sampling sites and the sampling procedure are described in the respective study and summarised in CA 7.5/2; CA 7.5/3 and CA 7.5/4.

3. Analytical Procedure

Selected samples were analysed for dimethenamid-P metabolites M23, M27 and M31 using a validated method which is described in the summary report CA 4.5/1 [*Jooß, S. (2012) "Determination of Dimethenamid-P and Its Metabolites M23, M27 and M31 in Water" BASF DocID 2012/1278546*].

The water specimens were also analysed for dimethenamid-P metabolites M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054 using a validated method which is described in the summary report CA 4.1.2/4 [*Mewis A., (2013) "Validation of an Analytical Method for Determination of Metabolites of Dimethenamid-P in Water" BASF DocID 2013/1349800*].

II. RESULTS AND DISCUSSION

A. Concurrent Method Validation

The analytical method for analysis of the dimethenamid-P metabolites M23, M27 and M31 described in CA 4.5/1 [*Jooß, S., 2012, BASF DocID 2012/1278546*] was concurrently validated with drinking water samples fortified at the limit of quantitation (LOQ) of 0.03 µg/L (5 replicates) and at 0.3 µg/L.

The calibration functions for all three analytes upon direct injection upon DI-LC-MS/MS were all linear up to a water concentration of 10 µg/L (ng/mL), resulting in consistent response factors over the entire calibration range of 0.009 to 10 µg/L (ng/mL).

Thus, the highest concentrations of M27 (approx. ≤ 1 µg/L) originating from Flechum were fully covered even that the highest fortification level of the concurrent recovery samples was with 0.3 µg/L by a factor of 3 below the actual water concentrations.

Concurrent overall mean recoveries for method validation were 99% (RSD = 3%, n = 10) for M23, 91% (RSD= 2%, n = 10) for M27 and 100% (RSD = 6%, n = 10) for M31.

Procedural recoveries using the method described in CA 4.1.2/4 [*Mewis A., (2013) BASF DocID 2013/1349800*] were concurrently determined with drinking water samples fortified at the limit of quantitation (LOQ) of 0.025 µg/L and at 0.1, 1 and 2 µg/L. The limit of detection was defined as 30 % of LOQ, i.e. 0.0075 µg/L.

Results of the recovery experiments indicated that the recovery efficiency and repeatability were within acceptable limits of 70 % - 110 % for mean recovery and < 20 % RSD (see Table 7.5-4). No peak interference occurred at the retention times of the analytes except mass transition 350-306 of analyte M656PH047 (in case of interferences the mass transition 350-121 was used as quantifier). A highly specific detection system was used (HPLC-MS/MS). The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev 4.

Table 7.5-4: Summary of procedural recoveries in groundwater

Substance	Fortification Levels (µg/L)	n	Overall Mean Recovery ± RSD (%)
M27 (Reg.No. 360714)	0.025, 0.1, 1, 2	12	93 ± 11
M23 (Reg.No. 360715)	0.025, 0.1, 1, 2	12	88 ± 11
M31 (Reg.No. 360712)	0.025, 0.1, 1, 2	12	91 ± 15
M656H032 (Reg.No. 395234)	0.025, 0.1, 1, 2	12	90 ± 14
M656PH043 (Reg.No. 5917262)	0.025, 0.1, 1, 2	12	88 ± 9
M656PH045 (Reg.No. 5917261)	0.025, 0.1, 1, 2	12	93 ± 12
M656PH047 (Reg.No. 5917260)	0.025, 0.1, 1, 2	12	91 ± 11
M656PH054 (Reg.No. 5920718)	0.025, 0.1, 1, 2	12	90 ± 10
M3 (Reg.No. 360717)	0.025, 0.1, 1, 2	12	88 ± 13
M656PH010 (Reg.No. 5931836)	0.025, 0.1, 1, 2	12	91 ± 10

A. **Analyses of Ground Water Samples**

Samples representing sampling events approximately every half year were selected for analysis from the available samples. Part of the samples were first analysed for metabolites M23, M27 and M31 by the method of Jooß. These results are described in CA 7.5/5.
Later on, these samples were analysed for the metabolites M3, M656PH010, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054 by the method of Mewis. Additionally, if residues of the major metabolites M23 or M27 were detected in the first round of analyses, further samples in between the half year raster were selected and analysed for all 10 metabolites (M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054). Furthermore, the samples originating from the last year of sampling (described in CA 7.5/4) were analysed for all 10 metabolites. The analytical results generated with the method of Mevis are described in CA 7.5/6.
Results are given in Table 7.5-5 to Table 7.5-44. Note that in these tables the corresponding results of the studies CA 7.5/5 and 7.5/6 are reported together.

The results from the entire body of work in the German monitoring study show that the metabolites of Dimethenamid-P were overall rarely observed in groundwater. Of the 20 sampling sites tested in Germany, 14 (70%) showed no detectable levels (and/or < LOQ) of all 10 metabolites of Dimethenamid-P. Six of the sites (Albersloh, Brekendorf, Flechum, Pfarrkirchen, Veltrup and Vinnen-Ahmsen) showed low levels of metabolites. The metabolite most often observed was M656PH027 which we well below the conservative estimates based on the lysimeter results. The second most frequently observed was M656PH023, but again in very low quantities. One site, Flechum, showed a higher degree of observeances for the metabolites of Diemthenamid-P. This site also gave the highest observed levels for M656PH027 (and highest level of any metabolite, for that matter) in the German groundwater monitoring study. Other than the Flechum site, the other observances of metabolites of Dimethenamid-P can only be described as sporadic and/or transient. This data strongly supports the conservative estimations for movement of metabolites from Dimethenamid-P to groundwater based on the lysimeter results.

Residues of M27 were found up to 1.680 µg/L mainly at the locations Pfarrkirchen, Flechum, Veltrup, Albersloh and Brekendorf.
Residues of M23 were found up to 0.379 µg/L mainly at the locations Flechum, Veltrup and Albersloh.
Residues of M656PH045 were found up to 0.045 µg/L mainly at the locations Veltrup and Albersloh.
Residues of M656PH047 were found up to 0.161 µg/L mainly at the locations Flechum and Albersloh.
Residues of M656PH054 were found up to 0.049 µg/L mainly at the location Flechum.
Residues of M31, M656PH010, M656H032, M656PH043 and M3 were not measurable (<LOQ; <0.025 µg/L) in all samples.

Table 7.5-5: Results of groundwater monitoring Albersloh

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Albersloh	07-05	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	07-11	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	08-05	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	08-11	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	09-05	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	09-11	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	10-01	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-03	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-06	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-09	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-12	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	11-03	13	n.d.	n.d.	n.d.	n.d.	<LOQ
Albersloh	11-06	13	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	11-09	13	n.d.	n.d.	n.d.	n.d.	<LOQ
Albersloh	11-12	13	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Albersloh	12-03	13	0.042	<LOQ	n.d.	n.d.	n.d.
Albersloh	12-06	13	0.217	0.064	n.d.	n.d.	n.d.
Albersloh	12-09	13	0.483	0.090	n.d.	n.d.	<LOQ
Albersloh	12-12	13	1.071	0.192	n.d.	n.d.	n.d.
Albersloh	13-03	13	1.277	0.246	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ, <LOQ*: <0.03 µg/L)

Table 7.5-6: Results of groundwater monitoring Albersloh (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Albersloh	07-05	13	n.d.	<LOQ	n.d.	n.d.	n.d.
Albersloh	07-11	13	<LOQ	0.026	n.d.	n.d.	n.d.
Albersloh	08-05	13	n.d.	0.031	n.d.	n.d.	n.d.
Albersloh	08-11	13	n.d.	0.026	n.d.	n.d.	n.d.
Albersloh	09-05	13	n.d.	0.030	n.d.	n.d.	n.d.
Albersloh	09-11	13	n.d.	0.028	n.d.	n.d.	n.d.
Albersloh	10-01	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-03	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-06	13	<LOQ	0.129	n.d.	n.d.	n.d.
Albersloh	10-09	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	10-12	13	<LOQ	0.118	n.d.	n.d.	n.d.
Albersloh	11-03	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	11-06	13	n.d.	<LOQ	n.d.	n.d.	n.d.
Albersloh	11-09	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	11-12	13	0.026	n.d.	n.d.	n.d.	n.d.
Albersloh	12-03	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	12-06	13	n.d.	0.149	n.d.	n.d.	n.d.
Albersloh	12-09	13	n.d.	n.d.	n.d.	n.d.	n.d.
Albersloh	12-12	13	0.027	0.143	n.d.	n.d.	n.d.
Albersloh	13-03	13	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ:<0.025 µg/L; -- not determined

Table 7.5-7: Results of groundwater monitoring Asing

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Asing	07-05	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	07-11	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	08-05	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	08-11	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	09-05	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	09-11	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	10-06	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	10-12	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	11-06	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	11-12	9	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Asing	12-06	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	12-12	9	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ; LOQ* = 0.03 µg/L)

Table 7.5-8: Results of groundwater monitoring Asing (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Asing	07-05	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	07-11	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	08-05	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	08-11	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	09-05	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	09-11	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	10-06	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	10-12	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	11-06	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	11-12	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	12-06	9	n.d.	n.d.	n.d.	n.d.	n.d.
Asing	12-12	9	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-9: Results of groundwater monitoring Brekendorf

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Brekendorf	07-05	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	07-11	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	08-05	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	08-11	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	09-05	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	09-11	20	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	10-06	20	0.121	n.d.	n.d.	n.d.	n.d.
Brekendorf	10-09	20	0.211	n.d.	n.d.	n.d.	n.d.
Brekendorf	10-12	20	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	11-03	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-06	20	0.034	n.d.*	n.d.*	n.d.	n.d.
Brekendorf	11-09	20	<LOQ	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-12	20	<LOQ*	n.d.*	n.d.*	n.d.	<LOQ
Brekendorf	12-03	20	<LOQ	n.d.	n.d.	n.d.	n.d.
Brekendorf	12-06	20	<LOQ	n.d.	n.d.	n.d.	n.d.
Brekendorf	12-12	20	<LOQ	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); < LOQ*: <0.03 µg/L

Table 7.5-10: Results of groundwater monitoring Brekendorf (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Brekendorf	07-05	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	07-11	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	08-05	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	08-11	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	09-05	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	09-11	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	10-06	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	10-09	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	10-12	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-03	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-06	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-09	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	11-12	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	12-03	20	<LOQ	n.d.	n.d.	n.d.	n.d.
Brekendorf	12-06	20	n.d.	n.d.	n.d.	n.d.	n.d.
Brekendorf	12-12	20	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L

Table 7.5-11: Results of groundwater monitoring Drewen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Drewen	07-05	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	07-11	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	08-05	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	08-11	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	09-05	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	09-11	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	10-06	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	10-12	12	n.d.*	n.d.*	n.d.*	n.d.	<LOQ
Drewen	11-06	12	n.d.*	n.d.*	n.d.*	--	--
Drewen	11-12	12	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Drewen	12-06	12	n.d.	n.d.	n.d.	n.d.	<LOQ
Drewen	12-12	12	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L; -- not determined
n.d.*= not detectable (<0.009 µg/L = 30% LOQ, LOQ* = 0.03 µg/L)

Table 7.5-12: Results of groundwater monitoring Drewen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Drewen	07-05	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	07-11	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	08-05	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	08-11	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	09-05	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	09-11	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	10-06	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	10-12	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	11-06	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	11-12	12	n.d.	n.d.	n.d.	n.d.	n.d.
Drewen	12-06	12	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-13: Results of groundwater monitoring Flechum

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Flechum	07-05	16	0.240*	<LOQ*	n.d.*	n.d.	n.d.
Flechum	07-07	16	0.595	0.073	n.d.	n.d.	n.d.
Flechum	07-09	16	0.629	0.082	n.d.	n.d.	n.d.
Flechum	07-11	16	0.570*	0.083*	n.d.*	n.d.	n.d.
Flechum	08-01	16	0.532	0.084	n.d.	n.d.	n.d.
Flechum	08-03	16	0.410	0.057	n.d.	n.d.	n.d.
Flechum	08-05	16	0.280*	0.048*	n.d.*	n.d.	n.d.
Flechum	08-07	16	0.526	0.062	n.d.	n.d.	n.d.
Flechum	08-09	16	0.643	0.065	n.d.	n.d.	n.d.
Flechum	08-11	16	0.510*	0.064*	n.d.*	n.d.	n.d.
Flechum	09-01	16	0.616	0.063	n.d.	n.d.	n.d.
Flechum	09-03	16	0.636	0.065	n.d.	n.d.	n.d.
Flechum	09-05	16	0.710*	0.073*	n.d.*	n.d.	n.d.
Flechum	09-07	16	0.808	0.084	n.d.	n.d.	n.d.
Flechum	09-09	16	0.565	0.048	n.d.	n.d.	n.d.
Flechum	09-11	16	0.650*	0.066*	n.d.*	n.d.	n.d.
Flechum	10-01	16	0.771	0.066	n.d.	n.d.	n.d.
Flechum	10-03	16	0.808	0.084	n.d.	n.d.	n.d.
Flechum	10-06	16	0.530	0.068	n.d.	n.d.	n.d.
Flechum	10-09	16	0.753	0.092	n.d.	n.d.	n.d.
Flechum	10-12	16	0.810*	0.093*	n.d.*	n.d.	n.d.
Flechum	11-03	16	0.987	0.106	n.d.	n.d.	n.d.
Flechum	11-06	16	0.990*	0.160*	n.d.*	n.d.	n.d.
Flechum	11-09	16	1.306	0.186	n.d.	n.d.	n.d.
Flechum	11-12	16	0.850*	0.150*	n.d.*	n.d.	n.d.
Flechum	12-03	16	1.538	0.379	<LOQ	n.d.	n.d.
Flechum	12-03	16	1.523	0.338	n.d.	n.d.	n.d.
Mean of Flechum 12-03 16			1.532	0.359	n.d.	n.d.	n.d.
Flechum	12-06	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	12-09	16	0.892	0.169	n.d.	n.d.	n.d.
Flechum	12-12	16	1.515	0.345	n.d.	n.d.	n.d.
Flechum	13-03	16	1.680	0.355	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ) <LOQ*: < 0.03 µg/L

Table 7.5-14: Results of groundwater monitoring Flechum (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Flechum	07-05	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	07-07	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	07-09	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	07-11	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	08-01	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	08-03	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	08-05	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	08-07	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	08-09	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	08-11	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	09-01	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	09-03	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	09-05	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	09-07	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	09-09	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	09-11	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	10-01	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	10-03	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	10-06	16	n.d.	n.d.	<LOQ	n.d.	n.d.
Flechum	10-09	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	10-12	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	11-03	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	11-06	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	11-09	16	n.d.	n.d.	n.d.	n.d.	n.d.
Flechum	11-12	16	n.d.	<LOQ	n.d.	n.d.	n.d.
Flechum	12-03	16	n.d.	n.d.	0.049	n.d.	n.d.
Flechum	12-03	16	n.d.	n.d.	n.d.	n.d.	n.d.
Mean of Flechum 12-03 16			n.d.	n.d.	<LOQ	n.d.	n.d.
Flechum	12-06	16	n.d.	0.038	n.d.	n.d.	n.d.
Flechum	12-09	16	n.d.	n.d.	0.036	n.d.	n.d.
Flechum	12-12	16	n.d.	0.084	0.047	n.d.	n.d.
Flechum	13-03	16	n.d.	0.069	0.028	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L

Table 7.5-15: Results of groundwater monitoring Gardelegen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Gardelegen	07-05	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	07-11	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	08-05	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	08-11	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	09-05	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	09-11	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	10-06	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	10-12	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	11-06	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	11-12	10	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Gardelegen	12-06	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	12-12	10	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-16: Results of groundwater monitoring Gardelegen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Gardelegen	07-05	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	07-11	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	08-05	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	08-11	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	09-05	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	09-11	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	10-06	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	10-12	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	11-06	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	11-12	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	12-06	10	n.d.	n.d.	n.d.	n.d.	n.d.
Gardelegen	12-12	10	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-17: Results of groundwater monitoring Glaslern

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Glaslern	07-05	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	07-11	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	08-05	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	08-11	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	09-05	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	09-11	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	10-06	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	10-12	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	11-06	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	11-12	5	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Glaslern	12-06	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	12-12	5	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-18: Results of groundwater monitoring Glaslern (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Glaslern	07-05	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	07-11	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	08-05	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	08-11	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	09-05	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	09-11	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	10-06	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	10-12	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	11-06	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	11-12	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	12-06	5	n.d.	n.d.	n.d.	n.d.	n.d.
Glaslern	12-12	5	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-19: Results of groundwater monitoring Hartheim

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Hartheim	07-05	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	07-11	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	08-05	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	08-11	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	09-05	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	09-11	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	10-06	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	10-12	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	11-06	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	11-12	4	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Hartheim	12-06	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	12-12	4	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-20: Results of groundwater monitoring Hartheim (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Hartheim	07-05	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	07-11	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	08-05	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	08-11	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	09-05	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	09-11	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	10-06	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	10-12	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	11-06	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	11-12	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	12-06	4	n.d.	n.d.	n.d.	n.d.	n.d.
Hartheim	12-12	4	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-21: Results of groundwater monitoring Ichenheim

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Ichenheim	07-05	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	07-11	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	08-05	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	08-11	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	09-05	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	09-11	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	10-06	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	10-12	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	11-06	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	11-12	2	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ichenheim	12-06	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	12-12	2	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-22: Results of groundwater monitoring Ichenheim (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Ichenheim	07-05	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	07-11	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	08-05	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	08-11	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	09-05	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	09-11	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	10-06	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	10-12	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	11-06	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	11-12	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	12-06	2	n.d.	n.d.	n.d.	n.d.	n.d.
Ichenheim	12-12	2	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-23: Results of groundwater monitoring Krogaspe

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Krogaspe	07-05	19	n.d.*	n.d.*	n.d.*	--	--
Krogaspe	07-11	19	n.d.*	n.d.*	n.d.*	--	--
Krogaspe	08-05	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	08-11	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	09-05	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	09-11	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	10-06	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	10-12	19	n.d.*	n.d.*	n.d.*	--	--
Krogaspe	11-06	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	11-12	19	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Krogaspe	12-06	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	12-12	19	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ:<0.025 µg/L; -- not determined
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-24: Results of groundwater monitoring Krogaspe (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Krogaspe	08-05	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	08-11	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	09-05	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	09-11	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	10-06	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	11-06	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	11-12	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	12-06	19	n.d.	n.d.	n.d.	n.d.	n.d.
Krogaspe	12-12	19	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-25: Results of groundwater monitoring Oberhausen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Oberhausen	07-05	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	07-11	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	08-05	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	08-11	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	09-05	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	09-11	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	10-06	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	10-12	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	11-06	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	11-12	3	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Oberhausen	12-06	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	12-12	3	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-26: Results of groundwater monitoring Oberhausen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Oberhausen	07-05	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	07-11	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	08-05	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	08-11	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	09-05	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	09-11	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	10-06	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	10-12	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	11-06	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	11-12	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	12-06	3	n.d.	n.d.	n.d.	n.d.	n.d.
Oberhausen	12-12	3	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-27: Results of groundwater monitoring Ostbevern

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Ostbevern	07-05	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	07-11	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	08-05	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	08-11	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	09-05	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	09-11	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	10-12	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	11-06	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	11-12	14	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Ostbevern	12-06	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	12-12	14	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-28: Results of groundwater monitoring Ostbevern (continued)

Sampling site	**Sampling Date (yy-mm)**	**Measuring point**	**M656PH045 (µg/L)**	**M656PH047 (µg/L)**	**M656PH054 (µg/L)**	**M3 (µg/L)**	**M656PH010 (µg/L)**
Ostbevern	07-05	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	07-11	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	08-05	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	08-11	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	09-05	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	09-11	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	10-12	14	n.d.	<LOQ	n.d.	n.d.	n.d.
Ostbevern	11-06	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	11-12	14	n.d.	<LOQ	n.d.	n.d.	n.d.
Ostbevern	12-06	14	n.d.	n.d.	n.d.	n.d.	n.d.
Ostbevern	12-12	14	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L

Table 7.5-29: Results of groundwater monitoring Osterholzen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Osterholzen	07-11	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	08-05	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	08-11	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	09-05	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	09-11	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	10-06	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	10-12	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	11-06	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	11-12	6	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Osterholzen	12-06	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	12-12	6	n.d.	n.d.	n.d.	n.d.	<LOQ

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-30: Results of groundwater monitoring Osterholzen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Osterholzen	07-11	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	08-05	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	08-11	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	09-05	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	09-11	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	10-06	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	10-12	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	11-06	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	11-12	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	12-06	6	n.d.	n.d.	n.d.	n.d.	n.d.
Osterholzen	12-12	6	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ = 0.025 µg/L

Table 7.5-31: Results of groundwater monitoring Pfarrkirchen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Pfarrkirchen	07-11	7	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	08-03	7	<LOQ	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-05	7	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	08-07	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-11	7	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	09-01	7	<LOQ	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-03	7	0.025	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-05	7	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	09-07	7	<LOQ	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-09	7	0.039	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-11	7	0.040*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	10-01	7	0.035	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-03	7	0.039	<LOQ	n.d.	n.d.	n.d.
Pfarrkirchen	10-06	7	0.036	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-09	7	0.056	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-12	7	0.040*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	11-03	7	0.049	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-06	7	0.039*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	11-09	7	0.046	<LOQ	n.d.	n.d.	n.d.
Pfarrkirchen	11-09	7	0.053	n.d.	n.d.	n.d.	n.d.
Mean of Pfarrkirchen 11-09 7			0.048	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-12	7	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Pfarrkirchen	12-03	7	0.049	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	12-06	7	0.042	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	12-12	7	0.037	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); <LOQ*: < 0.03 µg/L

Table 7.5-32: Results of groundwater monitoring Pfarrkirchen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Pfarrkirchen	07-11	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-03	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-05	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-07	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	08-11	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-01	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-03	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-05	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-07	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	09-11	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-01	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-03	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-06	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	10-12	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-03	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-06	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-09	7	n.d.	n.d.	n.d.	n.d.	n.d.
Mean of Pfarrkirchen 11-09 7			n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	11-12	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	12-03	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	12-06	7	n.d.	n.d.	n.d.	n.d.	n.d.
Pfarrkirchen	12-12	7	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-33: Results of groundwater monitoring Quadendambeck

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Quadendambeck	07-05	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	07-11	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	08-05	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	08-11	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	09-05	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	09-11	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	10-06	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	10-12	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	11-06	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	11-12	11	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Quadendambeck	12-06	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	12-12	11	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-34: Results of groundwater monitoring Quadendambeck (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Quadendambeck	07-05	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	07-11	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	08-05	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	08-11	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	09-05	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	09-11	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	10-06	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	10-12	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	11-06	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	11-12	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	12-06	11	n.d.	n.d.	n.d.	n.d.	n.d.
Quadendambeck	12-12	11	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-35: Results of groundwater monitoring Rheinau

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Rheinau	07-05	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	07-11	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	08-05	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	08-11	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	09-05	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	09-11	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	10-06	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	10-12	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	11-06	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	11-12	1	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rheinau	12-06	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	12-12	1	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-36: Results of groundwater monitoring Rheinau (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Rheinau	07-05	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	07-11	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	08-05	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	08-11	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	09-05	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	09-11	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	10-06	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	10-12	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	11-06	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	11-12	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	12-06	1	n.d.	n.d.	n.d.	n.d.	n.d.
Rheinau	12-12	1	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-37: Results of groundwater monitoring Rossbach

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Rossbach	07-05	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	07-11	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	08-03	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-05	8	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	08-07	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-09	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-11	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	09-01	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-03	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-05	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	09-07	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-11	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	10-06	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	10-12	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	11-06	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	11-12	8	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Rossbach	12-06	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	12-12	8	<LOQ	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); <LOQ*: <0.03 µg/L

Table 7.5-38: Results of groundwater monitoring Rossbach (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Rossbach	07-05	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	07-11	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-03	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-05	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-07	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-09	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	08-11	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-01	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-03	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-05	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-07	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	09-11	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	10-06	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	10-12	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	11-06	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	11-12	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	12-06	8	n.d.	n.d.	n.d.	n.d.	n.d.
Rossbach	12-12	8	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

Table 7.5-39: Results of groundwater monitoring Veltrup

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Veltrup	07-05	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	07-11	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	08-05	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	08-11	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	09-05	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	09-11	15	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Veltrup	10-06	15	0.054	<LOQ	n.d.	n.d.	n.d.
Veltrup	10-09	15	0.068	0.027	n.d.	n.d.	n.d.
Veltrup	10-12	15	0.091*	<LOQ*	n.d.*	n.d.	n.d.
Veltrup	11-03	15	0.267	0.036	n.d.	n.d.	n.d.
Veltrup	11-06	15	0.230*	0.049*	n.d.*	n.d.	n.d.
Veltrup	11-09	15	1.057	0.062	n.d.	n.d.	n.d.
Veltrup	11-12	15	0.140*	0.033*	n.d.*	n.d.	n.d.
Veltrup	12-03	15	0.267	0.054	n.d.	n.d.	n.d.
Veltrup	12-06	15	0.218	0.055	n.d.	n.d.	<LOQ
Veltrup	12-09	15	0.243	0.059	n.d.	n.d.	n.d.
Veltrup	12-12	15	0.182	0.046	n.d.	n.d.	n.d.
Veltrup	13-03	15	0.198	0.048	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); <LOQ*: <0.03 µg/L

Table 7.5-40: Results of groundwater monitoring Veltrup (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Veltrup	07-05	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	07-11	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	08-05	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	08-11	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	09-05	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	09-11	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	10-06	15	<LOQ	n.d.	n.d.	n.d.	n.d.
Veltrup	10-09	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	10-12	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	11-03	15	<LOQ	n.d.	n.d.	n.d.	n.d.
Veltrup	11-06	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	11-09	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	11-12	15	<LOQ	n.d.	n.d.	n.d.	n.d.
Veltrup	12-03	15	n.d.	n.d.	n.d.	n.d.	n.d.
Veltrup	12-06	15	0.029	n.d.	<LOQ	n.d.	n.d.
Veltrup	12-09	15	<LOQ	n.d.	n.d.	n.d.	n.d.
Veltrup	12-12	15	0.038	0.161	<LOQ	n.d.	n.d.
Veltrup	13-03	15	0.045	n.d.	<LOQ	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L

Table 7.5-41: Results of groundwater monitoring Vinnen-Ahmsen

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Vinnen-Ahmsen	07-05	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	07-11	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	08-05	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	08-11	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	09-05	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	09-11	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	10-06	17	<LOQ	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	10-12	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	11-03	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-06	17	<LOQ*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	11-09	17	<LOQ	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-12	17	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Vinnen-Ahmsen	12-03	17	<LOQ	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	12-06	17	<LOQ	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	12-12	17	<LOQ	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ:<0.025 µg/L;
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); <LOQ*: <0.03 µg/L

Table 7.5-42: Results of groundwater monitoring Vinnen-Ahmsen (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Vinnen-Ahmsen	07-05	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	07-11	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	08-05	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	08-11	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	09-05	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	09-11	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	10-06	17	n.d.	<LOQ	n.d.	n.d.	n.d.
Vinnen-Ahmsen	10-12	17	n.d.	0.027	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-03	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-06	17	n.d.	<LOQ	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-09	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	11-12	17	n.d.	<LOQ	n.d.	n.d.	n.d.
Vinnen-Ahmsen	12-03	17	n.d.	n.d.	n.d.	n.d.	n.d.
Vinnen-Ahmsen	12-06	17	n.d.	<LOQ	n.d.	n.d.	n.d.
Vinnen-Ahmsen	12-12	17	n.d.	<LOQ	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); <LOQ: <0.025 µg/L

Table 7.5-43: Results of groundwater monitoring Wedel

Sampling site	Sampling Date (yy-mm)	Measuring point	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
Wedel	07-05	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	07-11	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	08-05	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	08-11	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	09-05	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	09-11	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	10-06	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	10-12	18	n.d.*	n.d.*	n.d.*	--	--
Wedel	11-06	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	11-12	18	n.d.*	n.d.*	n.d.*	n.d.	n.d.
Wedel	12-06	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	12-12	18	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L; -- not determined
n.d.*= not detectable (<0.009 µg/L = 30% LOQ); LOQ* = 0.03 µg/L

Table 7.5-44: Results of groundwater monitoring Wedel (continued)

Sampling site	Sampling Date (yy-mm)	Measuring point	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
Wedel	07-05	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	07-11	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	08-05	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	08-11	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	09-05	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	09-11	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	10-06	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	11-06	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	11-12	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	12-06	18	n.d.	n.d.	n.d.	n.d.	n.d.
Wedel	12-12	18	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (<0.0075 µg/L for each analyte); LOQ = 0.025 µg/L

The analytical results on concentrations of Dimethenamid-P and its metabolites determined in selected samples regarding the monitoring well in the Netherlands are presented below.

Report:	CA 7.5/7 Mewis, F., 2013 Determination of Residues of BAS 656 PH and Metabolites in groundwater (monitoring Netherlands) 2013/1352173
Guidelines:	
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of this study was the determination of residues of metabolites of Dimethenamid-P (M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054) in groundwater samples in the Netherlands.
Groundwater specimens were sampled from groundwater monitoring wells located in corn producing areas selected from the monitoring network of the province North Brabant in the period from 08 January to 16 April 2013.

Samples were analysed using a validated method described in section CA 4.1.2/5 [*Mewis; A., 2013, Validation of an Analytical Method for Determination of Metabolites of Dimethenamid-P in Water, study S13-03461 BASF DocID 2013/1349800*].

The following results of analysis were obtained:
Residues of M27 were found up to 1.509 µg/L.
Residues of M23 were found up to 0.810 µg/L.
Residues of M656PH045 were found up to 0.213 µg/L.
Residues of M656PH047 were found up to 0.459 µg/L.
Residues of M31 were not detectable (<LOD; <0.0075 µg/L) except sample L0903330017 with 0.042 µg/L.
Residues of M10 were not measurable (<LOQ; <0.025 µg/L) except sample L0903330031 with 0.033 µg/L.
Residues of M656PH054 were found up to 0.076 µg/L.
Residues of M656H032, M656PH043 and M3 were not detectable (<LOD; <0.0075 µg/L) in all samples.

I. MATERIAL AND METHODS

A. MATERIALS

1. Reference items

Reference item 1	Dimethenamid-P Metabolite M23
IUPAC name:	N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid
Reg. No.	360715
Molar Mass:	271.3 g/mol
Chemical purity:	98.8%

Reference item 2	Dimethenamid-P Metabolite M27
IUPAC name:	Sodium[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonate
Reg. No.	360714
Molar Mass:	343.4 g/mol
Chemical purity	97.1%

Reference item 3	Dimethenamid-P Metabolite M31
IUPAC name:	[[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl]-acetic acid
Reg. No.	360712
Molar Mass:	347.5 g/mol
Chemical purity	98.7%

Reference item 4	Dimethenamid-P Metabolite M656PH032
IUPAC name:	({2-[(2,4-dimethyl-3-thienyl)(2-methoxy-1-methylethyl)amino]-2-oxoethyl}sulfanyl)acetic acid
Reg. No.	395234
Molar Mass:	331.5 g/mol
Chemical purity	92.1%

Reference item 4	Dimethenamid-P Metabolite M3
IUPAC name:	N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)acetamide
Reg. No.	360717
Molar Mass:	241.4 g/mol
Chemical purity	97.0%

Reference item 4	Dimethenamid-P Metabolite M656PH010
IUPAC name:	N-(2,4-dimethylthiophen-3-yl)-N-[(2S)-1-methoxypropan-2-yl]-2-(methylsulfonyl)acetamide
Reg. No.	5931836
Molar Mass:	319.4 g/mol
Chemical purity	94.0%

Reference item 4	Dimethenamid-P Metabolite M656PH047
IUPAC name:	3-{[(2S)-1-methoxypropan-2-yl](sulfoacetyl)amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917260
Molar Mass:	351.4 g/mol
Chemical purity	90.7%

Reference item 4	Dimethenamid-P Metabolite M656PH045
IUPAC name:	3-{(carboxycarbonyl)[(2S)-1-methoxypropan-2-yl]amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917261
Molar Mass:	301.3 g/mol
Chemical purity	99.7%

Reference item 4	Dimethenamid-P Metabolite M656PH054
IUPAC name:	N-(2,4-dimethylthiophen-3-yl)-N-(sulfoacetyl)-L-alanine
Reg. No.	5920718
Molar Mass:	321.4 g/mol
Chemical purity	85.1%

Reference item 4	Dimethenamid-P Metabolite M656PH043
IUPAC name:	3-{(hydroxyacetyl)[(2S)-1-methoxypropan-2-yl]amino}-4-methylthiophene-2-carboxylic acid
Reg. No.	5917262
Molar Mass:	287.3 g/mol
Chemical purity	94.6%

2. Test sites and monitoring wells

Groundwater specimens were sampled from groundwater monitoring wells located in corn producing areas selected from the monitoring network of the province North Brabant, The Netherlands (see Figure 7.5-2). North Brabant is a region in the South of the Netherlands. It is bordered by Belgium’s Antwerp and Limburg provinces in the South, the Meuse River in the North, Limburg in the East and Zeeland in the West. 80 measuring sites in close vicinity to corn fields were chosen for sampling of shallow groundwater. The wells selected for analysis are all located in areas with predominantly agricultural land usage and where over 25% of the agricultural area is used for maize production.

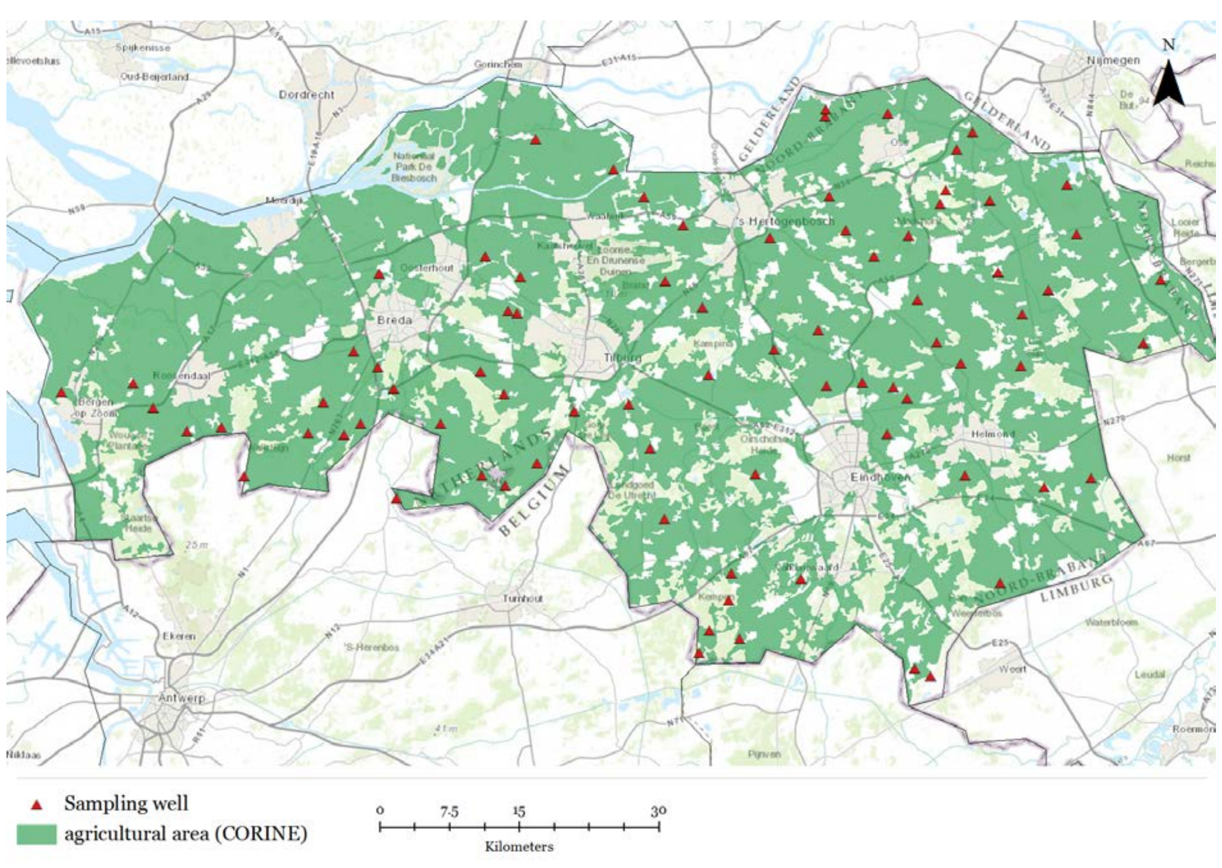


Figure 7.5-2: Distribution of agricultural land and locations of sampled monitoring wells in North Brabant province.

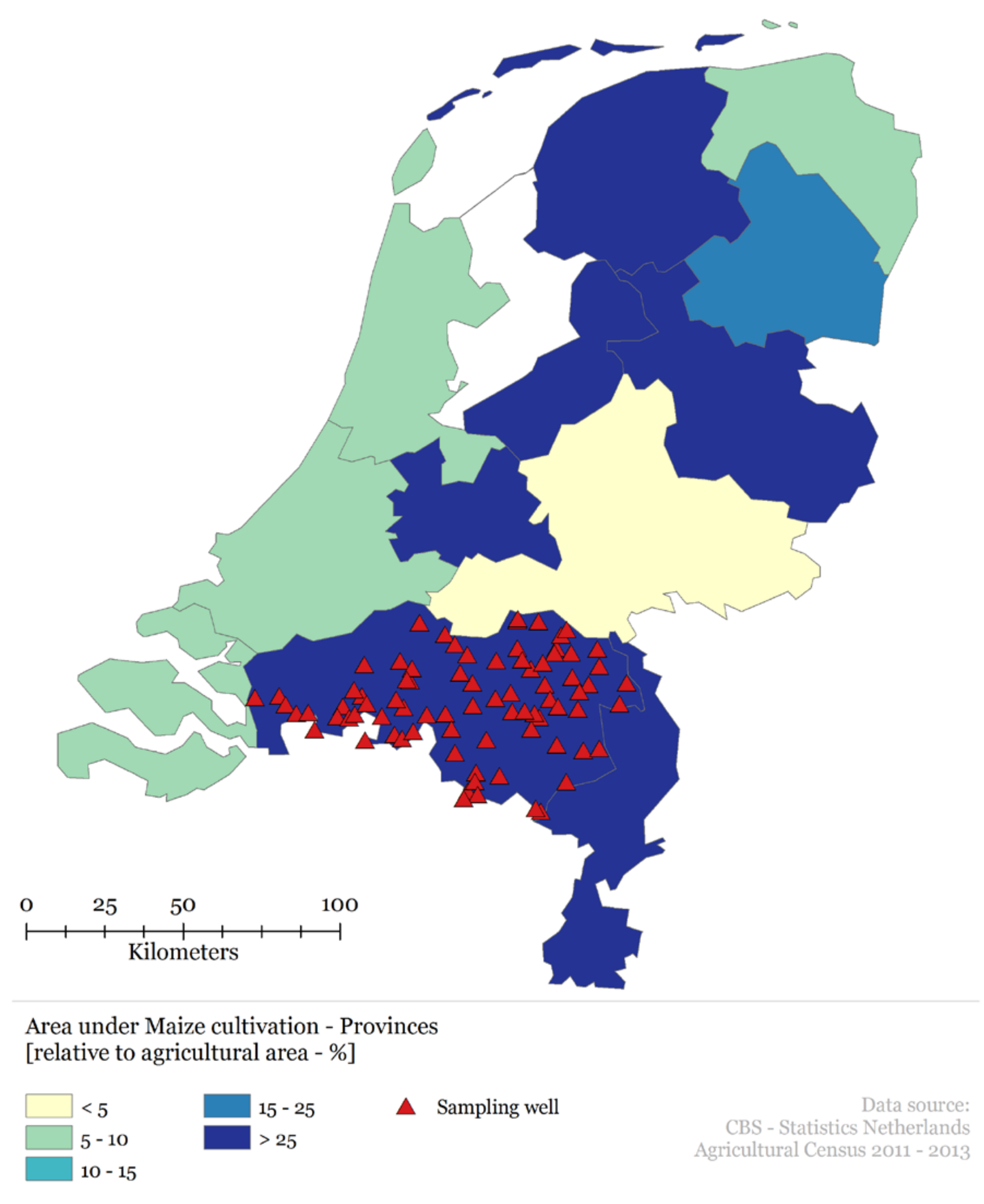


Figure 7.5-3: Distribution of maize cultivation on agricultural land in the Netherlands and locations of sampled monitoring wells in North Brabant province.

Groundwater samples were collected in the period from 08 January to 16 April 2013. Samples were collected in duplicate. The sampling was carried out according to Nederlandse norm NTA 8017 [*2008, Monsterneming van grondwater ten behoove van de monitoring van grondwaterkwaliteit (Sampling of ground water for the monitoring of ground water quality)*] and Stichting Infrastructuur Kwaliteitsborging Bodembeheer [*SIKB(2007) ver. 3.2:Het nemen van grondwatermonsters VKB – PROTOCOL 2002*]. The sampling points are listed in Table 7.5-45.

The sampling procedure can be summarised as follows:
First of all the lid of the measuring point was checked for damage and cleaned before being opened. After opening of the measuring point, the depth of the groundwater level was determined and a submersible pump with a flow rate of 500 mL/min was installed in the well. All components and instruments used were made of silicone or Teflon material to prevent influences on the sample. The stagnant water was pumped out and led away from the measuring point in order to prevent contamination. The sample containers (polyethylene [PE] bottles 500 mL) were rinsed three times with groundwater before the actual sample of groundwater was taken. While pumping out, the groundwater parameters pH, conductivity, redox potential and water temperature were measured and documented. When filling the containers, the tube of the pump was tilted to reduce turbulence. When containers were completely filled, they were sealed airtight, cooled to 4°C and stored in the dark.
The samples were kept at 4°C in an interim storage. Every Friday all samples collected within the week were transported to a refrigerated warehouse and stored at a temperature of -18°C until April 29th. Then the samples were sent frozen to the laboratories of BASF at Limburgerhof and were stored frozen.

Table 7.5-45: Sampling points for groundwater monitoring in the Netherlands

sample no	mpn[a]	filter	depth class	location	coordinates		filter		sampling date
					xc	yc	top [m]	bottom [m]	
95-1	95	1	ondiep	NULAND	158975	413725	8.10	10.10	17.01.
97-1	97	1	ondiep	HAAREN	145337	401807	6.00	8.00	25.02.
98-1	98	1	ondiep	VENKANT	143200	410625	8.00	10.00	25.02.
100-1	100	1	ondiep	MACHAREN	165225	422650	8.00	10.00	17.01.
101-1	101	1	ondiep	SCHAIJK	171500	414450	9.50	11.50	16.04.
103-1	103	1	ondiep	VEGHEL	168463	402625	8.10	10.10	04.02.
104-1	104	1	ondiep	ODILIAPEEL	177075	405600	6.60	8.60	12.02.
106-1	106	1	ondiep	LANDHORST	182500	403638	8.00	10.00	12.02.
107-1	107	1	ondiep	SAMBEEK	194616	404809	6.60	8.60	30.01.
108-1	108	1	ondiep	BIEST	137406	391354	7.05	9.05	08.01.
111-1	111	1	ondiep	OLLAND	157730	399394	9.05	11.05	15.04.
112-1	112	1	ondiep	SON	158616	393372	6.00	8.00	18.03.
115-1	115	1	ondiep	LIESHOUT	167341	392068	11.10	13.10	25.02.
116-1	116	1	ondiep	GEMERT	179573	395518	10.00	12.00	27.02.
122-1	122	1	ondiep	OVERLOON	192759	397966	8.05	10.05	30.01.
123-1	123	1	ondiep	VLIERDEN	182065	382505	11.70	13.20	06.03.
124-1	124	1	ondiep	WEEBOSCH	146068	367045	7.90	9.90	13.02.
125-1	125	1	ondiep	WESTERHOVEN	155915	372595	11.10	13.10	25.03.

Table 7.5-45: Sampling points for groundwater monitoring in the Netherlands

sample no	mpn[a]	filter	depth class	location	coordinates		filter		sampling date
					xc	yc	top [m]	bottom [m]	
128-1	128	1	ondiep	SOMEREN-HEIDE	177325	372138	12.00	14.00	11.03.
129-1	129	1	ondiep	BUDEL	169813	362163	10.05	12.05	10.04.
137-1	137	1	ondiep	ALMKERK	127360	419875	9.05	11.05	21.01.
138-1	138	1	ondiep	GENDEREN	135725	416615	7.95	9.95	15.01.
140-1	140	1	ondiep	RIJEN	125383	401139	9.00	11.00	23.01.
141-1	141	1	ondiep	HALSTEREN	76360	392710	8.00	10.00	20.03.
142-1	142	1	ondiep	WOUW	84060	393685	13.00	15.00	12.03.
146-1	146	1	ondiep	ACHTMAAL	96009	383629	4.50	6.50	27.03.
147-1	147	1	ondiep	RIJSBERGEN	104553	391617	6.50	8.50	20.02.
151-1	151	1	ondiep	GILZE	123975	392450	5.15	7.15	18.02.
420-1	420	1	ondiep	SPOORDONK	145965	394540	8.05	10.05	18.03.
423-1	423	1	ondiep	Cuijk	184520	415050	7.50	9.50	14.03.
425-1	425	1	ondiep	Lith	158526	422379	8.15	10.15	11.02.
426-1	426	1	ondiep	DINTHER	163802	407312	8.05	10.05	10.01.
1804-1	1804	1	ondiep	WOUW	86214	391031	8.20	10.20	12.03.
1806-1	1806	1	ondiep	NISPEN	89852	388453	4.00	6.00	19.02.
1807-2	1807	2	ondiep	RUCPHEN	93603	388908	7.00	9.00	19.02.
1808-2	1808	2	ondiep	ZUNDERT	102945	388291	8.00	10.00	22.01.
1809-1	1809	1	ondiep	ZUNDERT	106754	388142	5.00	7.00	22.01.
1810-3	1810	3	ondiep	OEKEL	108556	389331	11.50	13.50	20.02.
1811-1	1811	1	ondiep	BREDA	110523	405446	4.00	6.00	26.02.
1813-2	1813	2	ondiep	BREDA	110387	395400	7.00	9.00	04.02.
1814-2	1814	2	ondiep	BREDA	107775	397071	8.00	10.00	06.02.
1815-1	1815	1	ondiep	GALDER	112088	393084	5.00	7.00	04.02.
1816-2	1816	2	ondiep	OOSTERHOUT	121925	407312	9.00	11.00	21.02.
1817-2	1817	2	ondiep	DONGEN	125770	405092	7.00	9.00	04.03.
1818-2	1818	2	ondiep	RIJEN	124425	401425	9.00	11.00	23.01.
1819-1	1819	1	ondiep	GILZE	121413	394922	4.00	6.00	29.01.
1821-2	1821	2	midden	CHAAM	117174	389269	12.00	14.00	18.02.
1822-1	1822	1	ondiep	CASTELRE	112375	381325	5.00	7.00	25.03.
1823-1	1823	1	ondiep	BAARLE_ NASSAU	121559	383756	7.00	9.00	25.03.
1824-1	1824	1	ondiep	BAARLE_ HERTOG	124088	382593	8.00	10.00	03.04.
1825-1	1825	1	ondiep	KLEIN_ BEDAF	127489	385072	4.00	6.00	03.04.
1827-2	1827	2	ondiep	GOIRLE	131496	390651	5.50	7.50	08.01.
1831-2	1831	2	ondiep	BIEZENMORTEL	141314	404623	8.00	10.00	14.02.
1833-1	1833	1	ondiep	LIEMPDE	153045	397289	7.00	9.00	07.02.
1835-2	1835	2	ondiep	DIESSEN	139664	386590	6.00	8.00	09.01.
1837-2	1837	2	ondiep	H._EN_LAGE_ MIERDE	141244	379042	8.50	10.50	28.02.
1840-2	1840	2	ondiep	WINTELRE	150993	383906	9.00	11.00	07.02.

Table 7.5-45: Sampling points for groundwater monitoring in the Netherlands

sample no	mpn[a]	filter	depth class	location	coordinates		filter		sampling date
					xc	yc	top [m]	bottom [m]	
1841-2	1841	2	ondiep	EERSEL	148389	373234	8.00	10.00	28.03.
1842-2	1842	2	ondiep	EERSEL	148102	370333	8.00	10.00	28.03.
1843-2	1843	2	ondiep	LUYKSGESTEL	149331	366200	7.00	9.00	25.03.
1844-2	1844	2	ondiep	LUYKSGESTEL	144975	364702	7.00	9.00	13.02.
1847-1	1847	1	ondiep	BUDEL	168119	362997	7.00	9.00	10.04.
1850-2	1850	2	ondiep	DEURNE	187127	383420	7.00	9.00	06.03.
1851-1	1851	1	ondiep	WINKELSTRAAT	173563	383714	8.00	10.00	25.02.
1852-1	1852	1	ondiep	NUENEN	165140	388190	6.50	8.50	05.03.
1853-2	1853	2	ondiep	BREUGEL	165825	393241	9.00	11.00	22.01.
1854-2	1854	2	ondiep	WOLFWINKEL	162530	393755	8.00	10.00	12.02.
1855-2	1855	2	ondiep	ERP	170484	398068	8.00	10.00	24.01.
1856-2	1856	2	ondiep	DONK	173091	395851	6.00	8.00	24.01.
1858-1	1858	1	ondiep	VENHORST	179722	401094	5.00	7.00	31.01.
1860-1	1860	1	ondiep	WANROY	185536	409662	4.00	6.00	19.02.
1861-2	1861	2	ondiep	ZEELAND	176250	413300	8.00	10.00	31.01.
1862-1	1862	1	ondiep	SCHAYK	172695	418782	7.00	9.00	14.03.
1863-2	1863	2	ondiep	SCHAYK	170915	412915	9.00	11.00	04.02.
1864-1	1864	1	ondiep	LITH	158595	423097	9.00	10.00	11.02.
1865-1	1865	1	ondiep	NISTELRODE	167456	409564	4.50	6.50	10.01.
1866-1	1866	1	ondiep	HEESWIJK-DINTHER	160754	410089	8.00	10.00	31.01.
1868-2	1868	2	ondiep	ST.MICHIELS-GESTEL	152592	409272	8.00	10.00	14.02.
1870-1	1870	1	ondiep	RAVENSTEIN	174328	420661	7.50	8.50	07.03.
1871-1	1871	1	ondiep	HEUSDEN	139024	413661	7.00	8.00	21.01.

3. Analytical Procedure

The water specimens from sampling points were analysed for the Dimethenamid-P metabolites M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054 by using a validated method, which is described in the summary report CA 4.1.2/5 [*Mewis A., (2013) "Validation of an Analytical Method for Determination of Metabolites of Dimethenamid-P in Water" BASF DocID 2013/1349800*].

II. RESULTS AND DISCUSSION

A. Concurrent Method Validation

Procedural recoveries were concurrently determined with drinking water samples fortified at the limit of quantitation (LOQ) of 0.025 µg/L and at 1 and 2 µg/L. The limit of detection was defined as 30 % of LOQ, i.e. 0.0075 µg/L.

Results of the recovery experiments indicated that the recovery efficiency and repeatability were within acceptable limits of 70 % - 110 % for mean recovery and < 20 % RSD (see Table 7.5-46). No peak interference occurred at the retention times of the analytes. A highly specific detection system was used (HPLC-MS/MS). The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev 4.

Table 7.5-46: Summary of procedural recoveries in groundwater

Substance	Fortification Levels (µg/L)	n	Overall Mean Recovery ± RSD (%)
M27 (Reg.No. 360714)	0.025, 1, 2	8	84 ± 8
M23 (Reg.No. 360715)	0.025, 1, 2	8	81 ± 9
M31 (Reg.No. 360712)	0.025, 1, 2	8	84 ± 17
M656H032 (Reg.No. 395234)	0.025, 1, 2	8	81 ± 13
M656PH043 (Reg.No. 5917262)	0.025, 1, 2	8	84 ± 7
M656PH045 (Reg.No. 5917261)	0.025, 1, 2	8	86 ± 11
M656PH047 (Reg.No. 5917260)	0.025, 1, 2	8	83 ± 11
M656PH054 (Reg.No. 5920718)	0.025, 1, 2	8	87 ± 11
M3 (Reg.No. 360717)	0.025, 1, 2	8	87 ± 12
M656PH010 (Reg.No. 5931836)	0.025, 1, 2	8	88 ± 10

B. Analyses of Ground Water Samples

Groundwater samples were analysed for the following residues of metabolites of Dimethenamid-P: M3, M656PH010, M23, M27, M31, M656H032, M656PH043, M656PH045, M656PH047 and M656PH054. Results are given in Table 7.5-47 to Table 7.5-50.

Residues of M27 were found up to 1.509 µg/L.
Residues of M23 were found up to 0.810 µg/L.
Residues of M656PH045 were found up to 0.213 µg/L.
Residues of M656PH047 were found up to 0.459 µg/L.
Residues of M31 were not detectable (<LOD; <0.0075 µg/L) except sample 116-1 with 0.042 µg/L.
Residues of M10 were not measurable (<LOQ; <0.025 µg/L) except sample 151-1 with 0.033 µg/L.

Residues of M656PH054 were found up to 0.076 µg/L.
Residues of M656H032, M656PH043 and M3 were not detectable (<LOD; <0.0075 µg/L) in all samples.

Table 7.5-47: Results of groundwater monitoring in the Netherlands

Location	Sponsor code	Sample no.	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
NULAND	L0903330004	95-1	0.439	0.130	n.d.	n.d.	n.d.
HAAREN	L0903330005	97-1	<LOQ	n.d.	n.d.	n.d.	n.d.
VENKANT	L0903330006	98-1	n.d.	n.d.	n.d.	n.d.	n.d.
MACHAREN	L0903330007	100-1	<LOQ	n.d.	n.d.	n.d.	n.d.
SCHAIJK	L0903330008	101-1	n.d.	n.d.	n.d.	n.d.	n.d.
VEGHEL	L0903330009	103-1	n.d.	n.d.	n.d.	n.d.	n.d.
ODILIAPEEL	L0903330010	104-1	n.d.	n.d.	n.d.	n.d.	n.d.
LANDHORST	L0903330011	106-1	0.179	0.100	n.d.	n.d.	n.d.
SAMBEEK	L0903330012	107-1	0.323	n.d.	n.d.	n.d.	n.d.
BIEST	L0903330013	108-1	n.d.	<LOQ	n.d.	n.d.	n.d.
OLLAND	L0903330014	111-1	n.d.	n.d.	n.d.	n.d.	n.d.
SON	L0903330015	112-1	n.d.	n.d.	n.d.	n.d.	n.d.
LIESHOUT	L0903330016	115-1	n.d.	n.d.	n.d.	n.d.	n.d.
GEMERT	L0903330017	116-1	1.509	0.810	0.042	n.d.	n.d.
OVERLOON	L0903330018	122-1	n.d.	n.d.	n.d.	n.d.	n.d.
VLIERDEN	L0903330019	123-1	n.d.	n.d.	n.d.	n.d.	n.d.
WEEBOSCH	L0903330020	124-1	0.656	0.250	n.d.	n.d.	n.d.
WESTER-HOVEN	L0903330021	125-1	1.299	n.d.	n.d.	n.d.	n.d.
SOMEREN-HEIDE	L0903330022	128-1	n.d.	n.d.	n.d.	n.d.	n.d.
BUDEL	L0903330023	129-1	n.d.	n.d.	n.d.	n.d.	n.d.
ALMKERK	L0903330024	137-1	n.d.	n.d.	n.d.	n.d.	n.d.
GENDEREN	L0903330025	138-1	n.d.	n.d.	n.d.	n.d.	n.d.
RIJEN	L0903330026	140-1	n.d.	n.d.	n.d.	n.d.	n.d.
HALSTEREN	L0903330027	141-1	n.d.	n.d.	n.d.	n.d.	n.d.
WOUW	L0903330028	142-1	n.d.	n.d.	n.d.	n.d.	n.d.
ACHTMAAL	L0903330029	146-1	n.d.	n.d.	n.d.	n.d.	n.d.
RIJSBERGEN	L0903330030	147-1	0.113	0.105	n.d.	n.d.	n.d.
GILZE	L0903330031	151-1	0.275	0.251	n.d.	n.d.	n.d.
SPOORDONK	L0903330032	420-1	0.081	0.046	n.d.	n.d.	n.d.
CUIJK	L0903330033	423-1	0.066	n.d.	n.d.	n.d.	n.d.
LITH	L0903330034	425-1	n.d.	n.d.	n.d.	n.d.	n.d.
DINTHER	L0903330035	426-1	n.d.	n.d.	n.d.	n.d.	n.d.
WOUW	L0903330036	1804-1	n.d.	n.d.	n.d.	n.d.	n.d.
NISPEN	L0903330037	1806-1	<LOQ	n.d.	n.d.	n.d.	n.d.
RUCPHEN	L0903330038	1807-2	n.d.	n.d.	n.d.	n.d.	n.d.
ZUNDERT	L0903330039	1808-2	n.d.	n.d.	n.d.	n.d.	n.d.
ZUNDERT	L0903330040	1809-1	0.030	n.d.	n.d.	n.d.	n.d.
OEKEL	L0903330041	1810-3	0.048	<LOQ	n.d.	n.d.	n.d.
BREDA	L0903330042	1811-1	n.d.	n.d.	n.d.	n.d.	n.d.
BREDA	L0903330043	1813-2	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (0.0075 µg/L for each analyte)
LOQ = 0.025 µ/L

Table 7.5-48: Results of groundwater monitoring in the Netherlands (continued)

Location	Sponsor code	Sample no.	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
NULAND	L0903330004	95-1	0.057	n.d.	0.027	n.d.	n.d.
HAAREN	L0903330005	97-1	n.d.	n.d.	n.d.	n.d.	n.d.
VENKANT	L0903330006	98-1	n.d.	n.d.	n.d.	n.d.	n.d.
MACHAREN	L0903330007	100-1	n.d.	n.d.	n.d.	n.d.	n.d.
SCHAIJK	L0903330008	101-1	n.d.	n.d.	n.d.	n.d.	n.d.
VEGHEL	L0903330009	103-1	n.d.	n.d.	n.d.	n.d.	n.d.
ODILIAPEEL	L0903330010	104-1	n.d.	n.d.	n.d.	n.d.	n.d.
LANDHORST	L0903330011	106-1	0.211	n.d.	<LOQ	n.d.	<LOQ
SAMBEEK	L0903330012	107-1	n.d.	n.d.	n.d.	n.d.	n.d.
BIEST	L0903330013	108-1	n.d.	n.d.	n.d.	n.d.	n.d.
OLLAND	L0903330014	111-1	n.d.	n.d.	n.d.	n.d.	n.d.
SON	L0903330015	112-1	n.d.	n.d.	n.d.	n.d.	n.d.
LIESHOUT	L0903330016	115-1	n.d.	n.d.	n.d.	n.d.	n.d.
GEMERT	L0903330017	116-1	0.048	n.d.	0.076	n.d.	<LOQ
OVERLOON	L0903330018	122-1	n.d	n.d.	n.d.	n.d.	n.d.
VLIERDEN	L0903330019	123-1	n.d	n.d.	n.d.	n.d.	n.d.
WEEBOSCH	L0903330020	124-1	<LOQ	0.328	n.d.	n.d.	n.d.
WESTER-HOVEN	L0903330021	125-1	<LOQ	0.459	<LOQ	n.d.	n.d.
SOMEREN-HEIDE	L0903330022	128-1	n.d.	n.d.	n.d.	n.d.	n.d.
BUDEL	L0903330023	129-1	n.d.	n.d.	n.d.	n.d.	n.d.
ALMKERK	L0903330024	137-1	n.d.	n.d.	n.d.	n.d.	n.d.
GENDEREN	L0903330025	138-1	n.d.	n.d.	n.d.	n.d.	n.d.
RIJEN	L0903330026	140-1	n.d.	n.d.	n.d.	n.d.	n.d.
HALSTEREN	L0903330027	141-1	n.d.	n.d.	n.d.	n.d.	n.d.
WOUW	L0903330028	142-1	n.d.	n.d.	n.d.	n.d.	n.d.
ACHTMAAL	L0903330029	146-1	n.d.	n.d.	n.d.	n.d.	n.d.
RIJSBERGEN	L0903330030	147-1	0.061	n.d.	n.d.	n.d.	n.d.
GILZE	L0903330031	151-1	n.d.	n.d.	0.044	n.d.	0.033
SPOORDONK	L0903330032	420-1	n.d.	n.d.	n.d.	n.d.	n.d.
CUIJK	L0903330033	423-1	0.033	n.d.	n.d.	n.d.	n.d.
LITH	L0903330034	425-1	n.d.	n.d.	n.d.	n.d.	n.d.
DINTHER	L0903330035	426-1	n.d.	n.d.	n.d.	n.d.	n.d.
WOUW	L0903330036	1804-1	n.d.	n.d.	n.d.	n.d.	n.d.
NISPEN	L0903330037	1806-1	n.d.	n.d.	n.d.	n.d.	n.d.
RUCPHEN	L0903330038	1807-2	n.d.	n.d.	n.d.	n.d.	n.d.
ZUNDERT	L0903330039	1808-2	n.d.	n.d.	n.d.	n.d.	n.d.
ZUNDERT	L0903330040	1809-1	n.d.	n.d.	n.d.	n.d.	n.d.
OEKEL	L0903330041	1810-3	n.d.	n.d.	n.d.	n.d.	n.d.
BREDA	L0903330042	1811-1	n.d.	n.d.	n.d.	n.d.	n.d.
BREDA	L0903330043	1813-2	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (0.0075 µg/L for each analyte)
LOQ = 0.025 µ/L

Table 7.5-49: Results of groundwater monitoring in the Netherlands (continued)

Location	Sponsor code	Sample no.	M27 (µg/L)	M23 (µg/L)	M31 (µg/L)	M656H032 (µg/L)	M656PH043 (µg/L)
BREDA	L0903330044	1814-2	n.d.	n.d.	n.d.	n.d.	n.d.
GALDER	L0903330045	1815-1	n.d.	n.d.	n.d.	n.d.	n.d.
OOSTERHOUT	L0903330046	1816-2	0.156	0.147	n.d.	n.d.	n.d.
DONGEN	L0903330047	1817-2	n.d.	n.d.	n.d.	n.d.	n.d.
RIJEN	L0903330048	1818-2	0.189	0.145	n.d.	n.d.	n.d.
GILZE	L0903330049	1819-1	n.d.	n.d.	n.d.	n.d.	n.d.
CHAAM	L0903330050	1821-2	n.d.	n.d.	n.d.	n.d.	n.d.
CASTELRE	L0903330051	1822-1	0.879	0.044	n.d.	n.d.	n.d.
BAARLE_ NASSAU	L0903330052	1823-1	0.387	0.102	n.d.	n.d.	n.d.
BAARLE_ HERTOG	L0903330053	1824-1	0.040	<LOQ	n.d.	n.d.	n.d.
KLEIN_BEDAF	L0903330054	1825-1	0.247	0.087	n.d.	n.d.	n.d.
GOIRLE	L0903330055	1827-2	n.d.	n.d.	n.d.	n.d.	n.d.
BIEZEN-MORTEL	L0903330056	1831-2	n.d.	n.d.	n.d.	n.d.	n.d.
LIEMPDE	L0903330057	1833-1	0.040	0.036	n.d.	n.d.	n.d.
DIESSEN	L0903330058	1835-2	0.600	0.169	n.d.	n.d.	n.d.
H._EN_LAGE_ MIERDE	L0903330059	1837-2	n.d.	n.d.	n.d.	n.d.	n.d.
WINTELRE	L0903330060	1840-2	0.855	0.269	n.d.	n.d.	n.d.
EERSEL	L0903330061	1841-2	0.170	0.121	n.d.	n.d.	n.d.
EERSEL	L0903330062	1842-2	n.d.	n.d.	n.d.	n.d.	n.d.
LUYKSGESTEL	L0903330063	1843-2	1.021	0.530	n.d.	n.d.	n.d.
LUYKSGESTEL	L0903330064	1844-2	0.400	0.100	n.d.	n.d.	n.d.
BUDEL	L0903330065	1847-1	0.144	0.159	n.d.	n.d.	n.d.
DEURNE	L0903330066	1850-2	n.d.	n.d.	n.d.	n.d.	n.d.
WINKELSTRAAT	L0903330067	1851-1	<LOQ	<LOQ	n.d.	n.d.	n.d.
NUENEN	L0903330068	1852-1	n.d.	n.d.	n.d.	n.d.	n.d.
BREUGEL	L0903330069	1853-2	n.d.	n.d.	n.d.	n.d.	n.d.
WOLFWINKEL	L0903330070	1854-2	n.d.	n.d.	n.d.	n.d.	n.d.
ERP	L0903330071	1855-2	n.d.	n.d.	n.d.	n.d.	n.d.
DONK	L0903330072	1856-2	n.d.	n.d.	n.d.	n.d.	n.d.
VENHORST	L0903330073	1858-1	0.285	0.238	n.d.	n.d.	n.d.
WANROY	L0903330074	1860-1	0.173	0.109	n.d.	n.d.	n.d.
ZEELAND	L0903330075	1861-2	<LOQ	<LOQ	n.d.	n.d.	n.d.
SCHAYK	L0903330076	1862-1	0.038	0.028	n.d.	n.d.	n.d.
SCHAYK	L0903330077	1863-2	<LOQ	<LOQ	n.d.	n.d.	n.d.
LITH	L0903330078	1864-1	0.025	<LOQ	n.d.	n.d.	n.d.
NISTELRODE	L0903330079	1865-1	n.d.	n.d.	n.d.	n.d.	n.d.
HEESWIJK-DINTHER	L0903330080	1866-1	<LOQ	n.d.	n.d.	n.d.	n.d.
ST.MICHIELS-GESTEL	L0903330081	1868-2	n.d.	n.d.	n.d.	n.d.	n.d.
RAVENSTEIN	L0903330082	1870-1	0.102	0.054	n.d.	n.d.	n.d.
HEUSDEN	L0903330083	1871-1	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (0.0075 µg/L for each analyte)
LOQ = 0.025 µ/L

Table 7.5-50: Results of groundwater monitoring in the Netherlands (continued)

Location	Sponsor code	Sample no.	M656PH045 (µg/L)	M656PH047 (µg/L)	M656PH054 (µg/L)	M3 (µg/L)	M656PH010 (µg/L)
BREDA	L0903330044	1814-2	n.d.	n.d.	n.d.	n.d.	n.d.
GALDER	L0903330045	1815-1	n.d.	n.d.	n.d.	n.d.	n.d.
OOSTERHOUT	L0903330046	1816-2	0.070	n.d.	0.032	n.d.	n.d.
DONGEN	L0903330047	1817-2	n.d.	n.d.	n.d.	n.d.	n.d.
RIJEN	L0903330048	1818-2	0.097	n.d.	<LOQ	n.d.	n.d.
GILZE	L0903330049	1819-1	n.d.	n.d.	n.d.	n.d.	n.d.
CHAAM	L0903330050	1821-2	n.d.	n.d.	n.d.	n.d.	n.d.
CASTELRE	L0903330051	1822-1	0.159	n.d.	n.d.	n.d.	n.d.
BAARLE_ NASSAU	L0903330052	1823-1	0.051	n.d.	n.d.	n.d.	n.d.
BAARLE_ HERTOG	L0903330053	1824-1	n.d.	n.d.	n.d.	n.d.	n.d.
KLEIN_BEDAF	L0903330054	1825-1	n.d.	n.d.	<LOQ	n.d.	n.d.
GOIRLE	L0903330055	1827-2	n.d.	n.d.	n.d.	n.d.	n.d.
BIEZEN-MORTEL	L0903330056	1831-2	n.d.	n.d.	n.d.	n.d.	n.d.
LIEMPDE	L0903330057	1833-1	0.213	n.d.	n.d.	n.d.	n.d.
DIESSEN	L0903330058	1835-2	0.161	n.d.	<LOQ	n.d.	n.d.
H._EN_LAGE_ MIERDE	L0903330059	1837-2	n.d.	n.d.	n.d.	n.d.	n.d.
WINTELRE	L0903330060	1840-2	0.052	0.333	n.d.	n.d.	n.d.
EERSEL	L0903330061	1841-2	n.d.	n.d.	n.d.	n.d.	n.d.
EERSEL	L0903330062	1842-2	n.d.	n.d.	n.d.	n.d.	n.d.
LUYKSGESTEL	L0903330063	1843-2	n.d.	n.d.	0.032	n.d.	n.d.
LUYKSGESTEL	L0903330064	1844-2	n.d.	n.d.	n.d.	n.d.	n.d.
BUDEL	L0903330065	1847-1	0.038	n.d.	n.d.	n.d.	n.d.
DEURNE	L0903330066	1850-2	n.d.	n.d.	n.d.	n.d.	n.d.
WINKEL-STRAAT	L0903330067	1851-1	n.d.	n.d.	n.d.	n.d.	n.d.
NUENEN	L0903330068	1852-1	n.d.	n.d.	n.d.	n.d.	n.d.
BREUGEL	L0903330069	1853-2	<LOQ	n.d.	n.d.	n.d.	n.d.
WOLFWINKEL	L0903330070	1854-2	n.d.	n.d.	n.d.	n.d.	n.d.
ERP	L0903330071	1855-2	n.d.	n.d.	n.d.	n.d.	n.d.
DONK	L0903330072	1856-2	n.d.	n.d.	n.d.	n.d.	n.d.
VENHORST	L0903330073	1858-1	n.d.	n.d.	<LOQ	n.d.	n.d.
WANROY	L0903330074	1860-1	<LOQ	n.d.	n.d.	n.d.	n.d.
ZEELAND	L0903330075	1861-2	<LOQ	n.d.	n.d.	n.d.	n.d.
SCHAYK	L0903330076	1862-1	<LOQ	n.d.	n.d.	n.d.	n.d.
SCHAYK	L0903330077	1863-2	n.d.	n.d.	n.d.	n.d.	n.d.
LITH	L0903330078	1864-1	n.d.	n.d.	n.d.	n.d.	n.d.
NISTELRODE	L0903330079	1865-1	n.d.	n.d.	n.d.	n.d.	n.d.
HEESWIJK-DINTHER	L0903330080	1866-1	<LOQ	n.d.	n.d.	n.d.	n.d.
ST.MICHIELS-GESTEL	L0903330081	1868-2	n.d.	n.d.	n.d.	n.d.	n.d.
RAVENSTEIN	L0903330082	1870-1	<LOQ	n.d.	n.d.	n.d.	n.d.
HEUSDEN	L0903330083	1871-1	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable (0.0075 µg/L for each analyte)
LOQ = 0.025 µ/L

Report: CA 7.5/8
Haering T.,Miles B., 2014a
Evaluation of groundwater monitoring sites and context setting of groundwater monitoring data for metabolites of Dimethenamid-P
2013/1347948

Guidelines: <none>

GLP: no

Executive Summary

Within the context of the re-registration of dimethanamid-P in the EU analysis of groundwater samples has been undertaken to expand the groundwater concentration data basis for the metabolites of this substance.

From 2007 to 2012 a groundwater monitoring study involving BASF commercial herbicide products was conducted at 20 monitoring sites in the most significant corn production areas of Germany. This study was requested by German authorities as a post registration study for the active substance topramezone and its metabolite M670H05. The study was designed and conducted as a (mainly) prospective groundwater monitoring study starting about one year after first registration of formulations of a product family, containing the active ingredient topramezone together with dimethenamid-P.

The present report evaluates the validity of the Topramezone monitoring study in Germany for the dimethenamid-P metabolites, assessing both the retrospective aspect of the study (whether the study can show how shallow groundwater in the monitoring regions may have been affected by historical applications of dimethenamid-P, and previously the racemate dimethenamid) as well as the prospective aspect in which product applications were sponsored in the upstream vicinity of the monitoring wells. The monitoring sites and data from the study are then set into a European context. The representativeness of the German groundwater monitoring locations for agricultural areas in Europe is evaluated by applying a quantitative GIS-based spatial modelling approach to assess comparative leaching vulnerabilities.

The retrospective aspect of the study can be clearly shown to be valid for the metabolites of dimethenamid-P. The twenty monitoring sites were located in the most significant maize production areas of Germany. The monitoring sites can on the basis of available hydrogeological data be considered to be potentially vulnerable or highly vulnerable to leaching of agricultural chemicals. The depths of the well filters in relation to the groundwater surface are appropriate for sampling the upper portions of the shallow aquifers that are particularly relevant for such a study. Thus the study serves to show in a retrospective sense whether applications of the herbicide dimethenamid-P, which has been available for use in maize for many years (previously as dimethenamid), to maize fields in the monitoring regions may have affected the shallow groundwater.

The prospective aspect of the monitoring study shows that via leaching simulations, in conjunction with hydrogeological data for the monitoring sites, the timeframe of the groundwater sampling (2007-2013) would for nearly all of the wells be adequate to detect metabolites leaching from the treated fields in the different application years. Thus the prospective aspect of the groundwater monitoring study can be considered to be valid for the metabolites of dimethenamid-P.

The analysis of representativeness of groundwater monitoring wells in the European context is a comprehensive GIS analysis based on methods and data provided by the FOCUS groundwater report as well as the EFSA. The analysis considers pedoclimatic and agricultural aspects as well as for compounds properties. The resulting maps of representativeness show that the groundwater monitoring wells could be regarded as representative for most of Europe`s agricultural area, for both application times as well as for all three metabolites. There are some areas (e.g. northern Italy, southern France) which show a higher vulnerability compared to the German monitoring wells. However, since our proposed method follows a worst-case assumption by taking the 90th spatial percentile into account also for those NUTS2 areas only a small portion of the area could be considered as more vulnerable.

In the Netherlands, 80 groundwater monitoring wells located in corn producing areas, where the widespread usage of dimethenamid-P may be assumed, were selected from the monitoring network of the province Noord Brabant. This official monitoring network is specifically used for monitoring the quality of shallow groundwater in the province. Groundwater samples taken from these wells during official sampling in the first quarter of 2013 were analysed for the metabolites of dimethenamid-P. For the sampled wells an analysis is made of the sampling locations in relation to the maize producing areas in the Netherlands.
Based on the location and situation of the 80 monitoring wells and the sampling period, the single analysis for the dimethenamid-P metabolites provides a representative snapshot of the situation in an area with shallow groundwater and high levels of maize production at a time when both groundwater recharge and groundwater levels are expected to be around their annual maxima.

I. MATERIAL AND METHODS

Evaluation of the groundwater monitoring study in Germany
From 2007 to 2012 a groundwater monitoring study involving BASF commercial herbicide products containing Dimethenamid-P was conducted at 20 monitoring sites in Germany. A detailed description is provided in CA 7.5/2, a short summary relevant for this study is given below.

The study design of the ground water monitoring clearly covers a retrospective approach for the metabolites of dimethenamid-P. It serves to show whether applications of the herbicide dimethenamid-P, which has been available for use in maize for many years (previously as dimethenamid), to maize fields in the monitoring regions may have affected the shallow groundwater.

In addition, the study has also a significant prospective aspect in that it was designed to observe effects of known inputs of the target substance into groundwater in the area directly upgradient of the monitoring wells. To this end, products containing the target substances were applied specifically to fields upstream of the wells.

In order to ensure that the timeframe of the monitoring study was appropriate for the metabolites of Dimethenamid-P with regard to this prospective aspect of the monitoring study, leaching simulations were conducted for each monitoring site and used in conjunction with hydrogeological data to derive estimated potential arrival times at the monitoring wells for leachate from the known product applications.

Overview of the groundwater monitoring sites

A detailed description of the twenty groundwater monitoring sites is given in CA 7.5/2. The monitoring sites are located in four regions typical for maize cultivation and representing the prominent maize growing areas in Germany, namely the Nordwestdeutsches Tiefland / Geest (Northwest German Lowlands), the Altmark-Prignitz region, the Unterbayerisches Huegelland (Lower Bavarian Hilly Country) and the Suedliches Oberrheintal (Southern Upper Rhine Valley). The monitoring wells, which penetrate the upper groundwater layers at the respective sites, were selected following recommendations of the environmental or water authorities of the federal states. Selection criteria in the guidance paper from the German regulatory authorities for explanation of findings in groundwater and performance of groundwater monitoring studies (Aden et al. 2002) were applied. The monitoring sites themselves were all located downstream from major agricultural areas with intensive maize cultivation and represent situations vulnerable to leaching of agricultural chemicals, with shallow groundwater (generally at about 2 – 10 m depth) in unconsolidated sedimentary aquifers consisting mainly of moraine deposits and glaciofluvial or fluvial / fluvial terrace sands and gravels below sand or loam soils. All of the sites can on the basis of their hydrogeological characteristics be considered potentially vulnerable or highly vulnerable to leaching of agricultural chemicals, while the well filter screen depths are appropriate for detecting the effects of leaching in the upstream vicinity of the well.

Product applications

Product applications for the prospective aspect of the monitoring study were sponsored by BASF in the years 2007 – 2009. Substantial amounts of dimethenamid-P were applied in the course of the documented sponsored applications.

Table 7.5-51: Overview Product applications

year	product	[l/ha]	dimethenamid-p equivalent [g/ha]
2007	Clio® Super / Clio® Top Pack	1.8	968.4
2008	Clio® Super / Clio® Top Pack	1.8	968.4
2009	Clio® Super / Clio® Top Pack	1.5	807

Leaching simulations and evaluation of the prospective groundwater monitoring

In order to determine whether the timeframe of the monitoring study designed for Topramezone was also appropriate for the metabolites of dimethenamid-P with regard to the prospective aspect of the study, leaching simulations were conducted for each monitoring site to generate breakthrough curves for concentrations at the groundwater surface after a single product application. These were then used in conjunction with hydrogeological data to derive estimated potential arrival times at the monitoring wells for leachate from the known product applications.

Leaching simulations were carried out using the model PEARL 4.4.4. Estimates of travel times in groundwater from the treated fields to the monitoring well were derived from the hydrogeological data and distances to the well from treated fields.

Soil data for the PEARL leaching simulations

As the leaching behavior of the metabolites strongly depends on the makeup of the soil profile of the treated field, soil profile data were assembled to create realistic profiles for the individual sites. State authorities were asked for detailed soil profile data, which were located near the monitoring sites and which were representative for the soil conditions at the well. In most cases, representative soil profiles which characterize a high-resolution soil map unit, so called *Leitprofile*, were available. The scale of the soil maps ranges from 1:5.000 to 1:25.000. In areas, where no detailed soil map and no soil profiles were available, profile descriptions from coarser soil maps were used. In cases where more than one soil profile was available for a monitoring location modeling scenarios for each soil were set up.

Meteorological data for the PEARL leaching simulations

Data on temperature, precipitation, and evapotranspiration were obtained from the MARS-project of the AGRI4CAST and FOODSEC units of the Directorate General Joint Research Center (JRC) of the European Commission in Ispra (Italy). These data are the only source available to obtain homogeneous weather data with all parameters necessary to run groundwater leaching simulations with a daily temporal resolution.

Data are provided by MARS for 25x25 km grid units. In total eighteen units were relevant for the groundwater monitoring sites. Data were obtained for the period January 1st 1984 – December 31st 2012, which was the latest available date at the time of writing.

Application scenario for the PEARL simulations

The scenario characteristics and application dates are summarised in Table 7.5-52.

Table 7.5-52: Worst-case application scenario of dimethenamid-P considered for the PECgw calculations

Crop	Maize
Growth stage at application [BBCH]	Growth stage 1: Leaf development. 12-16
No. of applications	1
Application rate [g a.s. ha-1]	807
Crop interception [%]	25
Total yearly soil load [g a.s. ha-1]	600
Application dates	03-Jun-2007

Environmental fate parameters for the metabolites of dimethenamid-P

The substance-specific and default parameter values relevant to the leaching simulations are given in Table 7.5-53 and Table 7.5-54. Note that these parameters are endpoints used in the risk assessment before Annex I renewal. No change in the outcome is expected due to the generation of new endpoints in the framework of Annex I renewal and the conclusions drawn in this study.

Table 7.5-53: Summary of parameter specific and default input parameters used to simulate the leaching behavior of dimethenamid-p – old endpoints

Input parameter	Unit	Dimethenamid-p	Default value
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	275.8	-
Water solubility	[mg L^{-1}]	1449 (25°C)	-
Molar enthalpy of dissolution	[kJ mol^{-1}]	-	27
Saturated vapor pressure	[Pa]	2.51 x 10^{-3} (25°C)	-
Molar enthalpy of vaporisation	[kJ mol^{-1}]	-	95
Diffusion coefficient in water	[m² d^{-1}]	-	4.3 x 10^{-5} (20°C)
Diffusion coefficient in air	[m² d^{-1}]	-	0.43 (20°C)
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	10.8 (20°C, pF2)	-
Molar activation energy	[kJ mol^{-1}]	-	65.4
Exponent of moisture correction function	[-]	-	0.7
SORPTION PARAMETERS			
$K_{f,oc}$ value	[mL g^{-1}]	170.2	-
$K_{f,om}$ value	[mL g^{-1}]	98.7	-
Freundlich exponent 1/n	[-]	0.985	-
Method of subroutine description	[-]	-	pH-independent
CROP RELATED PARAMETERS			
PUF (plant uptake factor)	[-]	0.5	

Table 7.5-54: Summary of input parameters used to simulate the leaching behavior of the metabolites M656PH023, M656PH027 and M656PH031 – old endpoints

Input parameter	Unit	M656PH023	M656PH027	M656PH031
PHYSICO-CHEMICAL PARAMETERS				
Molecular weight	[g mol^{-1}]	271	321.4	347
Water solubility	[mg L^{-1}]	1000 (20°C)	1000 (20°C)	1000 (20°C)
Saturated vapor pressure	[Pa]	10^{-10} (20°C)	10^{-10} (20°C)	10^{-10} (20°C)
DEGRADATION PARAMETERS				
Half-life at reference conditions[1]	[d]	19.7 (20°C, pF2)	30.4 (20°C, pF2)	30.8 (20°C, pF2)
Formation fraction	[-]	0.154 from parent	0.143 from parent 1 from M656PH031	0.067 from parent
SORPTION PARAMETERS				
$K_{f,om}$ value	[mL g^{-1}]	6.9	5.9	2.5
Freundlich exponent 1/n[2]	[-]	0.72	1.01	0.93
CROP RELATED PARAMETERS				
PUF (plant uptake factor)	[-]	0.5	0.5	0.5

PEARL Simulations

Leaching simulations were carried out using the model FOCUS-Pearl 4.4.4. The simulation timeframe was defined as 01.01.2005 to 31.12.2012, allowing 2.5 years run-in time for the model before the herbicide application and continuing until the end of the available meteorological data time series. This was sufficient to obtain a clear peak solute breakthrough at the evaluation depth in all but two cases.

As the purpose of the simulations was to determine the potential leaching timeframes for solutes reaching groundwater, the evaluation depth for the PEARL simulations was for each site defined by the highest measured groundwater level. Where this was below the level of the defined soil profile, the lowest soil layer was assumed to extend to the lower boundary depth.

The FOCUS Hamburg Maize crop calendar with annual cropping was used for the simulations. The tillage depth was set to 20 cm.

Evaluation of the sampling timeframe for the prospective aspect of the groundwater monitoring study

Estimates of travel times in groundwater from the treated fields to the monitoring well were derived from the hydrogeological data (CA 7.5/2) and distances to the well from treated fields estimated from aerial photographs (*Google Inc. (2013): Google Earth v. 7.1.1.188*). These were then combined with the breakthrough times determined for the metabolites from a single application in the leaching simulations to derive approximate timescales for the potential arrivals and peak concentrations of the metabolites from the closest treated field in each application year.

Spatial Modelling approach to set monitoring locations in a European context

To determine the representativeness of the German groundwater monitoring locations for the agricultural area of Europe we apply a quantitative modelling approach. Representativeness with regard to pesticide leaching to groundwater has to consider several environmental as well as compound parameters which are relevant for groundwater leaching. Since the relevant environmental parameters vary in space and time, mapping approaches have been proposed to assess the spatial variability of groundwater vulnerability to pesticide leaching [*FOCUS (2009): Assessing Potential for Movement of Active Substances and their Metabolites to Groundwater in the EU Final Report of the Groundwater Workgroup of FOCUS, amending FOCUS (2000), EC Document Reference Sanco/13144/2010, 604 pp.*;
TIKTAK, A., BOESTEN, J.J.T.I., VAN DEN LINDEN, A.M.A. and VANCLOOSTER, M. (2006): Mapping ground water vulnerability to pesticide leaching with a process-based metamodel of EuroPEARL, J. Environ. Qual. 35: 1231-1226.;
FOCAZIO, M.J., REILLY, T.E., RUPERT, M.G., and HELSEL, D.R. (2002): Assessing ground-water vulnerability to contamination: Providing scientifically defensible information for decision makers. US Department of Interior and US Geological Survey, Reston, VA. US Geological Survey Circular No. 1224.].

Our assessment of representativeness of monitoring sites consists of two steps (Step 1 and Step 2). The comparison (Step 2) is done on the NUTS2 level, which is the official nomenclature of territorial units for statistics in the EU[1]. The workflow of the assessment is illustrated in Figure 7.5-4.

[1] http://epp.eurostat.ec.europa.eu/portal/page/portal/nuts_nomenclature/introduction

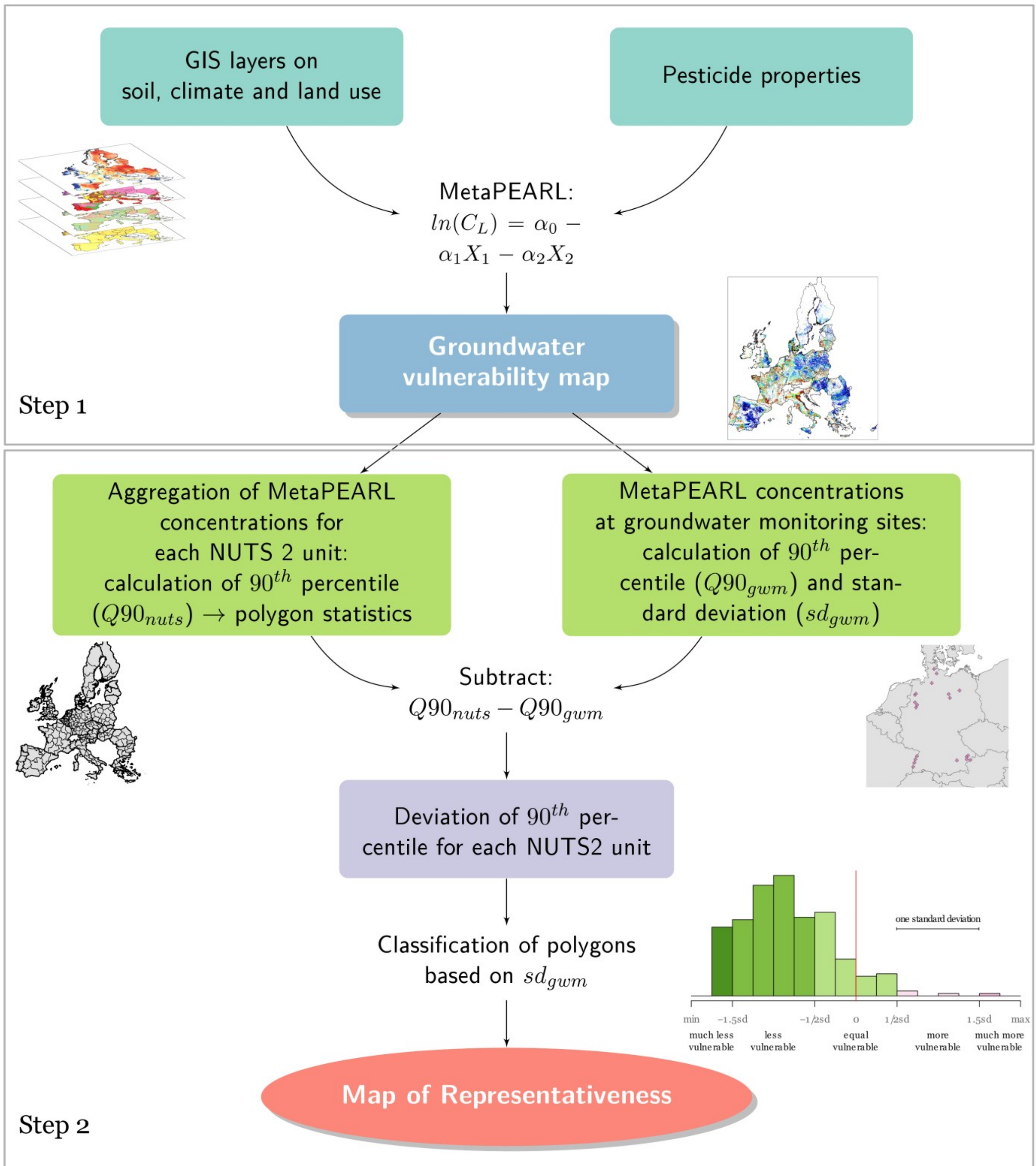


Figure 7.5-4: Workflow for the GIS analysis to assess the representativeness of the German groundwater monitoring sites for the entire EU.

II. RESULTS AND DISCUSSION

Retrospective

Results of the PEARL simulations for the metabolites of dimethenamid-P

As the aim of the simulations is to provide just the potential leaching timeframes at each site resulting from a single application of the active substance, the simulated concentrations for the metabolites are normalized to their respective maximum leachate concentrations during the simulation (C/C_{max}). Thus regardless of the actual magnitudes of their concentrations, the maximum normalized peak concentration is 1 for all metabolites. The times to the breakthrough and peak concentrations (defined respectively as C/C_{max} = 0.05 and C/C_{max} = 1 for the first arriving metabolite) at the evaluation depths in the simulations are given in Table 7.5-55.

Table 7.5-55: Calculated breakthrough times for metabolites of dimethenamid-P in leaching simulations

Simulation	Time after application [d]	
	Breakthrough (C/C_{max} = 0.05)	Peak (C/C_{max} = 1)
Albersloh	141	173
Asing1	1362	1812
Asing2	1329	1763
Brekendorf	347	621
Drewen	1305*	1925*
Flechum	251	674
Gardelegen	1661*	2037*
Glaslern1	752	1098
Glaslern2	460	1156
Glaslern3	447	787
Hartheim	735	1012
Ichenheim	382	710
Krogaspe	29	78
Oberhausen	715	1374
Ostbevern	192	264
Osterholzen	754	918
Pfarrkirchen	98	195
Quadendambeck	1186	1324
Rheinau	653	821
Rossbach1	446	667
Rossbach2	639	871
Veltrup	191	593
Vinnen Ahmsen	377	824
Wedel	248	385

* Peak not reached or not clearly reached before end of simulation

The concentration breakthrough occurs within 1 year or less at 10 of the monitoring sites, 2 years or less at 10 sites, and within 4 years at all but one of the sites (Quadendambeck). The peak concentrations occur within 1 year or less at 5 of the monitoring sites, 2 years or less at 16 sites, and within 5 years at all but two of the sites (Drewen, Quadendambeck). The sites with leaching times significantly longer than the others (Drewen, Gardelegen, Asing and Quadendambeck) have a combination of relatively deep groundwater and relatively high proportions of silt, and thus low vertical hydraulic conductivities, in their soil profiles, resulting in long breakthrough times.

Prospective

Approximate timescales for the potential arrivals and peak concentrations of the metabolites from the closest treated field in each application year based on travel times in groundwater from treated fields and the breakthrough times of the retrospective analysis are given in Table 7.5-56.

The estimated arrival times at the monitoring well can be considered as indicative only, as there are a number of sources of uncertainty. The most significant uncertainties are in the actual soil profiles and depths to groundwater beneath the treated fields compared to those used in the simulations and the hydrogeological parameters such as the hydraulic gradient and effective porosity that are key parameters determining the apparent groundwater flow velocity (solute transport velocity). Taking this uncertainty into account, estimated arrival times of up to 6 months after the end of the groundwater sampling period in March 2013 (i.e. up to October 2013) are considered as still being within the timeframe of the monitoring fields and only some concentration breakthroughs from the closest fields in the three treatment years may be expected in the sampling period.

Overall the estimated arrival times for concentrations at the monitoring wells from the product applications show that the prospective aspect of the Topramezone groundwater monitoring study is applicable to the metabolites of dimethenamid-P from the point of view of the sampling timeframe.

Table 7.5-56: Estimated arrival times for concentrations of metabolites at the monitoring wells from documented applications of dimethenamid-P

Monitoring site	Approximate depth to groundwater [m]	Leaching timescales for metabolites in PEARL simulations [days after application]		Apparent GW flow velocity** [m/d]	Approximate distance to well from closest treated field in each year [m]			Approximate travel time to well for closest treated field in each year [days]			Approximate potential arrival times at well for breakthrough and peak concentrations from closest treated field in each year					
		First arrival at groundwater surface	Peak concentration at groundwater surface		2007	2008	2009	2007	2008	2009	Arrival 2007 Application	Arrival PEAK 2007 Application	Arrival 2008 Application	Arrival PEAK 2008 Application	Arrival 2009 Application	Arrival PEAK 2009 Application
Southern Upper-Rhine Valley (Suedliches Oberrheintal)																
Rheinau	~ 2 5 - 3	653	821	0.3	300	300	10	1000	1000	33	Dec. 2011	May. 2012	Dec. 2012	May. 2013	Apr. 2011	Oct. 2011
Ichenheim	~ 2 - 2.5	382	710	1.4	500	10	10	357	7	7	Jun. 2009	May. 2010	Jun. 2009	May. 2010	Jun. 2010	May. 2011
Oberhausen	~ 3 5 - 4	715	1374	1.4	900	10	10	643	7	7	Feb. 2011	Dec. 2012	May. 2010	Mar. 2012	May. 2011	Mar. 2013
Hartheim	~ 6 - 7	735	1012	3.5	100	100	380	29	29	109	Jul. 2009	Apr. 2010	Jul. 2010	Apr. 2011	Sep. 2011	Jun. 2012
Northwest German Lowlands (Nordwestdeutsches Tiefland)																
Albersloh	~ 1 5 - 2	141	173	0.4	100	300	100	250	750	250	Jun. 2008	Jul. 2008	Nov. 2010	Dec. 2010	Jun. 2010	Jul. 2010
Ostbevern	~ 2 - 2.5	192	264	0.3	150	800	150	500	2667	500	Apr. 2009	Jul. 2009	Mar. 2016	Jun. 2016	Apr. 2011	Jul. 2011
Veltrup	~ 1 5 - 2	191	593	0.5	10	10	10	20	20	20	Dec. 2007	Feb. 2009	Dec. 2008	Feb. 2010	Dec. 2009	Feb. 2011
Flechum	~ 3 - 3.5	251	674	0.5	250	250	400	500	500	800	Jun. 2009	Aug. 2010	Jun. 2010	Aug. 2011	Apr. 2012	Jun. 2013
Vinnen-Ahmsen	~ 3 5 - 4	377	824	0.8	500	250	1000	625	313	1250	Mar. 2010	May. 2011	Apr. 2010	Jul. 2011	Nov. 2013	Feb. 2015
Wedel	~ 3 5 - 4	248	385	0.6	500	500	100	833	833	167	May. 2010	Oct. 2010	May. 2011	Oct. 2011	Jul. 2010	Dec. 2010
Krogaspe	~ 1 5 - 2	29	78	0.9	200	400	600	222	444	667	Feb. 2008	Mar. 2008	Sep. 2009	Nov. 2009	Apr. 2011	Jun. 2011
Brekendorf	~ 5 - 6	347	621	0.59	10	10	10	17	17	17	May. 2008	Mar. 2009	Jun. 2009	Mar. 2010	Jun. 2010	Mar. 2011
Lower Bavarian Hilly Country (Niederbayerisches Huegelland)																
Glaslern	~ 2 - 3	553**	1014**	1.5	900	300	1500	600	200	1000	Jul. 2010	Nov. 2011	Jun. 2010	Sep. 2011	Sep. 2013	Dec. 2014
Osterholzen	~ 9	754	918	0.5	50	300	600	100	600	1200	Oct. 2009	Mar. 2010	Feb. 2012	Jul. 2012	Oct. 2014	Mar. 2015
Pfarrkirchen	~ 1 5 - 2.5	98	195	0.6	200	300	200	333	500	333	Aug. 2008	Nov. 2008	Jan. 2010	Apr. 2010	Aug. 2010	Nov. 2010
Rossbach	~ 3 - 4	542**	769**	1	300	10	100	300	10	100	Sep. 2009	May. 2010	Dec. 2009	Jul. 2010	Mar. 2011	Oct. 2011
Asing	~ 8	1345**	3575**	1.3	300	600	300	231	462	231	Sep. 2011	Nov. 2017	May. 2013	Jun. 2019	Sep. 2013	Nov. 2019
Altmark and Prignitz																
Gardelegen	~ 8	1661*	not reached*	0.6	10	400	10	17	667	17	Jan. 2012	n/a	Oct. 2014	n/a	Jan. 2014	n/a
Quadendambeck	~ 6 5	1186	1324	0.26	300	500	200	1139	1899	760	Oct. 2013	Mar. 2014	Nov. 2016	Mar. 2017	Sep. 2014	Feb. 2015
Drewen	~ 3 5 - 4.5	1305*	not reached*	0.43	200	50	800	463	116	1852	Apr. 2012	Dec. 2013	Apr. 2012	Jan. 2014	Jan. 2018	Oct. 2019

* Peak concentrations not reached in simulation

** Mean value from range given for site

Estimated arrival or peak more than 6 months after end of sampling for closest field treated in year

Representativeness of groundwater monitoring wells in EU context

Following the approach outlined in the methods section above, vulnerability maps for spring and autumn application have been calculated for all three metabolites. Based on these maps the resulting maps of representativeness have been created. Only the maps of representativeness are presented here, since the vulnerability maps have been used only as input for these maps.
The resulting map for the autumn application of M656PH027 is illustrated in Figure 7.5-5. Only the autumn application representativeness is depicted, since the highest NUTS2 concentrations were modelled for this scenario, and as such the largest deviations which respect to representativeness are obtained.

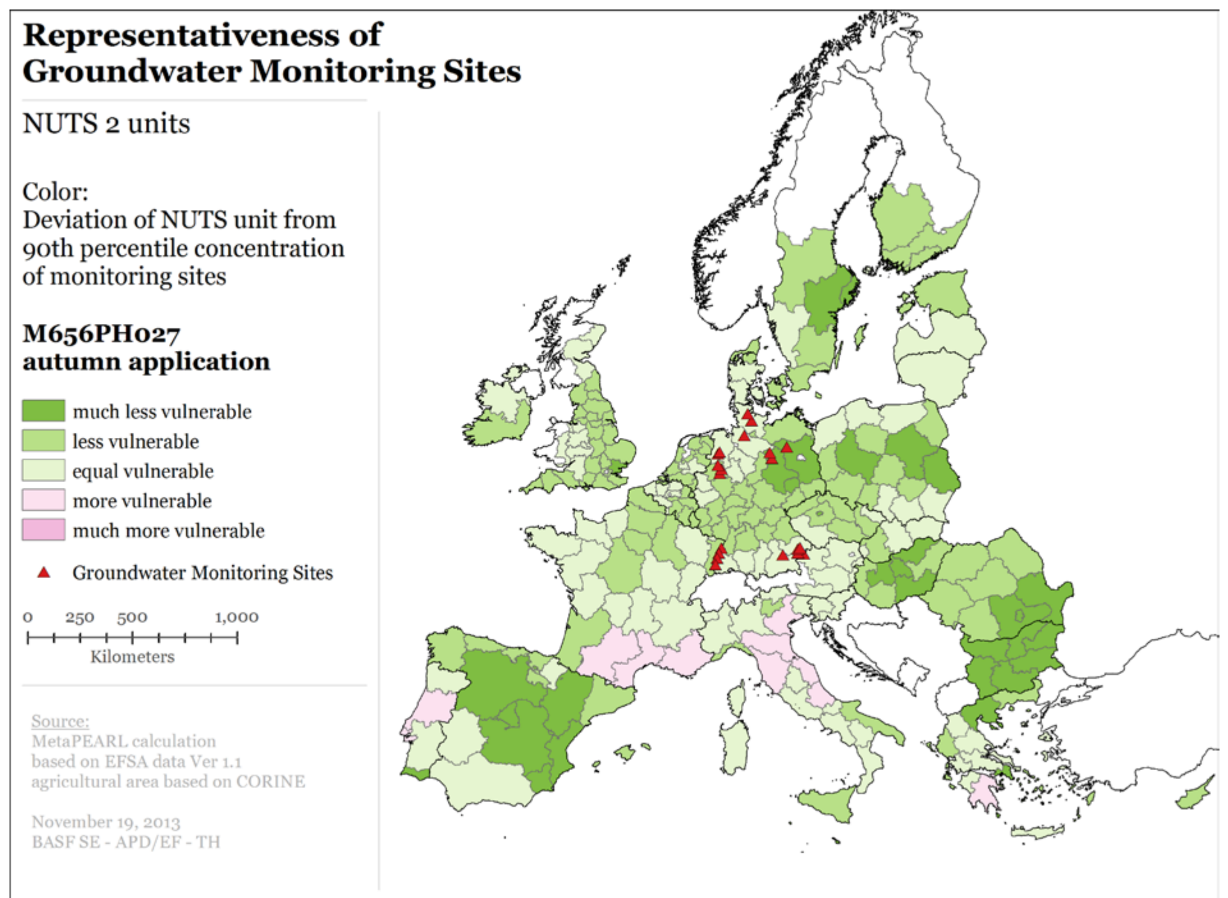


Figure 7.5-5: Map of representativeness of the German groundwater monitoring locations for the agricultural area of Europe: M656PH027 – autumn application.

Groundwater monitoring samples from the Netherlands

In the Netherlands, 80 groundwater monitoring wells located in corn producing areas, where the widespread usage of dimethenamid-P may be assumed, were selected from the monitoring network of the province Noord Brabant. Groundwater samples taken from these wells during official sampling in the period from January 8th 2013 to April 16th 2013 were analysed for the metabolites of dimethenamid -P.

Noord Brabant is a region in the south of the Netherlands. It is bordered by Belgium's Antwerp and Limburg provinces in the South, the Meuse River (Maas) in the North, Limburg in the East and Zeeland in the West. The wells selected for analysis (Figure 7.5-2) are all located in areas with predominantly agricultural land usage, and where over 25% of the agricultural area is used for maize production.
The official monitoring network from which the wells were selected is specifically used for monitoring the quality of shallow groundwater in the province. The majority of the wells are located in areas where the depth to groundwater is less than 3 meters. With regard to their filter depths, the wells are all classified as "ondiep" (shallow).
Based on the location and situation of the 80 monitoring wells and the sampling period, the single analysis for the dimethenamid-P metabolites should provide a representative snapshot of the situation in an area with shallow groundwater and high levels of maize production at a time when both groundwater recharge and groundwater levels are expected to be around their annual maxima.

III. CONCLUSION

Within the context of the re-registration of dimethanamid-P in the EU analysis of groundwater samples has been undertaken to expand the groundwater concentration data basis for the metabolites of this substance.

From 2007 to 2012 a groundwater monitoring study involving BASF commercial herbicide products was conducted at 20 monitoring sites in the most significant corn production areas of Germany. The validity of the monitoring study in Germany for the dimethenamid-P metabolites was assessed with regard to both the retrospective aspect of the study (whether the study can show how shallow groundwater in the monitoring regions may have been affected by historical applications of dimethenamid-P, and previously the racemate dimethenamid) as well as the prospective aspect in which product applications were sponsored in the upstream vicinity of the monitoring wells. **The retrospective and prospective aspect of the study can be clearly shown to be valid for the metabolites of dimethenamid-P. In addition, the resulting maps of representativeness show that the groundwater monitoring wells could be regarded as representative for most of Europe's agricultural area.**

In the Netherlands, 80 groundwater monitoring wells located in corn producing areas, where the widespread usage of dimethenamid-P may be assumed, were selected from the monitoring network of the province Noord Brabant. **Based on the location and situation of the 80 monitoring wells in the Netherlands and the chosen sampling period, the single analysis for the dimethenamid-P metabolites should provide a representative snapshot of the situation in an area with shallow groundwater and high levels of maize production at a time when both groundwater recharge and groundwater levels are expected to be around their annual maxima.**

The following citations are relevant data pulled from the open literature. Reults relevant for surface and groundwater are presented. All data reinforce the assertion of Dimethenamid-P as a safe plant protection product.

Report: CA 7.5/9
Chevre N. et al., 2007a
Risk assessment of herbicide mixtures in a large European lake
2013/1348573
Guidelines: <none>
GLP: no

Executive Summary

The aim of the study was to determine the levels of various pesticides of the Geneva Lake. A group of pesticides including RS-dimethenamid were monitored in 2004 and 2005. Water samples were collected at two different depths and different times from the lake. Samples were processed and analysed to determine pesticides concentrations. RS-dimethenamid was found only at one sampling date with similar average concentration (0.001 $\mu g\ L^{-1}$) in both depth ranges. The risk assessment results of the herbicides showed no risk of RS-dimethenamid contamination.

I. MATERIALS AND METHODS

1. Test material

Pesticides including RS-dimethenamid

2. Field study design

The study was performed in 2004 and 2005 to determine the levels of various pesticides of Geneva Lake. Samples were taken at different depths and different periods from a site situated in the middle of the lake. This site has been used for long time as a reference point, therefore it was considered as representative of the average contamination of the lake.

3. Water sampling and analysis

Water samples (2 L) were collected two times in April 26, 2004 and April 26, 2005, and one time in September 6, 2004. The samples were taken at nine different depths; Five at 0-10 m depths in the epilimnion-metalimnion (0, 1, 5, 7.5, and 10 m), and four at 10-309 m depth in the hypolimnion (30, 100, 305, and 309 m). An aliquot of 500 mL samples were extracted on an Oasis HLB cartridge (Waters) concentrated to 100 µL and analysed using HPLC/MS-MS. The risk for RS-dimethenamid was assessed by comparing the measured concentration to the water quality criteria (WQC) set by the authors.

II. RESULTS AND DISCUSSION

Method efficiency

A generic sample preparation for a wide range of pesticides was developed for water samples. High concentrated extracts of the water samples enabled reaching a limit of detection in the range from 1-100 ng $L^{-1}$for the different herbicides. The method recovery ranged from 50-120%. The method was validated with five concentration levels (1, 5, 20, 50, and 200 ng L^{-1}) using pure water, two repetitions for five days. The coefficient of variation for intra-day precision ranged from 8-18%, and for inter-day from 18-38%.

Residues in water

RS-dimethenamid was detected only at one sampling date at both depth ranges with an average concentration of 0.001 µg L^{-1}. The concentration of RS-dimethenamid was below the limit of detection at all other sampling dates and thus below the value for the WQC (0.16 µg/L) set by the authors, indicating no risk of RS-dimethenamid contamination.

Report: CA 7.5/10
Leu C. et al., 2004a
Simultaneous assessment of sources, processes, and factors influencing herbicide losses to surface waters in a small agricultural catchment
2013/1348574
Guidelines: <none>
GLP: no

Executive Summary

The aim of the study was to gain a detailed knowledge about all factors that control the losses of dimethenamid from a point source (farmyard) and from diffuse sources (fields). A small area of the catchment of the Lake Greifensee, located 25 km southeast of Zurich and into the river Aa Mönchaltorf was investigated over a period of 67 days after a controlled application of dimethenamid and two other pesticides. Dimethenamid was applied on 13 cornfields at 0.75 kg ha^{-1} using a boom sprayer. A high temporal sampling resolution approach was applied to collect water samples. Soil samples were also collected from all 13 fields. Thirteen rain events were recorded, causing loss of the herbicide mainly from diffuse sources by surface runoff and preferential flow into the subsurface drainage system.
The maximum concentration of the herbicide reached 1.5 µg L^{-1} at the discharge peak. Total mass losses of dimethenamid from the fields of the catchment accounted for 0.27% of its total amount applied.
The dissipation of dimethenamid from field was described by first-order kinetics with a DT_{50} of 13 days as a median value from 11 fields.

I. MATERIALS AND METHODS

4. Test material

Frontier 900 EC (emulsion, containing dimethenamid)

5. Field study design

The investigated area comprises about 2.1 km^2 of the catchment of the Lake Greifensee, located 25 km southeast of Zurich and drains into the river Aa Mönchaltorf. Agricultural land forms 91% of the catchment area; 7% of it are forest and the remaining area includes farmyard, buildings and roads. Soils in the northeast of catchment have developed from tertiary river deposits called Obere Süsswassermolasse. The underground of the remaining area is moraine material of the Würm glaciation. The annual precipitation is 1330 mm on average and higher rainfall may occur during the vegetation period. The average monthly temperatures range from -1°C in January to 18°C in July. In this area, 60% of the 7.2 km brooks length flow in subsurface concrete tubes. 12% of the catchment is systematically drained with tiles at 1.4 m depth and spaced at ~14 m. The study investigated 13 cornfields including poorly drained (cambic) Gley soils (73%) and well to relatively well drained (calcaric) Cambisol (27%). Five fields were systematically drained and at least five additional fields were non-systematically drained. Top soils of these fields are loamy to clay loamy with organic matter contents ranging from 2.8 to 8.5%. Dimethenamid was applied (in a mixture with two other herbicides) on 13 cornfields at 0.75 kg ha^{-1} using a boom sprayer on May 8, 2000. With each mixture a fourth pesticide (tracer pesticide) out of a selection of nine substances was applied to each field to identify losses from individual fields. Post- and pre-emergence field applications were performed. During the study year, dimethenamid was not used by other farmers in the catchment outside the test area.

6. Catchment sampling and analysis

Two rain gauges were used to record the amounts of rain every 10 min. Brook discharge at the outlet of the catchment was determined by two different methods. First, pressure at the bed of a flume was continuously recorded with a transducer connected to a data logger. The dilution method with NaCl was used to calibrate a pressure-discharge relation based on 16 calibration points over the whole discharge range, gauged during the sampling period. Second, the level and average flow velocity were continuously gauged by a sensor. Discharge data of both systems as well as conductivity were measured and stored in 5 min intervals. At the same station, water samples were taken from the brook using three portable automatic samplers. Two sampling approaches were followed. First, two samplers were used to collect time-proportional samples, triggered by elevated discharge levels. The sampling intervals were between 5 and 20 min and between 15 and 60 min, respectively. Second, a third sampler was set to take flow-proportional composite samples each composed of 9 subsamples.

A total of 596 water samples were analysed for dimethenamid using a SPME-GC/MS analytical method. Limits of quantification for the investigated substances were in the range from 0.02 to 0.12 $\mu g\ L^{-1}$ for the different pesticides. Tracer pesticides were determined with the same analytical method. Another analytical method was used to quantify ethansulfonic acid and oxanillic acid degradates of dimethenamid. The limit of quantification for this method ranged from 0.003 to 0.01 $\mu g\ L^{-1}$.

Soil samples from all cornfields were taken once prior to herbicides application and eleven times after application during a time period of 50 days. Fifteen to 20 cores were taken randomly on every field and mixed to one composite sample per field. Samples were weighted, milled, homogenised and divided into aliquots of 8 g before analysis. An internal standard of d_3-dimethenamid was added to the soil aliquots before extraction and dimethenamid was quantified using GC-MS. The limit of quantification ranged from 0.03 to 0.05 $\mu g\ g^{-1}$ dry matter.

II. RESULTS

Loss dynamics of dimethenamid

A total of 260 mm precipitation occurred during the 67 days from application until end of the sampling period. Thirteen rain events caused losses of herbicides from point sources (runoff from farmyards) or diffuse sources (runoff and preferential flow from fields) into the brook or subsurface drainage system. The first five rain events mainly caused loss of herbicides from point sources by very short runoff events. As dimethenamid never was used on the farmyard only very small concentrations were found at the outlet of the catchment.
Diffuse loss was the major source of herbicide removal from the fields into the brook. For dimethenamid, diffuse loss after rain events 6, 7, 9 and 13 accounted for 99% of the total brook loads lost until day 67 after application.
Table 7.5-57gives maximum dimethenamid concentrations, loads, and maximum discharge measured in the brook at the outlet of catchment during the four most important diffuse loss events.

Table 7.5-57: *Maximum dimethenamid concentr*ations, loads, and maximum discharge measured in the brook at the outlet of catchment during the four most important diffuse loss events (Leu *et al.* 2004)

Rain event	6	7	9	13
Days since application	23-29	29-33	37-41	61-67
Maximum concentrations ($\mu g\ L^{-1}$)	1.47	0.32	0.12	0.02
Load (g)	21.7 (93%)	1.2 (5%)	0.2 (1%)	nd [a]
Maximum flow ($m^3\ s^{-1}$)	0.63	0.22	0.19	0.49

[a] The dimethenamid load during event 13 was not quantified since concentrations were below the quantification limit of the SPME-GC/MS method. Note that the difference of 2 g resulting from treating values below the quantification limit as 0 as compared to treating them as 0.02 $\mu g\ L^{-1}$ (i.e., the quantification limit itself) is small as compared to the total load of 23 g. Parentheses list percentages of individual loads from the total load lost from the fields until day 67 after application

The load for dimethenamid metabolites dimethenamid ethansulfonic acid (ESA) and dimethenamid oxanillic acid (OXA) were 11.1 g and 15.5 g at the loss event 6. The ratio of molar amounts of metabolites to parent compound at this event was 0.54 and 0.73 for dimethenamid ESA and dimethenamid OXA.
Total mass losses of dimethenamid from the fields of the catchment accounted for 0.27% of its total amount applied.
The dissipation of dimethenamid from soil was described by first-order kinetics with a field DT_{50} of 13 days as a median value from 11 fields.

Report: CA 7.5/11
Hamer K.,Freudenberger U., 2011a
Pflanzenschutzrechtlich nicht relevante Metaboliten im Grundwasser
2013/1348575
Guidelines: <none>
GLP: no

Executive Summary

The authorisation process of plant protection products differentiates between relevant and non-relevant metabolites. Also non-relevant metabolites are considered as undesired in drinking water. The aim of the paper was to discuss the current legal situation and to assess whether the legal regulation covers the risk assessment of non-relevant metabolites occurring in the environment. The legal situation was evaluated and summarised from the Bund-/Länder-Arbeitsgemeinschaft Wasser (LAWA) on authority of the Umweltministerkonferenz (Conference of Environmental Ministers).

The paper presents a compilation of 24 substances investigated during groundwater monitoring of non-relevant metabolites in Germany from 2006 to 2008 (data obtained from secondary literature). Among these are data on two metabolites of Dimethenamid-P: dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23. Both metabolites did not exceed the maximum permissible level for groundwater of 1.0 µg/L (standard health orientation value recommended by the Umweltbundesamt [UBA]).

I. MATERIAL AND METHODS

A. MATERIALS

Dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23 were measured in the monitoring programm.

B. STUDY DESIGN

Results of regular measurements of dimethenamid-sulfonic acid M27 and dimethenamid acid M23 in Germany are given (228 and 232 monitoring points each in three federal states of Germany, respectively). The measurements were undertaken from 2006 to 2008 to evaluate the occurrence of non-relevant metabolites in groundwater. No information about sampling technique or sampling frequency was provided in the paper.

II. RESULTS AND CONCLUSION

Dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23 were not detected above the standard health orientation values (gesundheitliche Orientierungswerte GOW) in the water samples of all groundwater samples. For both metabolites a standard health orientation value of 1 µg/L was recommended by the UBA – the value recommended for substances without study of chronic toxicity.

Dimethenamid-P

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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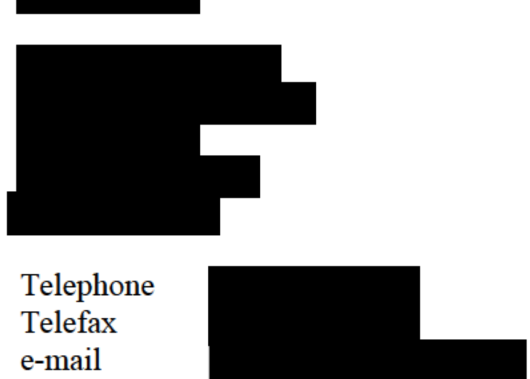

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Date	Data points containing amendments or additions and brief description	Document identifier and version number

[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 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

CA 8.1 Effects on birds and other terrestrial vertebrates

Information on Chapters MCA 8.1.1 and 8.1.2

In the context of chapters MCA 8.1.1 and 8.1.2, 'Effects on birds' and 'Effects on terrestrial vertebrates other than birds', **Table 8.1-1** provides information on the EU-reviewed and agreed toxicity endpoints. No additional toxicity studies are relevant for AIR3 for the active substance dimethenamid-p (BAS 656 PH) for assessing the risk to birds and mammals.

Table 8.1-1: Summary of EU-reviewed and agreed, relevant for AIR3 for the active substance dimethenamid-p (BAS 656 PH) for assessing the risk to birds and mammals [1)]

Test system	Test species	Reference [Author, BASF DocID]	EU-agreed
BIRDS			
Acute oral toxicity	*Colinus virginianus*	[redacted] 1996/5419	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Short-term dietary toxicity	*Colinus virginianus*	[redacted] 1996/5412	Yes (but no longer part of core data package according to EFSA/2009/1438)
	Anas platyrhynchos	[redacted] 1996/5410	Yes (but no longer part of core data package according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Colinus virginianus*	[redacted] 1994/11900	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	[redacted] 1994/11899	Yes (still valid for AIR 3 according to EFSA/2009/1438)
MAMMALS			
Acute oral toxicity	Rat	[redacted] 1996/11087	Yes (still valid for AIR 3 according to EFSA/2009/1438)
2-Generation reproductive toxicity	Rat	[redacted] 1990/11140	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	Rat	[redacted] 1987/11225	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rat	[redacted], 1997/5274	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rabbit	[redacted], 1988/11376	Yes (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 Review report for the active substance dimethenamid-p, SANCO/1402/2001-Final, July 2003

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

No new study available (cp Table 8.1-1).

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available (cp Table 8.1-1).

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available (cp Table 8.1-1).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available (cp Table 8.1-1).

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new study available (cp Table 8.1-1).

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 the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles. In the case of dimethenamid-p, no studies were found in the literature on the toxicity of this active ingredient on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibians should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and to the absence of standard guidelines for amphibian testing). 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 (Fryday and Thompson, 2012; Weltje *et al.,* 2013). Regulatory ecotoxicological information on terrestrial amphibians is scarce in general. However, in the few cases where terrestrial stages of amphibians were tested in the same study as birds and mammals the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 13 in Fryday and Thompson, 2012). This suggests that the quantitative risk assessment done for birds and mammals would be conservative for the terrestrial phase of 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 poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at 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. This uncertainty will remain until appropriate and validated testing methods are developed and validated and adequate regulatory risk assessment schemes are implemented in the EU.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994

CA 8.1.5 Endocrine disrupting properties

For mammals there is no concern for endocrine disrupting properties of dimethenamid-p as outlined in chapter CA 5.8.3.

For birds there is no indication from the reproductive toxicity studies for an endocrine disrupting potential of dimethenamid-p. Please refer to chapter CP 10.1.1.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of dimethenamid-P (BAS 656 PH), new toxicity studies on the active substance, its metabolites and several [redacted] 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 the sake of completeness, summaries are also provide for some older studies that have not been submitted during Annex I inclusion process of dimethenamid-P (*e.g.* no data requirement in the EU) and for EU agreed studies that have been amended in the meantime (*e.g.* due to recalculations of endpoints according to current guidelines). Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of dimethenamid-P are provided in the EU Review documents of the active substance (Monograph, Vol. 3, Annex B.9, September 2000; EC Review Report (SANCO/1402/2001-Final), March 2003).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1.

Full references used within the following chapters are given at the end of the document.

Table 8.2-1: Summary of the toxicity values for aquatic organisms obtained in the studies with the active substance dimethenamid-P (BAS 656-PH), its major metabolites and several [redacted]

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
active substance: dimethenamid-P				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	**6.3**	[redacted], 1996/5417	yes
Lepomis macrochirus	96 h LC_{50}	10.4	[redacted] 1996/5414	yes
Cyprinodon variegatus [1), 2)]	96 h LC_{50}	12.0	[redacted] 1996/5416	no (conducted for registrations outside of Europe)
Oncorhynchus mykiss [3)]	21 d NOEC	0.630	[redacted] 1991/11906	yes
Oncorhynchus mykiss [3)]	90 d NOEC (ELS)	**0.120**	[redacted] 1992/12456	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**12**	Graves & Swigert, 1996/5415	yes
Americamysis bahia [1),2)] (former name: *Mysidopsis bahia*)	48 h LC_{50}	**> 9.2** [+]	Graves & Swigert, 1996/5413	no (conducted for registrations outside of Europe)
Daphnia magna [3)]	21 d NOEC	1.36	Holmes & Swigert, 1992/12455	yes
Daphnia magna [3)]	21 d NOEC	**0.680**	Jenkins, 1991/11952	yes
Algae standard species [4)]				
Pseudokirchneriella subcapitata (syn. *Selenastrum capricornutum*)	72 h E_rC_{50} 72 h E_yC_{50}	0.0303 0.0191	Hoberg, 1997/10746 (= 1997/5170) Amendment: Kubitza, 2004/1025684 [5)]	yes, but recalculation of endpoints
	72 h E_rC_{50} 72 h E_yC_{50}	0.0873 0.0210	Backfisch, 2013/1078075 [1)]	no (new study)
	arithmetic mean 72 h E_rC_{50}	**0.0588** [6)]	--	(calculation based on new and old data)
Algae additional species [4)]				
Anabaena flos-aquae [5)]	72 h E_rC_{50} 72 h E_bC_{50}	> 0.860 (extrapolated: **1.340**) [$] 0.194	Hoberg, 1997/5173 Amendment: Kubitza, 2004/1025685	yes, but recalculation of endpoints
Ankistrodesmus bibraianus [1)]	72 h E_rC_{50} 72 h E_yC_{50}	**0.0370** 0.0097	Backfisch, 2012/1246639	no (new study)

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
Chlamydomonas reinhardtii 1)	72 h E_rC_{50} 72 h E_yC_{50}	**0.2245** 0.0854	Backfisch, 2013/1078084	no (new study)
Desmodesmus subspicatus 1)	72 h E_rC_{50} 72 h E_yC_{50}	> 0.0509 (extrapolated: **0.0857**) $ 0.0183	Hoffmann, 2012/1246638	no (new study)
Dictyococcus varians 1)	72 h E_rC_{50} 72 h E_yC_{50}	> 0.1000 (extrapolated: **0.1498**) $ 0.0141	Backfisch, 2013/1078080	no (new study)
Monoraphidium griffithii 1)	72 h E_rC_{50} 72 h E_yC_{50}	**0.0250** 0.0066	Backfisch, 2013/1078078	no (new study)
Navicula pelliculosa 5)	72 h E_rC_{50} 72 h E_bC_{50}	**0.287** 0.154	Hoberg, 1997/10745 Amendment: Kubitza, 2005/1003999	yes, but recalculation of endpoints
Neochloris aquatica 1)	72 h E_rC_{50} 72 h E_yC_{50}	**> 1.000** 0.3680	Backfisch, 2012/1246637	no (new study)
Pandorina morum 1)	72 h E_rC_{50} 72 h E_yC_{50}	**0.9238** 0.0978	Backfisch, 2013/1078083	no (new study)
Planktosphaeria botryoides 1)	72 h E_rC_{50} 72 h E_yC_{50}	**0.9120** 0.1110	Backfisch, 2013/1078081	no (new study)
Schroederia setigera 1)	72 h E_rC_{50} 72 h E_yC_{50}	**> 0.4055** 0.1267	Backfisch, 2013/1078077	no (new study)
Skeletonema costatum 1), 5)	72 h E_rC_{50} 72 h E_bC_{50}	**0.309** 0.109	Hoberg, 1997/10743 Amendment: Kubitza, 2005/1004000	no (conducted for registrations outside of Europe)
Staurastrum punctulatum 1)	72 h E_rC_{50} 72 h E_yC_{50}	**> 1.000** 0.1223	Backfisch, 2013/1078076	no (new study)
Aquatic macrophytes standard species 4)				
Lemna gibba	7 d E_rC_{50} 7 d E_yC_{50}	0.0429 7), 8) 0.0234 7), 8)	Hoberg, 1997/10742 Amendment Kubitza, 2004/1025686 5)	yes, but recalculation of endpoints
	7 d E_rC_{50} 7 d E_yC_{50}	0.0657 8) / 0.0505 9) 0.0197 8) / 0.0223 9)	Backfisch & Kubitza, 2012/1215555 1)	no (new study)
	arithmetic mean 7 d E_rC_{50}	**0.0543 6, 8)**	--	no (calculation based on new and old data)
Aquatic macrophytes additional species 4)				
Glyceria maxima 1)	14 d E_rC_{50} 14 d E_yC_{50}	> 1.0 9) / **0.184** 10) / 0.402 11) 0.934 9) / 0.109 10) / 0.221 11) / 0.318 12)	Janson, 2013/1286172	no (new study)
Lemna gibba 1) (with sediment)	7 d E_rC_{50} 7 d E_yC_{50}	**0.0990** 8) / > 0.160 9) 0.0332 8) / 0.0497 9)	Backfisch & Kubitza, 2012/1215555	no (new study)
Acorus calamus 15)	13 d E_rC_{50}	> 1.324 10), 11), 13)	Kubitza & Dohmen, 2002/1012788 Amendments: Kubitza, 2013/1361973 &	no (new study)
Iris pseudacorus 15)	13 d E_rC_{50}	> 1.324 10), 13) / **0.229** 11)		
Ludwigia palustris 15)	13 d E_rC_{50}	**0.047** 10) / 0.062 11)		

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
Mentha aquatica [15]	13 d E_rC_{50}	**0.278** [10] / > 1.472 [11]	2014/1082325	
Sparganium erectum [15]	13 d E_rC_{50}	> 0.572 [10), 13] / **0.455** [11]		
Veronica beccabunga [15]	13 d E_rC_{50}	**0.129** [10] / 0.431 [11]		
Ceratophyllum demersum [16]	9 d E_rC_{50}	**0.0157** [10] / 0.0428 [11]	Kubitza & Dohmen, 2002/1012789	no (new study)
Crassula recurva [16]	12 d E_rC_{50}	**0.0995** [10] / > 0.336 [11]		
Elodea densa [16]	12 d E_rC_{50}	**0.2044** [10] / > 0.336 [11]		
Myriophyllum spicatum [16]	9 d E_rC_{50}	**0.0972** [10] / > 0.336 [11]		
Potamogeton crispus [16]	9 d E_rC_{50}	**0.2839** [10] / > 0.336 [11]		
Vallisneria spiralis [16]	12 d E_rC_{50}	> 0.3360 [10), 11]		
Time-to-Effect Studies (TTE)- [4]				
Monoraphidium griffithii [1] (TTE study)	E_rC_{50} / E_yC_{50} (different exposure durations + 72 h growth phase)	> 2.4 (6 h exposure period) > 1.2 (24 h exposure period)	Backfisch, 2013/1299407	no (new study)
Pseudokirchneriella subcapitata [1] (TTE study)	E_rC_{50} / E_yC_{50} (different exposure durations + 72 h growth phase)	> 2.4 (6 h exposure period) **> 1.2** (extrapolated: 2.485) / 0.388 (24 h exposure period)	Backfisch, 2013/1299405	no (new study)
Lemna gibba [1] (TTE study)	Scenario A: E_rC_{50} / E_yC_{50} (different exposure durations + 7 d growth phase)	12 h exposure period: > 0.500 [8), 9] 24 h exposure period: **> 0.500** [8), 9] / 0.288 [9] 36 h exposure period: **0.458** [9] / 0.253 [9]	Hoffmann & Grund, 2012/1084264 Amendment: Hoffmann, 2012/1202274	no (new study)
	Scenario B: E_rC_{50} / E_yC_{50} (double peak exposure + 7 d growth phase)	"0.250 mg/L max. peak": > 0.250 peak [8), 9] "0.500 mg max. peak" > 0.500 peak [8), 9]		
Lemna gibba [1),14] (non-GLP TTE)	E_rC_{50} / E_yC_{50} (2 x 24 h peaks separated by non-exposure periods varying between 1 and 7 d + 6 d growth phase)	2x 24 h exposure peak (non-exposure period between peaks 1 - 7 d): > 0.250 mg/L [8]	Kubitza & Grund, 2013/1291744	no (new study)
Ceratophyllum demersum [1] (TTE study)	E_rC_{50} / E_yC_{50} (different exposure durations + 7 d growth phase)	> 3.0 [9), 10), 11] (24 h exposure period) > 3.0 [9), 10), 11] (48 h exposure period)	Janson, 2013/1286175	no (new study)

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
metabolite: M656H003 (Reg. No. 360717, M3)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	60.8	[redacted] 1997/10271	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 101.6	Gruetzner, 1997/10272	yes
Algae [4]				
Scenedesmus subspicatus	72 h E_rC_{50} 72 h E_bC_{50}	**97.4** 68.5	Gruetzner, 1997/10274	yes
metabolite: M656H023, (Reg. No. 360 715, M23)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 87	[redacted], 1995/11318	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 95	van der Kolk, 1995/11319	yes
Algae [4]				
Selenastrum capricornutum (syn. *Pseudokirchneriella subcapitata*)	72 h E_rC_{50} 72 h E_bC_{50}	**> 100** > 94	van der Kolk, 1995/11320	yes
metabolite: M656H027 (Reg. No. 360 714, M27)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 100	[redacted] 1995/11330	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	van der Kolk, 1995/11331	yes
Algae [4]				
Selenastrum capricornutum (syn. *Pseudokirchneriella subcapitata*)	72 h E_rC_{50} / E_bC_{50}	> 208	van der Kolk, 1995/11332	yes
metabolite: M656H031 (Reg. No. 360 712, M31) [18]				
Aquatic invertebrates				
Daphnia magna [1]	48 h EC_{50}	> 100	Janson, 2008/1042207	no (new study)
Algae [4]				
Pseudokirchneriella subcapitata [1]	72 h E_rC_{50} / E_yC_{50}	> 100	Hoffmann, 2008/1035874	no (new study)
Aquatic macrophytes [4]				

Organism	Endpoint	Value [mg/L]	Reference	EU agreed
Lemna gibba [1)]	7 d E_rC_{50} / E_yC_{50}	> 100 [8), 9)]	Hoffmann, 2008/1035918	no (new study)
metabolite: M656H062 (= **M62;** tested with Reg. No. 403 121) [17), 18)]				
Aquatic macrophytes [4)]				
Lemna gibba [1)]	7 d E_rC_{50} 7 d E_yC_{50}	**> 100** [8), 9)] 54.57 [8)] / 72.87 [9)]	Swierkot 2013/1191249	no (new study)
metabolite: M656PH043 (Reg. No. 5 917 262, M43) [17)]				
Aquatic macrophytes [4)]				
Lemna gibba [1)]	7 d E_rC_{50} / E_yC_{50}	> 100 [8), 9)]	Swierkot 2013/1191248	no (new study)
metabolite: M656H055 (Reg.No. 5 749 263, M55) [17)]				
Aquatic macrophytes [4)]				
Lemna gibba [1)]	7 d E_rC_{50} / E_yC_{50}	> 143 [8), 9)]	Swierkot 2013/1063800	no (new study)
Fish				
Oncorhynchus mykiss	96 h LC_{50}	9.0	2010/1123696	no (conducted for registrations outside of Europe)
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	4.87	Salinas, 2010/1212802	no (conducted for registrations outside of Europe
Algae [4)]				
Pseudokirchneriella subcapitata	72 h E_rC_{50} 72 h E_yC_{50}	**22.6** 17.6	Salinas, 2010/1185631	no (conducted for registrations outside of Europe
Algae [4)]				
Pseudokirchneriella subcapitata	72 h E_rC_{50} 72 h E_yC_{50}	**97.0** 58.9	Salinas, 2010/1079231	no (conducted for registrations outside of Europe
Algae [4)]				
Pseudokirchneriella subcapitata	72 h E_rC_{50} 72 h E_yC_{50}	**19.2** 6.32	Salinas, 2010/1154437 Amendment: Salinas, 2011/1255812	no (conducted for registrations outside of Europe

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), the relevant endpoint is used in the TER / SSD calculations presented in chapter 10.2 of the MCP dossier part for Annex I renewal.

ELS = early life stage; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

$ Extrapolated values are used for SSD calculations (chapter 10.2 of the MCP dossier part for Annex I renewal).

+ The 48-h LC_{50} obtained in the 96 h study is used in the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) on the data requirements for active substances and the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013).

1) Study has not been submitted during the Annex I inclusion process of dimethenamid-P; a study summary is provided below.

[2] marine / saltwater species

[3] Study conducted with dimethenamid (racemate) as acute studies with dimethenamid-P showed lower toxicity to fish and daphnids (worst-case approach).

[4] In accordance with recent guidelines for aquatic primary producers (*e.g.* EFSA Scientific Opinion (EFSA 2013); Aquatic Guidance Document (SANCO, 2002); OECD guideline 201 (OECD, 2011) and 221 (OECD, 2006)), only the EC_{50} values determined for the endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for algae and macrophytes if both “growth rate” and “biomass/yield” endpoints are available (for details also see Appendix II of chapter 10.2 of the MCP dossier part for Annex I renewal).

[5] The endpoints of some of the older (partly EU agreed) algae / aquatic plant studies have been (re-)calculated from original data (in particular, the more relevant E_rC_{50} values were calculated additionally to the E_yC_{50} values). The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the respective amendments. The summaries of these studies and the re-calculations are provided below.

[6] Two independent studies have been performed on the standard green alga *P. subcapitata* (Syn. *S. capricornutum*) and also on the standard aquatic plant species *L. gibba*. Both the old and the new studies are valid, however in the old studies the study duration is much longer as compared to the recent guideline, and only the endpoints based on biomass were generated. In the meantime the growth rate endpoints were calculated in addition and are presented in the corresponding amendments. Nevertheless, endpoints like dry weight for the current valid test duration cannot be recalculated. Thus, the arithmetic mean of the E_rC_{50} values (based on frond number) from the two studies is used for the risk assessment for these species.

[7] For *Lemna gibba,* the geometric mean of the recalculated six and nine days endpoints of the 14 d study is reported.

[8] based on frond number [9] based on dry weight [10] based on total length

[11] based on fresh / wet weight [12] based on number of leaves [13] based on root formation

[14] For *Lemna gibba* two consecutive 24 hour peaks of 0.250 mg dimethenamid-P/L can be considered toxicologically independent from each other if the interval between the single peaks is longer than 2 days. In this case, the second peak did not contribute to the magnitude of the response anymore.

[15] emergent aquatic plants

[16] submersed aquatic plants (slight deviations of the endpoints for the active substance reported in this table from the respective endpoints given in the study summary below results from minor differences in mathematical calculations (*i.e.* endpoints reported here were calculated based on nominal concentrations of the active substance whereas endpoints reported in the summary were calculated based on the analyzed content).

[17] The active substance is more than 100-fold less toxic to fish and daphnids than to green algae or *Lemna gibba*. Furthermore, none of the other metabolites was toxic to fish and daphnids. Since this metabolite showed no toxicity to *Lemna* and/or algae, which represent the most sensitive species for the a.s., as well as for animal welfare reasons, no additional studies on fish and/or daphnids were performed with this metabolite.

[18] The metabolite M656H062 (M62) cannot be synthesized; thus, the test was performed with Reg. No. 403 121 which is the hydrochloride of metabolite M656H039 (= PL 15-88) which itself is the putative metabolic precursor of M656H062.

CA 8.2.1 Acute toxicity to fish

The following acute toxicity study on marine sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance dimethenamid-P is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness.

Report: CA 8.2.1/1
1996a
SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With the Sheepshead Minnow (Cyprinodon variegatus)
1996/5416
Guidelines: 72-3
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, juvenile sheepshead minnow were exposed to nominal concentrations of 3.2, 5.4, 9.0, 15.0 and 25.0 mg dimethenamid-P/L (corresponding to mean measured concentrations of 3.4, 5.3, 9.2, 16.0 and 27.0 mg/L) and to a negative control and a solvent control in groups of 10 animals in aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortality was observed in the control and at mean measured concentrations of up to and including 9.2 mg dimethenamid-P/L, whereas 100% mortality was observed at the two highest test concentrations of 16.0 and 27.0 mg/L. Signs of toxicity such as discoloration (darker than control fish) was found in the test item concentration of 9.2 mg a.s./L.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimethenamid-P was 12 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.3 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H; Reg. No. 363 851), lot no. 6663-50-1; purity: 91.1%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*); juveniles, mean body length 2.1 cm (1.8 - 2.6 cm); mean body weight 0.31 g (0.20 - 0.50 g); collected from in-house culture.

Test design: Flow-through system (96 hours); 5 test item concentrations, a solvent control and a negative (saltwater) control, 10 fish per aquarium (loading 0.21 g fish/L) and per concentration; 2 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Negative control, solvent control (0.10 mL dimethylformamide/L), 3.2, 5.4, 9.0, 15.0 and 25.0 mg dimethenamid-P/L (nominal); corresponding to mean measured concentrations of 0, 0, 3.4, 5.3, 9.2, 16.0 and 27.0 mg a.s./L.

Test conditions: 25-L Teflon-lined polyethylene aquaria, test volume: 15 L, filtered natural seawater, diluted to a salinity of 20°‰ with well water; temperature: 22.1 °C - 22.9° C; pH 8.3 - 8.4; oxygen content: 5.0 - 6.6 mg/L; photoperiod: 16 h light : 8 h dark; light intensity: approx. 502 lux at test initiation; flow-rate: approx. 6 volume additions/day; no feeding, no aeration.

Analytics: Analytical verification of the test item was conducted using an HPLC with a ECD-detection (total dimethenamid) and HPLC with UV-detection (s-dimethenamid).

Statistics: Descriptive statistics; binomial method for calculation of the LC_{50}; determination of NOEC by visual interpretation of mortality and clinical observation data.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration (measured as total dimethenamid) was conducted in each concentration at the beginning of the test, after approximately 48 hours and, except for the highest concentration at the end of the test. The analyzed contents of dimethenamid-P ranged from 94% to 102% of nominal at test initiation and from 98% to 112% of nominal at test termination. Additionally, one sample from the low and high treatments was also analyzed concurrently for s-dimethenamid. Measured values for s-dimethenamid collected from the low and the high treatment groups at test initiation ranged from 110% to 118% and from 107% to 108% of nominal, respectively. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the controls and at mean measured concentrations of up to and including 9.2 mg dimethenamid-P/L, whereas 100% mortality was observed at the two highest test concentrations of 16.0 and 27.0 mg/L. Signs of toxicity such as discoloration (darker than control fish) was found in the test item concentration of 9.2 mg a.s./L. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of dimethenamid-P on sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Negative control**	**Solvent control**	**3.2**	**5.4**	**9.0**	**15.0**	**25.0**
Concentration [mg a.s./L] (mean measured)	**Negative control**	**Solvent control**	**3.4**	**5.3**	**9.2**	**16.0**	**27.0**
Mortality [%]	0	0	0	0	0	100	100
Symptoms *	none	none	none	none	D	n.d.	n.d.
	Endpoints [mg dimethenamid-P/L] (mean measured)						
LC_{50} (96 h)	12 (95% confidence limits: 9.2 - 16)						
NOEC (96 h)	5.3						

n.d. = not determined; all fish dead
* Symptoms after 96 hours: D = discoloration, darker than control fish

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimethenamid-P was 12 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.3 mg a.s./L (mean measured).

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with [redacted] of dimethenamid-P) was conducted due to U.S. data requirements. It has not been evaluated previously on EU level and it is provided for the sake of completeness.

Report:	CA 8.2.1/2 [redacted] 2010a [redacted] of BAS 656-PH, DMTA-P) - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2010/1123696
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 C.1, OECD 203, EPA 72-1, EPA 850.1075
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a semi-static acute toxicity laboratory study, juvenile rainbow trout were exposed to [redacted] of dimethenamid-P) at nominal concentrations of 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L and a water control in one replicate per concentration with 7 animals in glass flasks containing 10 L water. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 96 hours of exposure, no mortality occurred in the control and at the test item concentrations of up to and including 4.6 mg/L, whereas 86% mortality was observed at the highest tested concentration of 10 mg/L. At the two highest test item concentrations of 4.6 and 10 mg/L, proptosis and apathy were observed, respectively. No additional adverse effects or abnormal behavior were observed in any of the test treatments.

In a semi-static acute toxicity study with rainbow trout, the LC_{50} (96 h) of [redacted] of dimethenamid-P) was determined to be 9.0 mg/L based on nominal concentrations. The NOEC was 2.2 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: [redacted] of dimethenamid-P (BAS 656-PH; Reg. No. 363 851); batch no. B1210B01KE; purity: 91.6 area-%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*), approx. 3 months old; body length 4.7 cm (4.5 - 4.9 cm); body weight 0.77 g (0.63 - 0.91 g); supplied by 'Forellenzucht Trostadt GbR,' Troststadt, Germany.
Test design:	Semi-static system (96 hours); water exchange every 24 hours, one replicate with 7 fish per test group (loading: 0.54 g fish/L) and the control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L (nominal, based on test substance mass without correction for purity or composition).
Test conditions:	Glass flasks, test volume: 10 L, non-chlorinated charcoal filtered tap water (Frankenthal, Germany) mixed with deionized water and aerated; temperature: 13 °C; pH 8.0 - 8.4; oxygen content: 7.1 - 10.4 mg/L; total hardness: 1 mmol/L; conductivity: 250 µS/cm; photoperiod 16 h light : 8 h dark; approx. 75 - 446 lux; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a gas chromatographic procedure with MS-detection.
Statistics:	Descriptive statistics, probit analysis for determination of the LC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in fresh solutions at test initiation and after 72 h in samples of the control and all test item concentrations and in old test solutions for the test item treatments with concentrations ≥ 2.2 mg a.s./L. Measured concentrations could only be determined in the three highest concentrations of ≥ 2.2 mg a.s./L, since concentrations in the lower test groups were below the limit of quantification. In the ≥ 2.2 mg a.s./L treatments the measured concentrations ranged from 32% to 55% of the nominal concentrations in fresh test solutions. At the end of the 24 hour renewal interval, measured concentrations ranged from 45% to 115 % of the initial measured values. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical results, the effect concentration is expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 96 hours of exposure, no mortality occurred in the control and at the test item concentrations of up to and including 4.6 mg/L, whereas 86% mortality was observed at the highest tested concentration of 10 mg/L. At the two highest test item concentrations of 4.6 and

10 mg/L, proptosis and apathy were observed, respectively. No additional adverse effects or abnormal behavior were observed in any of the test treatments. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of [redacted] of dimethenamid-P) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	control	0.46	1.0	2.2	4.6	10.0
Mortality [%]	0	0	0	0	0	86
Symptoms *	none	none	none	none	E	A
	Endpoints [mg/L] (nominal)					
LC_{50} (96 h)	9.0 +					
NOEC (96 h)	2.2					

+ 95% confidence limits could not be calculated
* Symptoms after 96 hours: E = exophthalmos (proptosis); A = apathy

III. CONCLUSION

In a semi-static acute toxicity study with rainbow trout, the LC_{50} (96 h) of [redacted] of dimethenamid-P) was determined to be 9.0 mg/L based on nominal concentrations. The NOEC was 2.2 mg/L (nominal).

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.2.2 Fish full life cycle test

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.2.3 Bioconcentration in fish

No further studies required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance dimethenamid-P as well as based results of available studies there is no indication of endocrine disrupting properties of this active substance. Thus, no studies are required.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

The following acute toxicity study on *Daphnia magna* performed with the metabolite M656H031 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required due to new data requirements.

Report:	CA 8.2.4.1/1 Janson G.-M., 2008a Acute toxicity of Reg.No. 360 712 (metabolite of BAS 656 H) to Daphnia magna STRAUS in a 48 hour static test 2008/1042207
Guidelines:	OECD 202, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to M656H031 (metabolite of dimethenamid-P) at nominal concentrations of 0 (control), 6.25, 12.5, 25, 50 and 100 mg M656H031 /L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No immobility of daphnids was observed in the control or any of the test item treatment groups after 24 hour of exposure. After 48 hours of exposure, 5% immobility was observed at the three highest tested concentrations of 25, 50 and 100 mg M656H031/L.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M656H031 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4%.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from the Institute National de Recherche Chimique Appliquee, France), > 2 < 24 hours old at test initiation.
Test design:	Static system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Control, 6.25, 12.5, 25, 50 and 100 mg M656H031/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water: "M4" (Elendt medium); temperature: 19.3 °C - 21.6 °C; pH 7.87 - 8.04; oxygen content: 8.1 mg/L - 8.9 mg/L; total hardness: 2.27 mmol/L at test initiation; conductivity: 620 µS/cm at test initiation; photoperiod: 16 hours light : 8 hours dark; light intensity: 220 lux - 685 lux; no feeding and no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. Measured values for M656H031 ranged from 96.4% to 104.3% of nominal concentrations at test initiation and from 97.2% to 99.7% of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No immobility of daphnids was observed in the control or any of the test item treatment groups after 24 hour of exposure. After 48 hours of exposure, 5% immobility was observed at the three highest tested concentrations of 25, 50 and 100 mg M656H031/L. The results are summarized in Table 8.2.4.1-1.

Table 8.2.4.1-1: Effect of M656H031 (metabolite of dimethenamid-P) on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Immobility (24 h) [%]	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	5	5	5
Endpoints [mg M656H031/L] (nominal)						
EC_{50} (48 h)	> 100					
NOEC (48 h)	100					

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M656H031 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

The following acute toxicity study on water flea neonates performed with of dimethenamid-P) was conducted due to U.S. data requirements. It has not been evaluated previously on EU level and it is provided for the sake of completeness.

Report: CA 8.2.4.1/2
Salinas E., 2010b
of BAS 656-PH, DMTA-P) - Acute toxicity (immobilisation) study in the water flea Daphnia magna STRAUS
2010/1212802

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 C.2, OECD 202 (2004)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a semi-static acute toxicity laboratory study, water flea neonates were exposed to of dimethenamid-P) at nominal concentrations of 0.22, 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L and a water control in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 48 hours of exposure, no immobility of daphnids was observed in the control and at the three lowest tested concentrations, whereas 5% and 35% of the daphnids were immobile at 2.2 mg/L and 4.6 mg/L, respectively. At the highest test item concentration of 10 mg/L all daphnids were immobile after 24 and 48 hours of exposure. No additional adverse effects or abnormal behavior were observed in any of the test groups.

In a 48-hour semi-static acute toxicity study with *Daphnia magna* the EC_{50} of of dimethenamid-P) was determined to be 4.87 mg/L based on nominal concentrations. The EC_0 was 1.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: ███ of dimethenamid-P (BAS 656-PH, Reg. No. 363 851), batch no. B1210B01KE; purity: 91.0 area-%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), less than 24 hours old at test initiation.

Test design: Semi-static system (48 hours); water exchange after 24 hours, 6 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_0 and EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.22, 0.46, 1.0, 2.2, 4.6 and 10.0 mg/L (nominal).

Test conditions: Test tubes (glass) sealed with gas impermeable Teflon caps, test volume 23 mL, dilution water: “M4” (Elendt medium); temperature: 19.5 °C - 19.8 °C; pH 8.1 - 8.3; oxygen content: 8.5 mg/L - 9.0 mg/L; total hardness: 2.20 - 3.20 mmol/L; conductivity: 550 - 650 µS/cm; photoperiod: 16 hours light : 8 hours dark; light intensity: about 149 lux - 640 lux; no feeding and no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a gas chromatographic procedure with MS detection.

Statistics: Descriptive statistics, probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in fresh solutions at test initiation in samples of the control and all test item concentrations.

In old test solutions measurements were conducted at the end of each exposure interval in all test item treatments ≥ 1.0 mg a.s./L with mobile daphnids and the lowest concentration with 100% immobilization. Measured concentrations could only be determined in the four highest concentrations of ≥ 1.0 mg a.s./L, since concentrations in the lower test groups were below the limit of quantification. In the ≥ 1.0 mg a.s./L treatments the measured concentrations ranged from 69%% to 86% of the nominal concentrations in fresh test solutions. At the end of the 24 hour renewal interval, measured concentrations ranged from 46% to 51% of the initial measured values. Mean measured concentrations were between 55% and 77% of the nominal concentrations. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical

results, the effect concentration can be expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 48 hours of exposure, no immobility of daphnids was observed in the control and at the three lowest tested concentrations, whereas 5% and 35% of the daphnids were immobile at 2.2 mg/L and 4.6 mg/L, respectively. At the highest test item concentration of 10 mg/L all daphnids were immobile after 24 and 48 hours of exposure. No additional adverse effects or abnormal behavior were observed in any of the test groups. For results see Table 8.2.4.1-2.

Table 8.2.4.1-2: Effect [redacted] of dimethenamid-P) on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.22**	**0.46**	**1.0**	**2.2**	**4.6**	**10.0**
Immobility (24 h) [%]	0	0	0	0	0	10	100
Immobility (48 h) [%]	0	0	0	0	5	35	100
Endpoints [mg/L] (nominal)							
EC_{50} (48 h)	4.87 (95% confidence limits: 4.04 - 6.00)						
EC_0 (48 h)	1.0						

III. CONCLUSION

In a 48-hour semi-static acute toxicity study with *Daphnia magna* the EC_{50} of [redacted] of dimethenamid-P) was determined to be 4.87 mg/L based on nominal concentrations. The EC_0 was 1.0 mg/L (nominal).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on the saltwater mysid *Americamysis bahia* (former name: *Mysidopsis bahia*) performed with the active substance dimethenamid-P is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. The 48-h LC_{50} obtained in the 96 h study on the saltwater crustacean *Americamysis bahia* is used for the risk assessment of dimethenamid-P according to EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint. This harmonizes the duration of acute toxicity tests among aquatic arthropods.

Report:	CA 8.2.4.2/1 Graves W.,Swigert J., 1996b SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test With The Saltwater Mysid (Mysidopsis bahia) 1996/5413
Guidelines:	72-3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In an acute toxicity laboratory study under flow-through conditions, saltwater mysids were exposed to nominal concentrations of 0 (control), 1.0, 1.7, 2.9, 4.8 and 8.0 mg dimethenamid/L (corresponding to mean measured concentrations of 0, 1.2, 1.8, 3.0, 5.5 and 9.2 mg/L) in two replicate test chambers each with10 animals giving a total of 20 mysids per aquarium and per treatment. Saltwater mysids were observed for survival and symptoms of toxicity within 5 after start of exposure and 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 no other toxic effects were observed in the solvent control and at the lowest tested concentration of 1.2 mg dimethenamid/L. In the dilution water control, 5% mortality was observed after 96 hours. Mortality rates of 20%, 45%, 85% and 95% after 96 hours were observed in the 1.8, 3.0, 5.5 and 9.2 mg a.s./L test item groups, respectively. At concentrations of 1.8 mg/L and above, erratic swimming of the surviving mysids was observed after 96 hours of exposure.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for dimethenamid was determined to be 3.2 mg/L based on mean measured concentrations. The NOEC (96 h) was 1.2 mg/L (mean measured). The LC_{50} (48 h) for dimethenamid was determined to be > 9.2 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethenamid (BAS 656 H; Reg. No. 360 720), s-racemate; lot no. 6663-50-1, purity: 91.1% by weight for s-dimethenamid and 96.3% by weight for total dimethenamid.

B. STUDY DESIGN

Test species:	Saltwater mysid (*Americamysis bahia;* former name: *Mysidopsis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.
Test design:	Flow-through system (96 hours); 5 test concentrations plus control, 2 replicate test chambers per aquarium and treatment each containing 10 mysids, giving a total of 20 mysids per aquarium and per treatment ; assessment of mortality and symptoms of toxicity approx. 5, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50} (96 h), LC_{50} (48 h), NOEC (96 h), mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.10 mL/L dimethylformamide) and 1.0, 1.7, 2.9, 4.8 and 8.0 mg dimethenamid/L (nominal), corresponding to mean measured concentrations of 1.2, 1.8, 3.0, 5.5 and 9.2 mg dimethenamid/L.
Test conditions:	Polyethylene aquaria (8 L) containing two test chambers, test volume approx. 6.5 L; test chambers: 500 mL glass beakers with nylon mesh screen attached at each side of the beaker; dilution water: filtered and diluted seawater; flow rate: approx. 14 volume additions per 24 hours; salinity: 20‰; temperature: 24.8 °C - 25.0 °C; pH 8.3; oxygen content: 5.1 - 6.5 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 227 lux; feeding: juvenile mysids were fed daily with brine shrimps (*Artemia nauplii*).
Analytics:	Analytical verification of test item concentrations was conducted using gas chromatography with electron capture detection (total dimethenamid) and an HPLC-method with DAD detection (s-dimethenamid).
Statistics:	Descriptive statistics; probit analysis for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, once during the test after 48 hours and at test termination. Measured concentrations for total dimethenamid ranged from 103% to 113% of nominal at test initiation, from 101% to 114% after 48 hours and from 113% to 129% of nominal at test termination. Concentrations of s-dimethenamid were determined in the lowest and highest treatments and were in the range of 99% - 104% of nominal at test initiation and between 114% - 122% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and no other toxic effects were observed in the solvent control and at the lowest tested mean measured concentration of 1.2 mg dimethenamid/L. In the dilution water control, 5% mortality was observed after 96 hours. Mortality rates of 20%, 45%, 85% and 95% after 96 hours were observed in the 1.8, 3.0, 5.5 and 9.2 mg a.s./L test item groups, respectively. At concentrations of 1.8 mg/L and above, erratic swimming of the surviving mysids was observed. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity of dimethenamid to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**1.7**	**2.9**	**4.8**	**8.0**
Concentration [mg a.s./L] (mean measured)	**Control**	**Solvent control**	**1.2**	**1.8**	**3.0**	**5.5**	**9.2**
Mortality [%]	5	0	0	20	45	85	95
Symptoms *	none	none	none	E	E	E	E
	Endpoints [mg dimethenamid/L] (mean measured)						
LC_{50} (48 h)	> 9.2 (95% confidence limits: n.c.)						
LC_{50} (96 h)	3.2 (95% confidence limits: 2.7 - 3.9)						
NOEC (96 h)	1.2						

* Symptoms: E = erratically swimming
n.c. = confidence limits could not be calculated

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for dimethenamid was determined to be 3.2 mg/L based on mean measured concentrations. The NOEC (96 h) was 1.2 mg/L (mean measured). The LC_{50} (48 h) for dimethenamid was determined to be > 9.2 mg/L (mean measured).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.5.4 Sediment dwelling organisms

No study required; thus, this point is 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

The following toxicity studies on several green alga species performed with the active substance dimethenamid-P are provided in support of the aquatic risk assessment and have not been evaluated previously.

Report: CA 8.2.6.1/1
Backfisch K., 2013a
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Chlamydomonas reinhardtii
2013/1078084
Guidelines: OECD 201, EPA 850.5400
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Chlamydomonas reinhardtii* was investigated. The following nominal concentrations were applied: 0 (control), 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L.

In a 72-hour algae test with *Chlamydomonas reinhardtii*, the E_rC_{50} for dimethenamid-P was determined to be 0.2245 mg a.s./L and the E_yC_{50} was 0.0854 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Chlamydomonas reinhardtii;* specification: UTEX 2243; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.09 - 7.71 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 98% to 102% of nominal at test initiation and from 97% to 105% of nominal at test termination. As the analytical measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect of dimethenamid-P on the growth of green alga *Chlamydomonas reinhardtii*

Concentration [mg a.s./L] (nominal)	**Control**	**0.003**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	0.7	1.1	3.2	17.0	66.7	85.9
Inhibition in 72 h (yield) [%]	--	3.3	5.0	13.7	54.4	96.1	99.0
Endpoints [mg a.s./L] (nominal)							
E_rC_{50} (72 h)	0.2245 (95% confidence limits: 0.2049 - 0.2460)						
E_rC_{10} (72 h)	0.0620 (95% confidence limits: 0.0511 - 0.0751)						
E_yC_{50} (72 h)	0.0854 (95% confidence limits: 0.0797 - 0.0916)						
E_yC_{10} (72 h)	0.0273 (95% confidence limits: 0.0236 - 0.0317)						

III. CONCLUSION

In a 72-hour algae test with *Chlamydomonas reinhardtii*, the E_rC_{50} for dimethenamid-P was determined to be 0.2245 mg a.s./L and the E_yC_{50} was 0.0854 mg a.s./L, based on nominal concentrations.

Report:	CA 8.2.6.1/2 Backfisch K., 2013b Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Pandorina morum 2013/1078083
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Pandorina morum* was investigated. The following nominal concentrations were applied: 0 (control), 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L.

In a 72-hour algae test with *Pandorina morum*, the E_rC_{50} for dimethenamid-P was determined to be 0.9238 mg a.s./L and the E_yC_{50} was 0.0978 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pandorina morum*; specification: UTEX 18; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.25 - 7.88 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 98% to 104% of nominal at test initiation and from 98% to 112% of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-2.

Table 8.2.6.1-2: Effect of dimethenamid-P on the growth of green alga *Pandorina morum*

Concentration [mg a.s./L] (nominal)	**Control**	**0.003**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	3.1	3.2	3.8	19.9	40.4	47.0
Inhibition in 72 h (yield) [%]	--	11.9	11.8	14.1	54.3	80.1	85.0
Endpoints [mg a.s./L] (nominal)							
E_rC_{50} (72 h)	0.9238 (95% confidence limits: 0.7345 - 1.162)						
E_rC_{10} (72 h)	0.0329 (95% confidence limits: 0.0230 - 0.0471)						
E_yC_{50} (72 h)	0.0978 (95% confidence limits: 0.0799 - 0.1196)						
E_yC_{10} (72 h)	0.0120 (95% confidence limits: 0.0078 - 0.0183)						

III. CONCLUSION

In a 72-hour algae test with *Pandorina morum*, the E_rC_{50} for dimethenamid-P was determined to be 0.9238 mg a.s./L and the E_yC_{50} was 0.0978 mg a.s./L, based on nominal concentrations.

Report: CA 8.2.6.1/3
Backfisch K., 2013c
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Planktosphaeria botryoides
2013/1078081
Guidelines: EPA 850.5400, OECD 201
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Planktosphaeria botryoides* was investigated. The following nominal concentrations were applied: 0 (control), 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L.

In a 72-hour algae test with *Planktosphaeria botryoides*, the E_rC_{50} for dimethenamid-P was determined to be 0.9120 mg a.s./L and the E_yC_{50} was 0.1110 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Planktosphaeria botryoides*; specification: LB 951; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.43 - 7.59 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 107% to 110% of nominal at test initiation and from 85% to 97% of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-3.

Table 8.2.6.1-3: Effect of dimethenamid-P on the growth of green alga *Planktosphaeria botryoides*

Concentration [mg a.s./L] (nominal)	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	0.6	3.5	14.4	37.2	48.4
Inhibition in 72 h (yield) [%]	--	3.4	14.7	46.7	81.0	88.6
Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	0.9120 (95% confidence limits: 0.7420 - 1.121)					
E_rC_{10} (72 h)	0.0517 (95% confidence limits: 0.0370 - 0.0724)					
E_yC_{50} (72 h)	0.1110 (95% confidence limits: 0.0980 - 0.1250)					
E_yC_{10} (72 h)	0.0203 (95% confidence limits: 0.0157 - 0.0262)					

III. CONCLUSION

In a 72-hour algae test with *Planktosphaeria botryoides*, the E_rC_{50} for dimethenamid-P was determined to be 0.9120 mg a.s./L and the E_yC_{50} was 0.1110 mg a.s./L, based on nominal concentrations.

Report: CA 8.2.6.1/4
Backfisch K., 2013d
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Dictyococcus varians
2013/1078080
Guidelines: OECD 201, EPA 850.5400
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Dictyococcus varians* was investigated. The following nominal concentrations were applied: 0 (control), 0.001, 0.003, 0.01, 0.03 and 0.1 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg a.s./L.

In a 72-hour algae test with *Dictyococcus varians*, the E_rC_{50} for dimethenamid-P was determined to be > 0.10 mg a.s./L (extrapolated: 0.1498 mg a.s./L) and the E_yC_{50} was 0.0141 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Dictyococcus varians*; specification: CCALA 331; stock obtained from "Institute of Botany, v.v.i. Academy of Science of the Czech Republic", Trebon, Czech Republic.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.001, 0.003, 0.01, 0.03 and 0.1 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.75 - 8.04 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 105% to 112% of nominal at test initiation and from 98% to 109% of nominal at test termination. As the analytical measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-4.

Table 8.2.6.1-4: Effect of dimethenamid-P on the growth of green alga *Dictyococcus varians*

Concentration [mg a.s./L] (nominal)	Control	0.001	0.003	0.01	0.03	0.1
Inhibition in 72 h (growth rate) [%]	--	2.6	5.9	17.5	26.3	44.1
Inhibition in 72 h (yield) [%]	--	9.3	19.6	48.0	63.0	81.9
Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	> 0.100 extrapolated: 0.1498 (95% confidence limits: 0.1213 - 0.1851)					
E_rC_{10} (72 h)	0.0049 (95% confidence limits: 3.75 - 6.44)					
E_yC_{50} (72 h)	0.0141 (95% confidence limits: 0.0118 - 0.0168)					
E_yC_{10} (72 h)	0.0010 (95% confidence limits: 0.0007 - 0.0015)					

III. CONCLUSION

In a 72-hour algae test with *Dictyococcus varians*, the E_rC_{50} for dimethenamid-P was determined to be > 0.10 mg a.s./L (extrapolated: 0.1498 mg a.s./L) and the E_yC_{50} was 0.0141 mg a.s./L, based on nominal concentrations.

Report:	CA 8.2.6.1/5 Backfisch K., 2013e Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Monopaphidium griffithii 2013/1078078
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Monoraphidium griffithii* was investigated. The following nominal concentrations were applied: 0 (control), 0.001, 0.003, 0.01, 0.03 and 0.1 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg a.s./L.

In a 72-hour algae test with *Monoraphidium griffithii*, the E_rC_{50} for dimethenamid-P was determined to be 0.0250 mg a.s./L and the E_yC_{50} was 0.0066 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Monoraphidium griffithii*; specification: SAG 202-13; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.001, 0.003, 0.01, 0.03 and 0.1 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.78 - 8.15 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 104% to 110% of nominal at test initiation and from 55% to 67% of nominal at test termination. As the initially measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.1 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-5.

Table 8.2.6.1-5: Effect of dimethenamid-P on the growth of green alga *Monoraphidium griffithii*

Concentration [mg a.s./L] (nominal)	**Control**	**0.001**	**0.003**	**0.01**	**0.03**	**0.1**
Inhibition in 72 h (growth rate) [%] #	--	-0.1	2.4	33.3	63.4	66.3
Inhibition in 72 h (yield) [%] #	--	-0.6	9.6	75.9	94.2	95.1
Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	0.0250 (95% confidence limits: 0.0199 - 0.0313)					
E_rC_{10} (72 h)	0.0026 (95% confidence limits: 0.016 - 0.0042)					
E_yC_{50} (72 h)	0.0066 (95% confidence limits: 0.0061 - 0.0071)					
E_yC_{10} (72 h)	0.0030 (95% confidence limits: 0.0026 - 0.0035)					

Negative values indicate stimulated growth.

III. CONCLUSION

In a 72-hour algae test with *Monoraphidium griffithii*, the E_rC_{50} for dimethenamid-P was determined to be 0.0250 mg a.s./L and the E_yC_{50} was 0.0066 mg a.s./L, based on nominal concentrations.

Report: CA 8.2.6.1/6
Backfisch K., 2013f
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Schroederia setigera
2013/1078077
Guidelines: OECD 201, EPA 850.5400
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Schroederia setigeria* was investigated. The following nominal concentrations were applied: 0 (control), 0.010, 0.032, 0.10, 0.32 and 1.0 mg a.s./L (corresponding to geometric mean measured concentrations of 0, 0.0042, 0.0131, 0.0403, 0.1270 and 0.4055 mg a.s./L). Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.4055 mg a.s./L.

In a 72-hour algae test with *Schroederia setigera*, the E_rC_{50} for dimethenamid-P was determined to be > 0.4055 mg a.s./L and the E_yC_{50} was 0.1267 mg a.s./L, based on geometric mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Schroederia setigera*; specification: LB 2454; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.010, 0.032, 0.10, 0.32 and 1.0 mg a.s./L (nominal); corresponding to geometric mean measured concentrations of 0, 0.0042, 0.0131, 0.0403, 0.1270 and 0.4055 mg a.s./L.

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.96 - 7.84 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 3 x 10^3 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 38% to 43% of nominal at test initiation and from 39% to 42% of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.4055 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-6.

Table 8.2.6.1-6: Effect of dimethenamid-P on the growth of green alga *Schroederia setigera*

Concentration [mg a.s./L] (nominal)	**Control**	**0.010**	**0.032**	**0.10**	**0.32**	**1.0**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**0.0042**	**0.0131**	**0.0403**	**0.1270**	**0.4055**
Inhibition in 72 h (growth rate) [%]	--	1.5	6.0	11.7	22.6	29.5
Inhibition in 72 h (yield) [%]	--	5.6	18.2	32.7	54.2	64.3
Endpoints [mg a.s./L] (geometric mean measured)						
E_rC_{50} (72 h)	> 0.4055					
E_rC_{10} (72 h)	0.0287 (95% confidence limits: 0.0200 - 0.0411)					
E_yC_{50} (72 h)	0.1267 (95% confidence limits: 0.0981 - 0.1635)					
E_yC_{10} (72 h)	0.0051 (95% confidence limits: 0.0029 - 0.0089)					

III. CONCLUSION

In a 72-hour algae test with *Schroederia setigera*, the E_rC_{50} for dimethenamid-P was determined to be > 0.4055 mg a.s./L and the E_yC_{50} was 0.1267 mg a.s./L, based on geometric mean measured concentrations.

Report:	CA 8.2.6.1/7 Backfisch K., 2013g Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Staurastrum punctulatum 2013/1078076
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Staurastrum punctulatum* was investigated. The following nominal concentrations were applied: 0 (control), 0.010, 0.032, 0.10, 0.32 and 1.0 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L.

In a 72-hour algae test with *Staurastrum punctulatum*, the E_rC_{50} for dimethenamid-P was determined to be > 1.0 mg a.s./L and the E_yC_{50} was 0.1223 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Staurastrum punctulatum*; specification: UTEX 173; stock obtained from the "University of Texas", Austin, USA.
Test design:	Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.010, 0.032, 0.10, 0.32 and 1.0 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.82 - 7.99 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 99% to 104% of nominal at test initiation and from 98% to 103% of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-7.

Table 8.2.6.1-7: Effect of dimethenamid-P on the growth of green alga *Staurastrum punctulatum*

Concentration [mg a.s./L] (nominal)	**Control**	**0.010**	**0.032**	**0.10**	**0.32**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	2.1	7.9	23.7	32.8	34.9
Inhibition in 72 h (yield) [%]	--	7.1	24.2	56.8	69.0	71.3
	Endpoints [mg a.s./L] (nominal)					
E_rC_{50} (72 h)	> 1.0					
E_rC_{10} (72 h)	0.0227 (95% confidence limits: 0.0125 - 0.0410)					
E_yC_{50} (72 h)	0.1223 (95% confidence limits: 0.0936 - 0.1597)					
E_yC_{10} (72 h)	0.0055 (95% confidence limits: 0.0029 - 0.0106)					

III. CONCLUSION

In a 72-hour algae test with *Staurastrum punctulatum*, the E_rC_{50} for dimethenamid-P was determined to be > 1.0 mg a.s./L and the E_yC_{50} was 0.1223 mg a.s./L, based on nominal concentrations.

Report: CA 8.2.6.1/8
Backfisch K., 2013h
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Pseudokirchneriella subcapitata
2013/1078075
Guidelines: OECD 201, EPA 850.5400
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.100 mg a.s./L.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} for dimethenamid-P was determined to be 0.0873 mg a.s./L and the E_yC_{50} was 0.0210 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*; (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.83 - 8.02 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 92% to 96% of nominal at test initiation and from 44% to 63% of nominal at test termination. As the initially measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.100 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-8.

Table 8.2.6.1-8: Effect of dimethenamid-P on the growth of green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	Control	0.0031	0.00625	0.0125	0.0250	0.0500	0.100
Inhibition in 72 h (growth rate) [%] #	--	-1.5	-0.5	1.4	20.4	42.8	48.3
Inhibition in 72 h (yield) [%] #	--	-9.4	-3.3	7.6	68.2	92.2	94.5
Endpoints [mg a.s./L] (nominal)							
E_rC_{50} (72 h)	0.0873 (95% confidence limits: 0.0746 - 0.1022)						
E_rC_{10} (72 h)	0.0141 (95% confidence limits: 0.0107 - 0.0185)						
E_yC_{50} (72 h)	0.0210 (95% confidence limits: 0.0197 - 0.0224)						
E_yC_{10} (72 h)	0.0127 (95% confidence limits: 0.0109 - 0.0148)						

Negative values indicate stimulated growth.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} for dimethenamid-P was determined to be 0.0873 mg a.s./L and the E_yC_{50} was 0.0210 mg a.s./L, based on nominal concentrations.

Report: CA 8.2.6.1/9
Backfisch K., 2013i
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Ankistrodesmus bibraianus
2012/1246639
Guidelines: OECD 201, EPA 850.5400
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Ankistrodesmus bibraianus* was investigated. The following nominal concentrations were applied: 0 (control), 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L (corresponding to geometric mean measured concentrations of 0, 0.0015, 0.0031, 0.0060, 0.0122, 0.0241 and 0.0492 mg a.s./L). Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0492 mg a.s./L.

In a 72-hour algae test with *Ankistrodesmus bibraianus*, the E_rC_{50} for dimethenamid-P was determined to be 0.0370 mg a.s./L and the E_yC_{50} was 0.0097 mg a.s./L, based on geometric mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Ankistrodesmus bibraianus*; specification: SAG 278-1; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L (nominal); corresponding to geometric mean measured concentrations of 0, 0.0015, 0.0031, 0.0060, 0.0122, 0.0241 and 0.0492 mg a.s./L.

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.65 - 7.74 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 48% to 51% of nominal at test initiation and from 46% to 50% of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0492 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-9.

Table 8.2.6.1-9: Effect of dimethenamid-P on the growth of green alga *Ankistrodesmus bibraianus*

Concentration [mg a.s./L] (nominal)	**Control**	**0.0031**	**0.00625**	**0.0125**	**0.0250**	**0.0500**	**0.100**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**0.0015**	**0.0031**	**0.0060**	**0.0122**	**0.0241**	**0.0492**
Inhibition in 72 h (growth rate) [%]	--	0.6	4.9	9.5	36.9	43.6	50.7
Inhibition in 72 h (yield) [%]	--	2.0	14.7	26.5	70.9	77.2	82.5
Endpoints [mg a.s./L] (geometric mean measured)							
E_rC_{50} (72 h)	0.0370 (95% confidence limits: 0.0308 - 0.0444)						
E_rC_{10} (72 h)	0.0037 (95% confidence limits: 0.0027 - 0.0050)						
E_yC_{50} (72 h)	0.0097 (95% confidence limits: 0.0086 - 0.0109)						
E_yC_{10} (72 h)	0.0023 (95% confidence limits: 0.0018 - 0.0030)						

III. CONCLUSION

In a 72-hour algae test with *Ankistrodesmus bibraianus*, the E_rC_{50} for dimethenamid-P was determined to be 0.0370 mg a.s./L and the E_yC_{50} was 0.0097 mg a.s./L, based on geometric mean measured concentrations.

Report:	CA 8.2.6.1/10 Backfisch K., 2013a Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Desmodesmus subspicatus 2012/1246638
Guidelines:	OECD 201 (2006), EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Desmodesmus subspicatus* was investigated. The following nominal concentrations were applied: 0 (control), 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L (corresponding to geometric mean measured concentrations of 0.00160, 0.00315, 0.00625, 0.0127, 0.0246 and 0.0509 mg a.s./L). Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0509 mg a.s./L.

In a 72-hour algae test with *Desmodesmus subspicatus*, the E_rC_{50} for dimethenamid-P was determined to be > 0.0509 mg a.s./L (extrapolated: 0.0857 mg a.s./L) and the E_yC_{50} was 0.0183 mg a.s./L, based on geometric mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Desmodesmus subspicatus;* specification: SAG 86.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 6 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.0031, 0.00625, 0.0125, 0.0250, 0.0500 and 0.100 mg a.s./L (nominal); corresponding to geometric mean measured concentrations of 0.00160, 0.00315, 0.00625, 0.0127, 0.0246 and 0.0509 mg a.s./L

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.65 - 7.76 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 50% to 51% of nominal at test initiation and from 48% to 50% of nominal at test termination. Therefore, the following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 0.0509 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-10.

Table 8.2.6.1-10: Effect of dimethenamid-P on the growth of green alga *Desmodesmus subspicatus*

Concentration [mg a.s./L] (nominal)	**Control**	**0.0031**	**0.00625**	**0.0125**	**0.0250**	**0.0500**	**0.100**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**0.00160**	**0.00315**	**0.00625**	**0.0127**	**0.0246**	**0.0509**
Inhibition in 72 h (growth rate) [%] #	--	-1.7	9.4	6.5	9.8	24.6	38.9
Inhibition in 72 h (yield) [%] #	--	-7.8	31.1	21.7	28.3	62.5	79.3
Endpoints [mg a.s./L] (geometric mean measured)							
E_rC_{50} (72 h)	> 0.0509 extrapolated: 0.0857 (95% confidence limits: 0.0617 - 0.1190)						
E_rC_{10} (72 h)	0.00927 (95% confidence limits: 0.00682 - 0.0126)						
E_yC_{50} (72 h)	0.0183 (95% confidence limits: 0.0132 - 0.0255)						
E_yC_{10} (72 h)	0.0024 (95% confidence limits: 0.0012 - 0.0049)						

Negative values indicate stimulated growth.

III. CONCLUSION

In a 72-hour algae test with *Desmodesmus subspicatus*, the E_rC_{50} for dimethenamid-P was determined to be > 0.0509 mg a.s./L (extrapolated: 0.0857 mg a.s./L) and the E_yC_{50} was 0.0183 mg a.s./L, based on geometric mean measured concentrations.

Report: CA 8.2.6.1/11
Backfisch K., 2013j
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Neochloris aquatica
2012/1246637
Guidelines: EPA 850.5400, OECD 201
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Neochloris aquatica* was investigated. The following nominal concentrations were applied: 0 (control), 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L.

In a 72-hour algae test with *Neochloris aquatica*, the E_rC_{50} for dimethenamid-P was determined to be > 1.0 mg a.s./L and the E_yC_{50} was 0.3680 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Neochloris aquatica*; specification: UTEX 138; stock obtained from the "University of Texas", Austin, USA.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.01, 0.03, 0.1, 0.3 and 1.0 mg a.s./L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.89 - 8.03 at test termination; temperature: 22 °C ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values of dimethenamid-P ranged from 105% to 109% of nominal at test initiation and from 105% to 118% of nominal at test termination. As the analytically measured values confirm the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest tested concentration of 1.0 mg a.s./L. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-11.

Table 8.2.6.1-11: Effect of dimethenamid-P on the growth of green alga *Neochloris aquatica*

Concentration [mg a.s./L] (nominal)	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**
Inhibition in 72 h (growth rate) [%]	--	3.4	4.9	10.3	18.5	25.5
Inhibition in 72 h (yield) [%]	--	11.9	17.0	32.1	50.1	61.9
Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	> 1.0 (95% confidence limits: n.d.)					
E_rC_{10} (72 h)	0.0871 (95% confidence limits: 0.0689 - 0.1101)					
E_yC_{50} (72 h)	0.3680 (95% confidence limits: 0.3000 - 0.4510)					
E_yC_{10} (72 h)	0.0091 (95% confidence limits: 0.0059 - 0.0141)					

n.d. = not determined due to mathematical reasons

III. CONCLUSION

In a 72-hour algae test with *Neochloris aquatica*, the E_rC_{50} for dimethenamid-P was determined to be > 1.0 mg a.s./L and the E_yC_{50} was 0.368 mg a.s./L, based on nominal concentrations.

The following time-to-event studies on the green algae *Pseudokirchneriella subcapitata* and *Monoraphidium griffithii* performed with the active substance dimethenamid-P are provided in support of the aquatic risk assessment and have not been evaluated previously.

Report:	CA 8.2.6.1/12 Backfisch K., 2014k Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Pseudokirchneriella subcapitata after different exposure durations 2013/1299405
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated after two different exposure durations of 6 hours and 24 hours. Algae were exposed to 0 (control), 1.2 and 2.4 mg a.s./L (nominal) in the 6 h exposure scenario and to 0 (control), 0.3, 0.6 and 1.2 mg a.s./L (nominal) in the 24 h exposure scenario. At the end of the respective exposure time the cell densities were determined, alga cell were transferred to untreated test medium and incubated for a 72 h growth phase. Assessment of growth was conducted after the 72 hour growth phase.

The biological results are based nominal test concentrations, except for the 2.4 mg/L treatment in the 6-h exposure scenario where the measured concentration of 1.2 mg/L is considered. No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios.

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard studies on *P. subcapitata*. The E_rC_{50} and E_yC_{50} value for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 1.2 mg a.s./L (based on nominal and mean measured concentrations, respectively). Exposure over 24 h followed by a 72 h growth phase results in a E_rC_{50} of > 1.2 mg a.s./L (extrapolated value: 2.485 mg a.s./L) and E_yC_{50} of 0.388 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn.: *Selenastrum capricornutum* Prinz), SAG 61.81; in-house culture; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; exposure phase of 6 h and 24 h with 2 and 3 test concentrations, respectively, each with 5 replicates per treatment plus a control with 10 replicates; at the end of the exposure phase cell densities were determined, alga cell were transferred to untreated test medium and incubated for a 72 h growth phase; assessment of growth after 72 h growth phase.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over two different exposure phases followed by a 72 h growth phase; area under the curve (AUC) values.

Test concentrations: 6 h exposure scenario: control, 1.2 and 2.4 mg a.s./L (nominal).
24 h exposure scenario: control, 0.3, 0.6 and 1.2 mg a.s./L (nominal).

Test conditions: 250 mL glass Erlenmeyer dimple flasks; test volume: 100 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation of both exposure phases; pH 7.70 -7.91 and pH 7.69 - 8.00 after 72 h growth phase for the 6 h and 24 h exposure scenario, respectively; temperature: 22 °C ± 1 °C; initial cell densities: 1x 10^6 cells/mL for the exposure phase and 1x 10^4 cells/mL for the growth phase; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit and Weibull-analysis for 24 hour exposure scenario data; no statistical analysis was conducted for the 6-hour exposure data as only two concentrations were tested.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration of both exposure scenarios at test initiation and at the end of the exposure phase. For the 6 h exposure scenario, the mean measured values for dimethenamid-P at test initiation were 52% and 103% of nominal concentrations in the 2.4 mg/L and 1.2 mg/L treatment, respectively. At the end of the 6 h exposure period, the mean measured values were 46% of nominal in the 2.4 mg/L treatment and 96% of nominal in the 1.2 mg/L treatment. For the 24 h exposure scenario, the mean measured values for dimethenamid-P were between 103% and 107% of nominal in all treatments at test initiation. At the end of the 24 h exposure phase, the mean measured values were 29%, 40% and 58% of nominal in the 0.3, 0.6 and 1.2 mg a.s./L treatment, respectively. The initial recovery confirmed the correct application of the test item,

except for the concentration of 2.4 mg/L in a 6-hour exposure (recovery about 50%). Thus, the concentrations in both treatments of the 6-hour exposure scenario were (nearly) identical, *i.e.* 1.2 mg a.s./L. Therefore, the biological results of the 2.4 mg/L treatment will be reported based on the measured concentration of 1.2 mg/L. For all other treatments the results are based on nominal test item concentrations.

Biological results: No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios. The AUC allows comparing different peaks (variable concentration and exposure time) and the resulting effects. The calculated AUC of 0.3 mg/L*d results in effects in the same range for yield and growth rate for the concentration of 1.2 mg/L and exposure time of 6 hours as well as for the concentration of 0.3 mg/L and exposure time of 24 hours. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-12.

Table 8.2.6.1-12: Effect of dimethenamid-P on the growth of green alga *Pseudokirchneriella subcapitata* in different exposure scenarios followed by a growth phase of 72 h

Exposure scenario	**6 h exposure phase**		**24 h exposure phase**		
Concentration [mg a.s./L] (nominal)	**1.2**	**1.2 + (nominal: 2.4)**	**0.3**	**0.6**	**1.2**
Inhibition after 72 h growth phase (growth rate) [%]	10.7	9.9	11.1	15.6	30.7
Inhibition after 72 h growth phase (yield) [%]	43.4	40.8	45.9	57.8	84.4
AUC [mg/L*d] #	0.3	0.3	0.3	0.6	1.2
Endpoints [mg dimethenamid-P/L] (nominal)					
E_rC_{50}	> 1.2 1)		> 1.2 (extrapolated value: 2.485)		
E_yC_{50}	> 1.2 1)		0.388		

AUC = Area under the curve
+ The biological results in this treatment group are based on the mean measured concentration of 1.2 mg/L.
# Calculation of AUC (Area under the curve) values by multiplication of the test item concentration [mg/L] by the exposure time [d].
1) No statistical analysis was conducted for the 6-hour exposure data as only two concentrations were tested.

III. CONCLUSION

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard studies on *P. subcapitata*. The E_rC_{50} and E_yC_{50} value for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 1.2 mg a.s./L (based on nominal and mean measured concentrations, respectively). Exposure over 24 h followed by a 72 h growth phase results in a E_rC_{50} of > 1.2 mg a.s./L (extrapolated value: 2.485 mg a.s./L) and E_yC_{50} of 0.388 mg/L, based on nominal concentrations.

Report: CA 8.2.6.1/13
Backfisch K.,Kubitza J., 2014l
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the green alga Monoraphidium griffithii after different exposure durations
2013/1299407

Guidelines: OECD 201, EPA 850.5400

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static toxicity laboratory study, the effect of dimethenamid-P on the growth of the green alga *Monoraphidium griffithii* was investigated after two different exposure durations of 6 hours and 24 hours. Algae were exposed to 0 (control), 1.2 and 2.4 mg a.s./L (nominal) in the 6 h exposure scenario and to 0 (control), 0.3, 0.6 and 1.2 mg a.s./L (nominal) in the 24 h exposure scenario. At the end of the respective exposure time the cell densities were determined, alga cells were transferred to untreated test medium and incubated for a 72 h growth phase. Assessment of growth was conducted after the 72 hour growth phase.

The biological results are based nominal test concentrations. No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios.

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard study on *M. griffithii*. The E_rC_{50} and E_yC_{50} values for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 2.4 mg a.s./L, based on nominal concentrations. Exposure over 24 h followed by a 72 h growth phase results in an E_rC_{50} and an E_yC_{50} value of > 1.2 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Monoraphidium griffithii* , SAG 202-13; in-house culture; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; exposure phase of 6 h and 24 h with 2 and 3 test concentrations, respectively, each with 5 replicates per treatment plus a control with 10 replicates; at the end of the exposure phase cell densities

were determined, algae cells were transferred to untreated test medium and incubated for a 72 h growth phase; assessment of growth after 72 h growth phase.

Endpoints: EC_{50}, NOEC with respect to growth rate and yield after exposure over two different exposure phases followed by a 72 h growth phase; area under the curve (AUC) values.

Test concentrations: 6 h exposure scenario: control, 1.2 and 2.4 mg a.s./L (nominal).
24 h exposure scenario: control, 0.3, 0.6 and 1.2 mg a.s./L (nominal).

Test conditions: 250 mL glass Erlenmeyer dimple flasks; test volume: 100 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation of both exposure phases; pH 7.48 -7.53 and pH 7.47 - 7.68 after 72 h growth phase for the 6 h and 24 h exposure scenario, respectively; temperature: 22 °C ± 1 °C; initial cell densities: 1x 10^6 cells/mL for the exposure phase and 1x 10^4 cells/mL for the growth phase; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration of both exposure scenarios at test initiation and at the end of the exposure phase. For the 6 h exposure scenario, the mean measured values for dimethenamid-P were 96% of nominal concentrations in both treatments at test initiation and 92% and 94% of nominal in the 1.2 mg/L and 2.4 mg/L treatment, respectively, at the end of the exposure phase. For the 24 h exposure scenario, the mean measured values for dimethenamid-P were between 89% and 91% of nominal in all treatments at test initiation and between 23% and 48% at the end of the exposure phase. As initially mean measured concentrations confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test item concentrations.

Biological results: No morphological effects on alga cells were observed in the control and at any test item concentration in both exposure scenarios. The AUC allows comparing different peaks (variable concentration and exposure time) and the resulting effects. The calculated AUC of 0.3 mg/L*d results in effects in the same range for yield and growth rate for the concentration of 1.2 mg/L and exposure time of 6 hours as well as for the concentration of 0.3 mg/L and exposure time of 24 hours. The same is true for the treatment groups with AUC values of 0.6 mg/L*d. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-13.

Table 8.2.6.1-13: Effect of dimethenamid-P on the growth of green alga *Monoraphidium griffithii* in different exposure scenarios followed by a growth phase of 72 h

Exposure scenario	**6 h exposure phase**		**24 h exposure phase**		
Concentration [mg a.s./L] (nominal)	**1.2**	**2.4**	**0.3**	**0.6**	**1.2**
Inhibition after 72 h growth phase (growth rate) [%]	1.7	3.7	4.4	4.3	4.5
Inhibition after 72 h growth phase (yield) [%]	7.7	15.9	18.6	18.1	18.9
AUC [mg/L*d] #	0.3	0.6	0.3	0.6	1.2
Endpoints [mg dimethenamid-P/L] (nominal)					
E_rC_{50} / E_yC_{50}	> 2.4		> 1.2		

AUC = Area under the curve
# Calculation of AUC (Area under the curve) values by multiplication of the test item concentration [mg/L] by the exposure time [d].

III. CONCLUSION

The results of this study demonstrate that exposure to dimethenamid-P over time periods typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to more long-term constant exposure simulated in the standard study on *M. griffithii*. The E_rC_{50} and E_yC_{50} values for dimethenamid-P after exposure over 6 h followed by a 72 h growth phase are determined to be both > 2.4 mg a.s./L, based on nominal concentrations. Exposure over 24 h followed by a 72 h growth phase results in an E_rC_{50} and an E_yC_{50} value of > 1.2 mg/L (nominal).

The following toxicity study on the growth of the green alga *Pseudokirchneriella subcapitata* performed with the metabolite M656H031 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required due to new data requirements.

Report: CA 8.2.6.1/14
Hoffmann F., 2008a
Effect of Reg.No. 360 712 (M31, metabolite of Dimethenamid-P) on the growth of the green alga Pseudokirchneriella subcapitata
2008/1035874
Guidelines: OECD 201
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of M656H031 (metabolite of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 10, 18, 32, 56, 100 mg M656H031/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. Neither effects on growth rate and yield, nor morphological effects on algae were observed in the control group and at any of the concentrations tested.

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) were both determined to be > 100 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test concentrations with 5 replicates for each plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 10, 18, 32, 56, 100 mg M656H031/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume: 60 mL; nutrient solution (according to OECD 201); pH 8.1 at test initiation and pH 7.64 - 7.73 at test termination; temperature: 22 °C ± 1 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H031 ranged from 99.7% to 101.9% of nominal at test initiation and from 99.0% to 102.4% of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The effects on algal growth rate and yield are summarized in Table 8.2.6.1-14.

Table 8.2.6.1-14: Effect of M656H031 (metabolite of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**10**	**18**	**32**	**56**	**100**
Inhibition in 72 h (growth rate) [%] *	--	-1.5	-0.3	-0.3	1.5	3.1
Inhibition in 72 h (yield) [%] *	--	-8.2	-1.7	-1.8	7.7	15.1
Endpoints [mg M656H031/L] (nominal)						
E_rC_{50} (72 h)	> 100					
E_rC_{10} (72 h)	> 100					
E_yC_{50} (72 h)	> 100					
E_yC_{10} (72 h)	76.6 (95% confidence limits: 72.2 - 81.2)					

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) were both determined to be > 100 mg/L, based on nominal concentrations.

The following algal toxicity studies on the green alga *Pseudokirchneriella subcapitata* performed with several [redacted] of dimethenamid-P were conducted due to U.S. data requirements. They have not been evaluated previously on EU level and they are provided for the sake of completeness.

Report: CA 8.2.6.1/15
Salinas E., 2011a
[redacted] of BAS 656-PH, DMTA-P) - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV
2010/1079231

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 C.3, OECD 201 (2006)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 10, 22, 46, 100, 220 mg/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations of the test item. After 72 hours of exposure, no morphological effects on algae were determined in the control group and at the test item concentrations of up to and including 46 mg/L, whereas at 100 mg/L low cell density occurred and at 220 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 97.0 mg/L and the E_yC_{50} was 58.9 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	[redacted] of dimethenamid-P (BAS 656-PH, Reg. No. 363 851), batch no. B1112B01C4, purity: 98.3 corr. area-%; test substance is a mixture.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.
Test design:	Static system (72 hours); 5 test item concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 10, 22, 46, 100, 220 mg/L (nominal).
Test conditions:	250 mL Erlenmeyer dimple flasks plugged with gas permeable silicone sponge caps; test volume: 100 mL; nutrient solution according to OECD 201; pH 7.9 - 8.0 at test initiation and pH 7.9 - 8.5 at test termination; temperature: 22.9 °C - 23.3 °C; initial cell densities: 0.5 x 10^4 cells/mL; continuous light at about 5277 lux (± 15%), continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted by determination of total organic carbon (TOC) using an infrared gas analyser.
Statistics:	Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$) for calculation of NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured concentrations for the test item ranged from 91% to 103% of nominal concentrations at test initiation and from 88% to 101% of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 72 hours of exposure, no morphological effects on algae were determined in the control group and at the test item concentrations of up to and including 46 mg/L, whereas at 100 mg/L low cell density occurred and at 220 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$). The effects on algal growth rate and yield are summarized in Table 8.2.6.1-15.

Table 8.2.6.1-15: Effect of [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	Control	10	22	46	100	220
Inhibition in 72 h (growth rate) [%] *	--	-0.416	2.16	9.46*	51.6*	95.9**
Inhibition in 72 h (yield) [%] #	--	-14.1	10.0*	30.5*	91.8*	99.8*
Endpoints [mg/L] (nominal)						
E_rC_{50} (72 h)	97.0					
E_rC_{10} (72 h)	46.5					
NOE_rC (72 h)	22.0					
E_yC_{50} (72 h)	58.9					
E_yC_{10} (72 h)	22.0					
NOE_yC (72 h)	10.0					

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (Dunnett`s test, $p \leq 0.01$)

III. CONCLUSION

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 97.0 mg/L and the E_yC_{50} was 58.9 mg/L, based on nominal concentrations.

Report: CA 8.2.6.1/16
Salinas E., 2011b
[redacted] of BAS 656-PH, DMTA-P) - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV
2010/1154437

Guidelines: OECD 201 (2006), (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 C.3

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 8.2.6.1/17
Salinas E., 2011c
[redacted] of BAS 656-PH, DMTA-P) - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV
2011/1255812

Guidelines: OECD 201 (2006), (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 C.3

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of Reg. [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: (control), 1.0, 3.2, 10, 32 and 100 mg/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. After 72 hours of exposure, no morphological effects on algae were observed in the control group and at the test item concentrations of up to and including 3.2 mg/L, whereas at 10 mg/L low cell density occurred and at 32 and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data.

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 19.2 mg/L based on nominal concentrations and the E_yC_{50} was 6.32 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: [redacted] of dimethenamid-P (BAS 656-PH, Reg. No. 363 851), batch no. B1112B01TCK, purity 90.6 corr. area-%; test item is a mixture.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test item concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 1.0, 3.2, 10, 32, 100 mg/L (nominal).

Test conditions: 250 mL Erlenmeyer dimple flasks closed with glass plugs; test volume: 300 mL; nutrient solution (according to OECD 201); pH 7.0 - 7.1 at test initiation and pH 7.0 - 9.6 at test termination; temperature: 23 °C; initial cell densities: 0.3 x 10^4 cells/mL; continuous light at about 5158 lux (± 15%), continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted by determination of total organic carbon (TOC) using an infrared gas analyser.

Statistics: Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$) for calculation of NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each treatment group ≥ 10 mg/L at the beginning and at the end of the test since concentrations in the lower test groups were below the limit of quantification (*i.e.* LoQ = 2.8 - 5.9 mg/L). Measured concentrations for the test item in the test solutions of test groups ≥ 10 mg/L ranged from 85% to 99% of nominal concentrations at test initiation and from 83% to 98% of nominal at test termination. As the analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products.

Biological results: After 72 hours of exposure, no morphological effects on algae were observed in the control group and at the test item concentrations of up to and including 3.2 mg/L, whereas at 10 mg/L low cell density occurred and at 32 and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at the three highest concentrations based on growth rate data and at the four highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$). The effects on algal growth rate and yield are summarized in Table 8.2.6.1-16.

Table 8.2.6.1-16: Effect of Reg. No. [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**1.0**	**3.2**	**10**	**32**	**100**
Inhibition in 72 h (growth rate) [%]	--	1.63	2.79	21.4*	72.3*	100*
Inhibition in 72 h (yield) [%] *	--	-0.015	15.1*	73.6*	98.8*	100*
	Endpoints [mg/L] (nominal)					
E_rC_{50} (72 h)	19.2					
E_rC_{10} (72 h)	4.97					
NOE_rC (72 h)	3.2					
E_yC_{50} (72 h)	6.32					
E_yC_{10} (72 h)	2.16					
NOE_yC (72 h)	1.0					

[#] Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compared to control (Dunnett's test, $p \leq 0.01$).

III. CONCLUSION

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 19.2 mg/L based on nominal concentrations and the E_yC_{50} was 6.32 mg/L (nominal).

Report: CA 8.2.6.1/18
Salinas E., 2011d
[redacted] of BAS 656-PH, DMTA-P) - Growth inhibition study in unicellular green algae Pseudokirchneriella subcapitata KORSHIKOV
2010/1185631

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 C.3, OECD 201 (2006)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.32, 1.0, 3.2, 10, 32 and 100 mg/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. After 72 hours of exposure, no morphological effects on algae occurred in the control group and at test item concentrations of up to and including 10 mg/L, whereas in two highest test item concentrations of 32 mg/L and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at 3.2 mg/L and the two highest concentrations based on growth rate data and at the three highest concentrations based on yield data.

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 22.6 mg/L based on nominal concentrations and the E_yC_{50} was 17.6 mg/L (geometric mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: [redacted] of dimethenamid-P (BAS 656-PH, Reg. No. 363 851), batch no. B1210B01KE, purity 91.6 area%; the test substance is a mixture of chemical components.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; in-house culture; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 6 test concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L (nominal).

Test conditions: 250 mL Erlenmeyer dimple flasks closed with glass plugs; test volume: 300 mL; nutrient solution (according to OECD 201); pH 7.1 - 7.7 at test initiation and pH 7.5 - 10.1 at test termination; temperature: 23 °C; initial cell densities: 0.3 x 10^4 cells/mL; continuous light at about 5153 lux (± 15%), continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with MS-detection.

Statistics: Descriptive statistics, determination of EC_x values by interpolation, Dunnett's test ($p \leq 0.01$ and $p \leq 0.05$) for calculation of NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each treatment group at the beginning and at the end of the test. Measured concentrations for the test item in the test solutions of test item groups ≥ 1.0 mg/L ranged from 62% to 79% of nominal at test initiation, while the concentration in the lowest test group was below the limit of quantification (LoQ). At test termination, the measured concentrations in the two highest test item groups were both 15% of nominal, whereas in all lower test item groups the test item concentrations were below the LoQ. Since the concentration of test substance in test media could not be verified analytically, due to the high degree of uncertainty associated with the quantitative analytical results, the effect concentration can be expressed relative to the nominal concentration or loading rate. Due to the instability of the test substance, the results should be considered as the effect of the parent test substance and all degradation products. Thus, the following biological results are based on nominal concentration.

Biological results: After 72 hours of exposure, no morphological effects on algae occurred in the control group and at test item concentrations of up to and including 10 mg/L, whereas in two highest test item concentrations of 32 mg/L and 100 mg/L no algal cells could be observed. Statistically significant effects on algal growth compared to the control were observed at 3.2 mg/L and the two highest concentrations based on growth rate data and at the three highest concentrations based on yield data (Dunnett`s test, $p \leq 0.01$ or $p \leq 0.05$;for details see table below). The effects on algal growth rate and yield are summarized in Table 8.2.6.1-17.

Table 8.2.6.1-17: Effect of [redacted] of dimethenamid-P) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	Control	0.32	1.0	3.2	10	32	100
Inhibition in 72 h (growth rate) [%] #	--	-4.43	-9.72	6.10 *	2.81	70.0 **	100 **
Inhibition in 72 h (yield) [%] #	--	-34.8	-47.4	-0.771	2.95 *	99.4 **	100 **
Endpoints [mg/L] (nominal)							
E_rC_{50} (72 h)	22.6						
E_rC_{10} (72 h)	11.3						
E_yC_{50} (72 h)	17.6						
E_yC_{10} (72 h)	10.9						

\# Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (Dunnett`s test, $p \leq 0.05$).
** Statistically significantly different from the control (Dunnett`s test, $p \leq 0.01$).

III. CONCLUSION

In a 72-hour static toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of [redacted] of dimethenamid-P) was determined to be 22.6 mg/L based on nominal concentrations and the E_yC_{50} was 17.6 mg/L (geometric mean measured).

CA 8.2.6.2 Effects on growth of an additional algal species

The following algal studies on the diatom *Navicula pelliculosa,* the blue-green alga *Anabaena flos-aquae* and the freshwater green algae *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*) have already been submitted and accepted during Annex I renewal evaluation for dimethenamid-P. However, since then, new endpoints have been recalculated from original data and the recalculated results are now used in the aquatic risk assessment; for details on these calculations please refer to the respective amendments. Executive summaries of the originally submitted studies (plus endpoint tables) and the recalculations are provided below. For detailed summaries for the originally submitted studies reference is made to the EU dossier submitted during Annex I inclusion process for dimethenamid-P.

Report: CA 8.2.6.2/1
Kubitza J., 2005a
Amendment to study BASF DocID 1997/10745: SAN 1289H technical - Toxicity to the freshwater diatom, Navicula pelliculosa
2005/1003999
Guidelines: EPA 122-2, EPA 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study; BASF DocID 1997/10745)

In a 120-hour static toxicity laboratory study, the effect of dimethenamid-P on the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0 (control), 0.031, 0.063, 0.13, 0.25, 0.50, and 1.0 mg a.s./L (corresponding to mean measured concentrations of 0 (control), 0.028, 0.056, 0.10, 0.21, 0.41, and 0.89 mg a.s./L). Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation. Mean measured test concentrations during the course of the test ranged from 80% to 89% of nominal test concentrations. The biological results are based on mean measured concentrations. After 120 hours of exposure, no morphological effects on algae were observed in the control group and any test item concentration tested. Cell densities were statistically significant reduced compared to the control at test item concentrations of ≥ 0.10 mg a.s./L. The effects on alga cell densities are summarized in Table 8.2.6.2-1

Table 8.2.6.2-1: Effect (120 h) of dimethenamid-P on the growth of the diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**0.031**	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration [mg a.s./L] (mean measured)	**--**	**0.028**	**0.056**	**0.10**	**0.21**	**0.41**	**0.89**
Inhibition in cell density after 120 h [% of control]	--	0	1.3	15 *	36 *	58 *	75 *
	Endpoints [mg dimethenamid-P/L] (mean measured)						
EC_{50} (120 h)	0.34 (95% confidence limits: 0.17 - 0.71)						

* Statistically significantly different compare to the control (Williams` Test).

In the 120-hour static toxicity test with *Navicula pelliculosa*, the EC_{50} of dimethenamid-P based on cell density was determined to be 0.34 mg/L (mean measured).

Executive Summary (recalculations; BASF Doc ID 2005/1003999)

The study BASF DocID 1997/10745 with the freshwater diatom *Navicula pelliculosa* was conducted according to Good Laboratory Practice (GLP) following the U.S. EPA Guidelines 122-2 and 123-2 (also fulfilling the general requirements of OECD Guideline 201). However, in the original study report the endpoints were related to cell densities, only.
Additional endpoints related to growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The following 72-hour endpoints were obtained based on mean measured concentrations.

E_rC_{50} (72 h) = 0.287 mg/L (95% confidence limits: 0.277 - 0.297 mg/L)

E_rC_{10} (72 h) = 0.082 mg/L (95% confidence limits: 0.076 - 0.088 mg/L)

E_bC_{50} (72 h) = 0.154 mg/L (95% confidence limits: 0.148 - 0.160 mg/L)

E_bC_{10} (72 h) = 0.031 mg/L (95% confidence limits: 0.029 - 0.034 mg/L)

Report: CA 8.2.6.2/2
Kubitza J., 2004a
Amendment to study BASF DocID 1997/5173: SAN 1289H technical - Toxicity to the freshwater blue-green alga; Anabaena flos-aquae
2004/1025685

Guidelines: EPA 122-2, EPA 123-2

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

Executive Summary (originally submitted study; BASF DocID 1997/5173)

In a 120-hour static toxicity laboratory study, the effect of dimethenamid-P on the growth of the freshwater blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0 (control), 0.031, 0.065, 0.13, 0.25, 0.50, and 1.0 mg a.s./L (corresponding to mean measured concentrations of 0 (control), 0.028, 0.049, 0.11, 0.26, 0.41, and 0.86 mg a.s./L). Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation. Mean measured test concentrations during the course of the test ranged from 75% to 110% of nominal test concentrations. The biological results are based on mean measured concentrations. After 120 hours of exposure, no morphological effects on algae were observed in the control group and any test item concentration tested. Cell densities were statistically significant reduced compared to the control at test item concentrations of ≥ 0.049 mg a.s./L. The effects on alga cell densities are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect (120 h) of dimethenamid-P on the growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**0.031**	**0.065**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration [mg a.s./L] (mean measured)	**--**	**0.028**	**0.049**	**0.11**	**0.26**	**0.41**	**0.86**
Inhibition in cell density after 120 h [% of control]	--	1.5	11 *	17 *	38 *	58 *	68 *
	Endpoints [mg dimethenamid-P/L] (mean measured)						
EC_{50} (120 h)	0.38 (95% confidence limits: 0.18 - 0.83)						

* Statistically significantly different compare to the control (Williams` Test).

In the 120-hour static toxicity test with *Anabaena flos-aquae*, the EC_{50} of dimethenamid-P based on cell density was determined to be 0.38 mg/L (mean measured).

Executive Summary (recalculations; BASF DocID 2004/1025685)

The study BASF DocID 1997/5173 with the freshwater blue-green alga *Anabaena flos-aquae* was conducted according to Good Laboratory Practice (GLP) following the U.S. EPA Guidelines 122-2 and 123-2 (also fulfilling the general requirements of OECD Guideline 201). However, in the original study report the endpoints were related to cell densities, only.
Additional endpoints related to growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The following 72-hour endpoints were obtained based on mean measured concentrations.

E_rC_{50} (72 h) > 0.860
extrapolated values: E_rC_{50} (72 h) 1.340 mg/L (95% confidence limits: 1.136 - 1.581 mg/L)

E_rC_{10} (72 h) = 0.073 mg/L (95% confidence limits: 0.064 - 0.083 mg/L)

E_bC_{50} (72 h) = 0.194 mg/L (95% confidence limits: 0.182 - 0.206 mg/L)

E_bC_{10} (72 h) = 0.019 mg/L (95% confidence limits: 0.016 - 0.021 mg/L)

Report: CA 8.2.6.2/3
Kubitza J., 2004b
Amendment to study BASF DocID 1997/10746: SAN 1289H technical - Toxicity to the freshwater green alga Selenastrum capricornutum
2004/1025684
Guidelines: EPA 122-2, EPA 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study; BASF DocID 1997/10746 = 1997/5170)

In a 120-hour static toxicity laboratory study, the effect of dimethenamid-P on the growth of the unicellular fresh water green alga *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*) was investigated. The following nominal concentrations were applied: 0 (control), 0.0016, 0.0030, 0.0063, 0.013, 0.025 and 0.050 mg a.s./L (corresponding to mean measured concentrations of 0 (control), 0.0013, 0.0021, 0.0054, 0.0096, 0.021, and 0.044 mg a.s./L). Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation. Additionally, after 120 hours of exposure, recovery was investigated at a nominal concentration of 0.0030 mg/L for cells from the highest tested concentration over a period of up to 9 days. Mean measured test concentrations during the course of the test ranged from 72% to 88% of nominal test concentrations. The biological results are based on mean measured concentrations. After 120 hours of exposure, no morphological effects on algae were observed in the control group and at test item concentrations of up to and including 0.021 mg a.s./L. At the highest tested concentration of 0.044 mg a.s./L, bloated cells were observed. Cell densities were statistically significant reduced compared to the control at test item concentrations of ≥ 0.0054 mg a.s./L. After an 8-day recovery period at a nominal concentration of 0.0030 mg/l, cells from the highest test item concentration of 0.044 mg a.s./L (mean measured) exhibited signs of

recovery. This indicates that dimethenamid-P has an algistatic, rather than algicidal, effect at this concentration level. The effects on alga cell densities are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect (120 h) of dimethenamid-P on the growth of the green alga *Selenastrum capricornutum*

Concentration [mg a.s./L] (nominal)	**Control**	**0.0016**	**0.0030**	**0.0063**	**0.013**	**0.025**	**0.050**
Concentration [mg a.s./L] (mean measured)	**--**	**0.0013**	**0.0021**	**0.0054**	**0.0096**	**0.021**	**0.044**
Inhibition in cell density after 120 h [% of control]	--	25	2.3	18 *	31 *	73 *	99 *
	Endpoints [mg dimethenamid-P/L] (mean measured)						
EC_{50} (120 h)	0.017 (95% confidence limits: 0.0041 - 0.030)						

* Statistically significantly different compare to the control (Williams` Test).

In the 120-hour static toxicity test with *Selenastrum capricornutum*, the EC_{50} of dimethenamid-P based on cell density was determined to be 0.017 mg/L (mean measured).

Executive Summary (recalculations; BASF DocID 2004/1025684)

The study BASF DocID 1997/10746 (= 1997/5170) with the unicellular fresh water green alga *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*) was conducted according to Good Laboratory Practice (GLP) following the U.S. EPA Guidelines 122-2 and 123-2 (also fulfilling the general requirements of OECD Guideline 201). However, in the original study report the endpoints were related to cell densities, only; the results were based on mean measured test concentrations.
Additional endpoints related to growth rate (r) and biomass (b) after 72, 96 and 120 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The following 72-hour endpoints were obtained based on nominal concentrations, as the initial measured values confirmed the correct application of the test item.

E_rC_{50} (72 h) = 0.0303 mg/L (95% confidence limits: 0.0296 - 0.0310 mg/L)

E_rC_{10} (72 h) = 0.0156 mg/L (95% confidence limits: 0.0149 - 0.0163 mg/L)

E_bC_{50} (72 h) = 0.0191 mg/L (95% confidence limits: 0.0186 - 0.0197 mg/L)

E_bC_{10} (72 h) = 0.0076 mg/L (95% confidence limits: 0.0072 - 0.0080 mg/L)

The following algal toxicity study on the marine diatom *Skeletonema costatum* performed with the active substance dimethenamid-P is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness.

Report: CA 8.2.6.2/4
Hoberg J.R., 1997c
SAN 1289H technical - Toxicity to the marine diatom, Skeletonema costatum
1997/10743
Guidelines: EPA 122-2, EPA 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Report: CA 8.2.6.2/5
Kubitza J., 2005b
Amendment to study BASF DocID 1997/10743: SAN 1289H technical - Toxicity to the marine diatom, Skeletonema costatum
2005/1004000
Guidelines: EPA 122-2, EPA 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of dimethenamid-P on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0 (control), 0.013, 0.031, 0.065, 0.13, 0.25, 0.50 mg a.s./L (corresponding to mean measured concentrations of 0, 0.013, 0.030, 0.048, 0.11, 0.22 and 0.45 mg a.s./L). Assessment of growth was conducted daily.

The biological results are based on mean measured concentrations. No morphological effects on algae were observed in the control and at up to and including the highest test item concentration tested. Statistically significant effects compared to the control were observed at the four highest tested concentrations after exposure over 120 hours.

In a 120-hour algae test with *Skeletonema costatum* the E_bC_{50} of dimethenamid-P was determined to be 0.120 mg a.s./L based on mean measured concentrations.
Based on recalculations, the E_rC_{50} and the E_bC_{50} of dimethenamid-P after 72 hours were determined to be 0.309 and 0.109 mg/L, respectively (mean measured concentrations).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H; Reg. No. 363 851), batch no. 6663-50-1, purity: 91.1%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, strain CCMP 1332, Class Bacillariophyceae; obtained from Bigelow Laboratories, West Boothbay Harbor, Maine, maintain in stock culture at Springborn Laboratories, Inc.

Test design: Static system (5 days); 6 test concentrations plus a control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{25} and EC_{50} with respect to cell density, EC_{10} and EC_{50} with respect to growth rate and yield (recalculation), NOEC.

Test concentrations: Control, 0.013, 0.031, 0.065, 0.13, 0.25, 0.50 mg a.s./L (nominal) equivalent to mean measured concentrations of 0, 0.013, 0.030, 0.048, 0.11, 0.22 and 0.45 mg dimethenamid-P/L.

Test conditions: 250 mL Erlenmeyer flasks; test volume: 100 mL; nutrient solution (AES medium); pH 8.1 – 8.2 at test initiation and pH 8.2 – 9.0 at test termination; temperature: 20 °C ± 1 °C; initial cell densities: 1 x 10^4 cells/mL; photoperiod of 16 h light: 8 h darkness, light intensity: 3900 to 4700 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a gas chromatographic procedure.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values; Williams Test for determination of 120 h NOEC value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for dimethenamid-P ranged from 77% to 100% of nominal concentrations at test initiation and from 62% to 100% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control and at up to and including the highest test item concentration tested. Statistically significant effects compared to the control were observed at the four highest tested concentrations after exposure over 120 hours (William`s Test, $\alpha = 0.05$). The effects on algal growth rate and development of biomass are summarized in Table 8.2.6.2-4.

Table 8.2.6.2-4: Effect of dimethenamid-P on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Control**	**0.013**	**0.031**	**0.065**	**0.13**	**0.25**	**0.50**
Concentration [mg a.s./L] (mean measured)	**Control**	**0.013**	**0.030**	**0.048**	**0.11**	**0.22**	**0.45**
Inhibition in 120 h (cell density) [%]	--	2.2	4.6	24 *	36 *	76 *	89 *
Inhibition in 72 h (growth rate) [%] *	--	-0.9	2.6	7.9	19.1	51.6	53.1
Inhibition 72 h (biomass) [%] *	--	-3.3	8.9	24.4	48.9	84.1	86.2
Endpoints [mg a.s./L] (mean measured)							
EC_{50} (72 h)	0.120 (95% confidence limits: 0.054 - 0.260)						
NOEC (72 h)	0.030						
E_rC_{50} (72 h) #	0.309 (95% confidence limits: 0.290 - 0.330)						
E_rC_{10} (72 h) #	0.060 (95% confidence limits: 0.055 - 0.064)						
E_bC_{50} (72 h) #	0.109 (95% confidence limits: 0.105 - 0.114)						
E_bC_{10} (72 h) #	0.030 (95% confidence limits: 0.028 - 0.032)						

recalculated data (see study amendment)
* Statistically significant differences compared to the control (William`s Test, $\alpha = 0.05$).

III. CONCLUSION

In a 120-hour algae test with *Skeletonema costatum* the E_bC_{50} of dimethenamid-P was determined to be 0.120 mg a.s./L based on mean measured concentrations. Based on recalculations, the E_rC_{50} and the E_bC_{50} of dimethenamid-P after 72 hours were determined to be 0.309 and 0.109 mg/L, respectively (mean measured concentrations).

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity study on the aquatic plant *Lemna gibba* performed with the metabolite M656H031 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required due to new data requirements.

Report:	CA 8.2.7/1 Hoffmann F., 2008b Effect of Reg.No. 360712 (M31, metabolite of Dimethenamid-P) on the growth of Lemna gibba 2008/1035918
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of M656H031 (metabolite of dimethenamid-P) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 10, 18, 32, 56 and 100 mg M656H031/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and mean biomass data for the parameters frond number and dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth. Neither effects on growth rate and yield, nor morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) based on frond no. and dry weight were determined to be both > 100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	M656H031 (M31, Reg. No. 360 712); metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. RS-582TAS-050495, purity: 99.4%.

B. STUDY DESIGN

Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 10, 18, 31, 56 and 100 mg M656H031/L (nominal).

Test conditions: 400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.50 - 7.52 at test initiation and pH 8.62 - 8.87 at test termination; water temperature: 24.2 °C - 24.5 °C, continuous light, average light intensity: about 8200 lux.

Analytics: Analytical verification of the test item was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H031 ranged from 98.8% to 110.1% of nominal at test initiation and from 86.5% to 105.4% of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 222 fronds per vessel, corresponding to a 20.2 x multiplication. The dry weight increased from 2.1 mg to an average of 28.9 mg per vessel in the control at test termination. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Effects on growth rate and yield are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effect of M656H031 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**10**	**18**	**32**	**56**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	0.3	0.3	0.3	-0.1	-1.2
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.8	-1.7	0.1	-0.2	-3.4
Inhibition after 7 d [%] * (yield based on frond no.)	1.0	0.9	0.9	-0.4	-3.7
Inhibition after 7 d [%] * (yield based on dry weight)	2.4	-4.7	0.2	-0.9	-10.2
	Endpoints [mg M656H031/L] (nominal)				
E_rC_{50} (7 d) based on frond no and dry weight	> 100				
E_rC_{10} (7 d) based on frond no and dry weight	> 100				
E_yC_{50} (7 d) based on frond no and dry weight	> 100				
E_yC_{10} (7 d) based on frond no and dry weight	> 100				

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H031 (metabolite of dimethenamid-P) based on frond no. and dry weight were determined to be both > 100 mg/L (nominal).

The following time-to-event study on the aquatic plant *Lemna gibba* performed with the active substance dimethenamid-P is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report: CA 8.2.7/2
Hoffmann F.,Grund S., 2012a
Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba after different exposure scenarios
2012/1084264
Guidelines: OECD 221, EPA 850.4400, ASTM E 1415-91
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 8.2.7/3
Hoffmann F., 2012a
Report Amendment No. 1: Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba after different exposure scenarios
2012/1202274
Guidelines: OECD 221, EPA 850.4400, ASTM E 1415-91
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static toxicity laboratory study, the effect of dimethenamid-P on the growth of the duckweed *Lemna gibba* was investigated in two different exposure scenarios. In both scenarios, plants were exposed to different concentrations over different exposure durations followed by a growth phase over 7 days. In one scenario (A), plants were exposed to nominal concentrations of 0.100, 0.300 and 0.500 mg a.s./L over 12, 24 and 36 hours. In the other exposure scenario (B), plants were exposed in two different test designs, each simulating two exposure peaks with decreasing consecutive concentrations over distinct time intervals, which are separated by a "no-exposure period" over 13 h. Maximum peak concentrations in the two test designs are 0.250 mg/L (1st peak) and 0.090 mg/L (2nd peak), respectively 0.500 mg/L (1st peak) and 0.180 mg/L (2nd peak). After the respective exposure times, the plants were rinsed and transferred to fresh medium and cultivated for further seven days in both exposure scenarios. Assessment of plant growth and other effects was conducted at the end of the exposure phase, once during and at the end of the seven day growth phase. The percentage growth inhibition after the growth phase relative to the control was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight (biomass).

The biological results are based on nominal concentrations. For all exposure scenarios, good and continuous growth was achieved.
In scenario A, a 12 hour exposure period caused no negative impact on plant morphology after the growth phase at up to and including a test concentration of 0.300 mg dimethenamid-P/L. However, at the highest tested concentration of 0.500 mg a.s./L, single fronds appeared smaller than those in the controls at the end of the growth phase. Statistically significant effects on the growth of *Lemna gibba* compared to the control were observed at the two highest tested concentrations after exposure over 24 h and 36 h based on all tested parameters as well as in the 0.100 mg a.s./L treatment group after 36 h of exposure for the test parameters growth rate and yield based on dry weight. Furthermore, morphological effects like smaller fronds and single necrotic fronds were recorded after exposure over 24 hours at the two highest treatment levels. After exposure over 36 hours, morphological changes like smaller and concaved fronds were observed for all treatment levels.
In scenario B. there was no statistically significant difference between the growth of *Lemna gibba* in the control and the test item treatments in the "0.250 mg/L max. peak exposure" design based on all tested parameters and no morphological effects were determined at the end of the growth phase. For the "0.500 mg/L max. peak exposure" design, statistically significant differences between the control and the test item treatments could be determined for all test parameters. However, the effects were negligible with inhibition for the different parameters between 2% and 16%. Single fronds appeared smaller at day 4 and at termination of the growth phase.

The results of the study demonstrate that short-term exposure times typical for moving water bodies after run off events (hours to days) cause less effects than long-term constant exposure to dimethenamid-P simulated in the standard *Lemna* studies. A 12 h exposure period followed by a growth phase over 7 days caused no significant impact on the plants at up to and including concentrations of 0.500 mg a.s./L. Exposure periods of 24 h caused significant effects at the two highest concentrations and the EC_{50} value was > 0.500 mg/L for the parameter "growth rate" and 0.495 mg/L and 0.288 mg/L for the parameter "yield" based on frond number and dry weight, respectively. Plants exposed for 36 h showed statistically significant differences to the control at all treatments levels. The determined EC_{50} values for growth rate were > 0.500 mg/L and 0.458 mg/L based on frond number and dry weight, respectively. The corresponding EC_{50} value for yield was 0.317 mg/L based on frond number and 0.253 mg/L based on dry weight.
In the exposure scenarios simulating double peak exposure with maximum concentrations of 0.500 and 0.250 mg/L for the first peaks, there was no significant effect on the growth of *Lemna gibba* in the "0.250 mg/L max. peak exposure" design for all test parameters. The EC_{50} values were > 0.500 mg/L and > 0.250 mg/L for all test parameters in the respective exposure designs.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9% ± 1%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system, two different exposure scenarios each followed by a 7 day cultivation period (growth phase):

Scenario A: Three different concentration were tested over three different exposure durations, *i.e.* 12, 24, and 36 hours

Scenario B: Plants were exposed in two different exposure peak designs, each simulating two exposure peaks of the test item with decreasing consecutive concentrations over defined time intervals which are separated by a "no-exposure" period of 13 h; at the end of the respective exposure period after 34 h (see following table):

Consecutive exposure time [h]	"0.250 mg/L max. peak exposure" [mg/L]	"0.500 mg/L max. peak exposure" [mg/L]
0	0.250	0.500
5	0.175	0.350
9	0.050	0.100
12	0	0
25	0.090	0.180
31	0.050	0.100
34	start of 7 d growth phase	

In both scenarios, two plants with four fronds and one plant with three fronds were added impartially to each vessel under axenic conditions giving a total number of 11 fronds at initiation of the exposure phase. The tests were run with three replicates for the test item treatments and with six replicates for the control. After the respective exposure times the plants were transferred to fresh medium and afterwards cultivated for further seven days (growth phase). Assessment of growth and other effects was conducted at the end of the exposure periods, once during and at the end of the seven day growth phase. The yield based on the dry weight was determined at test beginning from a sample of the inoculum culture and at test termination with the plant material from each test concentration and control.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield after exposure over different exposure durations and at different exposure designs, followed by a 7 day cultivation period.

Test concentrations: Scenario A: Control, 0.100, 0.300, 0.500 mg a.s./L (nominal);
Scenario B: "0.250 mg/L maximum peak exposure" design with consecutive test concentrations of 0.250 - 0.175 - 0.050 - 0 - 0.090 - 0.050 mg/L; "0.500 mg/L maximum peak exposure" design with consecutive test concentrations of 0.500 - 0.350 - 0.100 - 0 - 0.180 - 0.100 mg/L (nominal).

Test conditions: 400 mL glass beakers, test volume: 160 mL, 20x-AAP nutrient medium, continuous light in both scenarios;
Scenario A: Temperature: 23.9 – 24.2 °C, pH 7.51 - 7.53 at test initiation and pH 7.63- 8.42 at test end, light intensity: approx. 8100 lux
Scenario B: Temperature: 24.0 – 24.2 °C, pH 7.50 - 7.55 at test initiation and pH 7.63- 7.71 at test end, light intensity: approx. 8400 lux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection. Samples from replicates of each test concentration were pooled.

Statistics: Descriptive statistics; Dunnett`s Multiple t-test for scenario A and Student`s t-test for scenario B for determination of the NOEC values; EC_{50} values were determined by Probit analysis using linear max. likelihood regression for scenario A data and estimated based on the raw data in scenario B.

II. RESULTS AND DISCUSSION

Analytical verification of the test item concentrations was carried out in the bulk solutions of each test concentration at test initiation and in mixed samples (pooled replicates) of each treatment at the end of the respective exposure period in scenario A. The mean measured values determined in the bulk solutions at test initiation were between 111.3% and 115.4% of nominal. At the end of the different exposure times (12 h, 24 h, 36 h) the means of the analytically determined values were between 82.7% and 105.0%. In scenario B, analytical verification of the test item concentrations in bulk solutions was performed at the start of exposure to each test concentration for both test designs. Measured values were between 92.7% and 113.3% for the "0.250 mg/L maximum peak exposure" design and between 93.5% and 117.4% of nominal for the "0.500 mg/L maximum peak exposure" design. As the analytically measured values confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test concentrations.

Biological results:
Scenario A: In the 12, 24 and 36 hour exposure treatment group, the duckweed population in the control vessels increased from 11 fronds per vessel at test initiation to an average of 128, 164 and 155 fronds per vessel in the control after 7.5 days, 8 days and 8.5 days, respectively. Thus, good and continuous exponential growth was achieved in all exposure scenarios. A 12 hour exposure period caused no negative impact on plant morphology after the growth phase at up to and including a test concentration of 0.300 mg dimethenamid-P/L. However, at the highest tested concentration of 0.500 mg a.s./L, single fronds appeared smaller than those in the controls at the end of the growth phase. Statistically significant effects on the growth of *Lemna gibba* compared to the control were observed at the two highest tested concentrations after exposure over 24 h and 36 h based on all tested parameters as well as in the 0.100 mg a.s./L treatment group after 36 h of exposure for the test parameters growth rate and yield based on dry weight (Dunnett`s Multiple t-test, $\alpha = 0.05$). Furthermore, morphological effects like smaller fronds and single necrotic fronds were recorded after exposure over 24 hours at the two highest treatment levels. After exposure over 36 hours, morphological changes like smaller and concaved fronds were observed for all treatment levels. The effects on plant growth in the exposure scenario A are summarized in Table 8.2.7-2.

Table 8.2.7-2: Exposure Scenario A - Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in different exposure scenarios followed by a 7 day cultivation period

Exposure scenario	**12 h exposure period**			**24 h exposure period**			**36 h exposure period**		
Concentration [mg a.s./L] (nominal)	**0.100**	**0.300**	**0.500**	**0.100**	**0.300**	**0.500**	**0.100**	**0.300**	**0.500**
Inhibition after 7 d cultivation [%] + (growth rate based on frond no.)	0.0	1.4	0.5	-0.6	8.2*	23.7*	1.7	22.1*	43.2*
Inhibition after 7 d cultivation [%] + (yield based on frond no.)	-4.3	3.1	-2.3	-1.5	21.4*	50.7*	4.8	47.5*	73.3*
Inhibition after 7 d cultivation [%] + (growth rate based on dry weight)	1.2	3.8	5.9	1.9	26.4*	44.7*	7.5*	23.5*	56.3*
Inhibition after 7 d cultivation [%] + (yield based on dry weight)	3.2	10.0	14.5	5.3	54.8*	74.8*	19.5*	49.8*	83.6*
	Endpoints [mg a.s./L] (nominal)								
E_rC_{50} based on frond no	> 0.500 #			> 0.500			> 0.500		
$NOEC_r$ based on frond no	≥ 0.500			0.100			0.100		
E_yC_{50} based on frond no	> 0.500 #			0.495			0.317		
$NOEC_y$ based on frond no	≥ 0.500			0.100			0.100		
E_rC_{50} based on dry weight	> 0.500 #			> 0.500			0.458		
$NOEC_r$ based on dry weight	≥ 0.500			0.100			< 0.100		
E_yC_{50} based on dry weight	> 0.500 #			0.288			0.253		
$NOEC_y$ based on dry weight	≥ 0.500			0.100			< 0.100		

+ Negative values indicate stimulated growth.
# EC_{50} values for the 12 h exposure period could not be determined by statistical analysis due to the lack of meaningful concentration-response-relationship; therefore, EC_{50} values for this exposure period were expressed as "> 0.500 mg/L".
* Statistically significant differences compared to the control (Dunnett`s Multiple t-test, $\alpha = 0.05$, one-sided smaller).

Scenario B: Both peak exposures designs were conducted with the same controls, due to the same test conditions and incubator. The duckweed population in the control vessels increased from 11 fronds per replicate at test initiation to an average of 255 fronds per vessel at the end of the growth phase, corresponding to a multiplication of 23.2. Thus, continuous exponential growth was achieved in all exposure scenarios during the growth phase. There was no statistically significant difference between the growth of *Lemna gibba* in the control and the test item treatments in the "0.250 mg/L max. peak exposure" design, referring to the test parameters growth rate and yield (both based on frond number and dry weight; Student`s t-test, $\alpha = 0.05$) and no morphological effects were determined at the end of the growth phase. For the "0.500 mg/L max. peak exposure" design, statistically significant differences between the control and the test item treatments could be determined for all test parameters. However, the effects were negligible with inhibition for the different parameters between 2% and 16%. Single fronds appeared smaller at day 4 and at termination of the growth phase. The effects on plant growth in the exposure scenario B are summarized in Table 8.2.7-3.

Table 8.2.7-3: Exposure Scenario B - Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in different exposure scenarios followed by a 7 day cultivation period

Exposure design	**"0.250 mg/L max. peak exposure"**	**"0.500 mg/L max. peak exposure"**
Inhibition after 7 d cultivation [%] [+] (growth rate based on frond no.)	1.0	3.0*
Inhibition after 7 d cultivation [%] [+] (yield based on frond no.)	3.2	9.4*
Inhibition after 7 d cultivation [%] [+] (growth rate based on dry weight)	-0.8	5.0*
Inhibition after 7 d cultivation [%] [+] (yield based on dry weight)	-2.7	15.9*
	Endpoints [mg a.s./L] (nominal) [#]	
E_rC_{50} based on frond no and dry weight	> 0.250	> 0.500
$NOEC_r$ based on frond no and dry weight	≥ 0.250	< 0.500
E_yC_{50} based on frond no and dry weight	> 0.250	> 0.500
$NOEC_y$ based on frond no and dry weight	≥ 0.250	< 0.500

[+] Negative values indicate stimulated growth.
[#] Endpoints were visually estimated based on raw data (E_xC_{50}) and based on statistical calculation ($NOEC_x$) respectively for
* Statistically significant differences compared to the control (Student`s t-test, $\alpha = 0.05$, one-sided smaller).

III. CONCLUSION

The results of the study demonstrate that short-term exposure times typical for moving water bodies after run off events (hours to days) cause less effects than long-term constant exposure to dimethenamid-P simulated in the standard *Lemna* studies. A 12 h exposure period followed by a growth phase over 7 days caused no significant impact on the plants at up to and including concentrations of 0.500 mg a.s./L. Exposure periods of 24 h caused significant effects at the two highest concentrations and the EC_{50} value was > 0.500 mg/L for the parameter "growth rate" and 0.495 mg/L and 0.288 mg/L for the parameter "yield" based on frond number and dry weight, respectively. Plants exposed for 36 h showed statistically significant differences to the control at all treatments levels. The determined EC_{50} values for growth rate were > 0.500 mg/L and 0.458 mg/L based on frond number and dry weight, respectively. The corresponding EC_{50} value for yield was 0.317 mg/L based on frond number and 0.253 mg/L based on dry weight.

In the exposure scenarios simulating double peak exposure with maximum concentrations of 0.500 and 0.250 mg/L for the first peaks, there was no significant effect on the growth of *Lemna gibba* in the "0.250 mg/L max. peak exposure" design for all test parameters. The EC_{50} values were > 0.500 mg/L and > 0.250 mg/L for all test parameters in the respective exposure designs.

The following toxicity study on the aquatic plant *Lemna gibba* performed with the active substance dimethenamid-P is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report:	CA 8.2.7/4 Backfisch K.,Kubitza J., 2012a Effect of Dimethenamid-P (BAS 656 H, Reg.No. 363851) on the growth of Lemna gibba in presence and absence of sediment 2012/1215555
Guidelines:	OECD 221, EPA 850.4400 (draft 1996), ASTM E 1415-91
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of dimethenamid-P on the growth of the duckweed *Lemna gibba* was investigated in the presence and absence of sediment. In the standard test without sediment, the following nominal concentrations were applied: 0 (control), 0.0050, 0.010, 0.020, 0.040 and 0.080 mg a.s./L. In the test with sediment, dimethenamid-P was applied at nominal concentrations of 0 (control), 0.010, 0.020, 0.040, 0.080 and 0.160 mg a.s./L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth in both tests with and without sediment. Morphological symptoms like smaller fronds and shorter roots were observed at the four highest test item concentrations in the test with and without sediment.

In the 7-day aquatic plant test on *Lemna gibba* without sediment, the E_rC_{50} of dimethenamid-P based on frond no. was determined to be the 0.0657 mg a.s./L and the E_yC_{50} was 0.0197 mg a.s./L (nominal). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the 0.0505 mg a.s./L and the E_yC_{50} was 0.0223 mg a.s./L (nominal).
In the test with sediment, the E_rC_{50} of dimethenamid-P based on frond no. was determined to be the 0.0990 mg a.s./L and the E_yC_{50} was 0.0332 mg a.s./L (nominal). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the $>$ 0.160 mg a.s./L (extrapolated: 0.223 mg a.s./L) and the E_yC_{50} was 0.0497 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 to 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system (7 days); two experiments: one with sediment and one without sediment, each with 6 treatment groups (5 test item concentrations, control) using 3 replicates for the test item treatments and 6 replicates for the control ; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Standard test without sediment: control, 0.0050, 0.010, 0.020, 0.040 and 0.080 mg a.s./L (nominal); test with sediment: 0.010, 0.020, 0.040, 0.080 and 0.160 mg a.s./L (nominal)

Test conditions: 400 mL glass beakers, test volume: 160 mL, 20x-AAP nutrient medium, in the test with sediment the glass beakers contained 100 g sediment (according to OECD 219; pH 7.41) corresponding to a layer of 1.0 - 1.5 cm; pH 7.48 - 7.51 at test initiation and pH 8.72 - 8.82 termination in the standard test without sediment; pH 7.48 - 7.51 at test initiation and pH 7.69 - 7.88 at test termination in the test with sediment; water temperature 24.8 °C - 24.9 °C, continuous light, light intensity: 7950 lux - 8970 lux.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for determination of the EC_x values based on frond no. and dry weight.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. In the standard test without sediment, the analyzed contents of dimethenamid-P ranged from 101.5% to 105.7% of nominal at test initiation and from 67.6% to 73.3% of nominal at test termination. In the test with sediment, the measured concentrations of dimethenamid-P were between 101.5% and 105.7% of nominal in samples taken at test initiation and between 52.2% and 58.5% of nominal at test termination. As the initially measured concentrations confirmed the correct application of the test item in both experimental setups, the following biological results are based on nominal concentrations.

Biological results:
Standard test without sediment: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 127.5 fronds per vessel, corresponding to a 12 x multiplication or a doubling time of 1.98 d. The dry weight increased from 1.01 mg to an average of 13.91 mg per vessel in the control at test termination. Morphological symptoms like smaller fronds and shorter roots were observed at the four highest test item concentrations. Effects on growth rate and yield in the standard test without sediment are summarized in Table 8.2.7-4.

Table 8.2.7-4: Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in the standard test without sediment

Concentration [mg a.s./L] (nominal)	**0.0050**	**0.010**	**0.020**	**0.040**	**0.080**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.7	11.8	35.3	43.8	48.0
Inhibition after 7 d [%] (yield based on frond no.)	1.9	27.6	63.4	72.0	75.7
Inhibition after 7 d [%] # (growth rate based on dry weight)	-6.7	5.1	28.6	37.3	66.6
Inhibition after 7 d [%] # (yield based on dry weight))	-20.4	13.6	56.8	67.3	89.0
	Endpoints [mg a.s./L] (nominal)				
E_rC_{50} (7 d) based on frond no	0.0657 (95% confidence limits: 0.0321 - > 0.0800)				
E_rC_{10} (7 d) based on frond no	0.0060 (95% confidence limits: 0.0 - 0.0158)				
E_yC_{50} (7 d) based on frond no	0.0197 (95% confidence limits: 0.0047 - 0.0804)				
E_yC_{10} (7 d) based on frond no	0.0042 (95% confidence limits: 0.0 - 0.0102)				
E_rC_{50} (7 d) based on dry weight	0.0505 (95% confidence limits: 0.0363 - 0.0832)				
E_rC_{10} (7 d) based on dry weight	0.0111 (95% confidence limits: 0.0028 - 0.0184)				
E_yC_{50} (7 d) based on dry weight	0.0223 (95% confidence limits: 0.0126 - 0.0401)				
E_yC_{10} (7 d) based on dry weight	0.0068 (95% confidence limits: 0.0005 - 0.0122)				

Negative values indicate stimulated growth compared to the control.

Test with sediment: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 242 fronds per vessel, corresponding to a 22 x multiplication or a doubling time of 1.57 d. The dry weight increased from 1.01 mg to an average of 29.38 mg per vessel in the control at test termination. Morphological symptoms like smaller fronds and shorter roots were observed at the four highest test item concentrations. Effects on growth rate and yield in the test with sediment are summarized in Table 8.2.7-5.

Table 8.2.7-5: Effect of dimethenamid-P on the growth of duckweed *Lemna gibba* in the test with sediment

Concentration [mg a.s./L] (nominal)	**0.010**	**0.020**	**0.040**	**0.080**	**0.160**
Inhibition after 7 d [%] # (growth rate based on frond no.)	-0.4	7.6	34.6	47.9	57.4
Inhibition after 7 d [%] # (yield based on frond no.)	-1.3	22.1	68.8	81.0	87.0
Inhibition after 7 d [%] # (growth rate based on dry weight)	-5.9	0.0	27.0	31.7	38.5
Inhibition after 7 d [%] # (yield based on dry weight))	-22.6	0.1	61.9	67.9	75.1
Endpoints [mg a.s./L] (nominal)					
E_rC_{50} (7 d) based on frond no	0.0990 (95% confidence limits: 0.0582 - > 0.160)				
E_rC_{10} (7 d) based on frond no	0.0152 (95% confidence limits: 0.0004 - 0.0320)				
E_yC_{50} (7 d) based on frond no	0.0332 (95% confidence limits: 0.0161 - 0.0681)				
E_yC_{10} (7 d) based on frond no	0.0124 (95% confidence limits: 0.0002 - 0.0216)				
E_rC_{50} (7 d) based on dry weight	> 0.160 (extrapolated: 0.223)				
E_rC_{10} (7 d) based on dry weight	0.0219 (95% confidence limits: n.d.)				
E_yC_{50} (7 d) based on dry weight	0.0497 (95% confidence limits: n.d.)				
E_yC_{10} (7 d) based on dry weight	0.0142 (95% confidence limits: n.d.)				

# Negative values indicate stimulated growth compared to the control.
n.d. = not determined

III. CONCLUSION

In the 7-day aquatic plant test on *Lemna gibba* without sediment, the E_rC_{50} of dimethenamid-P based on frond no. was determined to be the 0.0657 mg a.s./L and the E_yC_{50} was 0.0197 mg a.s./L (nominal). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the 0.0505 mg a.s./L and the E_yC_{50} was 0.0223 mg a.s./L (nominal).
In the test with sediment, the E_rC_{50} of dimethenamid-P based on frond no. was determined to be the 0.0990 mg a.s./L and the E_yC_{50} was 0.0332 mg a.s./L (nominal). The E_rC_{50} of dimethenamid-P based on dry weight was determined to be the > 0.160 mg a.s./L (extrapolated: 0.223 mg a.s./L) and the E_yC_{50} was 0.0497 mg a.s./L (nominal).

The following (non-GLP) time-to-event study on the aquatic plant *Lemna gibba* performed with the active substance dimethenamid-P is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report:	CA 8.2.7/5 Kubitza J.,Grund S., 2013a Effect of BAS 656P H (Dimethenamid-P, Reg.No. 363851) on the growth of Lemna gibba in different peak exposure scenarios 2013/1291744
Guidelines:	OECD 221, EPA 850.4400 (draft 1996), ASTM E 1415-91
GLP:	no

Executive Summary

In a static toxicity (non-GLP) laboratory study, the effect of dimethenamid-P on the growth of the duckweed *Lemna gibba* was assessed in eight different exposure scenarios ("A - H") to investigate the toxicological (in)dependence of two consecutive peaks of dimethenamid-P.
In scenarios "A - G", the plants were exposed to two consecutive 24 hour peaks at 0.250 mg dimethenamid-P/L (nominal) and a control. The peaks were separated by non-exposure periods ranging from 1 day (scenario "A") to 7 days (scenario "G"). After the second peak the plants were rinsed and transferred to fresh medium and afterwards cultivated for further 6 days (growth phase). In scenario "H", a single peak of 0.250 mg dimethenamid-P/L was tested over 24 hours followed by a 7 day growth phase.
Assessment of plant growth (by counting visible fronds) and other effects was conducted at the end of the exposure phase, at the end of the non-exposure period and at the end of the growth phase. In scenario "H", plant growth was additionally assessed 2 days after the single peak treatment. The percent growth inhibition relative to the control was calculated for each scenario based upon growth rates and final yield for the parameter frond number. The inhibition values of the different treatments with two peaks were then compared to the plant growth observed in the single peak treatment (scenario "H").

The biological results are based on nominal concentrations. For all exposure scenarios, good and continuous growth was achieved. In the scenarios with non-exposure periods of ≤ 2 days between two consecutive peaks (scenario "A" and "B"), plant growth was statistically significantly decreased based on both parameters growth rate and yield when compared to the single peak treatment (scenario "H"). Exposure of *Lemna gibba* to two consecutive peaks separated by > 2 days resulted in similar plant growth (scenarios "D and F", as well as scenarios "G and H") or statistically significantly increased plant growth (scenarios "C" and "F") when compared to the single peak treatment.

The results of this study show that the impact of consecutive peaks on the growth of *Lemna gibba* is influenced by the duration of the non-exposure period between the peaks. For *Lemna gibba* two consecutive 24 hour peaks of 0.250 mg dimethenamid-P/L can be considered toxicologically independent from each other if the interval between the single peaks is longer than 2 days. In this case, the second peak did not contribute to the magnitude of the response anymore.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656P H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9% ± 1%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula approximately 10 days old; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.

Test design: Static system, exposure over 1 or 2 x 24 h; 8 different exposure scenarios, 3 replicates for the test item treatment and the control in each scenario.

Scenario "A - G": two consecutive exposure peaks over 24 h; the consecutive peaks are separated by non-exposure periods varying between 1 and 7 days, after the second peak the plants were rinsed and transferred to fresh medium, the second peak was followed by a cultivation period over 6 days (growth phase).

Scenario "H": single exposure peak over 24 h followed by a 7 day cultivation period (growth phase).

In each scenario, two plants with four fronds and one plant with three fronds were added impartially to each vessel under axenic conditions giving a total number of 11 fronds at test initiation. Assessment of growth at the end of each exposure peak, at the end of the non-exposure period and at the end of the cultivation period; in scenario "H", plant growth was additionally assessed two days after the single peak treatment.

Endpoints: Effects on plant growth based on growth rate and yield; no endpoints values were calculated.

Test concentrations: 0 (control) and 0.250 mg dimethenamid-P/L (nominal) in all exposure scenarios.

Test conditions: 400 mL glass beakers, test volume: 160 mL, dilution water: 20 x-AAP medium; pH 7.50 - 7.54 at test initiation in all bulk solutions; temperature 20 °C - 22 °C; continuous light at approx. 10 klux.

Analytics: None.

Statistics: Descriptive statistics; ANOVA followed by Dunnett`s test (two-sided $p < 0.05$).

II. RESULTS AND DISCUSSION

No analytical verification of the test item concentrations was carried out. The following biological results are based on nominal test concentrations.

Biological results: In scenarios "A - G", the duckweed population in the control vessels increased from 11 fronds per vessel at test initiation to an average of 426, 656, 591, 729, 849, 931 and 1314 fronds per vessel in the control after 9, 10, 11, 12, 13, 14 and 15 days, respectively. In scenario "H", the frond number increased to an average of 680 fronds per vessel in the control after 9 days. Thus, good and continuous exponential growth was achieved in all exposure scenarios.

In the scenarios with non-exposure periods of ≤ 2 days between two consecutive peaks (scenario "A" and "B"), plant growth was statistically significantly decreased based on both parameters growth rate and yield when compared to the single peak treatment (scenario "H"; Dunnett`s test, $p < 0.05$) by about 6% based on growth rate and about 15% based on yield. The relative percent differences between the inhibition values in scenario "A" and "B" compared to those in scenario "H" were +62% and +63% based on growth rate and +33% and +43% based on yield. Exposure of *Lemna gibba* to two consecutive peaks separated by > 2 days resulted in similar plant growth (scenarios "D and F", as well as scenarios "G and H") or statistically significantly increased plant growth (scenarios "C" and "F") when compared to the single peak treatment (Dunnett`s test, $p < 0.05$).

The data show that the impact of consecutive peaks on the growth of *Lemna gibba* is influenced by the duration of the non-exposure period between the peaks. Non-exposure periods > 2 days between two consecutive 24 hour peaks of 250 mg BAS 656P H/L (scenario "C" to "G") caused no additional growth reduction as compared to the effects measured after a single peak treatment at the same concentration of BAS 656P H. In these scenarios, the second peak did not cause an increase of the magnitude of the effect.

Furthermore, there is no effect-addition between the number of exposure peaks and the magnitude of the measured effects (*i.e.* two consecutive peaks of the same test item concentration do not induce 2-fold of the effect caused by one single peak even if they are separated by only 1 day).

Effects on growth rate and yield are summarized in Table 8.2.7-6.

Table 8.2.7-6: Effects of dimethenamid-P on the growth of *Lemna gibba* based on frond numbers in different exposure scenarios

Exposure scenario				**Inhibition rel. to the control [%]** (rel. difference compared to single peak scenario "H")	
Serial code	Peak no. & duration	Peak concentration [mg a.s./L]	NEP between peaks [d]	growth rate	yield
"A"	2 x 24 h	0.250	1	16.3 * (+62)	46.1 * (+33)
"B"	2 x 24 h	0.250	2	16.4 * (+63)	49.6 * (+43)
"C"	2 x 24 h	0.250	3	8.0 ** (-21)	27.7 ** (-20)
"D"	2 x 24 h	0.250	4	10.2 (+2)	35.5 (+2)
"E"	2 x 24 h	0.250	5	9.6 (-4)	34.8 (+0.2)
"F"	2 x 24 h	0.250	6	7.3 ** (-28)	27.9 ** (-20)
"G"	2 x 24 h	0.250	7	8.3 (-17)	33.2 (-4)
"H"	1 x 24 h	0.250	--	10.0	34.7

NEP = Non-Exposure Period
Values in brackets give the relative percent differences of the inhibition values compared those of scenario "H".
* Statistically significant decrease in plant growth compared to the single peak exposure scenario "H" (Dunnett`s test $p < 0.05$).
** Statistically significant increase in plant growth compared to the single peak exposure scenario "H" (Dunnett`s test $p < 0.05$).

III. CONCLUSION

The results of this study show that the impact of consecutive peaks on the growth of *Lemna gibba* is influenced by the duration of the non-exposure period between the peaks. For *Lemna gibba* two consecutive 24 hour peaks of 0.250 mg dimethenamid-P/L can be considered toxicologically independent from each other if the interval between the single peaks is longer than 2 days. In this case, the second peak did not contribute to the magnitude of the response anymore.

The following time-to-event study on the aquatic plant *Ceratophyllum demersum* performed with the active substance dimethenamid-P is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report:	CA 8.2.7/6 Janson G.-M., 2013a Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant Ceratophyllum demersum after different exposure durations 2013/1286175
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static toxicity laboratory study with sediment, the effect of dimethenamid-P on the growth of the aquatic plant *Ceratophyllum demersum* was investigated after single peak exposure over two different exposure durations of 24 and 48 hours. The following nominal concentrations were applied in both exposure scenarios: 0.3, 1.0, and 3.0 mg a.s./L. After the respective exposure time, the plants were transferred to fresh sediment and medium and cultivated for further seven days. At test initiation, plants were weighted and the main shoot length was recorded. Assessment of total length of the plants (above sediment) was conducted at test initiation, at the end of the exposure periods, once during the cultivation period and at test end. The length of the side shoots were recorded at test initiation, once during the study and at test termination. Assessment of fresh weight was conducted at test initiation and test end and visual observations were performed once during the growth phase and at test end. In addition, plant dry weight was determined at test termination. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for all parameters.

The biological results are based on nominal concentrations. No statistically significant effects on plants were observed after exposure over 24 hours at concentrations of up to and including 3.0 mg a.s./L, except for the total length (based on growth rate and yield) in the highest tested concentration. After exposure over 48 hours, total length was statistically significantly impacted at the two highest test item concentrations of 1.0 and 3.0 mg a.s./L (based on growth rate data yield). Additionally, plant fresh weight based on growth rate was statistically significantly reduced after exposure over 48 hours at 3.0 mg a.s./L. Plant dry weight was not significantly impacted at any test item concentrations in both exposure scenarios. Generally, the overall impact was rather limited and none of the scenarios produced an impact strong enough to calculate an EC_{50}. At test end slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study.

The results of the study demonstrated that exposure times typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to long-term constant exposure simulated in the standard study on *Ceratophyllum demersum* (exposure over 9 days). No significant effects were observed on plants exposed to dimethenamid-P for 24 h at up to and including the concentration of 1.0 mg a.s./L (nominal). Significant effects on plants exposed for 48 h were observed at the test item concentrations of 1.0 and 3.0 mg a.s./L. However, the impacts were rather low and not sufficient to produce an EC_{50} endpoint; thus, all EC_{50} values for all measured parameters and all treatment groups are > 3.0 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H; Reg. no.: 363 851), batch no. COD-001509, purity: 95.9% ± 1%.

B. STUDY DESIGN

Test species: *Ceratophyllum demersum* (Ceratophyllaceae), a dicotyledonous aquatic plant species, cultivated in-house (non-GLP) after collected from natural running water.

Test design: Static system including sediment, three test item concentrations were tested over two different exposure durations, *i.e.* 24 and 48 hours, each followed by a 7 day cultivation period; 4 treatment groups (3 test item concentrations, control) for both exposure scenarios with 3 plants per replicate; the tests were run with 3 replicates for the test item treatments and 6 replicates for the control. After the respective exposure times the plants were transferred to fresh sediment and medium and cultivated for further seven days; assessment of total length of the plants (main shoot above the sediment plus side shoots) at test initiation, after the respective exposure phase, once during the cultivation period and at the end of the test; the length of the side shoots were recorded once during the study and at test termination; assessment of fresh weight at test initiation and test end; visual observations once during the growth phase and at test end; determination of dry weight at test termination. The starting dry weight was determined by calculating a mean factor based on the ratios of the final dry weights and lengths of the control replicates, which is then multiplied by the initial length data for each plant in all treatments.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield after exposure over two different exposure durations, each followed by a 7 day cultivation period.

Test concentrations: Control, 0.3, 1.0 and 3.0 mg a.s./L for both exposure scenarios.

Test conditions: 2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219) and 1.8 L Smart & Barko medium (pH 7.63 at test initiation); air temperature 20.0 ± 2°C; water temperature: 21.1 °C - 21.9 °C in the 24 h exposure scenario and 20.1 °C - 21.7 °C in the 48 h exposure scenario; oxygen saturation: 91.6% - 183.0% in the 24 h exposure scenario and 81.5% - 161.2% in the 48 h exposure scenario; pH 7.29 - 9.83 in the 24 h exposure scenario and pH 7.34 - 9.57 in the 48 h exposure scenario; light : dark - rhythm 16 : 8 h, light intensity: 10 - 12 klux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics; Probit analysis using linear max. likelihood regression for calculation of the EC_x values; ANOVA followed by Dunnett's Multiple Sequential t-test Procedure, Student-t test for Homogeneous Variances and Welch-t test for Inhomogeneous Variances ($\alpha = 0.05$) for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in the bulk solutions prepared for each concentration at test initiation, and in mixed samples (pooled replicates of each treatment) at the end of the different exposure periods. The mean measured concentrations of dimethenamid-P in the bulk solutions ranged from 93% to 95% of the nominal concentrations at test initiation. At the end of the different 24 h exposure phase, measured values were between 93% and 96% of nominal. At the end of the 48 h exposure time the measured values were between 89% and 94% of nominal. Since the analytical measured values confirmed the correct application of the test item, the following biological results based on nominal concentrations.

Biological results: No statistically significant effects on plants were observed after exposure over 24 hours at concentrations of up to and including 3.0 mg a.s./L, except for the total length (based on growth rate and yield) in the highest tested concentration (Student-t test, $\alpha = 0.05$). After exposure over 48 hours, total length was statistically significantly impacted at the two highest test item concentrations of 1.0 and 3.0 mg a.s./L (Student-t test for growth rate data and Welch-t test for yield data, $\alpha = 0.05$). Additionally, plant fresh weight based on growth rate was statistically significantly reduced after exposure over 48 hours at 3.0 mg a.s./L (Dunnett`s Multiple t-test Procedure, $\alpha = 0.05$). Plant dry weight was not significantly impacted at any test item concentrations in both exposure scenarios. Generally, the overall impact was rather limited and none of the scenarios produced an impact strong enough to calculate an EC_{50}. At test end slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study. The results are summarized in Table 8.2.7-7.

Table 8.2.7-7: Effect of dimethenamid-P on the aquatic plant *Ceratophyllum demersum* in different exposure scenarios followed by a 7 day cultivation period

Exposure scenario	**24 h exposure period + 7 d cultivation period**			**48 h exposure period + 7 d cultivation period**		
Concentration [mg a.s./L] (nominal)	**0.3**	**1.0**	**3.0**	**0.3**	**1.0**	**3.0**
Inhibition after 7 d cultivation [%] (growth rate based on total length)	4.2	15.9	19.6 *	17.9	32.4 *	35.3 *
Inhibition after 7 d cultivation [%] (yield based on total length)	5.3	25.3	31.9 *	22.4	42.0 *	46.7 *
Inhibition after 7 d cultivation [%] (growth rate based on fresh weight)	5.4	9.7	15.7	14.4	20.9	29.2 *
Inhibition after 7 d cultivation [%] (yield based on fresh weight)	2.0	11.2	16.3	16.1	25.3	31.2
Inhibition after 7 d cultivation [%] # (growth rate based on dry weight)	-14.6	12.1	14.4	10.9	8.9	12.0
Inhibition after 7 d cultivation [%] # (yield based on dry weight)	-23.6	13.9	22.4	5.5	10.3	16.0
	Endpoints [mg a.s./L] (nominal)					
E_rC_{50} based on total length, fresh weight and dry weight	> 3.0			> 3.0		
E_yC_{50} based on total length, fresh weight and dry weight	> 3.0			> 3.0		
NOE_rC / NOE_yC based on total length	1.0			0.3		
NOE_rC based on fresh weight	3.0			1.0		
NOE_yC based on fresh weight	3.0			3.0		
NOE_rC / NOE_yC based on dry weight	3.0			3.0		

Negative values indicate stimulated growth
* Statistically significant differences compared to the control (Dunnett's Multiple Sequential t-test Procedure, Student-t test for Homogeneous Variances or Welch-t test for Inhomogeneous Variances, α = 0.05, one-sided smaller).

III. CONCLUSION

The results of the study demonstrated that exposure times typical for running water bodies like streams or ditches (hours to days) cause less effects as compared to long-term constant exposure simulated in the standard study on *Ceratophyllum demersum* (exposure over 9 days). No significant effects were observed on plants exposed to dimethenamid-P for 24 h at up to and including the concentration of 1.0 mg a.s./L (nominal). Significant effects on plants exposed for 48 h were observed at the test item concentrations of 1.0 and 3.0 mg a.s./L. However, the impacts were rather low and not sufficient to produce an EC_{50} endpoint; thus, all EC_{50} values for all measured parameters and all treatment groups are > 3.0 mg a.s./L (nominal).

The following toxicity study on the aquatic plant *Glyceria maxima* performed with the active substance dimethenamid-P is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report:	CA 8.2.7/7 Janson G.-M., 2013b Effect of BAS 656 H (Dimethenamid-P, Reg.No. 363851) on the growth of the aquatic plant Glyceria maxima 2013/1286172
Guidelines:	OECD 221, OECD 219, ASTM E 1913-04
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of dimethenamid-P on the growth of the aquatic plant *Glyceria maxima* was studied in a 14-days static toxicity test including sediment. The following nominal concentrations were applied: 0 (control), 0.0030, 0.010, 0.030, 0.10, 0.30 and 1.0 mg a.s./L. Assessment of growth based on the number and length of the plant leaves was conducted at the beginning of the test, once during the test and at test end. Plant wet weight was measured at test initiation and at test termination. Visual observations were assessed once during the test and at the end of the test. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates and final yield for the parameter leave length, number of leaves (only yield) and plant weight.

The biological results are based on nominal concentrations. At test end, slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study.

In a 14-day aquatic-plant test with *Glyceria maxima*, the E_rC_{50} of dimethenamid-P was determined to be 0.184 mg a.s./L based on total length, > 1.0 mg a.s./L based on dry weight and 0.402 mg a.s./L based on wet weight (nominal). The E_yC_{50} was 0.109 mg a.s./L based on total length, 0.934 mg a.s./L based on dry weight and 0.221 mg a.s./L based on wet weight (nominal). The E_yC_{50} based on the number of leaves was 0.318 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851); batch no. COD-001509; purity: 95.9 ± 1%.

B. STUDY DESIGN

Test species: *Glyceria maxima* (Poaceae), a monocotyledonous aquatic plant species, cultivated in-house (non-GLP) after purchase from the plant nursery "Petrowsky" Eschede, Germany.

Test design: Static system (including sediment); test duration 14 days; 6 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; one grass blade with 2 - 4 leaves per replicate; one plant per replicate was potted to fresh sediment and medium and cultivated for 14 days; assessment of leaf number and length at test initiation, once during the and at the end of the test; assessment of fresh weight at test initiation and test end; visual observations once during the growth phase and at test end; determination of dry weight at test termination. The starting dry weight was determined by calculating a mean factor based on the ratios of the final dry weights and lengths of the control replicates, which is then multiplied by the initial length data for each plant in all treatments.

Endpoints: EC_{50} with respect to growth rate and yield related to wet weight, dry weight, total length as well as number of leaves after 14 days of exposure.

Test concentrations: Control, 0.0030, 0.010, 0.030, 0.10, 0.30 and 1.0 mg a.s./L (nominal).

Test conditions: 2.0 L glass beakers and flower pots (Ø 9 cm), standard artificial sediment (OECD 219 with slight modifications, pH 6.76) and 1000 mL Smart & Barko medium (pH 7.71 at test initiation); oxygen saturation: 88.5% - 94.0% at the test initiation and 102.6% - 123.6% at test termination; pH 7.60 - 7.63 at test initiation and 8.02 - 8.81 at test termination; conductivity: 286 - 287 µS/cm; water temperature: 20.3°C - 20.7 °C; light : dark - rhythm 16 : 8 h, light intensity: 10 klux ± 2 klux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection. At test initiation the analytical samples were taken from the respective bulk solutions and at the end from mixed samples (pooled replicates of each treatment).

Statistics: Descriptive statistics; probit analysis using linear max. likelihood regression for EC_{50} calculations.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of active substance concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured concentrations of dimethenamid-P ranged from 99% to 102% of nominal concentrations at test initiation and from 73% to 83% nominal at test termination. As the analytically measured values (at test initiation) confirmed the correct application of the test item in both exposure scenarios, the following biological results are based on nominal test concentrations.

Biological results: At test end, slight growth of algae was observed in all test item concentrations and the control; however, at levels not distorting the performance and the results of the study. The results are summarized in Table 8.2.7-8.

Table 8.2.7-8: Effect of dimethenamid-P on the growth of the aquatic plant *Glyceria maxima*

Concentration [mg a.s./L] (nominal)	**0.0030**	**0.010**	**0.030**	**0.10**	**0.30**	**1.0**
Inhibition in 14 d [%] # (growth rate based on total length)	5.9	0.4	-4.6	33.4	65.8	90.2
Inhibition in 14 d [%] (yield based on total length)	8.6	4.6	7.7	51.0	79.0	95.1
Inhibition in 14 d [%] # (growth rate based on dry weight)	-5.6	4.9	-14.8	6.5	28.0	28.0
Inhibition in 14 d [%] # (yield based on dry weight)	-17.3	12.1	-7.3	15.6	45.6	45.1
Inhibition in 14 d [%] (growth rate based on wet weight)	4.3	11.8	4.2	27.8	42.2	68.4
Inhibition in 14 d [%] # (yield based on wet weight)	-10.7	11.8	2.4	33.5	58.5	81.3
Inhibition in 14 d [%] # (yield based on number of leaves)	6.1	-6.1	2.0	26.5	38.8	87.8
Endpoints [mg a.s./L] (nominal)						
$E_rC_{50\ total\ length}$ (14 d)	0.184 (95% confidence limits: 0.136 - 0.250)					
$E_yC_{50\ total\ length}$ (14 d)	0.109 (95% confidence limits: 0.074 - 0.160)					
$E_rC_{50\ dry\ weight}$ (14 d)	> 1.0					
$E_yC_{50\ dry\ weight}$ (14 d)	0.934 (95% confidence limits: 0.315 - > 1.)					
$E_rC_{50\ wet\ weight}$ (14 d)	0.402 (95% confidence limits: 0.242 - 0.817)					
$E_yC_{50\ wet\ weight}$ (14 d)	0.221 (95% confidence limits: 0.136 - 0.375)					
$E_yC_{50\ no\ of\ leaves}$ (14 d)	0.318 (95% confidence limits: 0.179 - 0.608)					

Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 14-day aquatic-plant test with *Glyceria maxima*, the E_rC_{50} of dimethenamid-P was determined to be 0.184 mg a.s./L based on total length, > 1.0 mg a.s./L based on dry weight and 0.402 mg a.s./L based on wet weight (nominal). The E_yC_{50} was 0.109 mg a.s./L based on total length, 0.934 mg a.s./L based on dry weight and 0.221 mg a.s./L based on wet weight (nominal). The E_yC_{50} based on the number of leaves was 0.318 mg a.s./L, based on nominal concentrations.

The following toxicity studies on the aquatic plant *Lemna gibba* performed with the metabolites M656H055, Reg. No. 403 121 and M656PH043 are provided in support of the aquatic risk assessment and have not been evaluated previously. The studies are required due to new data requirements.

Report:	CA 8.2.7/8 Swierkot A., 2013a Reg.No. 5749263 (metabolite of BAS 656 H, Dimethenamid-P M656H055, M55) - Lemna gibba CPCC 310 growth inhibition test 2013/1063800
Guidelines:	OECD 221
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of M656H055 (metabolite of dimethenamid-P) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 13.6, 24.5, 44.1, 79.4 and 143 mg M656H055/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based on growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. No statistically significant effects on algal growth compared to the control were observed at any test item concentration tested.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H055 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 143 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M656H055 (M55; Reg. No. 5 749 263) metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L80-154, purity: 69.8 ± 1%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre", Ontario, Canada.

Test design: Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield based on frond number and dry weight after exposure over 7 days.

Test concentrations: Control, 13.6, 24.5, 44.1, 79.4 and 143 mg M656H055/L (nominal).

Test conditions: 600 mL glass beakers, test volume 400 mL, 20x-AAP nutrient medium, pH 7.54 - 7.83 at test initiation and pH 9.53 - 9.71 at test termination; water temperature: 23.8 °C - 24.3 °C, continuous light, light intensity: 8955 - 9248 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with UV-VIS detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values; Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$) for determination of the NOEC..

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656H055 ranged from 100.3% to 102.9% of nominal at test initiation and from 110.5% to 115.0% of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 108 fronds per vessel, corresponding to a 12 x multiplication. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. No statistically significant effects on algal growth compared to the control were observed at any test item concentration tested (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$). Effects on growth rate and yield are summarized in Table 8.2.7-9.

Table 8.2.7-9: Effect of M656H055 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna gibba*

Concentration [mg/L] (nominal)	**13.6**	**24.5**	**44.1**	**79.4**	**143**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.0	0.0	0.0	0.0	2.23
Inhibition after 7 d [%] (yield based on frond no.)	0.0	0.0	0.0	0.0	5.88
Inhibition after 7 d [%] (growth rate based on dry weight)	0.0	0.0	0.0	0.0	0.0
Inhibition after 7 d [%] (yield based on dry weight)	0.0	0.0	0.0	0.0	0.0
	Endpoints [mg M656H055/L] (nominal)				
E_rC_{50} / E_yC_{50} (7 d) based on frond no and dry weight	> 143				
E_rC_{10} / E_yC_{10} (7 d) based on frond no and dry weight	> 143				
NOE_rC / NOE_yC (7 d) based on frond no and dry weight	≥ 143				

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656H055 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 143 mg/L (nominal).

The metabolite M656H062 (M62) cannot be synthesized; thus, the following test on *L. gibba* was performed with Reg. No. 403 121 which is the hydrochloride of metabolite M656H039 (= PL 15-88) which itself is the putative metabolic precursor of M656H062.

Report:	CA 8.2.7/9 Swierkot A., 2013b Reg.No. 403121 (metabolite of BAS 656 H, Dimethenamid-P, M39) - Lemna gibba CPCC 310 growth inhibition test 2013/1191249
Guidelines:	OECD 221
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 7-day semi-static toxicity laboratory study, the effect of Reg. No. 403 121 (metabolite of dimethenamid-P) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.41, 1.23, 3.70, 11.11, 33.33 and 100 mg Reg. No. 403 121/L. Assessment of growth and other effects was conducted 2, 4, 6 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based on growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth. No morphological effects on algae were observed in the control group and at test item concentrations of up to and including 33.33 mg/L. At the highest test item concentration of 100 mg/L, smaller fronds, shorter roots and plants with single fronds were observed. Statistically significant effects on algal growth rate and yield compared to the control were determined in the two highest test item concentrations based on frond numbers and in the highest concentration based on dry weight.

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} of Reg. No. 403 121 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on both frond no. and dry weight (nominal). The E_yC_{50} was 54.57 mg/L based on frond no. and 72.87 mg/L based on dry weight (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 403 121, metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L74-88, purity: 99.1 ± 1%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from “University of Waterloo, Canadian Phycological Culture Centre, Department of Biology”, Ontario, Canada.

Test design: Semi-static system (7 days); renewal of test solutions on days 2, 4 and 6; 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 4, 6 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control, 0.41, 1.23, 3.7, 11.11, 33.33 and 100 mg Reg. No. 403 121/L (nominal).

Test conditions: Glass vessels, test volume 150 mL, 20x-AAP nutrient medium, pH 7.24 - 7.67 at test initiation and pH 8.35 - 8.99 at test termination; water temperature: 24.2 °C - 24.6 °C, continuous light, average light intensity 8350 lux - 9225 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with DAD detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values; Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure (both $\alpha = 0.05$) for determination of the NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in the fresh and old solutions of each test concentration at the beginning of the test, on day 2, 4 and 6 and at the end of the test, except for the 0.41 mg/L treatment since this concentration was below the limit of detection (LoD = 0.50 mg/L). Mean measured values for Reg. No. 403 121 ranged from 85.4% to 102.4% of nominal concentrations in fresh solutions and from 36.5% to 102.0% of nominal in old solutions. The determined mean concentrations in old solutions were in the range of < LoD and 77.2% of initial concentrations, what shows that the test item was not stable under test condition. However, as initially measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 198 fronds per vessel, corresponding to a 22 x multiplication. After 7 days of exposure, no morphological effects on algae were observed in the control group and at test item concentrations of up to and including 33.33 mg/L. At the highest test item concentration of 100 mg/L, smaller fronds, shorter roots and plants with single fronds were observed. Statistically significant effects on algal growth rate and yield compared to the control were determined in the two highest test item concentrations based on frond numbers and in the highest concentration based on dry weight (Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure, both α = 0.05). Effects on growth rate and yield are summarized in Table 8.2.7-10.

Table 8.2.7-10: Effect of Reg. No. 403 121 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna* gibba

Concentration [mg/L] (nominal)	**0.41**	**1.23**	**3.70**	**11.11**	**33.33**	**100**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.00	0.01	0.00	1.45	10.44 *	40.68 *
Inhibition after 7 d [%] (yield based on frond no.)	0.00	0.00	0.00	4.67	28.99 *	74.98 *
Inhibition after 7 d [%] (growth rate based on dry weight)	0.00	0.62	0.00	0.00	5.83	34.67 *
Inhibition after 7 d [%] (yield based on dry weight)	0.00	2.97	0.00	0.00	16.69	65.27 *
	Endpoints [mg Reg. No. 403 121/L] (nominal)					
E_rC_{50} (7 d) based on frond no	> 100					
E_rC_{10} (7 d) based on frond no	10.88 (95% confidence limits: 1.68 - 36.15)					
E_yC_{50} (7 d) based on frond no	54.57 (95% confidence limits: 53.39 - 55.78)					
E_yC_{10} (7 d) based on frond no	17.07 (95% confidence limits: 6.28 - 17.85)					
NOE_rC / NOE_yC (7 d) based on frond no	11.11					
E_rC_{50} (7 d) based on dry weight	> 100					
E_rC_{10} (7 d) based on dry weight	14.22 (95% confidence limits: 1.22 - > 100)					
E_yC_{50} (7 d) based on dry weight	72.87 (95% confidence limits: 67.37 - 78.93)					
E_yC_{10} (7 d) based on dry weight	26.05 (95% confidence limits: 21.42 - 30.25)					
NOE_rC / NOE_yC (7 d) based on dry weight	33.33					

* Statistically significant differences compared to the control (Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment and Williams Multiple Sequential t-test Procedure; both α = 0.05).

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} of Reg. No. 403 121 (metabolite of dimethenamid-P) was determined to be > 100 mg/L based on both frond no. and dry weight (nominal). The E_yC_{50} was 54.57 mg/L based on frond no. and 72.87 mg/L based on dry weight (nominal).

Report:	CA 8.2.7/10 Swierkot A., 2013c Reg.No. 5917262 (metabolite of BAS 656 H, Dimethenamid-P, M43) - Lemna gibba CPCC 310 growth inhibitation test 2013/1191248
Guidelines:	OECD 221 (2006)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of M656PH043 (metabolite of dimethenamid-P) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 2.56, 6.4, 16, 40 and 100 mg M656PH043/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based on growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} and the E_yC_{50} of M656PH043 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M656PH043 (M43, Reg. No. 5 917 262), metabolite of dimethenamid-P (BAS 656 PH, Reg. No. 363 851), batch no. L82-113, purity: 94.6 ± 1%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula 7 days old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre", Ontario, Canada.

Test design: Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 3, 5 and 7.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield based on frond number and dry weight after exposure over 7 days.

Test concentrations: Control, 2.56, 6.4, 16, 40 and 100 mg M656PH043/L (nominal).

Test conditions: 600 mL glass beakers, test volume 400 mL, 20x-AAP nutrient medium, pH 7.58 - 7.76 at test initiation and pH 9.56 - 9.98 at test termination; water temperature: 24.0 °C - 24.4 °C, continuous light, light intensity: 7674 - 7910 lux.

Analytics: Analytical verification of the test item was conducted using a liquid chromatography-method with DAD detection.

Statistics: Descriptive statistics, probit analysis for calculation of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for M656PH043 ranged from 98.1% to 105.8% of nominal at test initiation and from 104.6% to 108.1% of nominal at test termination. As analytical data confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 9 fronds per vessel to an average of 136 fronds per vessel, corresponding to a 15 x multiplication. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Effects on growth rate and yield are summarized in Table 8.2.7-11.

Table 8.2.7-11: Effect of M656PH043 (metabolite of dimethenamid-P) on the growth of duckweed *Lemna* gibba

Concentration [mg/L] (nominal)	**2.56**	**6.4**	**16**	**40**	**100**
Inhibition after 7 d [%] (growth rate based on frond no.)	1.00	2.60	6.98	10.43	12.30
Inhibition after 7 d [%] (yield based on frond no.)	2.87	7.31	18.54	25.85	30.29
Inhibition after 7 d [%] (growth rate based on dry weight)	0.00	0.00	1.49	3.18	6.28
Inhibition after 7 d [%] (yield based on dry weight)	0.00	0.00	4.53	8.73	17.03
	Endpoints [mg M656PH043/L] (nominal)				
E_rC_{50} (7 d) based on frond no and dry weight	> 100				
E_rC_{10} (7 d) based on frond no	50.5 (95% confidence limits: 24.3 - > 100)				
E_rC_{10} (7 d) based on dry weight	> 100				
E_yC_{50} (7 d) based on frond no and dry weight	> 100				
E_yC_{10} (7 d) based on frond no	7.0 (95% confidence limits: 0.5 - 15.6)				
E_yC_{10} (7 d) based on dry weight	48.3 (95% confidence limits: 35.6 - 60.0)				

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_yC_{50} of M656PH043 (metabolite of dimethenamid-P) based on both frond no. and dry weight were determined to be both > 100 mg/L (nominal).

The following toxicity study on the aquatic plant *Lemna gibba* has already been submitted and accepted during Annex I renewal evaluation for dimethenamid-P. However, since then, the endpoints of the study have been recalculated from original data and the recalculated results (*i.e.* the geometric mean of the recalculated six and nine days endpoints of the 14 d study) are now used in the aquatic risk assessment; for details on these calculations please refer to the respective amendment. Executive summaries of the originally submitted study (plus endpoint table) and the re-calculations are provided below. For detailed summary for the originally submitted study reference is made to the EU dossier submitted during Annex I inclusion process for dimethenamid-P.

Report:	CA 8.2.7/11 Kubitza J., 2004c Amendment to study BASF DocID 1997/10742: SAN 1289H technical - Toxicity to duckweed; Lemna gibba 2004/1025686
Guidelines:	EPA 122-2, EPA 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary (originally submitted study; BASF DocID 1997/10742)

In a 14-day semi-static toxicity laboratory study, the effect of dimethenamid-P on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.0010, 0.0030, 0.0089, 0.027, and 0.081 mg a.s./L (corresponding to mean measured concentrations of 0 (control), 0.0012, 0.0032, 0.0073, 0.026, and 0.074 mg a.s./L). Assessment of growth (by counting fronds) and other effects was conducted 3, 6, 9, 12 and 14 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon frond density and frond dry weight (biomass) at test end.

Measured concentrations of dimethenamid-P ranged from 82% to 120% of nominal concentrations at test initiation. Measured concentrations at test solution renewal (days 3, 6, 9, and 12) decreased significantly and ranged from 15% to 36% of nominal concentrations. The biological results are based on initial measured test concentrations.

The duckweed population in the control vessels showed sufficient growth. After 14 days of exposure, the number of fronds was statistically significantly reduced compared to the control at the two highest test item concentrations of 0.026 and 0.074 mg a.s./L. Statistically significant effects on frond dry weight (biomass) were observed at test item concentrations of ≥ 0.0032 mg a.s./L. No morphological effects were observed in the control and at the lowest tested concentration of 0.0012 mg a.s./L throughout the test duration. At test termination, fronds exposed to 0.0032 and 0.0073 mg a.s./L were smaller and had less root formation compared to the control fronds. In addition, fronds exposed to the 0.0073 mg a.s./L were observed to be slightly chlorotic and curled. Fronds exposed to the two highest test item concentrations of 0.026 and 0.074 mg a.s./L were curled, slightly chlorotic, had very little root formation and were smaller compared to the fronds in the control.

The effects on plant growth (based on frond densities and frond dry weight) are summarized in Table 8.2.7-12

Table 8.2.7-12: Effect (14 d) of dimethenamid-P on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	Control	0.0010	0.0030	0.0089	0.027	0.081
Concentration [mg a.s./L] (initial measured)	**--**	**0.0012**	**0.0032**	**0.0073**	**0.026**	**0.074**
Inhibition after 14 d [%] # (based on frond density)	--	4.9	-7.9	8.1	90 *	96 *
Inhibition after 14 d [%] * (based on frond dry weight (biomass))	--	11	18 *	28 *	90 *	93 *
	Endpoints [mg dimethenamid-P/L] (initial measured)					
EC_{50} (14 d) based on frond density	0.016 (95% confidence limits: 0.0055 - 0.048)					
EC_{50} (14 d) based on frond dry weight	0.0089 (95% confidence limits: 0.0025 - 0.032)					

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compare to the control (Williams' Test).

In the 14-day semi-static toxicity test with the aquatic plant *Lemna gibba,* the EC_{50} of dimethenamid-P was determined to be 0.016 mg a.s./L based on frond density and 0.0089 mg a.s./L based on frond dry weight (initial measured).

Executive Summary (recalculations; BASF DocID 2004/1025686)

The study BASF DocID 1997/10742 with the duckweed *Lemna gibba* was conducted over 14 days according to Good Laboratory Practice (GLP) following the U.S. EPA Guidelines 122-2 and 123-2. However, in the original study report the endpoints were related to frond densities and frond dry weight after 14 days, only; the results were based on initial measured test concentrations.
Additional endpoints related to growth rate (r) and biomass (b) based on frond numbers after 3, 6, 9, 12 and 14 days of exposure were recalculated according to current recommendations (OECD 221, July, 2011). The following 6-day and 9-day endpoints were obtained based on nominal concentrations, as the initial measured values confirmed the nominal concentrations.

E_rC_{50} (6 d) = 0.0473 mg/L (95% confidence limits: 0.0444 - 0.0503 mg/L)
E_rC_{50} (9 d) = 0.0390 mg/L (95% confidence limits: 0.0372 - 0.0409 mg/L)

E_rC_{10} (6 d) = 0.0112 mg/L (95% confidence limits: 0.0103 - 0.0122 mg/L)
E_rC_{10} (9 d) = 0.0125 mg/L (95% confidence limits: 0.0116 - 0.0135 mg/L)

E_bC_{50} (6 d) = 0.0256 mg/L (95% confidence limits: 0.0244 - 0.0268 mg/L)
E_bC_{50} (9 d) = 0.0211 mg/L (95% confidence limits: 0.0204 - 0.0219 mg/L)

E_bC_{10} (6 d) = 0.0083 mg/L (95% confidence limits: 0.0077 - 0.0089 mg/L)
E_bC_{10} (9 d) = 0.0110 mg/L (95% confidence limits: 0.0103 - 0.0116 mg/L)

The following two toxicity studies on several emergent and submersed aquatic plant species performed with the formulated product BAS 656 08 H (containing 720 g dimethenamid-P/l, nominally) are provided in support of the aquatic risk assessment. The studies have already been submitted within several dossier submissions in the past, however, they have not been evaluated previously on EU level. Thus, study summaries are provided below.

Report:	CA 8.2.7/12 Kubitza J.,Dohmen G.-P., 2003a Effect of Dimethenamid-P - Tested as formulated product - BAS 656 08 H - On emergent aquatic plants 2002/1012788
Guidelines:	HARAP, CLASSIC
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 8.2.7/13 Kubitza J., 2013a Report amendment no. 1 to Effect of Dimethenamid-P - Tested as formulated product BAS 656 08 H - On emergent aquatic plants 2013/1361973
Guidelines:	HARAP (Campbell et al 1999) Guidance Document on Higher-tier Aquatic Risk Assessment of Pesticides, CLASSIC (Workshop on Community Level Aqiuatic System Studies May-June 1999)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 8.2.7/14 Kubitza J., 2014a Report amendment no. 2 to Effect of Dimethenamid-P - Tested as formulated product - BAS 656 08 H - On emergent aquatic plants 2014/1082325
Guidelines:	HARAP (Campbell et al 1999) Guidance Document on Higher-tier Aquatic Risk Assessment of Pesticides, CLASSIC (Workshop on Community Level Aqiuatic System Studies May-June 1999)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The effect of dimethenamid-P (tested with the formulated product BAS 656 08 H) on the growth of the emergent aquatic plant species *Acorus calamus, lris pseudacorus, Sparganium erectum, Mentha aquatica, Ludwigia palustris and Veronica beccabunga* was studied in a 13-days static toxicity test including sediment. Application of the test item was done using a laboratory spray track system simulating a realistic spraying event. The following amounts of BAS 656 08 H were applied with an amount of water corresponding to 200 L/ha: 0 (control), 0.010, 0.023, 0.052, 0.118, 0.270, 0.614 and 1.4 L/ha (corresponding to 0, 0.007, 0.016, 0.037, 0.084, 0.192, 0.437 and 0.996 kg dimethenamid-P/ha). The application rates for both the product and the active substance were recalculated to nominal and initial measured water concentrations. Assessments of growth were conducted at the beginning and at the end of the test based on wet weight, leave length and root weight for monocotyledonous plants and based on wet weight and shoot length for dicotyledonous plants. Visual observations were made one day after treatment, one week after treatment and at the end of the test. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates for the respective test parameters.

The biological results are based on recalculated nominal water concentrations for all tested plant species except *S. erectum*, for which the biological results are based on the recalculated initial measured water concentrations.No morphological effects were observed on the monocotyledonous plants in the control and at any of the concentrations tested. At test termination some plants of the dicotyledonous species *M. aquatica* were yellowish and cambered at test item concentrations of 0.197 mg BAS 656 08 H/L (0.124 mg a.s./L) and higher, furthermore the plants appeared smaller with increasing test item concentrations. The root formation of *L. palustris* and *V. beccabunga* was affected at concentrations of 0.086 mg BAS 656 08 H/L (0.054 mg a.s./L) and higher at test termination. For *V. beccabunga* the effect was not uniform and some replicates at the two highest test item concentrations showed strong root formation. The most sensitive species in this test was the dicotyledonous *L. palustris* which showed statistically significant effects compared to the control at the 6 highest test item concentrations based on both wet weight and length data after exposure over 13 days.

In a 13-day static toxicity test with six emergent aquatic plant species, *Ludwigia palustris* was the most sensitive species generating an E_rC_{50} of 0.098 mg BAS 656 08 H/L (0.062 mg dimethenamid-P/L) based on wet weight and an E_rC_{50} of 0.075 mg BAS 656 08 H/L (0.047 mg a.s./L) based on length data (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 08 H, batch no. 2001-1; content of a.s.: dimethenamid-P (BAS 656 H, Reg. no. 363 851): 711.4 g/L (nominal: 720.0 g/L); density: 1.127 g/cm^3.

B. STUDY DESIGN

Test species: Emergent aquatic plants; monocotyledonous: *Acorus calamus* (Araceae), *lris pseudacorus* (Iridaceae), *Sparganium erectum* (Sparganiaceae); dicotyledonous: *Mentha aquatic* (Lamiaceae), *Ludwigia palustris* (Onagraceae), *Veronica beccabunga* (Scrophulariaceae). Monocotyledonous plants purchased from the garden centre “Germann”, dicotyledonous plants purchased from "Harster", both resident in Speyer, Germany; *Sparganium erectum* cultivated in-house.

Test design: Static system (including sediment); test duration 13 days, 7 test item concentrations, each with 3 replicates plus a control with 6 replicates; the number of plants per replicate (one to several) varied depending on the test species; plants were treated using a laboratory spray track system simulating a realistic spraying event; visual observations one day after treatment, one week after treatment and at the end of the test; determination of wet weight, leave length and root weight for monocotyledonous plants and determination of wet weight and shoot length as well as visual assessment of root formation for dicotyledonous plants at test end. The test with *S. erectum* had been repeated because of high variability of results, which were probably due to the very differing growth stages of the initial plants at the start of the first test (only the results of the repeated test are reported here).

Endpoints: EC_{50} with respect to growth rate related to wet weight, length data and, for monocotyledonous plants, root formation after 13 days of exposure.

Test concentrations: The amounts of BAS 656 08 H (and respective values for dimethenamid-P) applied *via* spray application with 200 L/ha as well as the recalculated nominal and initial measured water concentrations are presented in the following table.

nominal application rates		**nominal water concentrations**				**initial measured water concentrations**	
all tested species		**monocotyledonous** (*A. calamus, I. pseudacorus, S. erectum)*		**dicotyledonous** *(M. aquatica, L. palustris, V. beccabunga)*		***S. erectum***	
BAS 656 08 H [L/ha]	dimethen-amid-p [kg/ha]	BAS 656 08 H [mg/L]	dimethenamid-P [mg/L]	BAS 656 08 H [mg/L]	dimethen-amid-P [mg/L]	BAS 656 08 H [mg/L]	dimethen-amid-P [mg/L]
0.010	0.007	0.015	0.010	0.017	0.011	0	0
0.023	0.016	0.034	0.022	0.038	0.024	0.009	0.006
0.052	0.037	0.078	0.049	0.086	0.054	0.023	0.014
0.118	0.084	0.177	0.112	0.197	0.124	0.085	0.054
0.270	0.192	0.404	0.255	0.449	0.283	0.257	0.162
0.614	0.437	0.921	0.581	1.023	0.646	0.506	0.319
1.400	0.996	2.098	1.324	2.332	1.472	0.905	0.572

Test conditions: 0.8 L and 1 L glass beakers, standard artificial sediment (OECD 219) and 3x- AAP medium (pH: 7.2); oxygen content: 6.9 mg/L - 8.3 mg/L; pH: 7.93 - 10.13; conductivity: 265 - 275 µS/cm; water temperature: 19.6 °C - 25.2 °C; air temperature: 21 ± 1 °C; light : dark - rhythm 16 : 8 h, light intensity: approx. 8.5 ± 1.5 klux.

Analytics: Analytical verification of test item concentrations was conducted using a SPME-method with GC/MS detection.

Statistics: Descriptive statistics; determination of EC_{50} values by probit analysis, William's, Dunnett`s and Bonferroni test ($p \leq 0.05$) for determination of NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: The nominal test item concentrations in samples taken from additional plant-free vessels were confirmed by analytical analysis showing recovery of 106.6% ± 11%. Test item recovery in the repeated part of the study was 108.6% ± 16%, except for the lowest test concentration at which 33.9% was found. In addition, analytical verification of test item concentrations was conducted in treated vessels for each plant species in each concentration at the beginning and at the end of the test. The measured concentrations in samples taken from the test vessels with *A. calamus*, *I. pseudacorus*, *M. aquatic*a and *L. palustris* ranged from 84.2% to 134.0% of nominal concentrations at test initiation and from 30.6% to 120.0% of nominal at test termination. The following biological results for these species are based on nominal (recalculated) water concentrations of the test item. Regarding analytical measurements for *V. beccabunga,* 5 out of 7 samples showed very high recoveries (470.1% - 10719.1%) at test initiation, but since the other samples showed normal recovery (110.6% and 156.2%) and concentrations at test termination were in the range of 49.8% - 76.2% results for *V. beccabunga* are based on nominal water concentrations, too. The measured concentrations in samples from vessels with *S. erectum* ranged from 26.0% to 63.6% of nominal at test initiation and from 15.2% to 51.6% of nominal at test termination. The low recovery could be only explained by interception of the leaves as verification samples of the additional plant free vessels demonstrated very good recovery (*i.e.* 83.5% - 121.7%). The biological results for this plant species are thus based on recalculated, initial measured water concentrations.

Biological results: No morphological effects on the monocotyledonous plants were observed in the controls and at any of the concentrations tested. At test termination some plants of the dicotyledonous species *Mentha aquatica* were yellowish and cambered at test item concentrations of 0.197 mg BAS 656 08 H/L (0.124 mg a.s./L) and higher, furthermore the plants appeared smaller with increasing test item concentrations. The root formation of *Ludwigia palustris* and *Veronica beccabunga* was affected at concentrations of 0.086 mg BAS 656 08 H/L (0.054 mg a.s./L) and higher at test termination. For *Veronica beccabunga* the effect was not uniform and some replicates at the two highest test item concentrations showed strong root formation.

The most sensitive species in this test was the dicotyledonous *Ludwigia palustris* which showed statistically significant effects compared to the control at the 6 highest test item concentrations (William's test, $p \leq 0.05$) based on both wet weight and length data after exposure over 13 days. Effects on plant growth are summarized in Table 8.2.7-13 to Table 8.2.7-18 separately for each tested plant species.

Table 8.2.7-13: Effect of dimethenamid-P on the growth of the aquatic plant *Acorus calamus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.015**	**0.034**	**0.078**	**0.177**	**0.404**	**0.921**	**2.098**
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Inhibition in 13 d [%] # (growth rate based on wet weight)	-13.6	-27.5	-33.0	-12.9	15.9	11.5	13.0
Inhibition in 13 d [%] # (growth rate based on leave length)	-18.7	-6.1	4.5	-15.5	-19.7	-23.3	0.6
Inhibition in 13 d [%] # (growth rate based on root weight)	-4.8	-13.6	-11.4	-3.6	0.1	1.1	4.0

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_rC_{50} based on wet weight, leave length and root formation (13 d)	> 2.098	> 1.324
NOEC based on wet weight, leave length and root weight (13 d)	≥ 2.098	≥ 1.324

Negative values indicate stimulated growth compared to the control.

Table 8.2.7-14: Effect of dimethenamid-P on the growth of the aquatic plant *Iris pseudacorus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.015**	**0.034**	**0.078**	**0.177**	**0.404**	**0.921**	**2.098**
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Inhibition in 13 d [%] # (growth rate based on wet weight)	8.4	-13.7	41.7	37.9	64.8 *	44.3 *	82.9 *
Inhibition in 13 d [%] # (growth rate based on leave length)	9.3	9.7	16.0	26.3	23.4	47.6 *	36.6 *
Inhibition in 13 d [%] # (growth rate based on root weight)	7.8	-15.5	26.3 *	18.6 *	24.4 *	25.1 *	38.1 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_rC_{50} based on wet weight (13 d)	0.363 (95% confidence limits: 0.337 - 0.392)	0.229 (95% confidence limits: 0.213 - 0.247)
E_rC_{50} based on leave length and root formation (13 d)	> 2.098	> 1.324
NOEC based on wet weight (13 d)	0.177	0.112
NOEC based on leave length (13 d)	0.404	0.255
NOEC based on root formation (13 d)	0.034	0.022

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-15: Effect of dimethenamid-P on the growth of the aquatic plant *Sparganium erectum*

Concentration [mg BAS 656 08 H/L] (nominal)	0.015	0.034	0.078	0.177	0.404	0.921	2.098
Concentration [mg dimethenamid-P/L] (nominal)	**0.010**	**0.022**	**0.049**	**0.112**	**0.255**	**0.581**	**1.324**
Concentration [mg BAS 656 08 H/L] (initial measured)	**0**	**0.009**	**0.023**	**0.085**	**0.257**	**0.506**	**0.905**
Concentration [mg dimethenamid-P/L] (initial measured)	**0**	**0.006**	**0.014**	**0.054**	**0.162**	**0.319**	**0.572**
Inhibition in 13 d [%] # (growth rate based on wet weight)	-5.7	-8.2	15.2	-14.1	32.7 *	42.6 *	53.7 *
Inhibition in 13 d [%] # (growth rate based on leave length)	-3.7	19.2	26.0	-20.6	33.7	63.7 *	13.9 *
Inhibition in 13 d [%] # (growth rate based on root weight)	-14.4	-12.7	-8.7	-17.7	26.4 *	25.7 *	26.7 *

Endpoints	**related to BAS 656 08 H [mg/L] (initial measured)**	**related to dimethenamid-P [mg/L] (initial measured)**
E_rC_{50} based on wet weight (13 d)	0.720	0.455
E_rC_{50} based on leave length and root weight (13 d)	> 0.905	> 0.572
NOEC based on wet weight and root weight (13 d)	0.085	0.054
NOEC based on leave length (13 d)	0.257	0.162

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-16: Effect of dimethenamid-P on the growth of the aquatic plant *Mentha aquatica*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg dimethenamid-P/L] (nominal)	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Inhibition in 13 d [%] # (growth rate based on wet weight)	-27.3	-4.5	-23.0	18.3	41.6	-12.9	30.6
Inhibition in 13 d [%] # (growth rate based on shoot length)	-20.1	17.2	34.3	40.5	85.5 *	44.8 *	56.6 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_rC_{50} based on wet weight (13 d)	> 2.332	> 1.472
E_rC_{50} based on shoot length (13 d)	0.441 (95% confidence limits: 0.400 - 0.487)	0.278 (95% confidence limits: 0.252 - 0.307)
E_rC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	≥ 2.332	≥ 1.472
NOEC based on shoot length (13 d)	0.197	0.124
NOEC based on root weight (13 d)	0.086 +	0.054 +

n.d. = not determined
# Negative values indicate stimulated growth compared to the control.
+ based on visual observation
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-17: Effect of dimethenamid-P on the growth of the aquatic plant *Ludwigia palustris*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg dimethenamid-P/L] (nominal)	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Inhibition in 13 d [%] (growth rate based on wet weight)	12.1	39.3 *	59.8 *	59.8 *	71.8 *	85.2 *	76.6 *
Inhibition in 13 d [%] (growth rate based on shoot length)	10.4	36.5 *	61.3 *	68.3 *	82.3 *	98.3 *	96.6 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_rC_{50} based on wet weight (13 d)	0.098 (95% confidence limits: 0.089 - 0.107)	0.062 (95% confidence limits: 0.056 - 0.068)
E_rC_{50} based on shoot length (13 d)	0.075 (95% confidence limits: 0.072 - 0.080)	0.047 (95% confidence limits: 0.045 - 0.050)
E_rC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight and shoot length (13 d)	0.017	0.011
NOEC based on root weight (13 d)	0.017 $^{+}$	0.011 $^{+}$

n.d. = not determined
$^{+}$ based on visual observation
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-18: Effect of dimethenamid-P on the growth of the aquatic plant *Veronica beccabunga*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.017**	**0.038**	**0.086**	**0.197**	**0.449**	**1.023**	**2.332**
Concentration [mg dimethenamid-P/L] (nominal)	**0.011**	**0.024**	**0.054**	**0.124**	**0.283**	**0.646**	**1.472**
Inhibition in 13 d [%] (growth rate based on wet weight)	1.9	10.7	35.6 *	42.8 *	44.5 *	50.4 *	63.4 *
Inhibition in 13 d [%] (growth rate based on shoot length)	10.0	35.2 *	43.3 *	46.6 *	55.2 *	67.0 *	90.0 *

Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**	**related to dimethenamid-P [mg/L] (nominal)**
E_rC_{50} based on wet weight (13 d)	0.683 (95% confidence limits: 0.615 - 0.756)	0.431 (95% confidence limits: 0.388 - 0.477)
E_rC_{50} based on shoot length (13 d)	0.205 (95% confidence limits: 0.188 - 0.222)	0.129 (95% confidence limits: 0.118 - 0.140)
E_rC_{50} based on root weight (13 d)	n.d.	n.d.
NOEC based on wet weight (13 d)	0.038	0.024
NOEC based on shoot length (13 d)	0.017	0.011
NOEC based on root weight (13 d)	0.038 $^{+}$	0.024 $^{+}$

n.d. = not determined
$^{+}$ based on visual observation
* Statistically significantly different from the control (William's test, p ≤ 0.05).

III. CONCLUSION

In a 13-day static toxicity test with six emergent aquatic plant species, *Ludwigia palustris* was the most sensitive species generating an E_rC_{50} of 0.098 mg BAS 656 08 H/L (0.062 mg dimethenamid-P/L) based on wet weight and an E_rC_{50} of 0.075 mg BAS 656 08 H/L (0.047 mg a.s./L) based on length data (nominal).

Report: CA 8.2.7/15
Kubitza J.,Dohmen G.P., 2003a
Effect of Dimethenamid-P - Tested as formulated product BAS 656 08 H - on submersed aquatic plants
2002/1012789
Guidelines: HARAP, CLASSIC
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The effect of dimethenamid-P (tested with the formulated product BAS 656 08 H) on the growth of the submersed aquatic plant species *Elodea densa, Vallisneria spiralis, Potamogeton crispus, Ceratophyllum demersum, Myriophyllum spicatum* and *Crassula recurva* was studied in a static toxicity test including sediment over 9 to 12-days. The following nominal concentrations were applied: 0 (control), 0.0015, 0.0030, 0.0063, 0.0146, 0.0634 and 0.526 mg BAS 656 08 H/L, (corresponding to 0, 0.00096, 0.00189, 0.0040, 0.0092, 0.040 and 0.332 mg dimethenamid-P/L (nominal)). Assessment of growth was conducted at the beginning and at the end of the test based on wet weight and plant length. Visual observations were made one day after treatment, one week after treatment and at the end of the test. The percentage inhibition relative to the control was calculated for each test concentration based upon growth rates for the parameters wet weight and plant length.

The biological results are based on nominal concentrations of the test item. At the highest test item concentration plant tips of *E. densa* appeared slightly dark colored at test termination the plants in one replicate had no side branches. Root formations and side branch development of *E. densa* was generally normal except for the highest test concentration. The leaves of *P. crispus* were necrotic in the highest test item concentration one week after treatment and in the three highest test item concentrations at test termination. Furthermore, no root formation was observed, neither in the controls nor in the test item treatments. Regarding *V. spiralis*, no particular test substance related visual assessments were made throughout the test. At test termination shoot tips of *C. demersum* were slightly dark colored at 0.0146 mg BAS 656 08 H/L (0.0092 mg a.s./L) and shoot-tips at the highest test item concentration were necrotic. At test termination leaves of *M. spicatum* tended to drop off when handled and the root formation was poor; however both effects were independent of the treatment level. No root formation was observed in two replicates at 0.0146 mg BAS 656 08 H/L (0.0092 mg a.s./L). Some plants of *C. recurva* showed necrotic leaf parts at the two highest test item concentrations one week after treatment and plants appeared dark colored at the highest test item concentration at test termination.
The most sensitive species in this test was the dicotyledonous plant species *C. demersum* which showed statistically significant effects compared to the control at the 4 highest test item concentrations based on plant length development after exposure over 9 days.

In a static toxicity test with six submersed aquatic plant species, *Ceratophyllum demersum* was the most sensitive species generating an E_rC_{50} of 0.067 mg BAS 656 08 H/L (0.042 mg dimethenamid-P/L) based on wet weight and an E_rC_{50} of 0.025 mg BAS 656 08 H/L (0.016 mg a.s./L) based on length data (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 08 H, batch no. 2001-1; content of a.s.: dimethenamid-P (BAS 656 H, Reg. no. 363 851): 711.4 g/L (nominal: 720.0 g/L); density: 1.127 g/cm^3.

B. STUDY DESIGN

Test species: Submersed aquatic plants;
monocotyledonous: *Elodea densa* (Hydrocharitaceae) (source: "Aquaristic Reier", Frankfurt, Germany), *Potamogeton crispus* (Potamogetonaceae) (source: field collected from river "Eisbach" near Bachern, Germany), *Vallisneria spiralis* (Hydrocharitaceae) (source: first test, "Aquaristic Reier", Frankfurt, Germany; repeated test: in-house cultivation);
dicotyledonous: *Ceratophyllum demersum* (Ceratophyllaceae) (source: field collected from a pond in "Fischach" near Augsburg, Germany), *Myriophyllum spicatum* (Haloragaceae) (in-house cultivation), *Crassula recurva* (Crassulaceae) (source: garden market "Glaß", Augsburg, Germany).

Test design: Static system (including sediment), test duration 9 - 12 days (plant dependent), 6 test item concentrations, each with 3 replicates plus a control with 6 replicates; the number of plants per replicate (one to several) varied depending on the test species; the plants (except *C. demersum*) were potted in sediment; *C. demersum* was kept free floating within the vessels; nevertheless pots with the same sediment were placed on the bottom of the vessels; visual observations one day after treatment, one week after treatment and at the end of the test; determination of wet weight and plant length at test end, visual assessment of root formation. At test end the potential for recovery was tested in the control and the three highest test item concentrations for all tested species (except for *V. spiralis*) over 9, respectively 10 days.

Endpoints: EC_{50} with respect to growth rate related to wet weight and length data after exposure over 9 to 12 days (plant dependent).

Test concentrations: Control, 0.0015, 0.0030, 0.0063, 0.0146, 0.0634 and 0.526 mg BAS 656 08 H/L (nominal), corresponding to 0, 0.00096, 0.00189, 0.0040, 0.0092, 0.040 and 0.332 mg dimethenamid-P/L (nominal).

Test conditions: 2 L glass beakers, standard artificial sediment (OECD 219 amended with 1 g plant fertilizer per pot) and 3 x AAP medium (amended with 2.24 mg/L $FeCl_3$, pH 6.5); oxygen content: 7.5 mg/L - 9.4 mg/L: 80.0% - 248.0%; pH: 7.27 - 10.95; conductivity: 268 - 283 µS/cm; carbonate hardness: 0.33 - 0.35 mmol/L; water temperature: 19.8 °C - 24.0 °C; air temperature: 21 ± 1 °C; light : dark - rhythm 16 : 8 h, light intensity: approx.. 8.5 klux.

Analytics: Analytical verification of test item concentrations was conducted using a SPME-method with GC/MS detection.

Statistics: Descriptive statistics; determination of EC_{50} values by probit analysis, William's, Dunnett`s and Bonferroni test ($p \leq 0.05$) for determination of NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted for each tested plant species in all test item concentrations at the beginning and at the end of the test, except for the two lowest test concentrations which were below the limit of quantification. The measured concentrations of the test item for all plant species ranged from 80.2% to 112.0% of nominal concentrations at test initiation and from 46.7% to 99.8% of nominal at test termination. As the analytically measured values at test initiation confirmed the correct application of the test item, the following biological results are based on nominal test concentrations.

Biological results: At the highest test item concentration plant tips of *E. densa* appeared slightly dark colored at test termination the plants in one replicate had no side branches. Root formations and side branch development of *E. densa* was generally normal except for the highest test concentration. The leaves of *P. crispus* were necrotic in the highest test item concentration one week after treatment and in the three highest test item concentrations at test termination. Furthermore, no root formation was observed, neither in the control nor in the test item treatments. Regarding *V. spiralis*, no particular test substance related visual assessments were made throughout the test. At test termination shoot tips of *C. demersum* were slightly dark colored at 0.0146 mg BAS 656 08 H/L (0.0092 mg a.s./L) and shoot-tips at the highest test item concentration were necrotic. At test termination leaves of *M. spicatum* tended to drop off when handled and the root formation was poor; however both effects were independent of the treatment level. No root formation was observed in two replicates at 0.0146 mg BAS 656 08 H/L (0.0092 mg a.s./L). Some plants of *C. recurva* showed necrotic leaf parts at the two highest test item concentrations one week after treatment and plants appeared dark colored at the highest test item concentration at test termination.
The most sensitive species in this test was the dicotyledonous *C. demersum* which showed statistically significant effects compared to the control at the 4 highest test item concentrations (William's test, $p \leq 0.05$) based on plant length development after exposure over 9 days. The recovery test showed that all plant species were able to recover rapidly from a previous treatment with the test item.
Effects on plant growth are summarized in Table 8.2.7-19 to Table 8.2.7-24 separately for each plant species.

Table 8.2.7-19: Effect of dimethenamid-P on the growth of the aquatic plant *Elodea densa*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 12 d [%] # (growth rate based on wet weight)	-36.5	-9.4	-10.4	-10.3	15.4	32.2 *
Inhibition in 12 d [%] # (growth rate based on length)	-18.4	-55.5	-78.9	-51.4	-0.9	66.3 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight (12 d)	> 0.526			> 0.332		
E_rC_{50} based on length (12 d)	0.320 +			0.202 +		
NOEC based on wet weight and length (12 d)	0.0634			0.040		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).
+ Graphical determination

Table 8.2.7-20: Effect of dimethenamid-P on the growth of the aquatic plant *Potamogeton crispus*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 9 d [%] # (growth rate based on wet weight)	-1.8	-16.6	6.9	-8.5	2.8	3.1
Inhibition in 9 d [%] # (growth rate based on length)	-9.8	-26.9	2.7	-4.2	8.6	56.4 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight (9 d)	> 0.526			> 0.332		
E_rC_{50} based on length (9 d)	0.444 (95% confidence limits: 0.396 - 0.498)			0.280 (95% confidence limits: 0.250 - 0.314)		
NOEC based on wet weight (9 d)	≥ 0.526			≥ 0.332		
NOEC based on length (9 d)	0.0634			0.040		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-21: Effect of dimethenamid-P on the growth of the aquatic plant *Vallisneria spiralis*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 12 d [%] # (growth rate based on wet weight)	-3.3	-3.8	1.3	-3.8	3.8	-1.8
Inhibition in 12 d [%] # (growth rate based on length)	12.0	10.9	5.7	0.2	8.0	7.3
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight and length (12 d)	> 0.526			> 0.332		
NOEC based on wet weight and length (12 d)	≥ 0.526			≥ 0.332		

Negative values indicate stimulated growth compared to the control.

Table 8.2.7-22: Effect of dimethenamid-P on the growth of the aquatic plant *Ceratophyllum demersum*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 9 d [%] # (growth rate based on wet weight)	-3.8	-7.7	10.6	33.2 *	64.8 *	72.8 *
Inhibition in 9 d [%] # (growth rate based on length)	13.7	3.3	27.7 *	31.4 *	74.8 *	94.0 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight (9 d)	0.067 (95% confidence limits: 0.062 - 0.074)			0.042 (95% confidence limits: 0.039 - 0.047)		
E_rC_{50} based on length (9 d)	0.025 (95% confidence limits: 0.023 - 0.026)			0.016 (95% confidence limits: 0.015 - 0.016)		
NOEC based on wet weight (9 d)	0.0063			0.0040		
NOEC based on length(9 d)	0.0030			0.0019		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-23: Effect of dimethenamid-P on the growth of the aquatic plant *Myriophyllum spicatum*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 9 d [%] # (growth rate based on wet weight)	-18.6	-16.0	-15.3	-16.0	-38.6	-17.3
Inhibition in 9 d [%] # (growth rate based on length)	-20.7	-49.8	-55.2	16.3	43.3 *	66.8 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight (9 d)	> 0.526			> 0.336		
E_rC_{50} based on length (9 d)	0.152 (95% confidence limits: 0.138 - 0.168)			0.096 (95% confidence limits: 0.087 - 0.106)		
NOEC based on wet weight (9 d)	≥ 0.526			≥ 0.332		
NOEC based on length (9 d)	0.0146			0.0092		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

Table 8.2.7-24: Effect of dimethenamid-P on the growth of the aquatic plant *Crassula recurva*

Concentration [mg BAS 656 08 H/L] (nominal)	**0.0015**	**0.0030**	**0.0063**	**0.0146**	**0.0634**	**0.526**
Concentration [mg dimethenamid-P/L] (nominal)	**0.00096**	**0.00189**	**0.0040**	**0.0092**	**0.040**	**0.332**
Inhibition in 12 d [%] # (growth rate based on wet weight)	-27.0	7.2	24.8	9.4	18.6	7.7
Inhibition in 12 d [%] # (growth rate based on length)	9.0	-5.1	-23.3	-14.5	33.8	79.2 *
Endpoints	**related to BAS 656 08 H [mg/L] (nominal)**			**related to dimethenamid-P [mg/L] (nominal)**		
E_rC_{50} based on wet weight (12 d)	> 0.526			> 0.336		
E_rC_{50} based on length (12 d)	0.156 (95% confidence limits: 0.141 - 0.173)			0.098 (95% confidence limits: 0.089 - 0.109)		
NOEC based on wet weight (12 d)	≥ 0.526			≥ 0.336		
NOEC based on length (12 d)	0.0634			0.040		

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different from the control (William's test, $p \leq 0.05$).

III. CONCLUSION

In a static toxicity test with six submersed aquatic plant species, *Ceratophyllum demersum* was the most sensitive species generating an E_rC_{50} of 0.067 mg BAS 656 08 H/L (0.042 mg dimethenamid-P/L) based on wet weight and an E_rC_{50} of 0.025 mg BAS 656 08 H/L (0.016 mg a.s./L) based on length data (nominal).

CA 8.2.8 Further testing on aquatic organisms

No further studies required; thus, this point is not addressed *via* new toxicity studies.

REFERENCES

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SANCO (2002) Guidance document on Aquatic Ecotoxicology. Working document in the context of the Directive 91/414/EEC. European Commission, Health & Consumer Protection Directorate-General. SANCO/3268/2001 rev. 4 (final), 17 October 2002.

CA 8.3 Effects on arthropods

Since Annex I inclusion of dimethenamid-P (BAS 656 PH), new toxicity studies on the active substance on bees have been performed and as a result there are new endpoints which are now used in the risk assessment. Summaries of these new studies are provided below.

Table 8.3-1 Toxicity to arthropods of dimethenamid-P

Test substance	Test species	Endpoint	Value	Reference	Study EU agreed?
dimethenamid-P	honeybee	24 h acute oral LD_{50}	> 1000 µg a.s./bee	Donat 1986/11170	Yes [1)]
		24 h acute contact LD_{50}	94 µg a.s./bee		
	honeybee	48 h acute oral LD_{50}	118.8 µg a.s./bee	Zenker 2010/1126065	No, new study
		48 h acute contact LD_{50}	93.8 µg a.s./bee		
	honeybee larvae	96 h oral LD_{50} 96 h oral LC_{50}	69.6 µg a.s./larva 2.054 g a.s./kg food.	Kleebaum 2013/1132510	No, new study
	bumblebee	48 h acute oral LD_{50}	> 158 µg a.s./bumblebee	Roehlig, 2013/1275562	No, new study
		48 h acute contact LD_{50}	> 200 µg a.s./bumblebee		

[1)] In the risk assessment endpoints obtained in the 48 h acute contact and oral study replace the endpoints from the 24 h study.

CA 8.3.1 Effects on bees

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report: CA 8.3.1.1.1/1
Zenker K., 2011a
Acute toxicity of BAS 656-H (Reg.No. 363 851, Dimethenamid-P) to the honeybee Apis mellifera L. under laboratory conditions
2010/1126065
Guidelines: OECD 213 (1998), OECD 214 (1998)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an oral dose response toxicity test, young adult worker bees (*Apis mellifera* L.) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.9, 25.8, 51.7, 103.3 and 206.6 µg test item/bee, resulting in an actual uptake of 12.9, 25.8, 51.1, 101.8 and 193.2 µg test item/bee. This results in an uptake of dimethenamid-P of 12.5, 25.0, 49.5, 98.6 and 187.0 µg a.s./bee. Additionally, honeybees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.063 to 0.500 µg dimethoate/bee (analysed) or with a solution of water and sucrose or of water, sucrose and acetone as controls. The test was conducted with 3 replicates each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, a mortality of 3.3% was observed in both controls. In the test item treated groups, mortalities between 0.0% and 90.0% were observed. At the highest tested doses of 98.6 µg and 187.0 µg dimethenamid-P/bee statistically significant differences compared to the control were observed. Some of the surviving bees treated with 98.6 µg or 187.0 µg dimethenamid-P/bee showed an abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.

In an acute oral toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 118.8 µg dimethenamid-P/bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. 6261B01BH, purity: 96.8% (± 1%).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera carnica* P.), young adult worker bees, age: 3 -5 weeks, deriving from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany; collected in the morning of use.

Test design: Dose response test for oral toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioral abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: 50% (w/v) aqueous sucrose solution, 50% (w/v) aqueous sucrose solution + 1% v/v Acetone; test item: 12.9, 25.8, 51.7, 103.3 and 206.6 µg/bee, resulting in an actual uptake of 12.9, 25.8, 51.1, 101.8 and 193.2 µg/bee. This results in an uptake of dimethenamid-P of 12.5, 25.0, 49.5, 98.6 and 187.0 µg a.s./bee.

Test conditions: Temperature: 24.8 – 25.2 °C; relative humidity: 59% - 61%, photoperiod: 24 h darkness. Food: 50% w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction ($\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, a mortality of 3.3% was observed in both controls. In the test item treated groups, mortalities between 0.0% and 90.0% were observed. At the highest tested doses of 98.6 µg and 187.0 µg dimethenamid-P/bee statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed. Some of the surviving bees treated with 98.6 µg and 187.0 µg dimethenamid-P/bee showed an abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.
The results are summarized in Table 8.3.1.1-1.

Table 8.3.1.1-1: Toxicity of dimethenamid-P to honeybees (*Apis mellifera carnica* P.) in an oral toxicity test

Treatment		Uptake of test item		Mortality [%]		Corrected mortality [%] [1)]	
[µg test item/bee]	[µg dimethenamid-P/bee]	[µg test item/bee]	[µg dimethenamid-P/bee]	24 h	48 h	24 h	48 h
Control (sucrose solution)	Control (sucrose solution)	--	--	3.3	3.3	--	--
Control (sucrose sol. + acetone)	Control (sucrose sol. + acetone)	--	--	0.0	3.3	--	--
12.9	12.5	12.9	12.5	3.3	3.3	--	0.0
25.8	25.0	25.8	25.0	0.0	0.0	--	-3.4
51.7	50.0	51.1	49.5	0.0	0.0	--	-3.4
103.3	100.0	101.8	98.6	33.3 *	33.3 *	--	31.0
206.6	200.0	193.2	187.0	86.7 *	90.0 *	--	89.7
Endpoint [µg/bee]							
LD_{50} (95% CL) [2)] (48 h)			based on test item		based on analysed content of dimethenamid-P in test item		
			122.7 (106.9 – 140.7)		118.8 (103.5 – 136.2)		

1) according to Abbott (1925)
2) Median lethal dose after 48 hours of exposure calculated by Probit analysis (with 95% Confidence Limits)
* Statistically significant differences compared to the control (Fisher's Exact Binominal Test, α = 0.05).

The LD_{50} value (24 h) for the toxic reference item in the oral toxicity test was LD_{50} = 0.196 µg a.s./bee (95% confidence limits: 0.158 - 0.244 µg a.s./bee).

III. CONCLUSION

In an acute oral toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 118.8 µg dimethenamid-P/bee.

Report: CA 8.3.1.1.1/2
Roehlig U., 2014a
Acute toxicity of BAS 656 H (Dimethenamid-P) to the bumblebee Bombus terrestris L. under laboratory conditions
2013/1275562

Guidelines: OECD 213 (1998), OECD 214 (1998), EFSA Guidance Document on bees (2013), Van der Steen (2009), Hanewald et al. (2013)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an oral dose response toxicity test, young adult worker bumblebees (*Bombus terrestris*) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100, and 200 µg a.s./bumblebee (based on analysed purity) and resulting in an actual uptake of 11.1, 19.7, 44.6, 87.8 and 158 µg a.s./bumblebee. Additionally, bumblebees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.25 to 1.998 µg dimethoate/bumblebee (analysed) or with a solution of water and sucrose or of water, sucrose and acetone as controls. The test was conducted with 30 replicates each contained 1 bumblebee. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of oral exposure, a mortality of 3.3% was observed in both controls. In the test item treated groups, mortalities between 0.0% and 10.0% were observed. No statistically significant differences compared to the control were observed in all treatment groups. No behavioral abnormalities of surviving bumblebees occurred throughout the oral toxicity test.

In an acute oral toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 158 µg a.s./bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. COD-001509, purity: 95.9%.

B. STUDY DESIGN

Test species: Bumblebee (*Bombus terrestris*) young adult worker bumblebees deriving from a healthy and queen-right micro-hive; source: Biobest Belgium N.V., Westerlo, Belgium and delivered by Katz Biotech AG, Baruth, Germany; individuals were collected in the morning prior to use.

Test design: Dose response test for oral toxicity; duration 48 h, 30 replicates, each replicate consisting of 1 bumblebee per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value and behavioral abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: 50% (w/v) aqueous sucrose solution, 50% (w/v) aqueous sucrose solution + 1% v/v acetone; test item: 12.5, 25.0, 50.0, 100, and 200 µg a.s./bumblebee (based on analysed purity) and resulting in an actual uptake of 11.1, 19.7, 44.6, 87.8 and 158 µg a.s./bumblebee.

Test conditions: Temperature: 24.6 – 25.6 °C; relative humidity: 58% - 62%, photoperiod: 24 h darkness. Food: 50% w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction (one-sided greater α = 0.05), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, a mortality of 3.3% was observed in both controls. In the test item treated groups, mortalities between 0.0% and 10.0% were observed. No statistically significant differences compared to the control (Fisher's Exact Binominal test, α = 0.05) were observed in all treatment groups. No behavioral abnormalities of surviving bumblebees occurred throughout the oral toxicity test. The results are summarized in Table 8.3.1.1-2.

Table 8.3.1.1-2: Toxicity of dimethenamid-P to bumblebees (*Bombus terrestris* L.) in an oral toxicity test

Treatment	Uptake of test item	Mortality [%]		Corrected mortality [%] [1)]	
[µg a.s./bumblebee]	[µg a.s./bumblebee]	24 h	48 h	24 h	48 h
Control (sucrose solution)	--	0.0	3.3	--	--
Control (sucrose sol. + acetone)	--	0.0	3.3	--	--
12.5	11.1	0.0	0.0	0.0	0.0
25.0	19.7	3.3	3.3	0.0	0.0
50.0	44.6	3.3	6.7	0.0	3.4
100	87.8	6.7	10.0	0.0	6.9
200	158	3.3	10.0	0.0	6.9
Endpoint [µg a.s./bumblebee]					
LD_{50} (48 h)	> 158				

[1)] Calculated from mean mortality data in the 1 % acetone control group, according to the formula of Abbott (1925), corrected by Schneider-Orelli (1947); negative values are given as 0.0.

The LD_{50} value (24 h) for the toxic reference item in the oral toxicity test was LD_{50} = 0.504 µg a.s./bumblebee (95% confidence limits: 0.269 - 0.943 µg a.s./bumblebee).

III. CONCLUSION

In an acute oral toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 158 µg a.s./bumblebee.

CA 8.3.1.1.2 Acute contact toxicity

Report: CA 8.3.1.1.2/1
Zenker K., 2011a
Acute toxicity of BAS 656-H (Reg.No. 363 851, Dimethenamid-P) to the honeybee Apis mellifera L. under laboratory conditions
2010/1126065
Guidelines: OECD 213 (1998), OECD 214 (1998)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a contact dose response toxicity test, young adult worker bees (*Apis mellifera* L.) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg dimethenamid-P/bee (based on analysed purity). Additionally, honeybees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.063 to 0.500 µg dimethoate/bee (analysed) and with deionized water, Tween solution and Acetone as control. The test was conducted with 3 replicates each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of contact exposure, a mortality of 0.0% was observed in all controls. In the test item treated groups, mortalities between 0.0% and 100.0% were observed. At the highest tested doses of 100.0 µg and 200.0 µg dimethenamid-P/bee statistically significant differences compared to the control were observed. 92% of the surviving bees treated with 100.0 µg dimethenamid-P/bee showed some abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.

In an acute contact toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 93.8 µg dimethenamid-P/bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. 6261B01BH, purity: 96.8% (± 1%).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera carnica* P.), young adult worker bees, age: 3 -5 weeks, deriving from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany; collected in the morning of use.

Test design: Dose response test for contact toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioral abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: deionised water, Tween control (1.0% (v/v) Tween solution), Acetone control (undiluted); test item: 12.9, 25.8, 51.7, 103.3 and 206.6 µg/bee, equivalent to 12.5, 25.0, 50.0, 100.0 and 200.0 µg dimethenamid-P/bee (based on analysed purity).

Test conditions: Temperature: 24.8 – 25.2 °C; relative humidity: 59% - 61%, photoperiod: 24 h darkness. Food: 50% w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction ($\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, a mortality of 0.0% was observed in all controls. In the test item treated groups, mortalities between 0.0% and 100.0% were observed. At the highest tested doses of 100.0 µg and 200.0 µg dimethenamid-P/bee statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed. 92% of the surviving bees treated with 100.0 µg dimethenamid-P/bee showed some abnormal behaviour after 48 hours, like uncoordinated movements or lying on the back.
The results are summarized in Table 8.3.1.1-3.

Table 8.3.1.1-3: Toxicity of dimethenamid-P to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment [µg test item/bee]	Treatment [µg dimethenamid-P/bee]	Mortality [%]		Corrected mortality [%]	
		24 h	48 h	24 h	48 h
Control (water)	--	0.0	0.0	--	--
Tween control	--	0.0	0.0	--	--
Acetone control	--	0.0	0.0	--	--
12.9	12.5	0.0	0.0	--	--
25.8	25.0	0.0	3.3	--	--
51.7	50.0	0.0	3.3	--	--
103.3	100.0	46.7 *	56.7 *	--	--
206.6	200.0	100.0 *	100.0 *	--	--
206.6		**Endpoint [µg/bee]**			
LD_{50} (95% CL) [1)] (48 h)		based on test item		based on analysed content of dimethenamid-P in the test item	
		96.9 (76.2 – 123.2)		93.8 (73.8 – 119.3)	

[1)] Median lethal dose after 48 hours of exposure calculated by Probit analysis (with 95% Confidence Limits)
* Statistically significant differences compared to the control (Fisher's Exact Binominal Test α = 0.05).

The LD_{50} value (24 h) for the toxic reference item in the contact toxicity test was LD_{50} = 0.147 µg a.s./bee (95% confidence limits: 0.113 - 0.190 µg a.s./bee).

III. CONCLUSION

In an acute contact toxicity study with dimethenamid-P on honeybees the LD_{50} value (48 h) was determined to be 93.8 µg dimethenamid-P/bee.

Report: CA 8.3.1.1.2/2
Roehlig U., 2014b
Acute toxicity of BAS 656 H (Dimethenamid-P) to the bumblebee Bombus terrestris L. under laboratory conditions
2013/1275562

Guidelines: OECD 213 (1998), OECD 214 (1998), EFSA Guidance Document on bees (2013), Van der Steen (2009), Hanewald et al. (2013)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a contact dose response toxicity test, young adult worker bumblebees (*Bombus terrestris*) were exposed to dimethenamid-P. The toxicity of the test item was determined at doses of 12.5, 25.0, 50.0, 100, and 200 µg a.s./bumblebee (based on analysed purity). Additionally, bumblebees were treated with Dimethoate EC 400 as toxic reference item at doses ranging from 0.25 to 1.999 µg dimethoate/bee (analysed) and with deionized water, Tween solution and Acetone as control. The test was conducted with 3 replicates each of the test cages contained 10 bumblebees. Assessment of mortality was done after 4, 24 and 48 hours.

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionized water or tween solution; 3.3 % mortality occurred in the acetone control group. In the test item treated groups, mortalities between 0.0% and 3.3% were observed. No statistically significant differences compared to the control were observed in all treatment groups. No behavioral abnormalities of surviving bumblebees occurred throughout the contact toxicity test.

In an acute contact toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 200 µg a.s./bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, Reg. No. 363 851), batch no. COD-001509, purity: 95.9%.

B. STUDY DESIGN

Test species: Bumblebee (*Bombus terrestris*) young adult worker bumblebees deriving from a healthy and queen-right micro-hive; source: Biobest Belgium N.V., Westerlo, Belgium and delivered by Katz Biotech AG, Baruth, Germany; collected in the morning prior to use.

Test design: Dose response test for contact toxicity; duration 48 h, 3 replicates, each replicate consisting of 10 bumblebees per cage, assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioral abnormalities.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Controls: deionised water, Tween control (deionised water plus 1.0% (v/v) Tween solution), acetone control (pure acetone); test item: 12.5, 25.0, 50.0, 100.0, and 200 µg a.s./bumblebee (based on analysed purity).

Test conditions: Temperature: 24.6 – 25.6 °C; relative humidity: 58% - 62%, photoperiod: 24 h darkness. Food: 50% w/v sucrose solution.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni correction (one-sided greater $\alpha = 0.05$), Probit analysis for determination of the LD_{50} values.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionized water or tween solution; 3.3 % mortality occurred in the acetone control group. In the test item treated groups, mortalities between 0.0% and 3.3% were observed. No statistically significant differences compared to the control (Fisher's Exact Binominal test, $\alpha = 0.05$) were observed in all treatment groups. No behavioral abnormalities of surviving bumblebees occurred throughout the contact toxicity test. The results are summarized in Table 8.3.1.1-4.

Table 8.3.1.1-4: Toxicity of dimethenamid-P to bumblebees (*Bombus terrestris* L.) in a contact toxicity test

Treatment [µg a.s./bumblebee]	Mortality [%]		Corrected mortality [%]	
	24 h	48 h	24 h	48 h
Control (water)	0.0	0.0	--	--
Tween control	0.0	0.0	--	--
Acetone control	0.0	3.3	--	--
12.5	0.0	0.0	0.0	0.0
25.0	0.0	0.0	0.0	0.0
50.0	0.0	3.3	0.0	0.0
100	0.0	0.0	0.0	0.0
200	0.0	3.3	0.0	0.0
	Endpoint [µg a.s./bumblebee]			
LD_{50} (48 h)	> 200			

[1)] Calculated from mean mortality data in the 1 % acetone control group, according to the formula of Abbott (1925), corrected by Schneider-Orelli (1947);, negative values are given as 0.0.

The LD_{50} value (24 h) for the toxic reference item in the contact toxicity test was LD_{50} = 1.122 µg a.s./bumblebee (95% confidence limits: 0.907 - 1.387 µg a.s./bumblebee).

III. CONCLUSION

In an acute contact toxicity study with dimethenamid-P on bumblebees the LD_{50} value (48 h) was determined to be > 200 µg a.s./bumblebee.

CA 8.3.1.2 Chronic toxicity to bees

No new studies are available.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report: CA 8.3.1.3/1
Kleebaum K., 2014a
Acute toxicity of BAS 656 H (Dimethenamid-P) to honeybee larvae (Apis melifera L.) under laboratory conditions (in vitro)
2013/1132510

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 toxicity test, honeybee larvae (*Apis mellifera carnica* P.) were exposed to BAS 656 H (dimethenamid-P). The toxicity of the test item was determined at doses of 12.4, 24.8, 49.6, 99.2 and 198.5 µg a.s./larva. The corresponding concentrations of the test item in the diet were 0.366, 0.732, 1.464, 2.927 and 5.855 g a.s./kg.
Additionally, honeybee larvae were treated with dimethoate as reference item or with an untreated control and a solvent control.

After 72 hours of oral exposure, a mortality of 5.6 % was observed in both controls. In the test item group, mortalities ranged between 2.8 and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg a.s./larva with mortalities of each 100%.
After 96 hours of oral exposure, a mortality of 8.3 % was observed in both controls. In the test item group, mortalities ranged between 8.3 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg a.s./larva with mortalities of each 100%..

After 72 hours of exposure 3.0 %, 13.0 % and 30.3 % of the surviving larvae showed deviations to the normal food consuming behavior. These deviations occurred proportionally to the test item doses (being 12.4, 24.6 and 49.6 µg a.s./larva, respectively).
96 hours after treatment with 12.4, 24.6 and 49.6 µg a.s./larva, 3.3 %, 0.0 % and 12.1 % of the surviving larvae showed deviations to the normal food consuming behavior and correspondingly to develop into an average sized larva. Still, a dose-relation could be detected. Nevertheless, if compared to the previous day it becomes obvious, that development of some larvae was only slightly delayed and not irreversibly disturbed.

In an acute larval toxicity test with BAS 656 H (dimethenamid-P), the LD_{50} (72 h) was determined to be 65.8 µg a.s./larva, which is equivalent to an LC_{50} (72 h) of 1.941 g a.s./kg food. The LD_{50} (96 h) was determined to be 69.6 µg a.s./larva, which is equivalent to a LC_{50} (96 h) of 2.054 g a.s./kg food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 H (dimethenamid-P) (Reg. No. 363851), batch no.: COD-001509; Content of active substance: analyzed purity of 95.9 % (*w/w*) (tolerance ± 1.0 %).

Test species: *Apis mellifera carnica* P. (honeybee), first instar larvae; derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

B. STUDY DESIGN

Test design: One day old honeybee larvae (D1) of *Apis mellifera carnica* P. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. Afterwards, in a 72 hour acute test, the 4 day old (D4) larvae were exposed to a single application of BAS 656 H (dimethenamid-P) diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 3 treatment groups were set up: 5 doses of the test item, two untreated control groups (with and without solvent) and 4 doses of the reference item with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 hours (D5) and 48 hours later (D6). Assessments of larval mortality were done after 24, 48, 72 and 96 hours (respectively D5, D6, D7 and D8). Additionally, other observations as small body size or large quantities of remaining food on D7 and D8 were noted. In an analytical phase of the study the concentration of the active substance in the test item stock solution A was determined.

Endpoint: Mortality, body size, food uptake.

Reference item: Dimethoate (99.8 % *w/w* analyzed).

Test concentrations: control (untreated diet: aqueous sugar solution with gelee royal, 1:1) and solvent control (aqueous sugar solution with gelee royal, 1:1 including 1% (*v/v*) acetone); BAS 656 H: 12.4, 24.8, 49.6, 99.2 and 198.5 µg a.s./larva, corresponding to 0.366, 0.732, 1.464, 2.927 and 5.855 g a.s./kg food (nominal); reference item: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/larva.

Test conditions: Temperature: 34.0 °C – 34.5 °C, relative humidity: 92 % - 96 % with two short periods of lower humidity, photoperiod: darkness (except during assessments), food: aqueous sugar solution mixed with gelee royal (1:1).

Statistics: Descriptive statistics; Fisher's Exact Binomial test with Bonferroni Correction for mortality data and no effect levels (one-sided greater, $\alpha = 0.05$); Probit and Weibull analysis for calculation of LC_{50} and LD_{50} values.

II. RESULTS AND DISCUSSION

After 72 hours of oral exposure, a mortality of 5.6 % was observed in both controls. In the test item group, mortalities ranged between 2.8 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg a.s./larva with mortalities of each 100% (Fisher's Exact Binomial test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
After 96 hours of oral exposure, a mortality of 8.3 % was observed in both controls. In the test item group, mortalities ranged between 8.3 % and 100.0 %. Statistically significant effects on survival occurred at the two highest test item doses of 99.2 and 198.5 µg a.s./larva with mortalities of each 100% (Fisher's Exact Binomial test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
After 72 hours of exposure 3.0 %, 13.0 % and 30.3 % of the surviving larvae showed deviations to the normal food consuming behavior. These deviations occurred proportionally to the test item doses (being 12.4, 24.6 and 49.6 µg a.s./larva, respectively).
96 hours after treatment with 12.4, 24.6 and 49.6 µg a.s./larva, 3.3 %, 0.0 % and 12.1 % of the surviving larvae showed deviations to the normal food consuming behavior and correspondingly to develop into an average sized larva. Still a dose-relation could be detected. Nevertheless, if compared to the previous day it becomes obvious, that development of some larvae was only slightly delayed and not irreversibly disturbed.

The LD_{50} (96 h) was determined to be 69.6 µg a.s./larva, which is equivalent to a LC_{50} (96 h) of 2.054 g a.s./kg food.

The results are summarized in Table 8.3.1.3-1.

Table 8.3.1.3-1: Toxicity of BAS 656 H (Dimethenamid-P) to *Apis mellifera carnica* P. in an acute larval toxicity test

Treatment		Mortality after 72 hours			Mortality after 96 hours		
Dosage [µg a.s./larva]	Concentration [g a.s./kg food]	mean mortality [%]	corrected mortality	mean other observations [%] 1)	mean mortality [%]	corrected mortality	mean other observations [%] 1)
Control	--	5.6	--	0.0	8.3	--	0.0
Solvent control	--	5.6	--	0.0	8.3	--	0.0
12.4	0.366	2.8	0.0	3.0	8.3	0.0	3.3
24.8	0.732	8.3	2.9	13.0	13.9	6.1	0.0
49.6	1.464	8.3	2.9	30.3	8.3	0.0	12.1
99.2	2.927	100.0 *	100.0	--	100.0 *	100.0	--
198.5	5.855	100.0 *	100.0	--	100.0 *	100.0	--

Endpoints		72 hours	96 hours
Test item dose [µg a.s./larva]	LD_{50} (95% CL)	65.8 (45.9 – 94.3)	69.6 (48.8 – 99.4)
	NOED	49.6	49.6
Test item concentrations [g a.s./kg food]	LC_{50} (95% CL)	1.941 (1.353 – 2.783)	2.054 (1.438 – 2.933)
	NOEC	1.464	1.464

* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher`s Exact Binominal Test with Bonferroni Correction; α = 0.05; one sided greater).
1) Other observations (large quantities of remaining food, smaller body size of larva).

The LD_{50} value (72 h) for the reference item in the acute larval toxicity test could not be determined. Mortality at 8.8 µg/larvae was above 50 % across all replicates (D7/72 h), being 55.6 % (corrected for control mortality 52.9 %.)

III. CONCLUSION

In an acute larval toxicity test with BAS 656 H (Dimethenamid-P), the LD_{50} (72 h) was determined to be 65.8 µg a.s./larva, which is equivalent to an LC_{50} (72 h) of 1.941 g a.s./kg food. The LD_{50} (96 h) was determined to be 69.6 µg a.s./larva, which is equivalent to a LC_{50} (96 h) of 2.054 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 dimethenamid-P (BAS 656 PH), new toxicity studies on the active substance and its metabolites have been performed and as a result there are new endpoints which are now used in the risk assessment. Summaries of these new studies are provided below. For codes and synonyms of the metabolites of dimethenamid-P please refer to DOCUMENT N3.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of dimethenamid-P and relevant metabolites

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	Reference	Study EU agreed?
dimethenamid-P	*Eisenia fetida*	LC_{50}	294.4 147.2 [1)]	van Dijk 1988/11372	Yes
M656H023	*Eisenia fetida*	LC_{50}	> 1264 > 632 [1)]	Krieg 1998/10299	Yes
M656H027	*Eisenia fetida*	LC_{50}	> 1264 > 632 [1)]	Krieg 1998/10300	Yes
dimethenamid-P	*Eisenia fetida*	NOEC	25.4	Friedrich 2012/1129456	No, new study
M656H023	*Eisenia fetida*	NOEC	≥ 8.32	Luehrs 2007/1037731	No, new study
M656H027	*Eisenia fetida*	NOEC	≥ 10.56	Luehrs 2007/1037732	No, new study
M656H031	*Eisenia fetida*	NOEC	≥ 100	Luehrs 2008/1070910	No, new study
dimethenamid-P	*Folsomia candida*	NOEC	25	Friedrich 2011/1000481	No, new study
M656H023	*Folsomia candida*	NOEC	≥ 200	Friedrich 2012/1129536	No, new study
M656H027	*Folsomia candida*	NOEC	≥ 200	Friedrich 2012/1129537	No, new study
M656H031	*Folsomia candida*	NOEC	≥ 200	Friedrich 2011/1000222	No, new study
dimethenamid-P	*Hypoaspis aculeifer*	NOEC	500	Schulz 2012/1129457	No, new study
M656H023	*Hypoaspis aculeifer*	NOEC	100	Schulz 2012/1129538	No, new study
M656H027	*Hypoaspis aculeifer*	NOEC	≥ 200	Schulz 2012/1129539	No, new study
M656H031	*Hypoaspis aculeifer*	NOEC	≥ 500	Schulz 2013/1103674	No, new study

[1)] Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil, since the log P_{ow} of the substance is > 2 and the test was carried in 10% peat.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2012a
Sublethal toxicity of BAS 656 H (Dimethenamid-P) to the earthworm Eisenia fetida in artificial soil with 5% peat
2012/1129456
Guidelines: OECD 222 (2004)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects BAS 656 H (dimethenamid-P) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five application rates (15.0, 19.5, 25.4, 33.0 and 42.8 mg a.s./kg dry soil) were incorporated into the soil (5% peat only) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure the mortality between 0% and 5.0% in the test item groups and 1.3% in the control. No statistically significant mortality compared to the control was observed at any test item concentration. Body weight was not statistically significantly different from the control up to highest concentration of 42.8 mg a.s./kg dry soil. The reproduction rate was significantly different from the control in the two highest concentration of 33.0 and 42.8 mg a.s./kg dry soil. No behavioral abnormalities were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BAS 656 H (dimethenamid-P), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be 25.4 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 H (dimethenamid-P), batch no. COD-001509, Reg. No. 363 851, purity: 95.9%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight of 300 mg – 448 mg), approximately 3 months old; source: "W. Neudorff GmbH KG" followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioral effects (feeding activity) and biomass development after 28 days; assessment of reproduction rate after additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 Flow (carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test rates: Control, 15.0, 19.5, 25.4, 33.0, 42.8 mg a.s./kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 6.12 – 6.19 at test initiation and pH 5.82 – 5.88 at test termination; water content: 58.3% – 58.5% of maximum water holding capacity (WHC) at test initiation and 56.9% – 58.3% of WHC at test termination; temperature: 18.0 °C – 21.1 °C; photoperiod: 16 h light : 8 h dark, light intensity: 610 lux.

Statistics: Descriptive statistics. Fisher's Exact Binominal test for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller), Probit analysis.

II. RESULTS AND DISCUSSION

After 28 days of exposure the mortality between 0% and 5.0% in the test item groups and 1.3% in the control. No statistically significant mortality compared to the control was observed at any test item concentration (Fisher's Exact Binominal test with Bonferroni correction, $\alpha = 0.05$). Body weight was not statistically significantly different from the control up to highest concentration of 42.8 mg a.s./kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). The reproduction rate was significantly different from the control in the two highest concentration of 33.0 and 42.8 mg a.s./kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups.

Table 8.4.1-1: Effect of BAS 656 H on earthworms (Eisenia fetida) in a 56-day reproduction study

BAS 656 H [mg/kg dry soil]	Control	15.0	19.5	25.4	33.0	42.8
Mortality (day 28) [%]	1.3	2.5	0.	5.0	5.0	5.0
Weight change (day 28) [%]	46.7	46.4	49.6	46.6	43.5	39.1
No. of juveniles (day 56)	84.1	77.5	82.3	70.3	43.3 *	23.0 *
Reproduction in [%] of control (day 56)	100	92.1	97.8	83.5	51.4	27.3
	Endpoints [mg a.s./kg dry soil]					
NOEC (day 28 mortality and weight)	42.8					
NOEC (day 56 reproduction)	25.4					
EC_{50} (95% confidence limits)	34.3 (31.3 – 37 6)					

* Statistically significantly different from control (Williams t-test; $\alpha = 0.05$, one-sided smaller.

In a separate study the reference item inhibited the reproduction rate by 72.7% and 98.8% compared to a control at 5 and 10 mg product/kg dry soil.

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 656 H (dimethenamid-P), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be 25.4 mg a.s./kg dry soil.

Report: CA 8.4.1/2
Luehrs U., 2007a
Effects of Reg.No. 360715 on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat
2007/1037731
Guidelines: OECD 222, ISO 11268-2 (1998)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

The effects of M23, a metabolite of dimethenamid-P, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five application rates (0.52, 1.04, 2.08, 4.16 and 8.32 mg M23/kg dry soil) were incorporated into the soil (5% peat only) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes and reproduction of the earthworms exposed to M23, a metabolite of dimethenamid-P, were not statistically significantly different compared to the control up to the highest test item concentration of 8.32 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

In a 56-day earthworm reproduction study with M23, a metabolite of dimethenamid-P, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 8.32 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M23 (metabolite of dimethenamid-P), batch no. L59-52, Reg. No. 360 715, purity: 97.1%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight of 301 mg – 575 mg), 9 - 10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioral effects (feeding activity) and biomass development after 28 days; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (Carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 0.52, 1.04, 2.08, 4.16 and 8.32 mg M23/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 6.0 – 6.1 at test initiation and termination; water content: 48.5 – 53.4% of maximum water holding capacity (WHC) at test initiation and 52.8 - 58.8% WHC at test termination; temperature: 19 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 490 lux - 710 lux.

Statistics: Descriptive statistics. Two-sided Bonferroni-Welch-t-test for weight change and one-sided Bonferroni-Welch-t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes and reproduction of the earthworms exposed to M23, a metabolite of dimethenamid-P, were not statistically significantly different compared to the control up to the highest test item concentration of 8.32 mg/kg dry soil (Bonferroni-Welch-t-test, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized in Table 8.4.1-2.

Table 8.4.1-2: Effect of M23, a metabolite of dimethenamid-P, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

M23 [mg/kg dry soil]	**Control**	**0.52**	**1.04**	**2.08**	**4.16**	**8.32**
Mortality (day 28) [%]	0	0	0	0	0	0
Weight change (day 28) [%]	29.7	29.7	27.2	29.6	29.4	26.8
No. of juveniles (day 56)	243	241	241	249	247	212
Reproduction in [%] of control (day 56)	--	99.3	99.3	102.6	101.6	87.1
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
NOEC (day 28 mortality and weight)	≥ 8.32					
NOEC (day 56 reproduction)	≥ 8.32					

III. CONCLUSION

In a 56-day earthworm reproduction study with M23, a metabolite of dimethenamid-P, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 8.32 mg/kg dry soil.

Report: CA 8.4.1/3
Luehrs U., 2007b
Effects of Reg.No. 360714 on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat
2007/1037732
Guidelines: OECD 222, ISO 11268-2 (1998), EEC 96/12, EEC 91/414
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

The effects of M27, a metabolite of dimethenamid-P, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five application rates (0.66, 1.32, 2.64, 5.28 and 10.56 mg M27/kg dry soil) were incorporated into the soil (5% peat only) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days and assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure no mortality was observed in any treatment group except for a mortality of 2.5% at 10.56 mg M27/kg dry soil, which was not statistically significant different. Body weight changes of earthworms exposed to M27 were not statistically significantly different compared to the control up to the highest test item concentration of 10.56 mg/kg dry soil. No statistically significant differences on reproduction were observed in any of the treatment groups. No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the one in the control.

In a 56-day reproduction study with M27, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 10.56 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum, weight: 304 mg - 600 mg), approx.10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days of exposure; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 0.66, 1.32, 2.64, 5.28 and 10.56 mg M27/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with reduced content of peat (5%); pH 6.3 – 6.4 at test initiation, 5.9 – 6.1 at test termination; water content: 51.8% – 55.0% of maximum water holding capacity (WHC) at test initiation and 50.8%- 63.8% WHC at test termination; temperature: 19 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 750 lux.

Statistics: Descriptive statistics. Fisher's exact test for mortality data, Dunnett's test for weight change data and Bonferroni-Welch-t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed in any treatment group except for a mortality of 2.5% at 10.56 mg M27/kg dry soil, which was not statistically significant different (Fisher's exact test, $\alpha = 0.05$). Body weight changes of earthworms exposed to M27 were not statistically significantly different compared to the control up to the highest test item concentration of 10.56 mg/kg dry soil (Dunnett's test, $\alpha = 0.05$). No statistically significant differences on reproduction were observed in any of the treatment groups (Bonferroni-Welch-test, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all treated groups was comparable to the one in the control. The results are summarized in Table 8.4.1-3.

Table 8.4.1-3: Effect of M27, a metabolite of dimethenamid-P, on earthworms (Eisenia fetida) in a 56-day reproduction study

M27 [mg/kg dry soil]	**Control**	**0.66**	**1.32**	**2.64**	**5.28**	**10.56**
Mortality (day 28) [%]	0	0	0	0	0	2.5
Weight change (day 28) [%]	8.3	11.7	5.5	11.8	9.5	20.8
No. of juveniles (day 56)	254	229	256	277	194	254
Reproduction in [%] of control (day 56)	--	90.1	101	109	76.4	100
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
$NOEC_{mortality, weight}$ (day 28)	≥ 10.56					
$NOEC_{reproduction}$ (day 56)	≥ 10.56					

III. CONCLUSION

In a 56-day reproduction study with M27, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 10.56 mg/kg dry soil.

Report: CA 8.4.1/4
Luehrs U., 2009a
Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat
2008/1070910
Guidelines: OECD 222, ISO 11268-2 (1998)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

The effects of M31, a metabolite of dimethenamid-P, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five application rates (6.25, 12.5, 25, 50 and 100 mg M31/kg dry soil) were incorporated into the soil (5% peat only) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days and assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes of earthworms exposed to M31 were not statistically significantly different compared to the control up to the highest test item concentration of 100 mg/kg dry soil. No statistically significant differences on reproduction were observed in any of the treatment groups. No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all treated groups was comparable to the one in the control.

In a 56-day reproduction study with M31, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 100 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum, weight: 305 mg - 596 mg), approx.10 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects (feeding activity) and biomass development after 28 days of exposure; assessment of reproduction rate after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 6.25, 12.5, 25, 50 and 100 mg M31/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with reduced content of peat (5%); pH 6.4 – 6.5 at test initiation, 6.1 – 6.5 at test termination; water content: 50.5% – 54.5% of maximum water holding capacity (WHC) at test initiation and 53.4% – 60.0% WHC at test termination; temperature: 18 - 21 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 640 lux.

Statistics: Descriptive statistics. Dunnett's test for weight change and reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed in any treatment group. Body weight changes of earthworms exposed to M31 were not statistically significantly different compared to the control up to the highest test item concentration of 100 mg/kg dry soil (Dunnett's test, $\alpha = 0.05$). No statistically significant differences on reproduction were observed in any of the treatment groups (Dunnett's test, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the control. The results are summarized in Table 8.4.1-4.

Table 8.4.1-4: Effect of M31, a metabolite of dimethenamid-P, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

M31 [mg/kg dry soil]	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality (day 28) [%]	0	0	0	0	0	0
Weight change (day 28) [%]	42.1	52.6	49.3	47.4	44.8	39.1
No. of juveniles (day 56)	167	177	226	162	230	185
Reproduction in [%] of control (day 56)	--	106.2	135.6	97.1	138.0	110.9
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg dry soil]					
NOEC (day 28 mortality and weight)	≥ 100					
NOEC (day 56 reproduction)	≥ 100					

III. CONCLUSION

In a 56-day reproduction study with M31, a metabolite of dimethenamid-P, on earthworms, the NOEC for mortality, biomass, reproduction, and feeding activity was ≥ 100 mg/kg dry soil.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Report: CA 8.4.2.1/1
Friedrich S., 2011a
Effects of BAS 656 H (Reg.No. 363 851, Dimethenamid-P) on the reproduction of the collembolans Folsomia candida
2011/1000481
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of dimethenamid-P on mortality and reproduction of the springtail *Folsomia candida* were investigated in an extended laboratory study according OECD 232 over 28 days.

In the test item treatments mortality rates of 5% to 47.5% were observed, compared to 5% mortality in the control. Statistically significant differences compared to the control were observed at concentrations of 25, 50 and 100 mg test item/kg dry soil.
In the control, a mean of 637.9 juveniles was counted. In the treatment groups, a mean number of juveniles between 138.5 to 664.8 was counted corresponding to a reproduction rate between 21.7% and 104.2%. Statistically significant differences on reproduction compared to the control were recorded at concentrations tested of 50 and 100 mg test item/kg dry soil.

In a 28 day reproduction study with dimethenamid-P on the collembolan *Folsomia candida* the NOEC for reproduction was determined to be 25.0 mg a.s./kg dry soil. The NOEC for mortality was determined to be 12.5 mg a.s./kg dry soil. The EC_{50} was 41.6 mg a.s./kg dry soil and the LC_{50} was 118.3 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethenamid-P (BAS 656 H, batch no. 6261B01BH, Reg. No. 363 851, purity: 96.8 ± 1.0%.

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), age: 9-12 days; source: in-house culture.

Test design: In a 28-day test, adults of *Folsomia candida* were exposed to six soil concentrations of dimethenamid-P. The test substrate was artificial soil according to OECD 232 (5% peat). In total, 7 treatment groups were set up (6 concentrations of the test item and an untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, each with 10 collembolans. The artificial soil was treated and filled into glass vessels before collembolans were introduced on the top of the soil. Assessment of adult collembolans mortality, behavioral effects and reproduction (number of juveniles) was done after 28 days.

Endpoints: Mortality and reproduction rate.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a content of 5% peat; pH 5.89 - 6.00 at test initiation and 5.79 – 6.08 at test termination; water content at test initiation 57.8% – 58.0 % of the max. water holding capacity (WHC) and 56.8% – 57.5 % of the max. WHC at test termination; temperature: 18.0 - 21.4 °C; photoperiod: 16 h light : 8 h dark, light intensity: 680 lux.

Statistics: Descriptive statistics. Fisher's exact test with Bonferroni Correction for mortality data, Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data (α = 0.05). Probit analysis for determination of LC_{50} and EC_{50}.

II. RESULTS AND DISCUSSION

In the test item treatments mortality rates of 5% to 47.5% were observed, compared to 5% in the control. Statistically significant differences compared to the control were observed at concentrations of 25, 50 and 100 mg test item/kg dry soil (Fisher's exact test with Bonferroni Correction, α = 0.05).
In the control, a mean of 637.9 juveniles was counted. In the treatment groups, a mean number of juveniles between 138.5 to 664.8 was counted corresponding to a reproduction rate between 21.7% and 104.2%. Statistically significant differences on reproduction compared to the control were recorded at concentrations tested of 50 and 100 mg test item/kg dry soil (Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data, α = 0.05). The results are summarized in Table 8.4.2.1-1.

Table 8.4.2.1-1: Effects of BAS 656 H on Collembola (Folsomia candida) in 28-day reproduction study

Dimethenamid-P **[mg/kg dry soil]**	**Control**	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality [%] (day 28)	5.0	5.0	10.0	7.5	27.5*, [1)]	30.0*, [1)]	47.5*, [1)]
No. of juveniles (day 28)	637.9	606.3	611.8	664.8	443.5	237.3*, [2)]	138.5*, [2)]
Reproduction in [%] of control (day 28)	100.0	95.0	95.9	104.2	69.5	37.2	21.7
	Endpoints [mg BAS 656 H/kg dry soil]						
NOEC (mortality)	12.5						
LC_{50} (mortality)	118.3 (95% confidence limit: 77.1 – 251.1)						
NOEC (reproduction)	25.0						
EC_{50} (reproduction)	41.6 (95% confidence limit: 31.3 – 56.2)						

* Statistically significantly different compared to the control.
1) Fisher's exact test with Bonferroni Correction for mortality data ($\alpha = 0.05$).
2) Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction data ($\alpha = 0.05$).

III. CONCLUSION

In a 28 day reproduction study with dimethenamid-P on the collembolan *Folsomia candida* the NOEC for reproduction was determined to be 25.0 mg a.s./kg dry soil. The NOEC for mortality was determined to be 12.5 mg a.s./kg dry soil. The EC_{50} was 41.6 mg a.s./kg dry soil and the LC_{50} was 118.3 mg a.s./kg dry soil.

Report: CA 8.4.2.1/2
Friedrich S., 2011b
Effects of Reg.No. 360 712 (metabolite of BAS 656 H, M31) on the reproduction of the collembolans Folsomia candida
2011/1000222
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of M31, a metabolite of dimethenamid-P, on mortality and reproduction of the springtail *Folsomia candida* were investigated in an extended laboratory study according OECD 232 over 28 days.

In the test item treatments mortality rates of 0.0% to 7.5% were observed, compared to 3.8% in the control. No statistically significant differences compared to the control were observed at any test item concentration.
In the control groups, a mean of 830.5 (untreated control) and 824.5 (solvent control) juveniles was counted. In the treatment groups, a mean number of juveniles between 805.5 to 880.5 was counted. This is corresponding to a reproduction relative to the control between 97.7% and 106.8%. No statistically significant differences on reproduction compared to the control were observed at any concentration tested.

In a 28 day reproduction study with M31, a metabolite of dimethenamid-P, on the collembolan, *Folsomia candida*, the NOEC for reproduction and mortality was determined to be ≥ 200 mg M31/kg dry soil. The EC_{50} and the LC_{50} could not be calculated, but it can be concluded that the EC_{50} and the LC_{50} are > 200.0 mg M31/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7 ± 1.0%.

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), age: 9-12 days; source: in-house culture.

Test design: In a 28-day test, adults of *Folsomia candida* were exposed to five soil concentrations of M31. The test substrate was artificial soil according to OECD 232 (5% peat). In total, 7 treatment groups were set up (5 concentrations of the test item, an untreated control and a solvent control) with 4 replicates for the test item treatments and 8 replicates for the control groups, each with 10 collembolans. The artificial soil was treated and filled into glass vessels, before the collembolans were introduced on the top of the soil. Assessment of adult collembolans mortality, behavioral effects and reproduction (number of juveniles) was done after 28 days.

Endpoints: Mortality and reproduction rate.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 12.5, 25, 50, 100 and 200 mg M31/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a content of 5% peat; pH 5.73 – 5.88 at test initiation and 5.64 – 6.03 at test termination; water content at test initiation 58.2% - 58.5 % of the max. water holding capacity (WHC) and 57.7%- 58.2% of WHC at test termination; temperature 18.0 - 20.1 °C; photoperiod: 16 h light : 8 h dark, light intensity: 710 lux.

Statistics: Descriptive statistics. Fisher's Exact Binomial test with Bonferroni Correction for mortality data, Student-t-test, Dunnett-t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

In the test item treatments mortality rates of 0.0% to 7.5% were observed, compared to 3.8% in the control. No statistically significant differences compared to the control were observed at any test item concentration (Fisher's exact binomial test with Bonferroni Correction $\alpha = 0.05$).
In the control groups, a mean of 830.5 (untreated control) and 824.5 (solvent control) juveniles was counted. In the treatment groups, a mean number of juveniles between 805.5 to 880.5 was counted corresponding to a reproduction relative to the control between 97.7% and 106.8%. No statistically significant differences on reproduction compared to the control were observed at any concentration tested (Student-t-test, Dunnett-t-test for reproduction data, $\alpha = 0.05$). The results are summarized in Table 8.4.2.1-2.

Table 8.4.2.1-2:Effects of M31 on Collembola (Folsomia candida) in 28-day reproduction study

M31 [mg/kg dry soil]	Control	Solvent control	12.5	25	50	100	200
Mortality [%] (day 28)	3.8	3.8	5.0	2.5	2.5	7.5	0.0
No. of juveniles (day 28)	830.5	824.5	880.5	812.3	871.5	805.5	857.0
Reproduction in [%] of control (day 28)	--	100.0	106.8	98.5	105.7	97.7	103.9
	Endpoints [mg M31/kg dry soil]						
NOEC (reproduction/mortality)	≥ 200						
LC_{50} (mortality)	> 200						
EC_{50} (reproduction)	> 200						

III. CONCLUSION

In a 28 day reproduction study with M31, a metabolite of dimethenamid-P, on the collembolan, *Folsomia candida*, the NOEC for reproduction and mortality was determined to be ≥ 200 mg M31/kg dry soil. The EC_{50} and the LC_{50} could not be calculated, but it can be concluded that the EC_{50} and the LC_{50} are > 200.0 mg M31/kg dry soil.

Report:	CA 8.4.2.1/3 Schulz L., 2012a BAS 656 H (Dimethenamid-P) - Effects of BAS 656 H (Dimethenamid-P) on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129457
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 656 H (dimethenamid-P) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 62.5, 125, 250, 500 and 1000 mg BAS 656 H/kg dry soil. Test item treatments were replicated four times each. As a control treatment, the soil was left untreated (without vehicle acetone) in eight replicates. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure. No differences in behavior between mites in the control and the test item treatments could be observed.

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. The mortality rate in the untreated and the solvent control was 2.5 % and 0.0 %, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 262.9 and 258.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 184.5 and 257.8. BAS 656 H showed no statistically significantly adverse effects on reproduction up to and including 500 mg BAS 656 H/kg dry soil. At the highest concentration of the test item at 1000 mg BAS 656 H/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed.

In a 14-day reproduction study with BAS 656 H on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for reproduction was determined to be 500 mg BAS 656 H (dimethenamid-P)/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 656 H, batch no. COD-001509 (BAS 656 H, Reg. No. 360 720): 95.9 % (±1.0 %).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 2 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of BAS 656 H on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated control, solvent control, 62.5, 125, 250, 500 and 1000 mg BAS 656 H/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 5.5 – pH 5.7 at test initiation, pH 5.4 - 5.6 at test termination; water content at test initiation 45.99 % - 52.35 % of maximum water holding capacity (WHC) and 44.40 % - 52.19 % of maximum WHC at test termination; temperature: 19.5°C - 21.4°C; photoperiod: 16 h light : 8 h dark; light intensity: 470 lux. food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), William`s t-test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. The mortality rate in the untreated and the solvent control was 2.5 % and 0.0 %, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 262.9 and 258.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 184.5 and 257.8. BAS 656 H showed no statistically significantly adverse effects on reproduction up to and including 500 mg BAS 656 H/kg dry soil. At the highest concentration of the test item at 1000 mg BAS 656 H/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed (William`s t-test, $\alpha = 0.05$, one-sided smaller). The results are summarized in Table 8.4.2.1-3.

Table 8.4.2.1-3:Effects of BAS 656 H on predatory mites (Hypoaspis aculeifer) in a 14-day reproduction study

BAS 656 H (dimethenamid-P) [mg/kg dry soil]	Control	Solvent control	62.5	125	250	500	1000
Mortality (day 14) [%]	2.5	0.0	5.0	0.0	2.5	5.0	5.0
No. of juveniles (day 14)	262.9	258.1	257.8	240.8	240.3	243.5	184.5 *
Reproduction [% of solvent control] (day 14)	--	100	100	93	93	94	72
Endpoint [mg BAS 656 H (dimethenamid-P)/kg dry soil]							
NOEC $_{mortality}$	1000						
NOEC $_{reproduction}$	500						
LC_{50}	> 1000						
EC_{50}	> 1000						

* statistically significantly different from the solvent control (William`s t-test, $\alpha = 0.05$, one-sided smaller).

The reference item dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil. The EC_{50} (reproduction) for dimethoate EC 400 was calculated to be 6.87 mg a.s./kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with BAS 656 H on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for reproduction was determined to be 500 mg BAS 656 H (dimethenamid-P)/kg dry soil.

Report:	CA 8.4.2.1/4 Friedrich S., 2012b Reg.No. 360715 (metabolite of BAS 656 H, Dimethenamid-P, M23) on the reproduction of the collembolan Folsomia candida 2012/1129536
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a chronic laboratory study over 28 days. Five test concentrations (12.5, 25, 50, 100 and 200 mg test item/kg dry soil) were incorporated into the soil (5 % peat only) with 4 replicates per treatment. As control treatments, one untreated control and one prepared with acetone as solvent control, each with 8 replicates, was included. Each treatment contained 10 juvenile collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

Mortalities of 8.8 % and 6.3 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 7.5 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration. In the untreated and the solvent control group, mean numbers of 823 and 781 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 749 and 829. No statistically significant effect on the number of juveniles was found at any concentration tested.

In a 28-day collembolan reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23), theLC_{50} and EC_{50} was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg test item/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23, batch no. L81-76; analyzed purity: 98.8 % (±1.0 %).

B. STUDY DESIGN

Test species:	Collembola (*Folsomia candida*), juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 and ISO 11267 (5% peat only); 5 different concentrations of the test item were homogenously mixed into artificial soil which was then filled in glass vessels before collembolans were introduced on top of the soil. 7 treatment groups (5 test item concentrations, untreated and solvent control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Assessment of adult mortality, reproduction rate (number of juveniles) and behavioral effects was carried out after 28 days.

Endpoints: Mortality, reproduction rate.

Reference item: Boric acid (100 %, analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5 % peat); pH 6.11 – 6.20 at test initiation, pH 5.97 – 6.01 at test termination; water content at test initiation 58.5 % – 58.9 % of maximum water holding capacity (WHC) and 57.0 % – 57.7 % of maximum WHC at test termination; temperature: 18.5 °C – 20.7 °C; photoperiod: 16 h light : 8 h dark; light intensity: 640 lx; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics. Fisher`s Exact Test with Bonferroni Correction for mortality data (α = 0.05), Williams t-test for reproduction (α = 0.05).

II. RESULTS AND DISCUSSION

Mortalities of 8.8 % and 6.3 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 7.5 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration (Fisher`s Exact Test with Bonferroni Correction, α = 0.05). In the untreated and the solvent control group, mean numbers of 823 and 781 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 749 and 829 No statistically significant effect on the number of juveniles was found at any concentration tested (Williams t-test, α = 0.05). The results are summarized in Table 8.4.2.1-4.

Table 8.4.2.1-4:Effect of Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-p, M23) on collembola (*Folsomia candida)* mortality and reproduction (28 d)

Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	8.8	6.3	7.5	7.5	7.5	5.0	2.5
No. of juveniles [28 d]	823	781	774	779	749	781	829
Reproduction (28 d) [% of control]	--	100.0	99.2	99.8	96.0	100.1	106.2
Endpoint [mg Reg. No. 360 715/kg dry soil]							
$NOEC_{mortality, reproduction}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

III. CONCLUSION

In a 28-day collembolan reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23), the LC_{50} and EC_{50} was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg test item/kg dry soil, the highest concentration tested.

Report:	CA 8.4.2.1/5 Schulz L., 2012b Effects of Reg.No. 360715 (metabolite of BAS 656 H, Dimethenamid-P, M23) on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129538
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 12.5, 25, 50, 100 and 200 mg test item/kg dry soil. Test item treatments were replicated four times each. As control treatments, one untreated control and one prepared with acetone as solvent control, each with 8 replicates, was included. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure. No differences in behavior between mites in the solvent control and the test item treatments could be observed.

Test item treatment groups had mortality rates of between 0.0 % - 2.5 %. No mortality could be observed in the untreated and the solvent control group, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 348.3 and 335.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 252.0 and 351.8. Reg. No. 360 715 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 100 mg/kg dry soil. At the highest concentration of the test item at 200 mg/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed.

In a 14-day reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on predatory soil mites (*Hypoaspis aculeifer*), the LC50 and EC50 values were determined to be > 200 mg/kg dry soil. The NOEC for mortality was determined to be ≥ 200 mg Reg. No. 360 715/kg dry soil. The NOEC for reproduction was determined to be 100 mg Reg. No. 360 715/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23, batch no. L81-76; analyzed purity: 98.8 % (±1.0 %).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 3 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 715 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 6.3 – pH 6.4 at test initiation, pH 6.3 – 6.4 at test termination; water content at test initiation 48.30 % – 51.85 % of maximum water holding capacity (WHC) and 48.12 % – 50.80 % of maximum WHC at test termination; temperature: 19.1°C – 20.5°C; photoperiod: 16 h light : 8 h dark; light intensity: 545 lux. food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), William`s t-test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Test item treatment groups had mortality rates of between 0.0 % - 2.5 %. No mortality could be observed in the untreated and the solvent control group, respectively. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 348.3 and 335.1 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 252.0 and 351.8. Reg. No. 360 715 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 100 mg/kg dry soil. At the highest concentration of the test item at 200 mg/kg dry soil, a statistically significant effect on reproduction compared to the solvent control was observed (William`s t-test, $\alpha = 0.05$, one-sided smaller). The results are summarized in Table 8.4.2.1-5.

Table 8.4.2.1-5:Effects of Reg. No. 360 715 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	0.0	0.0	2.5	2.5	0.0	0.0	2.5
No. of juveniles [14 d]	348.3	335.1	346.8	351.8	339.5	297.0	252.0 *
Reproduction (14 d) [% of control]	--	100	103	105	101	89	75
Endpoint [mg Reg. No. 360 715/kg dry soil]							
NOEC $_{mortality}$	≥ 200						
NOEC $_{reproduction}$	100						
LC_{50}	> 200						
EC_{50}	> 200						

* statistically significantly different from the solvent control (William`s t-test, $\alpha = 0.05$, one-sided smaller).

The reference item dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil. The EC_{50} (reproduction) for dimethoate EC 400 was calculated to be 6.87 mg a.s./kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with Reg. No. 360 715 (metabolite of BAS 656 H, dimethenamid-P, M23) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 200 mg/kg dry soil. The NOEC for mortality was determined to be ≥ 200 mg Reg. No. 360 715/kg dry soil. The NOEC for reproduction was determined to be 100 mg Reg. No. 360 715/kg dry soil.

Report: CA 8.4.2.1/6
Friedrich S., 2012c
Effects of Reg.No. 360714 (metabolite of BAS 656 H, Dimethenamid-P, M27) on the reproduction of the collembolans Folsomia candida
2012/1129537
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a chronic laboratory study over 28 days. Five test concentrations (12.5, 25, 50, 100 and 200 mg test item/kg dry soil) were incorporated into the soil (5 % peat only) with 4 replicates per treatment. As control treatments, one untreated control and one prepared with acetone as solvent control, each with 8 replicates, was included. Each treatment contained 10 juvenile collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

Mortalities of 3.8 % and 5.0 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 5.0 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration. In the untreated and the solvent control group, mean numbers of 890 and 948 juveniles were counted, respectively. In the treatment groups, the mean number of juveniles was between 897 and 970. No statistically significant effect on the number of juveniles was found at any concentration tested compared to the solvent control.

In a 28-day collembolan reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27), theLC$_{50}$ and EC$_{50}$ was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg test item/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27, batch no. 1213-32; analyzed purity: 97.4 % (±1.0 %).

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 and ISO 11267 (5% peat only); 5 different concentrations of the test item were homogenously mixed into artificial soil which was then filled in glass vessels before collembolans were introduced on top of the soil. 7 treatment groups (5 test item concentrations, untreated and solvent

control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Assessment of adult mortality, reproduction rate (number of juveniles) and behavioral effects was carried out after 28 days.

Endpoints: Mortality, reproduction rate.

Reference item: Boric acid (100 %, analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 /kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5 % peat); pH 6.18 – 6.25 at test initiation, pH 6.00 – 6.04 at test termination; water content at test initiation 56.6 % – 57.0 % of maximum water holding capacity (WHC) and 55.4 % – 56.6 % of maximum WHC at test termination; temperature: 18.9 °C – 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 650 lx; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics; Fisher`s Exact Binominal Test for mortality, α = 0.05, one-sided greater data; Williams test for reproduction, α = 0.05, one-sided smaller.

II. RESULTS AND DISCUSSION

Mortalities of 3.8 % and 5.0 % were observed in the untreated control and the solvent control groups compared to 2.5 % to 5.0 % mortality in the test item treatment groups. No statistically significant effect on mortality was found in any test concentration (Fisher`s Exact Binominal Test, α = 0.05, one-sided greater). In the untreated and the solvent control group, mean numbers of 890 and 948 juveniles were counted, respectively. In the treatment groups, the mean number of juveniles was between 897 and 970. No statistically significant effect on the number of juveniles was found at any concentration tested compared to the solvent control (Williams test, α = 0.05, one-sided smaller). The results are summarized in Table 8.4.2.1-6.

Table 8.4.2.1-6:Effect of Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on collembola (*Folsomia candida)* mortality and reproduction (28 d)

Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 [mg/kg dry soil]	**Control**	**Solvent control**	**12.5**	**25**	**50**	**100**	**200**
Mortality [%]	3.8	5.0	5.0	5.0	2.5	5.0	5.0
No. of juveniles [28 d]	890	948	897	921	952	906	970
Reproduction (28 d) [% of control]	--	100	94.6	97.2	100.5	95.6	102.4
Endpoint [mg Reg. No. 360 517/kg dry soil]							
$NOEC_{\text{mortality, reproduction}}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

III. CONCLUSION

In a 28-day collembolan reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27), theLC_{50} and EC_{50} was determined to be > 200 mg test item/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg test item/kg dry soil, the highest concentration tested.

Report: CA 8.4.2.1/7
Schulz L, 2012a
Effects of Reg.No. 360714 (metabolite of BAS 656 H, Dimethenamid-P, M27) on the reproduction of the predatory mite Hypoaspis aculeifer
2012/1129539

Guidelines: OECD 226 (2008)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 12.5, 25, 50, 100 and 200 mg test item/kg dry soil. Test item treatments were replicated four times each. As control treatments, one untreated control and one prepared with acetone as solvent control, each with 8 replicates, was included. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure. No differences in behavior between mites in the solvent control and the test item treatments could be observed.

Test item treatment groups had mortality rates of between 2.5 % - 12.5 %. In the untreated control and the solvent control group, mortalities of 7.5 % and 1.3 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 241.8 and 229.9 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 217.8 and 268.8. Reg. No. 360 714 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 200 mg/kg dry soil, the highest concentration tested.

In a 14-day reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 200 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg Reg. No. 360 714/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27, batch no. 1213-32; analyzed purity: 97.4 % (±1.0 %).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 2 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 714 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 12.5, 25, 50, 100 and 200 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 6.2 – pH 6.3 at test initiation, pH 6.2 – 6.3 at test termination; water content at test initiation 56.72 % – 57.01 % of maximum water holding capacity (WHC) and 54.29 % – 56.16 % of maximum WHC at test termination; temperature: 19.0°C – 20.4°C; photoperiod: 16 h light : 8 h dark; light intensity: 473 lux. food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher`s Exact Binominal Test with Bonferroni Correction for mortality ($\alpha$ = 0.05, one-sided greater), William`s t-test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Test item treatment groups had mortality rates of between 2.5 % - 12.5 %. In the untreated control and the solvent control group, mortalities of 7.5 % and 1.3 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group (Fisher`s Exact Binominal Test with Bonferroni Correction, α = 0.05, one-sided greater).

In the untreated and the solvent control group, mean numbers of 241.8 and 229.9 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 217.8 and 268.8. Reg. No. 360 714 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 200 mg/kg dry soil, the highest concentration tested (William`s t-test, α = 0.05, one-sided smaller). The results are summarized in Table 8.4.2.1-7.

Table 8.4.2.1-7: Effects of Reg. No. 360 714 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27 [mg/kg dry soil]	Control	Solvent control	12.5	25	50	100	200
Mortality [%]	7.5	1.3	5.0	7.5	2.5	2.5	12.5
No. of juveniles [14 d]	241.8	229.9	247.0	268.8	256.8	230.5	217.8
Reproduction (14 d) [% of control]	--	100	107	117	112	100	95
Endpoint [mg Reg. No. 360 714/kg dry soil]							
NOEC $_{mortality + reproduction}$	≥ 200						
LC_{50}	> 200						
EC_{50}	> 200						

The reference item dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil. The EC_{50} (reproduction) for dimethoate EC 400 was calculated to be 6.87 mg a.s./kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with Reg. No. 360 714 (metabolite of BAS 656 H, dimethenamid-P, M27) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be> 200 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 200 mg Reg. No. 360 714/kg dry soil.

Report: CA 8.4.2.1/8
Schulz L., 2014a
Effects of Reg.No. 360712 (Metabolite of BAS 656 H, Dimethenamid-P) on the reproduction of the predatory mite Hypoaspis aculeifer
2013/1103674
Guidelines: OECD 226 (2008)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 31.25, 62.5, 125, 250 and 500 mg Reg. No. 360 712/kg dry soil. Test item treatments were replicated four times each. As control treatments, one untreated control and one prepared with acetone as solvent control, each with 8 replicates, was included. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure. No differences in behavior between mites in the solvent control and the test item treatments could be observed.

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. In the untreated control and the solvent control group, mortalities of 0.0 % and 3.8 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group.

In the untreated and the solvent control group, mean numbers of 249.6 and 215.0 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 183.3 and 256.5. Reg. No. 360 712 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 500 mg/kg dry soil, the highest concentration tested.

In a 14-day reproduction study with Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 500 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 500 mg Reg. No. 360 712/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31, batch no. L81-46; analyzed purity: 98.7 % (±1.0 %).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult predatory mites (age difference 2 days); source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 360 712 on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5 % peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test item concentrations); 8 replicates for the control treatments and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Untreated and solvent control, 31.25, 62.5, 125, 250 and 500 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 5.8 – pH 5.9 at test initiation, pH 5.8 – 6.0 at test termination; water content at test initiation 50.49 % –

52.12 % of maximum water holding capacity (WHC) and 50.48 % – 52.32 % of maximum WHC at test termination; temperature: 19.5°C – 21.4°C; photoperiod: 16 h light : 8 h dark; light intensity: 522 lux; food: cheese mites (*Tyrophagus putrescentiae*) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), Dunnett-t-test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Test item treatment groups had mortality rates of between 0.0 % - 5.0 %. In the untreated control and the solvent control group, mortalities of 0.0 % and 3.8 % were observed. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in solvent control group (Fisher's Exact Binominal Test with Bonferroni Correction, α = 0.05, one-sided greater).
In the untreated and the solvent control group, mean numbers of 249.6 and 215.0 juveniles were counted, respectively. In the test item treatment groups, the mean number of juveniles was between 183.3 and 256.5. Reg. No. 360 712 showed no statistically significantly adverse effects on reproduction up to and including a concentration of 500 mg/kg dry soil, the highest concentration tested (Dunnett-t-test, α = 0.05, one-sided smaller). The results are summarized in Table 8.4.2.1-8.

Table 8.4.2.1-8: Effects of Reg. No. 360 712 on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 360 712 [mg/kg dry soil]	Control	Solvent control	31.25	62.5	125	250	500
Mortality [%]	0.0	3.8	5.0	0.0	2.5	5.0	5.0
No. of juveniles (day 14)	249.6	215.0	256.5	183.3	197.3	186.0	209.8
Reproduction (day 14) [% of solvent control]	--	100	119	85	92	87	98
Endpoints [mg Reg. No. 360 712/kg dry soil]							
$NOEC_{mortality + reproduction}$	≥ 500						
LC_{50}	> 500						
EC_{50}	> 500						

The reference item Dimethoate EC 400 was tested in a separate study at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil. The EC_{50} (reproduction) for Dimethoate EC 400 was calculated to be 6.64 mg a.s./kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with Reg. No. 360 712 (metabolite of BAS 656 H, dimethenamid-P, M31) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 500 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 500 mg Reg. No. 360 712/kg dry soil.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of dimethenamid-P (BAS 656 PH), new toxicity studies on its metabolites have been performed and as a result there are new endpoints which are now used in the risk assessment. Summaries of these new studies are provided below. For codes and synonyms of the metabolites of dimethenamid-P please refer to DOCUMENT N3.

Table 8.5-1 Toxicity to nitrogen transformation of dimethenamid-P and metabolites

Test substance	Endpoint	NOEC [mg/kg dry soil]	Reference	Study EU agreed?
dimethenamid-P	Effects on nitrogen transformation	8.4	EFSA Scientific Report 53, 2005; DAR, Vol. 3, Annex B.9, January 2005; SANCO/1402/2001-Final	Yes
M656H023	Effects on nitrogen transformation	0.67	Schulz, 2008/1065117	No, new study
M656H027	Effects on nitrogen transformation	0.85	Schulz, 2008/1065119	No, new study
M656H031	Effects on nitrogen transformation	0.60	Schulz, 2008/1065115	No, new study

Report: CA 8.5/1
Schulz L., 2008a
Effects of Reg.No. 360715 (metabolite of BAS 656 H, M23) on the activity soil microflora (Nitrogen transformation test)
2008/1065117
Guidelines: OECD 216 (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M23, a metabolite of dimethenamid-P, on nitrogen transformation were investigated in a loamy sand soil. M23 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M23 treated soils and control soil treatments were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of mineral nitrogen 0, 7, 14 and 28 days after application. There were no significant effects on the rate of conversion of NH_4-N to NO_3-N at any application rate.

Based on the results of this study, M23, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M23/kg dry soil (deviation from control < 25%, OECD 216).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M23 (metabolite of dimethenamid-P), batch no. L59-90, Reg. No. 360 715, purity: 98.4%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer II (Bran and Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M23/kg dry soil and 1.0 mg M23/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its max. WHC; measured water content: 17.97 – 18.46 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M23 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -2.0% (application rate 0.2 mg/kg dry soil) and -1.0% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.5-2.

Table 8.5-2: Effects of M23 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M23/kg dry soil		1.0 mg M23/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	10.3	10.9	+ 5.5	10.6	+ 2.6
Loamy sand soil (7 d)	30.8	30.4	- 1.3	30.9	+ 0.3
Loamy sand soil (14 d)	36.2	36.2	± 0.0	35.9	- 0.9
Loamy sand soil (28 d)	50.1	49.1	- 2.0	49.6	-1.0

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7%, + 60.8% and +68.1% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M23, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M23/kg dry soil (deviation from control < 25%, OECD 216).

Report: CA 8.5/2
Schulz L., 2008b
Effects of Reg.No. 360 714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Nitrogen transformation test)
2008/1065119
Guidelines: OECD 216 (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M27, a metabolite of dimethenamid-P, on nitrogen transformation were investigated in a loamy sand soil. M27 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M27 treated soils and control soil treatments were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of mineral nitrogen 0, 7, 14 and 28 days after application. There were no significant effects on the rate of conversion of NH_4-N to NO_3-N at any application rate.

Based on the results of this study, M27, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M27/kg dry soil (deviation from control < 25%, OECD 216).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).

Test design: Determination of N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer II (Bran and Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M27/kg dry soil and 1.0 mg M27/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its maximum water holding capacity; measured water content: 17.82 – 18.79 g/100 g dry soil; pH 6.3 – 6.5. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M27 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +1.5% (application rate 0.2 mg/kg dry soil) and +3.2% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.5-3.

Table 8.5-3: Effects of M27 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M27/kg dry soil		1.0 mg M27/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	11.3	11.2	- 1.5	11.7	+ 2.9
Loamy sand soil (7 d)	31.3	33.0	+ 5.4	31.7	+ 1.1
Loamy sand soil (14 d)	34.2	36.7	+ 7.3	37.6	+ 10.1
Loamy sand soil (28 d)	50.1	50.9	+ 1.5	51.7	+ 3.2

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7%, + 60.8% and 68.1% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M27, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M27/kg dry soil (deviation from control < 25%, OECD 216).

Report:	CA 8.5/3 Schulz L., 2008c Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Nitrogen transformation test) 2008/1065115
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M31, a metabolite of dimethenamid-P, on the nitrogen transformation were investigated in a loamy sand soil. M31 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M31 treated soils and control soil treatments were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of mineral nitrogen 0, 7, 14 and 28 days after application. There were no significant effects on the rate of conversion of NH_4-N to NO_3-N at any application rate.

Based on the results of this study, M31, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M31/kg dry soil (deviation from control < 25%, OECD 216).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).

Test design: Determination of N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer II (Bran and Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M31/kg dry soil and 1.0 mg M31/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its max. WHC; measured water content: 16.00 – 18.70 g/100 g dry soil; pH 6.3 – 6.5. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M31 on nitrogen transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +2.9% (application rate 0.2 mg/kg dry soil) and +3.5% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.5-4.

Table 8.5-4: Effects of M31 on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M31/kg dry soil		1.0 mg M31/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control 1)	NO_3-N [mg/kg dry soil]	% Deviation from control 1)
Loamy sand soil (0 d)	16.3	16.2	- 0.6	15.9	- 2.3
Loamy sand soil (7 d)	36.7	36.2	- 1.3	37.1	+ 1.3
Loamy sand soil (14 d)	40.3	40.0	- 0.6	39.5	- 1.8
Loamy sand soil (28 d)	54.6	56.2	+ 2.9	56.5	+ 3.5

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of + 27.7%, + 60.8% and 68.1% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M31, a metabolite of dimethenamid-P, caused no short-term and long-term effects on nitrogen transformation in a field soil tested up to a concentration of 1.0 mg M31/kg dry soil (deviation from control < 25%, OECD 216).

CA 8.6 Effects on terrestrial non-target higher plants

Table 8.6-1 Toxicity to terrestrial non-target higher plants

Test substance	Endpoint	NOER * [g/ha]	Reference	Study EU agreed?
M31	Effects on seedling emergence	≥ 1008	Dutille, 2008/1068011	No, new study

* Biological activity (phytotoxic effects)

CA 8.6.1 Summary of screening data

No new studies are available.

CA 8.6.2 Testing on non-target plants

Herbicidal activity of the dimethenamid-P metabolite M31 was tested in a pre-emergence greenhouse study on ten different plant species. For codes and synonyms of the metabolite of dimethenamid-P please refer to DOCUMENT N3.

Report: CA 8.6.2/1
Dutillie H.,Sack D., 2008a
Effects of Reg.No. 360712 (M31, metabolite of BAS 656 H) on non-target plants in the greenhouse
2008/1068011
Guidelines: OECD 227 July 2006, OECD 208 (2006)
GLP: no

Executive Summary

In a seedling emergence test, four species of dicotyledonous plants (shepherd's purse, fat hen, common chickweed, scentless mayweed) and six species of monocotyledonous plants (hairy crabgrass, green foxtail, italian ryegrass, giant foxtail, barnyard grass, annual meadow grass) were exposed to M31, metabolite of BAS 656 H. The phytotoxic potential of the metabolite was compared to two reference items, BAS 656 H and BAS 656 08 H, additionally to a blank formulation and a water treated control. The test and the reference items were applied at rates of 648 and 1008 g a.s./ha at a water rate of 750 L/ha.

The plants were cultivated for 21 days under greenhouse conditions. Assessment of phytotoxicity (e.g. chlorosis, necrosis etc.) was carried out 7 and 21 days after application (DAA). Fresh weight was determined at study termination 21 DAA.

Based on the results of this study it can be concluded that M31, metabolite of BAS 656 H, applied pre-emergence up to rate of 1008 g/ha shows no biological activity on fresh weight and plant damage in all tested species.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of BAS 656 H; Reg. No 360 712), batch No. L81-46.

Reference items: BAS 656 H (Dimethenamid-P; Reg. No. 363 851), batch No. 6261B01BH; BAS 656 08 H, batch No. FRE-000484.

B. STUDY DESIGN

Test species: Hairy crabgrass (*Digitaria sanguinalis*), Green foxtail (*Setaria viridis*), Italian ryegrass (*Lolium multiflorum*), Giant foxtail (*Setaria faberi*), Barnyard grass (*Echinocloa crus-galli*), Annual meadow grass (*Poa annua*), Shepherd´s purse (*Capsella bursa-pastoris*), Fat hen (*Chenopodium album*), Scentless mayweed (*Matricaria inodora*), Common chickweed (*Stellaria media*).

Test design: 5 treatment groups (2 rates for test item and both reference items, blank formulation, water treated control); 4 replicates/treatment; 1 pot/replicate, number of plants is related to standard sowing depending on species, pre-emergence applications using a laboratory spray cabin at a water rate of 750 L/ha. Following the application the plants were cultivated for 21 days in the greenhouse. Assessments for phytotoxicity (e.g. chlorosis, necrosis etc.) were done 7 and 21 days after application (DAA) for all plants. Shoot fresh weight was determined at study termination 21 DAA.

Endpoints: Fresh weight and phytotoxicity.

Test rates: Water control, blank formulation, 648 g a.s./ha and 1008 g a.s./ha for M31, BAS 656 H and BAS 656 08 H.

Test conditions: Greenhouse conditions, average temperature: 14 °C - 31 °C, average humidity: about 80%; photoperiod: 16 h light : 8 h dark; additional light when outdoor illumination was less than 4500 lux.

Statistics: Descriptive statistics. Dunnett-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Phytotoxicity: BAS 656 08 H resulted in 83% - 100% plant damage in all tested species in the lower rate and in 98% - 100% plant damage in the higher rate. BAS 656 H caused plant damages up to 88% - 100% in the lower rate and 96% - 100% in the higher rate. No effects were observed in the plants treated with M31, metabolite of BAS 656 H, in both treatment rates. Also the water treated control and the blank formulation did not cause plant damages.

Biomass (fresh weight): BAS 656 08 H resulted in 100% reduction of mean plant weight in all tested species in both tested rates, except for the species fat hen which showed a reduction of 75.81% in the lower test rate and 96.30% in the higher test rate. BAS 656 H resulted in 100% reduction of mean plant weight in all tested species in both tested rates, except for the species fat hen which showed a reduction of 92.85% in the lower test rate and 95.51% in the higher test rate. The metabolite, tested at the higher rate, caused maximum 12.38% reduction in plant weight. Even though in one case this reduction was statistically significant, it is not considered to be caused by the metabolite M31, as the same reduction was also observed by the blank formulation and it is well within the normal variability of the test system. Also at the lower test rate statistically significant differences were observed but it is not considered to be caused by the metabolite for the same reasons. The coincidence of significance of differences in plant biomass is supported by the fact that it was not observed at the higher test rate.

The results are summarized in Table 8.6-2.

Table 8.6-2 Effects of M31 (metabolite of BAS 656 H) on fresh weight and plant damage 21 DAA

Test substance	Test rate [g a.s./ha]	hairy crabgrass	green foxtail	italian ryegrass	giant foxtail	barnyard grass
Phytotoxicity [% chlorosis/necrosis/stunting/deformation]						
Control	--	0	0	0	0	0
Blank formulation	--	0	0	0	0	0
M31	648	0	0	0	0	0
	1008	0	0	0	0	0
BAS 656 H	648	100	100	100	100	100
	1008	100	100	100	100	100
BAS 656 08 H	648	100	100	100	100	100
	1008	100	100	100	100	100
Reduction of fresh weight [% of control]						
Control	--	0	0	0	0	0
Blank formulation	--	4.45	22.17*	-7.79	31.45*	-10.56
M31	648	14.94*	27.22*	-9.87	21.94*	1.52
	1008	-7.44	12.04	-31.58	11.74	-23.09
BAS 656 H	648	100*	100*	100*	100*	100*
	1008	100*	100*	100*	100*	100*
BAS 656 08 H	648	100*	100*	100*	100*	100*

Table 8.6-2 Effects of M31 (metabolite of BAS 656 H) on fresh weight and plant damage 21 DAA

Test substance	Test rate [g a.s./ha]	hairy crabgrass	green foxtail	italian ryegrass	giant foxtail	barnyard grass
	1008	100*	100*	100*	100*	100*
Test substance	**Test rate [g a.s./ha]**	**annual meadowgrass**	**shepherd´s purse**	**fat hen**	**scentless mayweed**	**chickweed**
Phytotoxicity [% chlorosis/necrosis/stunting/deformation]						
Control	--	0	0	0	0	0
Blank formulation	--	0	0	0	0	0
M31	648	0	0	0	0	0
	1008	0	0	0	0	0
BAS 656 H	648	100	100	88	99	95
	1008	100	100	96	100	100
BAS 656 08 H	648	100	100	83	98	99
	1008	100	100	98	100	99
Reduction of fresh weight [% of control]						
Control	--	0	0	0	0	0
Blank formulation	--	-2.84	-65.38	12.70*	14.07	-18.20
M31	648	14.18	-42.89	23.76*	29.95*	-15.77
	1008	-6.22	-74.01	12.38*	0.88	-35.90
BAS 656 H	648	100*	100*	92.85*	99.92*	99.80*
	1008	100*	100*	95.51*	100*	100*
BAS 656 08 H	648	100*	100*	75.81*	98.96*	99.95*
	1008	100*	100*	96.30*	100*	99.95*

* Statistically significant difference compared to the control (Dunnett-test, $\alpha = 0.05$).

III. CONCLUSION

Based on the results of this study it can be concluded that M31, metabolite of BAS 656 H, applied pre-emergence up to a rate of 1008 g/ha, shows no biological activity on plants in any of the tested species.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

The following studies are no data requirement for the risk assessment and are presented as additional information to the dossier.

Report:	CA 8.7/1 Schulz L., 2008g Effects of Reg.No. 360715 (metabolite of BAS 656 H, M23) on the activity of soil microflora (Carbon transformation test) 2008/1065116
Guidelines:	OECD 217 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M23, a metabolite of dimethenamid-P, on the carbon transformation were investigated in a loamy sand soil. M23 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M23 treated soils and controls were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of carbon content 0, 7, 14 and 28 days after application. There were no significant effects on the carbon transformation at any application rate.

Based on the results of this study, M23, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M23/kg dry soil (deviation from control < 25%, OCD 217).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	M23 (metabolite of dimethenamid-P), batch no. L59-90, Reg. No. 360 715, purity: 98.4%.

B. STUDY DESIGN

Test soil:	Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).
Test design:	Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M23/kg dry soil and 1.0 mg M23/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its max. WHC; measured water content: 17.14 – 18.54 g/100 g dry soil; pH 6.2 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M23 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -4.4% (application rate 0.2 mg/kg dry soil) and -2.7% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.7-1.

Table 8.7-1: Effects of M23 on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M23/kg dry soil		1.0 mg M23/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	15.47	15.66	+ 1.3	15.24	- 1.5
Loamy sand soil (7 d)	14.85	14.80	- 0.3	14.25	- 4.0
Loamy sand soil (14 d)	13.82	13.49	- 2.4	13.57	- 1.8
Loamy sand soil (28 d)	12.77	12.21	- 4.4	12.42	- 2.7

[1)] Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of -24.8%, -42.0% and -49.0% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M23, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M23/kg dry soil (deviation from control < 25%, OCD 217).

Report: CA 8.7/2
Schulz L., 2008h
Effects of Reg.No. 360714 (metabolite of BAS 656 H, M27) on the activity of soil microflora (Carbon transformation test)
2008/1065118

Guidelines: OECD 217 (2000)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M27, a metabolite of dimethenamid-P, on the carbon transformation were investigated in a loamy sand soil. M27 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M27 treated soils and controls were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of carbon content 0, 7, 14 and 28 days after application. There were no significant effects on the carbon transformation at any application rate.

Based on the results of this study, M27, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M27/kg dry soil (deviation from control < 25%, OCD 217).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M27 (metabolite of dimethenamid-P), batch no. 01311-28, Reg. No. 360 714, purity: 97.1%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M27/kg dry soil and 1.0 mg M27/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its max. WHC; measured water content: 17.56 – 18.56 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M27 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +0.9% (application rate 0.2 mg/kg dry soil) and -0.6% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.7-2.

Table 8.7-2: Effects of M27 on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M27/kg dry soil		1.0 mg M27/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	16.34	15.89	- 2.8	16.16	- 1.1
Loamy sand soil (7 d)	15.76	15.76	± 0.0	15.36	- 2.6
Loamy sand soil (14 d)	14.26	14.40	+ 1.0	14.13	- 0.9
Loamy sand soil (28 d)	13.65	13.76	+ 0.9	13.56	- 0.6

1) Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of -24.8%, - 42.0% and – 49.0% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M27, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M27/kg dry soil (deviation from control < 25%, OCD 217).

Report: CA 8.7/3
Schulz L., 2008i
Effects of Reg.No. 360712 (metabolite of BAS 656 H, M31) on the activity of soil microflora (Carbon transformation test)
2008/1065109
Guidelines: OECD 217 (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of M31, a metabolite of dimethenamid-P, on the carbon transformation were investigated in a loamy sand soil. M31 was applied to samples of the soil at test concentrations of 0.2 mg/kg and 1.0 mg/kg dry soil. M31 treated soils and controls were incubated at approx. 20 °C in the dark for 28 days.

Triplicate samples of each treatment were removed for analysis of carbon content 0, 7, 14 and 28 days after application. There were no significant effects on the carbon transformation at any application rate.

Based on the results of this study, M31, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M31/kg dry soil (deviation from control < 25%, OCD 217).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, Reg. No. 360 712, purity: 98.7%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.5, 1.49% C_{org}, 38.11% water holding capacity (WHC).

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.4%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.2 mg M31/kg dry soil and 1.0 mg M31/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.8, 16.0 and 27.0 mg/kg dry soil in a separate study.

Test conditions: Soil moisture: approx. 45% of its max. WHC; measured water content: 17.77 – 18.71 g/100 g dry soil; pH 6.3 – 6.4. Soil samples were incubated at 20.2 °C – 21.9 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of M31 on carbon transformation in soil could be observed in both test item concentrations (0.2 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -1.7% (application rate 0.2 mg/kg dry soil) and -2.7% (application rate 1.0 mg/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarized in Table 8.7-3.

Table 8.7-3: Effects of M31 on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	0.2 mg M31/kg dry soil		1.0 mg M31/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	16.23	16.28	+ 0.3	16.17	- 0.4
Loamy sand soil (7 d)	15.76	15.64	- 0.8	15.23	- 3.3
Loamy sand soil (14 d)	14.62	14.61	- 0.1	14.17	- 3.1
Loamy sand soil (28 d)	14.42	14.17	- 1.7	14.03	- 2.7

[1)] Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of -24.8%, -42.0% and -49.0% at 6.8, 16.0 and 27.0 mg/kg dry soil.

III. CONCLUSION

Based on the results of this study, M31, a metabolite of dimethenamid-P, caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 1.0 mg M31/kg dry soil (deviation from control < 25%, OCD 217).

Report: CA 8.7/4
Krome K., 2008a
Acute toxicity (14 days) of Reg.No. 360712 (Metabolite of BAS 656 H, M31) to the earthworm Eisenia fetida in artificial soil
2008/1052695

Guidelines: OECD 207, DIN ISO 11268-1 (April 1997)

GLP: yes
(certified by Staatliches Gewerbeaufsichtsamt, Hildesheim, Germany)

In an acute toxicity laboratory study adult earthworms, species *Eisenia fetida* Savigny (Annelida: Oligochaeta), were exposed to M31, a metabolite of dimethenamid-P. The test item was mixed into artificial soil (10% peat) at rates of 62.5, 125, 250, 500, and 1000 mg M31/kg dry soil. For the control treatments, the soil was left untreated.
The worms were placed on the surface of the soil. Four replicates were prepared for each treatment group, each containing 10 worms. Assessment of mortality was carried out 7 and 14 days after treatment. Assessment of behaviour and weight change as sub-lethal parameters was carried out after 14 days.
After 14 days of exposure, no mortality was observed in any test item group and the control. The biomass development was not statistically significant different compared to the control at all test item concentrations.

In a 14-day toxicity study to earthworms (*Eisenia fetida)* with M31, a metabolite of dimethenamid-P, the LC_{50} was > 1000 mg/kg dry soil. The NOEC related to mortality and biomass was determined to be ≥ 1000 mg/kg soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M31 (metabolite of dimethenamid-P), batch no. L81-46, purity of a.s.: [[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbomoyl]-methanesulfinyl]-acetic acid, Reg. No. 360 712): 98.7 ± 1%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight of 300 – 600 mg), age: between 2 and 12 months; source: in-house.

Test design: 14-d exposure in treated artificial soil (10% peat) according to OECD 207; different concentrations of the test item were mixed homogeneously into the soil, which was then used to fill glass vessels after which the earthworms were introduced on top of the soil; 6 treatment groups (5 test item concentrations, water control); 4 replicates/treatment group with 10 worms each. Assessment of worm mortality was carried out after 7 and 14 d, measurement of behavioral effects and weight change as sub-lethal parameter after 14 d.

Endpoints: LC50 (50% mortality of earthworms after exposure over 14 days), behavioural effects, weight change.

Test concentrations: Control (Water); 62.5, 125, 250, 500 and 1000 mg M31/kg dry soil (nominal).

Reference item: 2-Chloroacetamide. The effects of the reference item were investigated in a separate study.

Test conditions: Artificial soil according to OECD 207 (10% peat); pH 5.58 – 5.87 at test initiation, pH 5.40 – 5.63 at test termination; water content: approx. 54% of water holding capacity (WHC); temperature: 18 °C – 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 544 ± 22 lux.

Statistics: Descriptive statistics, Normality and Equal Variance test followed by one-way ANOVA ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LC_{50} was determined to be > 1000 mg M31/kg dry soil.

After 14 days of exposure, no mortality was observed in any test item group and the control. The biomass development was not statistically significant different compared to the control at all test item concentrations (ANOVA, $\alpha = 0.05$). The results are summarized in Table 8.7-4.

Table 8.7-4: Effect of M31, a metabolite of dimethenamid-P, on earthworm (*Eisenia fetida*) mortality and biomass (14 d)

M31 [mg/kg dry soil]	Control	62.5	125	250	500	1000
Mortality [%]	0	0	0	0	0	0
Weight change [%]	-3	-3	-5	-2	-6	-4
	Endpoint [mg/kg dry soil]					
LC_{50}	> 1000					
NOEC	≥ 1000					

III. CONCLUSION

In a 14-day toxicity study to earthworms (*Eisenia fetida)* with M31, a metabolite of dimethenamid-P, the LC_{50} was > 1000 mg/kg dry soil. The NOEC related to mortality and biomass was determined to be ≥ 1000 mg/kg soil.

CA 8.8 Effects on biological methods for sewage treatment

Since Annex I inclusion of dimethenamid-P (BAS 656 PH), no new studies have been performed for investigation of the effects on biological methods for sewage treatment.

Therefore, the results of the already submitted studies are still valid. For better transparency and traceability of the active substance history, the results of already submitted and accepted studies are summarized in Table 8.8-1.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	**Study type**	**Endpoint [mg a.s./L]**	**Reference** (Author (Year), BASF DocID)	**EU agreed**
BAS 656 H (dimethenamid)[#]	*Pseudomonas* cell multiplication inhibition test (determination of the inhibitory effect on bacteria)	EC_{50} > 400 EC_{10} > 400	Scholtz R. (1994), 1994/11901	Yes
BAS 656 H (dimethenamid)[#]	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	EC_{50} > 100 EC_{10} > 100	Desmares-Koopmans M.J.E. (1995), 1995/11327	Yes

[#] Study was performed with technical dimethenamid (BAS 656 H, Reg. No. 360 720).

CA 8.9 Monitoring data

No monitoring studies assessing ecotoxicological effects of dimethenamid-P are available.

Dimethenamid-P

DOCUMENT M-CA, Section 9

LITERATURE DATA

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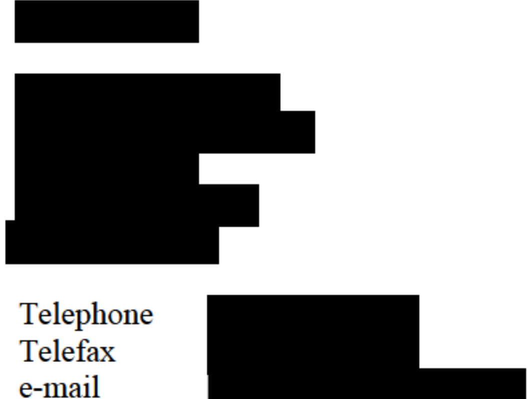

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CA 9 LITERATURE DATA

A literature search on Dimethenamid-P and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on Dimethenamid-P describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The complete search report is provided in K-CA 9 (BASF DocID 2014/1103028).

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; a list is available upon request.

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:

Product Chemistry:	Dimethenamid-P - Literature Product Chemistry
Toxicology:	Dimethenamid-P - Literature Toxicology
Consumer Safety:	Dimethenamid-P - Literature Consumer Safety
E-fate:	Dimethenamid-P - Literature E-fate,
Ecotoxicology:	Dimethenamid-P - Literature Ecotoxicology general
	Dimethenamid-P - Literature Ecotoxicology aquatic
	Dimethenamid-P - Literature Ecotoxicology terrestric
	Dimethenamid-P - Literature Ecotoxicology wildlife

The hits in Product Chemistry, Ecotoxicology or Analytical Methods did not contribute to the risk assessment and were therefore not further discussed in the dossier.

Report: CA 9/1
Coscolla C. et al., 2010b
Occurrence of currently used pesticides in ambient air of Centre Region (France)
2010/1229759
Guidelines: none
GLP: no

Executive Summary

The study aimed to improve the knowledge about the atmospheric behaviour of current-used pesticides in the central region of France. A group of 56 pesticides including dimethenamid was monitored in this region during 2006 to 2008. Weekly, air samples were collected at three rural and two urban sites on quartz fibre filters plus polyurethane foam plugs. Targeted pesticides were extracted from the samples and analysed to determine their concentrations. In 262 samples analysed, dimethenamid was detected at a frequency of 2%. The overall concentration of dimethenamid ranged from 0.16-0.74 ng m^{-3}.

MATERIALS AND METHODS

1. Test material

In the study, three groups of pesticides were tested:
A group of herbicides including dimethenamid
A group of insecticides
A group of fungicides

2. Field study design

The study was carried out within the central region of France on three rural sites (Saint Martin d'Auxigny, Oysonville and Saint Aignan) and two urban sites (Tour and Orléans). The area of the region is about 40000 km^2 and about 57% of the surface is used for agricultural activities. Pesticides are used intensively in the farming activities in this region.

Air Sampling and analysis

Sampling was performed in three campaigns from 2006 to 2008 [2006: 14 March12 September; 2007: 11 April-11 July, except for Saint Martin d'Auxigny (until 11 September). 2008: 9 April - 2 July, except for Saint Martin d'Auxigny (until 5 November)) using a low-volume sampler (Partisol 2000). Gas samples and particulate matter were collected together on quartz fibre filters (47 mm diameter) followed by polyurethane foam plugs (26 mm diameter x 76 mm length). Sampling was performed on a weekly basis by exposing filters and polyurethane plugs at a flow rate of 1 m^3 h^{-1}. A total volume of 168 m^3 was collected approximately. Samples were analysed after sampling or after a storage period at -18 °C. The studied pesticides were extracted from the samples and the concentrations were determined using LC-MS/MS and GC-MS techniques.

RESULTS AND DISCUSSION

Method efficiency

The retention capacity for the polyurethane foam plugs ranged from 60-120% and the obtained recoveries were in the range from 70-110% for PUF plugs plus quartz filters.

Residues in Air

In total, 262 air samples were tested for herbicides along the study period. Dimethenamid was detected at a low frequency of 2%. The overall concentration of Dimethenamid ranged from 0.16-0.74 ng m^{-3}.

Report: CA 9/2
Chevre N. et al., 2007a
Risk assessment of herbicide mixtures in a large European lake
2013/1348573
Guidelines: none
GLP: no

Executive Summary

The aim of the study was to determine the levels of various pesticides of the Geneva Lake. A group of pesticides including RS-Dimethenamid were monitored in 2004 and 2005. Water samples were collected at two different depths and different times from the lake. Samples were processed and analysed to determine pesticides concentrations. RS-Dimethenamid was found only at one sampling date with similar average concentration (0.001 µg L^{-1}) in both depth ranges. The risk assessment results of the herbicides showed no risk of RS-Dimethenamid contamination.

MATERIALS AND METHODS

Test material

Pesticides including RS-Dimethenamid

Field study design

The study was performed in 2004 and 2005 to determine the levels of various pesticides of Geneva Lake. Samples were taken at different depths and different periods from a site situated in the middle of the lake. This site has been used for long time as a reference point, therefore it was considered as representative of the average contamination of the lake.

Water sampling and analysis

Water samples (2 L) were collected two times in April 26, 2004 and April 26, 2005, and one time in September 6, 2004. The samples were taken at nine different depths; Five at 0-10 m depths in the epilimnion-metalimnion (0, 1, 5, 7.5, and 10 m), and four at 10-309 m depth in the hypolimnion (30, 100, 305, and 309 m). An aliquot of 500 mL samples were extracted on an Oasis HLB cartridge (Waters) concentrated to 100 µL and analysed using HPLC/MS-MS. The risk for RS-Dimethenamid was assessed by comparing the measured concentration to the water quality criteria (WQC) set by the authors.

RESULTS AND DISCUSSION

Method efficiency
A generic sample preparation for a wide range of pesticides was developed for water samples. High concentrated extracts of the water samples enabled reaching a limit of detection in the range from 1-100 ng $L^{-1}$for the different herbicides. The method recovery ranged from 50-120%. The method was validated with five concentration levels (1, 5, 20, 50, and 200 ng L^{-1}) using pure water, two repetitions for five days. The coefficient of variation for intra-day precision ranged from 8-18%, and for inter-day from 18-38%.

Residues in water
RS-Dimethenamid was detected only at one sampling date at both depth ranges with an average concentration of 0.001 µg L^{-1}. The concentration of RS-Dimethenamid was below the limit of detection at all other sampling dates and thus below the value for the WQC (0.16 µg/L) set by the authors, indicating no risk of RS-Dimethenamid contamination.

Report: CA 9/3
Leu C. et al., 2004a
Simultaneous assessment of sources, processes, and factors influencing herbicide losses to surface waters in a small agricultural catchment
2013/1348574
Guidelines: none
GLP: no

Executive Summary

The aim of the study was to gain a detailed knowledge about all factors that control the losses of Dimethenamid from a point source (farmyard) and from diffuse sources (fields). A small area of the catchment of the Lake Greifensee, located 25 km southeast of Zurich and into the river Aa Mönchaltorf was investigated over a period of 67 days after a controlled application of Dimethenamid and two other pesticides. Dimethenamid was applied on 13 cornfields at 0.75 kg ha^{-1} using a boom sprayer. A high temporal sampling resolution approach was applied to collect water samples. Soil samples were also collected from all 13 fields. Thirteen rain events were recorded, causing loss of the herbicide mainly from diffuse sources by surface runoff and preferential flow into the subsurface drainage system.
The maximum concentration of the herbicide reached 1.5 µg L^{-1} at the discharge peak. Total mass losses of Dimethenamid from the fields of the catchment accounted for 0.27% of its total amount applied.
The dissipation of Dimethenamid from field was described by first-order kinetics with a DT_{50} of 13 days as a median value from 11 fields.

I. MATERIALS AND METHODS

3. Test material

Frontier 900 EC (emulsion, containing dimethenamid)

4. Field study design

The investigated area comprises about 2.1 km^2 of the catchment of the Lake Greifensee, located 25 km southeast of Zurich and drains into the river Aa Mönchaltorf. Agricultural land forms 91% of the catchment area; 7% of it are forest and the remaining area includes farmyard, buildings and roads. Soils in the northeast of catchment have developed from tertiary river deposits called Obere Süsswassermolasse. The underground of the remaining area is moraine material of the Würm glaciation. The annual precipitation is 1330 mm on average and higher rainfall may occur during the vegetation period. The average monthly temperatures range from -1°C in January to 18°C in July. In this area, 60% of the 7.2 km brooks length flow in subsurface concrete tubes. 12% of the catchment is systematically drained with tiles at 1.4 m depth and spaced at ~14 m. The study investigated 13 cornfields including poorly drained (cambic) Gley soils (73%) and well to relatively well drained (calcaric) Cambisol (27%). Five fields were systematically drained and at least five additional fields were non-systematically drained. Top soils of these fields are loamy to clay loamy with organic matter contents ranging from 2.8 to 8.5%. Dimethenamid was applied (in a mixture with two other herbicides) on 13 cornfields at 0.75 kg ha^{-1} using a boom sprayer on May 8, 2000. With each mixture a fourth pesticide (tracer pesticide) out of a selection of nine substances was applied to each field to identify losses from individual fields. Post- and pre-emergence field applications were performed. During the study year, Dimethenamid was not used by other farmers in the catchment outside the test area.

5. Catchment sampling and analysis

Two rain gauges were used to record the amounts of rain every 10 min. Brook discharge at the outlet of the catchment was determined by two different methods. First, pressure at the bed of a flume was continuously recorded with a transducer connected to a data logger. The dilution method with NaCl was used to calibrate a pressure-discharge relation based on 16 calibration points over the whole discharge range, gauged during the sampling period. Second, the level and average flow velocity were continuously gauged by a sensor. Discharge data of both systems as well as conductivity were measured and stored in 5 min intervals. At the same station, water samples were taken from the brook using three portable automatic samplers. Two sampling approaches were followed. First, two samplers were used to collect time-proportional samples, triggered by elevated discharge levels. The sampling intervals were between 5 and 20 min and between 15 and 60 min, respectively. Second, a third sampler was set to take flow-proportional composite samples each composed of 9 subsamples.

A total of 596 water samples were analysed for Dimethenamid using a SPME-GC/MS analytical method. Limits of quantification for the investigated substances were in the range from 0.02 to 0.12 µg L^{-1} for the different pesticides. Tracer pesticides were determined with the same analytical method. Another analytical method was used to quantify ethansulfonic acid and oxanillic acid degradates of Dimethenamid. The limit of quantification for this method ranged from 0.003 to 0.01 µg L^{-1}.

Soil samples from all cornfields were taken once prior to herbicides application and eleven times after application during a time period of 50 days. Fifteen to 20 cores were taken randomly on every field and mixed to one composite sample per field. Samples were weighted, milled, homogenised and divided into aliquots of 8 g before analysis. An internal standard of d_3-dimethenamid was added to the soil aliquots before extraction and Dimethenamid was quantified using GC-MS. The limit of quantification ranged from 0.03 to 0.05 µg g^{-1} dry matter.

II. RESULTS

Loss dynamics of Dimethenamid

A total of 260 mm precipitation occurred during the 67 days from application until end of the sampling period. Thirteen rain events caused losses of herbicides from point sources (runoff from farmyards) or diffuse sources (runoff and preferential flow from fields) into the brook or subsurface drainage system. The first five rain events mainly caused loss of herbicides from point sources by very short runoff events. As Dimethenamid never was used on the farmyard only very small concentrations were found at the outlet of the catchment.
Diffuse loss was the major source of herbicide removal from the fields into the brook. For Dimethenamid, diffuse loss after rain events 6, 7, 9 and 13 accounted for 99% of the total brook loads lost until day 67 after application.
Table: 9-1 gives maximum Dimethenamid concentrations, loads, and maximum discharge measured in the brook at the outlet of catchment during the four most important diffuse loss events.

Table : 9-1 Maximum Dimethenamid concentrations, loads, and maximum discharge measured in the brook at the outlet of catchment during the four most important diffuse loss events (Leu *et al.* 2004)

Rain event	**6**	**7**	**9**	**13**
Days since application	23-29	29-33	37-41	61-67
Maximum concentrations (µg L^{-1})	1.47	0.32	0.12	0.02
Load (g)	21.7 (93%)	1.2 (5%)	0.2 (1%)	nd [a]
Maximum flow ($m^3 s^{-1}$)	0.63	0.22	0.19	0.49

[a] The dimethenamid load during event 13 was not quantified since concentrations were below the quantification limit of the SPME-GC/MS method. Note that the difference of 2 g resulting from treating values below the quantification limit as 0 as compared to treating them as 0.02 µg L^{-1} (i.e., the quantification limit itself) is small as compared to the total load of 23 g. Parentheses list percentages of individual loads from the total load lost from the fields until day 67 after application

The load for Dimethenamid metabolites Dimethenamid ethansulfonic acid (ESA) and Dimethenamid oxanillic acid (OXA) were 11.1 g and 15.5 g at the loss event 6. The ratio of molar amounts of metabolites to parent compound at this event was 0.54 and 0.73 for Dimethenamid ESA and Dimethenamid OXA.
Total mass losses of Dimethenamid from the fields of the catchment accounted for 0.27% of its total amount applied.
The dissipation of Dimethenamid from soil was described by first-order kinetics with a field DT_{50} of 13 days as a median value from 11 fields.

Report: CA 9/4
Hamer K.,Freudenberger U., 2011a
Pflanzenschutzrechtlich nicht relevante Metaboliten im Grundwasser
2013/1348575
Guidelines: none
GLP: no

Executive Summary

The authorisation process of plant protection products differentiates between relevant and non-relevant metabolites. Also non-relevant metabolites are considered as undesired in drinking water. The aim of the paper was to discuss the current legal situation and to assess whether the legal regulation covers the risk assessment of non-relevant metabolites occurring in the environment. The legal situation was evaluated and summarised from the Bund-/Länder-Arbeitsgemeinschaft Wasser (LAWA) on authority of the Umweltministerkonferenz (Conference of Environmental Ministers).

The paper presents a compilation of 24 substances investigated during groundwater monitoring of non-relevant metabolites in Germany from 2006 to 2008 (data obtained from secondary literature). Among these are data on two metabolites of Dimethenamid-P: dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23. Both metabolites did not exceed the maximum permissible level for groundwater of 1.0 µg/L (standard health orientation value recommended by the Umweltbundesamt [UBA]).

MATERIALS AND METHODS

6. Test material

Dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23 were measured in the monitoring programm.

7. Study design

Results of regular measurements of dimethenamid-sulfonic acid M27 and dimethenamid acid M23 in Germany are given (228 and 232 monitoring points each in three federal states of Germany, respectively). The measurements were undertaken from 2006 to 2008 to evaluate the occurrence of non-relevant metabolites in groundwater. No information about sampling technique or sampling frequency was provided in the paper.

RESULTS AND CONCLUSION

Dimethenamid-sulfonic acid M27 and dimethenamid oxalic acid M23 were not detected above the standard health orientation values (gesundheitliche Orientierungswerte GOW) in the water samples of all groundwater samples. For both metabolites a standard health orientation value of 1 µg/L was recommended by the UBA – the value recommended for substances without study of chronic toxicity.

Dimethenamid-P

DOCUMENT M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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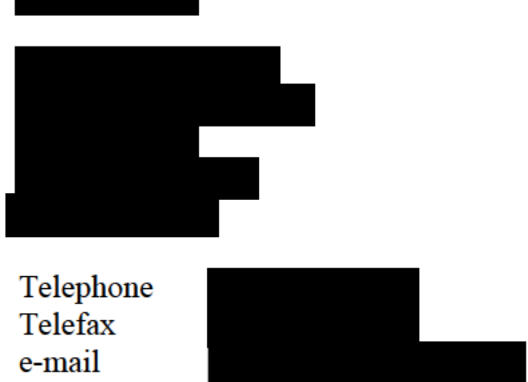

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Version history[1]

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 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for Dimethenamid-P:

Legislation	Classification	Labelling	Concentration limits
Directive 67/548/EEC	Xn; N R:22-43 R:50/53	Xn; N R: 22-43-50/53 S: (2-)24-37-60-61	N; R50-53: C ≥ 2,5 % N; R51-53: 0,25 % ≤ C < 2,5 % R52-53: 0,025 % ≤ C < 0,25 %
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 4(oral) Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H302,H317, H400, H410	Pictogram signal word code: GHS07 GHS09 Warning Hazard statement code: H302,H317, H400, H410	M-factor = 10