We create chemistry

Metrafenone

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

Amended by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
19 October 2015	Original submission	BASF Doc ID 2015/1170518
04 December 2015	CA 1.1 New Contact Person as of Nov. 2015	BASF DocID 2015/1253679

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

Table of Contents

CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF SE
Crop Protection Division
P.O. Box 120
67117 Limburgerhof
Germany

Contact person:

Until Oct. 2015

[redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany

Telephone: [redacted]
Telefax:
E-mail:

As of Nov. 2015

[redacted]
BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

(b) Alternative: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany

Telephone: [redacted]
Telefax:
E-mail:

CA 1.2 Producer

Confidential information. Refer to document J-CA.

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Metrafenone

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC	3'-bromo-2,3,4,6'-tetramethoxy-2',6-dimethylbenzophenone
CAS	Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 560 F

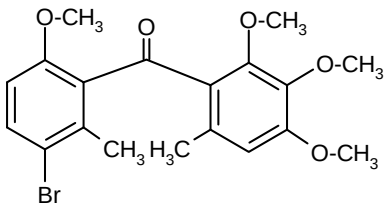
CA 1.6 CAS, EC and CIPAC Numbers

CAS number:	220899-03-6
EC number:	Not allocated
CIPAC number:	752

CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{19}H_{21}BrO_5$

Structural formula:



Molecular mass: 409.27 g/mol

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

CONFIDENTIAL information - data provided separately (Document J-CA)

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

Minimum purity: 980 g/kg (see Document J-CA)

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)

CA 1.10.2 Significant impurities

CONFIDENTIAL information - data provided separately (Document J-CA)

CA 1.10.3 Relevant impurities

CONFIDENTIAL information - data provided separately (Document J-CA)

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J-CA)

BASF
We create chemistry

Metrafenone

Document M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

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		99.5%	99.2 – 100.8°C		EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
		99.5%	The pure a.s. did not show an indicationof a boiling point up to a temperature of approx. 310°C, where the test substance turned to a black tar accompanied by a smell of decomposition		
CA 2.2 Vapour pressure, volatility		99.7%	1.53×10^{-4} Pa (1.53×10^{-6} mbar) at 20°C 2.56×10^{-4} Pa (2.56×10^{-6} mbar) at 25°C		EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
		-	Based on the vapour pressure at 20°C and the water solubility at 20°C: KH = 0.132 Pa m^3 mol^{-1}		
CA 2.3 Appearance (Physical state, colour)		99.5%	White crystalline solid		EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
		95.9%	Powdery, yellow-white crystalline solid		

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV-Vis OECD 101	99.7%	Solvent / pH / λmax (nm) / Absorbance / ε (dm^3/mol/cm) Purified water / 6.9 / 226(sh) / 0.9447 / 24300 290 / 0.3598 / 9200 0.1M HCl / 1.4 / 226(sh) / 0.9544 / 24500 290 / 0.3608 / 9300 0.1M NaOH / 13.2 / 226(sh) / 0.9169 / 23600 290 / 0.3453 / 8900 *sh = shoulder	Y	Cowlyn, N. (2015d) [see 2014/1036915]
	1H NMR	99.5%	Simple proton spectrum. Well resolved singlets for the separate methyl and methoxy protons and the isolated aromatic proton. Doublets for the adjacent aroamtic protons.	Y	Metrafenone DAR, UK, July 2005
	^{13}C NMR	99.5%	More complex spectrum but with distinct signals for all 19 carbon atoms.	Y	Metrafenone DAR, UK, July 2005

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
	Infra-red	99.5%	3084 cm^{-1}: aromatic C-H stretch 3000 – 2800 cm^{-1}: aliphatic C-H stretch 1667 cm^{-1}: C=O stretch 1572, 1460 cm^{-1}: aromatic C=C stretch 1280, 1265 cm^{-1}: C-O-C stretch 834 cm^{-1}: aromatic C-H deformation Complex IR fingerprint between 1620-600 cm^{-1} in which few peaks can be confidently assigned.	Y	Metrafenone DAR, UK, July 2005
	Mass spec	99.5%	Electron Impact MS: 408/410 [M^+], 393/395, 377/370, 227/229, 209 Positive Chemical Ionisation MS: 409/411 $[M+H]^+$, 331, 227/229, 209 Atmospheric Pressure Chemical Ionisation MS: 409/411 $[M+H]^+$, 227/229, 209 All listed ions were structurally assigned in the study report.	Y	Metrafenone DAR, UK, July 2005
CA 2.5 Solubility in water	EEC Method A6 OECD 105	99.7%	0.55 mg/L in purified water at 20°C	Y	Cowlyn, N. (2015d) [see 2014/1036915]
CA 2.6 Solubility in organic solvents	EEC Method A6	100.0%	Solvent — Solubility (g/L) n-Heptane — 4.1 Xylene — 200 1,2-Dichloroethane — >250 Methanol — 31 Acetone — >250 Ethyl acetate — 230	Y	Cowlyn, N. (2015e) [see 2014/1036914]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.7 Partition co-efficient n-octanol/water		Metrafenone 99.5%	pH4 at 25°C: $\log_{10}P_{ow}$ = 4.3 The effect of pH was not investigated, as the test substance shows no dissociation in water.	Y	EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
	EC Method A8	CL 377160 96.9%	pH4: $\log_{10}P_{ow}$ = 3.2 pH7: $\log_{10}P_{ow}$ = 3.2 pH10: $\log_{10}P_{ow}$ = 1.3	N	Cowlyn, N. (2014f) [see 2014/1036916]
	EC Method A8	CL 375816 99.5%	pH4: $\log_{10}P_{ow}$ = < 1 pH7: $\log_{10}P_{ow}$ = < 1 pH10: $\log_{10}P_{ow}$ = < 1	N	Cowlyn, N. (2014g) [see 2014/1036917]
	EC Method A8	CL 4084564 98.2%	pH4: $\log_{10}P_{ow}$ = 1.8 pH7: $\log_{10}P_{ow}$ = 1.8 pH10: $\log_{10}P_{ow}$ = 1.3	N	Cowlyn, N. (2014h) [see 2014/1036918]
	EC Method A8	CL 3000402 99.6%	$\log_{10}P_{ow}$ = 3.0	N	Cowlyn, N. (2014i) [see 2014/1036919]
	EC Method A8	CL 434223 98.8%	pH4: $\log_{10}P_{ow}$ = 3.3 pH7: $\log_{10}P_{ow}$ = 3.2 pH10: $\log_{10}P_{ow}$ = < 1	N	Cowlyn, N. (2015f) [see 2014/1036920]
	EC Method A8	CL 376991 99.9%	$\log_{10}P_{ow}$ = 3.4	N	Cowlyn, N. (2014j) [see 2014/1036921]
CA 2.8 Dissociation in water		99.5%	No dissociation		EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
CA 2.9 Flammability and self-heating		95.86%	Flammability: Not highly flammable	Y	EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
		95.86%	Self-heating: No indication of an exothermic reaction was observed at temperatures up to 400°C at at heating rate of 0.5°C/min.	Y	Metrafenone DAR, UK, July 2005

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.10 Flash point			Not applicable, as the test substance is a solid		Metrafenone DAR, UK, July 2005
CA 2.11 Explosive properties		95.86%	Not explosive.		EFSA Scientific Report (2006) 58, 1 – 72, List of Endpoints
CA 2.12 Surface Tension	EEC Method A5 OECD 115	99.7%	Not rquired, as the water solubility is less than 1 mg/L.	Y	Cowlyn, N. (2015d) [see 2014/1036915]
CA 2.13 Oxidising properties		95.86%	Not oxidising.	Y	Metrafenone DAR, UK, July 2005
CA 2.14 Other studies			None.		

Summary of Data Points CA 2.1 to CA 2.14

Metrafenone is a white, crystalline solid with a melting point of 99.2 to 100.8°C. It has a very low vapour pressure and is very slightly volatile. It has low solubility in water (0.55 mg/L) but is soluble in organic solvents. It does not dissociate in water and has a partition coefficient (log Pow) of 4.3, indicating a potential for bioaccumulation. Metrafenone does not possess explosive or oxidising properties and is neither flammable nor auto-flammable, indicating that it does not present problems during transport or storage. There are no implications for classification.

We create chemistry

Metrafenone

DOCUMENT M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Metrafenone, a benzophenone fungicide, is used in various countries for the control of powdery mildew in a range of crops and for the control of eyespot in cereals. After application to the plant, the active ingredient is taken up via the leaf and then translocated via the transpiration flow. Due its mobility, it shows systemic and translaminar activity. By that, it can control fungal stages which have already become established in deeper tissue layers. Metrafenone is thus suitable for preventative and curative treatments.
Since the vapour pressure of metrafenone is low to moderate, a certain level of activity via gas phase could be confirmed which allows protection of plant parts which were not directly treated.

CA 3.2 Function

Metrafenone is used as a fungicide.

CA 3.3 Effects on Harmful Organisms

Morphological observations on the mode of action in cereal powdery mildew show that it inhibits growth of mycelium on the leaf surface, leaf penetration, formation of haustoria and sporulation.

CA 3.4 Field of Use Envisaged

Agriculture and horticulture.

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

Powdery mildew *(Erysiphe graminis)* and eyespot (*Pseudocersporella herpotrichioides)* in cereals, and powdery mildew (*Uncinula necator and Erysiphe necator)* in grapevines.

CA 3.6 Mode of Action

Preventative treatments with metrafenone reduce spore germination and block development beyond formation of appressoria, which penetrate less often. Additionally, metrafenone affects fungal survival by causing swelling, bursting and collapse of hyphal tips resulting in the release of globules of cytoplasm. Bifurcation of hyphal tips, secondary appressoria and hyperbranching were also frequently observed (Opalski et al. 2006). A histochemical analysis showed that metrafenone causes disruption of the apical actin cap and apical vesicle transport as well as weakening of the cell wall at hyphal tips. Finally, metrafenone strongly reduces sporulation. Reduced sporulation is associated with malformation of conidiophores that show irregular septation, multinucleate cells and delocalization of actin. Microtubules seem to be only secondarily affected in metrafenone-treated *B. graminis*. The results suggest that the mode of action of metrafenone interferes with hyphal morphogenesis, polarized hyphal growth, and establishment and maintenance of cell polarity. Metrafenone likely disturbs a pathway regulating organization of the actin cytoskeleton.

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

First evidence of resistance came from sensitivity monitoring studies on *Blumeria graminis* f.sp. *tritici* in 2008 (Felsenstein *et al.* 2010, Stammler *et al.* 2014). In the following years, monitoring was intensified and airborne isolates were randomly collected in different regions of the most important European cereal growing regions. The sensitivities of the majority of the isolates were comparable to the baseline sensitivity, which was determined in 2000 before market launch of the compound. Three different sensitivity phenotypes were identified for wheat powdery mildew (Figure 1), those with wild type sensitivity (EC_{50} <0.02 mgl^{-1}), moderately adapted isolates (EC_{50}=0.1-0.5 mgl^{-1}) and resistant isolates (EC_{50}>10 mgl^{-1}). Sensitive reference isolates were completely inhibited at 1/3 of the registered dose rate at any application time point (2 days preventive as well as 2 days curative treatment scheme) and also moderately adapted strains were controlled well at full rate in any trial lay-out. A strain classified as resistant showed a decreased response to increasing fungicide concentrations and was not completely controlled even at high dose rates (Figure 2).
Spores from the 3-day curative trial derived from lesions of untreated leaves and from leaves treated with 1/9 rate (for sensitive isolates) or 1/3 rate (for moderately adapted isolates and resistant isolate) were used as inoculum to investigate the viability of spores from treated leaves. The results indicate that the spores from treated leaves infected with the sensitive and moderately adapted isolates cannot infect untreated leaves, while spores from treated leaves infected with the resistant isolate are able to infect untreated leaves (Table 1).

Frequency of moderately adapted isolates slightly increased from 2009 to 2012 (up to 25%) with a stabilisation in the last 2 years. Resistant isolates remained at low frequency (~1%), despite their high resistance levels (Figure 3). Competition trials, where sensitive, moderately adapted and resistant isolates were mixed in various constellations and grown for several cycles on untreated wheat leaves, showed that the sensitive isolates dominated after several transfers in mixtures with moderately adapted or resistant isolates (Figure 4).
The findings on the frequency development of less sensitive strains over Europe and the competition experiments indicate fitness penalties for the moderately adapted and resistant phenotypes.

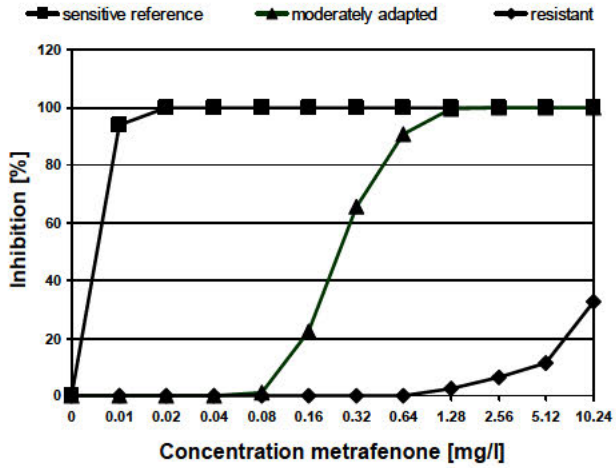


Figure 1. Inhibition curves of a sensitive, moderately adapted and resistant isolate of wheat powdery mildew in detached leaf assay.

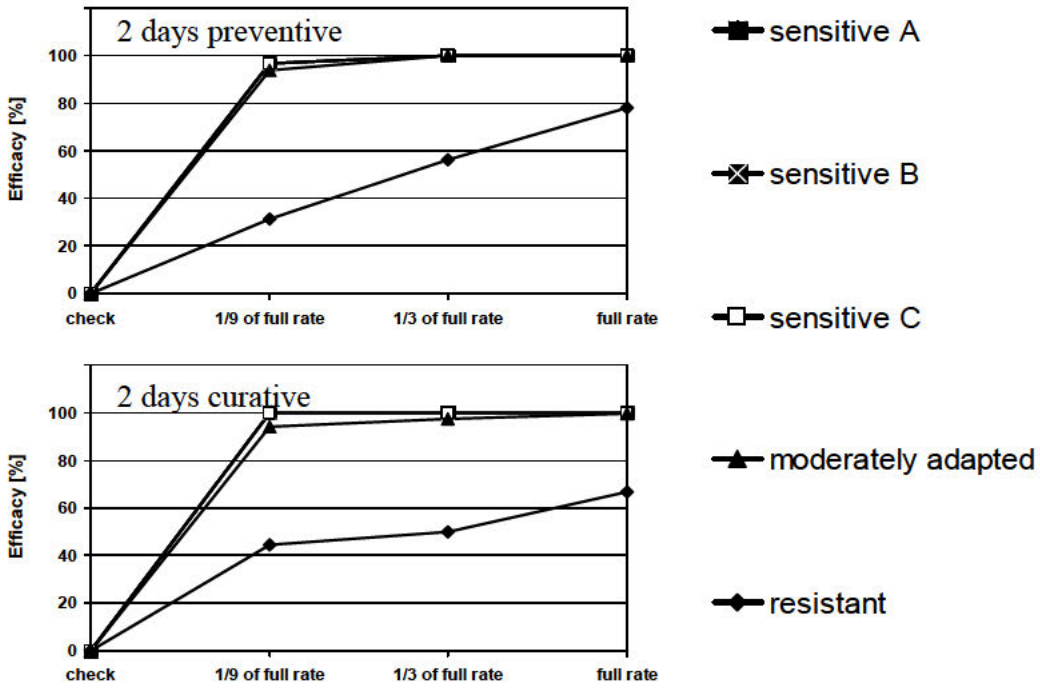


Figure 2. Preventive and curative activity of metrafenone on isolates of wheat powdery mildew with different sensitivities to metrafenone. Three sensitive isolates (labeled with squares), 1 moderately adapted isolate (triangle) and 1 resistant isolate (rhombus) were included. All three sensitive isolates were nearly completely inhibited at lowest concentration and share therefore the same lines.

Table 1. Capability of infection of spores from different isolates taken from metrafenone-treated leaves. Spores of sensitive and moderately adapted isolates from treated leaves were not able to infect untreated leaves. Spores of a resistant isolate from treated leaves infected untreated leaves. Spores of all isolates from untreated leaves infected untreated leaves (+ = spores infected untreated leaves, - = spores did not infect untreated leaves).

Origin of spores	Sensitive isolate	Moderately adapted isolate	Resistant isolate
Spores from untreated leaves	+	+	+
Spores from treated leaves*	-	-	+

* Spores taken from 1/9 reg. rate for sensitive and 1/3 reg. rate for moderately adapted and resistant isolate.

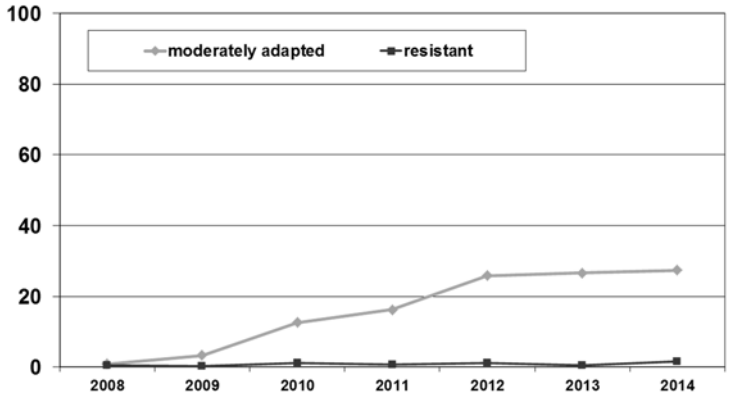


Figure 3. Frequency of metrafenone moderately adapted and resistant isolates of wheat powdery mildew in Europe from 2008 to 2014.

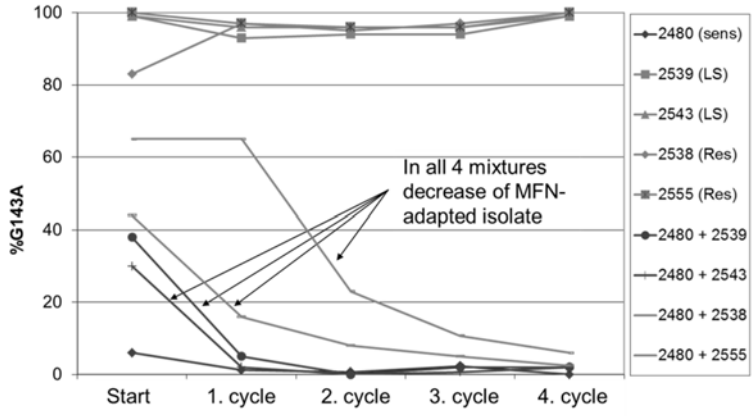


Figure 4. Competitive growth of sensitive (2480), moderately adapted (2539 or 2543) and resistant (2538 or 2555) isolates on untreated leaves for 4 cycles. Moderately adapted and resistant isolates contained G143A in cytochrome *b*, which was used as marker and detected by qPCR. "Start"= initial suspension value. Curves show high values for single isolates 2539, 2543, 2538 and 2555. Four curves of the mixtures 2480+2538, 2480+2555, 2480+2539 and 2480+2538 showed decreasing tendency (MFN = metrafenone). Curve at the bottom represents the sensitive isolate without G143A mutation.

Studies on the sensitivity of other target pathogens to metrafenone

European sensitivity monitoring studies for barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) showed no adaptation to metrafenone up to now (last monitoring data from 2012) besides 1 isolate (out of 75) with metrafenone resistance which was found in 2010. In the years afterwards until last season (2014) no isolates with a moderately adapted or resistant phenotype have been found again.

The situation for grape powdery mildew (*Erysiphe necator*) is currently under investigation. One strain (out of 160) with a resistant phenotype had been detected in 2010 but not in the year after. Resistance of this strain decreased rapidly after several transfers indicating that adaptation was not stable or that the strain was a mix of sensitive and adapted isolates, and that the sensitive isolates dominated the population more and more during propagation steps. The hypothesis is that without selection pressure the sensitive isolates show a higher fitness than the resistant one as it has been also found for wheat powdery mildew. In 2012, a single isolate (out of 170) with a resistant phenotype was detected again, and also in the following years 2013 and 2014 single cases of metrafenone resistant isolates were detected in single vineyards in different areas of the most important grape growing regions of Europe. A recent paper from Kunova *et al.* (2015) reported a case of resistance in an Italian vineyard and that frequency decreased after metrafenone applications had ended.

All strains included in the cucurbit powdery mildew (*Podosphaera xanthii*, *Golovinomyces cichoracearum*) sensitivity monitoring studies from the last years (last monitoring done in 2014) were sensitive to metrafenone.

There is no rapid and reliable sensitivity monitoring method available for eye spot (*Oculimacula* species). However, there are no reports of a field failure in the last years.

No data are available for powdery mildews in strawberries (*Podosphaera aphanis* syn. *Sphaerotheca macularis*), hops (*Podosphaera macularis*), tomato, aubergine and pepper (*Leveillula taurica*), ornamentals (different species) and cobweb mold in mushrooms (*Hypomyces rosellus* anamorph *Dactylium dendroides).*

Mechanism of resistance

The mechanism of resistance is not yet known. Baseline and monitoring studies with different isolates from wheat, barley, cucurbit and grape powdery mildews showed that there is no cross-resistance to QoI, SDHIs, azanapthalenes, DMIs and morpholines.

Management strategy

The objective of anti-resistance management strategies is the reduction of selection pressure to avoid or delay the occurrence and spread of resistance.
This can be achieved by good agricultural practice which leads to less infection pressure (e.g. phytosanitary measurements, cultivation of less susceptible varieties, appropriate crop cultivation unfavourable for the target pathogens such as optimal sowing time, ploughing, optimised seed rate, optimal fertilization, crop rotation).
Limiting the number of sprays is an important factor in delaying the build-up of resistant pathogen populations. Number of applications of metrafenone containing products will is restricted depending on the product and target pathogens.
Since population size of pathogens is lower at disease onset than when already established in the field, selection pressure is less when using preventive applications rather than curative or eradicative spray schemes. Therefore, metrafenone containing products should be applied at early stages of disease development following the recommendations on the label and highly curative applications should be avoided.

A resistance monitoring programme for *Blumeria graminis* f.sp. *tritici, Blumeria graminis* f.sp. *hordei, Erysiphe necator, Podosphaera xanthii* and *Golovinomyces cichoracearum* is running since several years and will be continued in all regions where metrafenone products are used.

References

Felsenstein, F., Semar, M. and Stammler, G. (2010) Sensitivity of wheat powdery mildew (*Blumeria graminis* f.sp. *tritici*) towards metrafenone. *Gesunde Pflanzen* **62**, 29-33

Opalski, K.S., Tresch, S., Kogel, K.H., Grossmann, K., Köhle, H., Hückelhoven, R., (2006) Metrafenone: studies on the mode of action of a novel cereal powdery mildew fungicide. *Pest Management Science* **62,** 393-401

Stammler, G. Semar, M. and Strobel, D. (2014) Resistance management of metrafenone in powdery mildews. In: Dehne, HW.; Deising HB.; Fraaije, B.; Gisi, U.; Hermann, D.; Mehl, A.; Oerke, E.C.; Russell, PE.: Stammler, G.; Kuck KH. and Lyr H. (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VII,, pp. 1079-184. Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-13-6

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

Precautions for safe handling
No special measures necessary if stored and handled correctly.
Protection against fire and explosion:
Avoid dust formation. Avoid deposition of dust. Dust can form an explosive mixture with air. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Conditions for safe storage, including any incompatibilities
Segregate from foods and animal feeds. Segregate from bases. Segregate from acids.
Further information on storage conditions: Protect against moisture. Keep away from heat. Protect from direct sunlight.

Storage stability:
Storage duration: 24 Months
Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

Transport Information

Land transport
ADR

Hazard class:	9
Packing Group:	III
Identification Number:	UN 3077
Hazard label :	9, EHSM
Proper shipping Name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE SOLID NOS (Contains METRAFENONE 90%)

RID

Hazard class:	9
Packing Group:	III
Identification Number:	UN 3077
Hazard label :	9, EHSM
Proper shipping Name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE SOLID NOS (Contains METRAFENONE 90%)

Inland waterway transport
ADNR

Hazard class:	9
Packing Group:	III
Identification Number:	UN 3077
Hazard label :	9, EHSM
Proper shipping Name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE SOLID NOS (Contains METRAFENONE 90%)

Sea transport
IMDG

Hazard class:	9
Packing Group:	III
Identification Number:	UN 3077
Hazard label :	9, EHSM
Marine Pollutant	YES
Proper shipping Name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE SOLID NOS (Contains METRAFENONE 90%)

Fire fighting measures

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

Special hazards arising from the substance or mixture
Carbon monoxide, carbon dioxide, halogenated hydrocarbons, dibenzofuran, bromine compounds, hydrocarbons, brominated dibenzodioxins.
The substances/groups of substances mentioned can be released in case of fire.

Advice for fire-fighters
Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

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

CA 3.9 Procedures for Destruction or Decontamination

Unwanted quantities of BAS 560 F TGAI are best destroyed by combustion in a licensed incinerator.

Decontamination of equipment, packing etc. is achieved by washing with water.

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. Use breathing apparatus if exposed to vapours/dust/aerosol.

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

Methods and material for containment and cleaning up:
For small amounts: Sweep/shovel up.
For large amounts: Sweep/shovel up.
Cleaning operations should be carried out only while wearing breathing apparatus.
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
We create chemistry

Metrafenone

Document M-CA, Section4

ANALYTICAL METHODS

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 4 ANALYTICAL METHODS

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

A method for the determination of the pure active substance in the active substance as manufactured was reviewed in the metrafenone DAR (UK, July 2005) and remains acceptable (BASF DocID's 1998/1001703 and 1998/7000365).

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

Confidential information – see Document JCA.

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

No new environmental fate studies for which analytical methods are required are being submitted in support of the Annex I renewal of metrafenone.

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

No new efficacy studies for which analytical methods are required are being submitted in support of the Annex I renewal of metrafenone.

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

No new toxicology studies for which analytical methods are required are being submitted in support of the Annex I renewal of metrafenone.

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

No new exposure studies for which analytical methods are required are being submitted in support of the Annex I renewal of metrafenone.

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

Method number 535 (535/0, 535/1 or 535/2) is used for the determination of metrafenone in a number of the residue studies. The method was originally validated in BASF Doc ID 2004/1010553 and this study is summarised in CA 4.1.2/1 below. Where additional validation data are available in the residue studies, these are also summarised below.

Report:	CA 4.1.2/1 Benz A.,Mackenroth C., 2005a Validation of the analytical method No. 535/0: Determination of Metrafenone BAS 560 F (Reg.No. 4037710) in plant matrices 2004/1010553
Guidelines:	EPA 860.1340, EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 6.1.2, 6.1.6, 6.3.1/1, 6.3.1/2, 6.3.2/23 and 6.3.2/24

Principle of the Method (Method No. 535/0)
Samples (5g) are weighed into wide neck bottles (250 mL) and methanol/water/2N HCL, 70:25:5, v/v/v (100 mL) is added. The samples are homogenised for 2 minutes at 5000 rpm, before an aliquot (10 mL) is centrifuged for 5 minutes at 4000 rpm. An aliquot (1 mL) of the supernatant is transferred to a culture tube (10 mL) containing 0.2 N NaOH (1 mL), cyclohexane (5 mL) is added and the samples are shaken for 15 minutes. An aliquot (2 mL) of the cyclohexane phase is transferred to a culture tube and evaporated to dryness using a nitrogen steam at 40°C. The residues are dissolved in methanol (0.5 mL) and water is added (0.5 mL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411.0 > 209.0 is used for quantification and the ion transition m/z 411.0 > 229.0 is used for confirmation.

Recovery Findings
Recovery data were generated from five samples fortified at the LOQ and five samples fortified at a higher concentration for each matrix. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-1 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.050 to 1.000 ng/mL. The correlation coefficient (r) was determined to be 0.9995 (slope = 59560.6633, intercept = 995.7479).

Specificity
HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at a higher concentration for each matrix. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-1 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-1: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Wheat whole plant	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	98.0, 101.2, 93.3, 89.2, 83.8	93.1	7.4
	5.0	92.9, 91.9, 96.0, 92.0, 93.9	93.3	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	94.7, 93.4, 92.0, 95.5, 94.3	94.0	1.4
	5.0	90.4, 94.0, 95.7, 93.8, 95.4	93.9	2.3
Wheat grain	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	88.4, 86.4, 83.7, 86.0, 89.3	86.8	2.5
	0.1	85.0, 86.8, 84.9, 85.3, 83.0	85.0	1.6
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	90.0, 90.8, 88.8, 87.4, 85.4	88.5	2.4
	0.1	84.1, 93.1, 90.8, 80.0, 74.4	84.5	9.1
Wheat straw	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	87.1, 89.7, 90.3 91.5*, 90.0	89.3	1.6
	5.0	97.1, 93.3, 94.1, 93.2, 93.0	94.1	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	94.4, 86.7, 86.4, 90.8, 90.5	89.5	4.2
	5.0	96.7, 96.2, 91.8, 87.6, 93.2	93.1	4.0
Grape, fruit	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	91.7, 89.0, 89.1, 87.9, 86.5	88.8	2.1
	0.1	78.4, 81.7, 82.7, 84.2, 89.9	83.4	5.1
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	82.8, 89.0, 89.5, 96.6, 86.7	88.9	5.7
	0.1	82.7, 76.1, 85.8, 79.2, 81.0	80.9	4.5

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Lemon fruit	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	88.1, 87.8, 87.7, 90.2, 83.7	87.5	2.7
	0.1	89.4, 90.1, 88.2, 89.8, 92.5	90.0	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	81.9, 78.2, 88.5, 88.5, 82.2	83.9	5.4
	0.1	89.0, 88.8, 92.1, 88.5, 88.6	89.4	1.7
Oilseed, rape seed	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	84.6, 85.8, 83.4, 83.5, 89.5	85.4	3.0
	0.1	83.4, 83.9, 82.8, 84.3, 86.3	84.1	1.6
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	85.1, 81.7, 82.4, 88.4, 88.0	85.1	3.6
	0.1	79.4, 84.3, 85.5, 77.7, 80.9	81.6	4.0

Method number 933/0 (also referred to as RLA 12619.00, .01V, .02V and .03V) is used for the determination of metrafenone in a number of the residue studies. The method was originally validated in BASF Doc ID 2001/7001048 and BASF DocID 2001/7001770, and these studies are summarised in CA 4.1.2/2 and CA 4.1.2/3 below. Where additional validation data are available in the residue studies, these are also summarised below.

Report:	CA 4.1.2/2 Smalley R., 2001a Method validation of RLA 12619.00 - Determination of CL 375839, CL 3000402, CL 434223, and CL 376991 residues in cereals (whole plant and straw) using LC-MS 2001/7001048
Guidelines:	EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Cross reference to M-CA Section 6.1/5, 6.3.2/22 and 6.5.3/8

Principle of the Method (993/0, also referred to as RLA 12619.00)
Samples are extracted with methanol/water (80:20) and filtered. Aliquots are taken and the methanol evaporated. The residue is partitioned with dichloromethane and cleaned up through a strong anion exchange cartridge. The samples are analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using a Phenomenex Ultracarb 5 ODS (30) column (100 x 4.6 mm) and gradient elution with mobile phases of 99/1, v/v, water/acetic acid and 99/1, v/v, methanol/acetic acid.

Recovery Findings
Recovery data was generated from five samples fortified at the LOQ and 10 x LOQ for each matrix. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in
Table 4.1.2-2 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.005 to 0.1 µg/mL. The coefficient of determination (R^2) was determined to be 0.9994 (slope = 10.98 x 10^8, intercept = 1558013).

Specificity
No interferences at or above the LOQ were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard and by the monitoring of several ion transitions (409 > 209, 409 > 227, 411 > 209 and 411 > 229).

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.1 mg/kg.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and 10 x LOQ for each matrix. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in
Table 4.1.2-2 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-2: Accuracy and Precision Data

Analyte	Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Metrafenone	Wheat whole plant	0.1	81 – 106 (5)	88	11.7
		1.0	84 – 90 (5)	88	2.8
	Wheat straw	0.1	78 – 95 (5)	86	9.1
		1.0	83 – 101 (5)	91	8.2

Report:	CA 4.1.2/3 Kang J., 2001a Validation of RLA 12619.02V for CL 375839 in wheat grain to a limit of quantification of 0.01 mg/kg 2001/7001770
Guidelines:	EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Cross reference to M-CA Section 6.1/5, 6.3.2/22 and 6.5.3/8

Principle of the Method (993/0, also referred to as RLA 12619.00)
Samples are extracted with methanol/water (80:20) and filtered. Aliquots are taken and the methanol evaporated. The residue is partitioned with dichloromethane and cleaned up through a strong anion exchange cartridge. The samples are analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using a MSLCC013 Luna C18 column (50 x 4.6 mm, 3 µm) and gradient elution with mobile phases of 99/1, v/v, water/acetic acid and 99/1, v/v, methanol/acetic acid. The three ions m/z = 411.3, 365 and 209.0 were selected for monitoring.

Recovery Findings
Recovery data was generated from five samples fortified at the LOQ and 10 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-3 below.

Linearity
Linearity of detector response was demonstrated using six external standard solutions across the concentration range of 0.5 to 100 ng/mL. The coefficient of determination (R^2) was determined to be 1.000 (slope = 99789, intercept = -16239).

Specificity
HPLC-MS monitoring three ions with m/z > 100 is considered to be a highly specific technique.
No interferences at or above the LOQ were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-3 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-3: Accuracy and Precision Data

Analyte	Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Metrafenone	Wheat grain	0.01	75 – 110 (5)	95	13.3
		0.1	94 – 103 (5)	98	3.4

Report:	CA 4.1.2/4 Jordan J.M.,Kasiri A., 2006a Magnitude of BAS 560 F residues in grapes and grape processed fractions following applications of BAS 560 00 F (amended final report) 2006/7007012
Guidelines:	EPA 860.1500, EPA 860.1520, EPA 860.1380
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 6.1/2 and 6.5.3/6

Principle of the Method (Method No. 535/0)
See CA 4.1.2/1 above.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and samples fortified at higher fortification levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-4 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.025 to 0.5 ng/mL. The correlation coefficient (r) was determined to be 0.9972 (slope = 48073.3039, intercept = 1137.1969).

Specificity
No interferences at or above the LOQ were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and samples fortified at higher fortification levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-4 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-4: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	SD	RSD (%)
Grape RAC					
Fresh Fruit	0.01	83 – 125 (13)	96	13	13.5
	0.10	82 – 127 (11)	101	13	12.9
	20.00	91, 125 (2)	108	-	-
Overall	0.01 – 20.0	82 – 127 (26)	99	13	13.1
Grape Processed Fractions					
Juice	0.01, 0.10	83, 91 (2)	87	-	-
Must	0.01, 0.10	84, 84 (2)	84	-	-
Unwashed Raisins	0.01	92 (1)	92	-	-
Washed Raisins	0.01, 1.00	88, 112 (2)	100	-	-
Wet Pomace	0.01, 2.00	80, 91 (2)	86	-	-
Wine	0.01, 0.10	94, 80 (2)	87	-	-
Yeast	0.01, 1.00	95, 98 (2)	96	-	-
Young Wine	0.01, 0.10	93, 100 (2)	96	-	-
Overall	0.01 – 2.00	80 - 112 (15)	91	9	9.9

Report:	CA 4.1.2/5 Lehmann A.,Mackenroth C., 2012a Investigation of the storage stability of BAS 560 F in plant matrices 2012/1166088
Guidelines:	EEC 7032/VI/97 rev. 5, 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), EPA 860.1380
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 6.1/6

Principle of the Method (Method No. L0076/01, also known as 535/1)
See CA 4.1.2/1 above.

Recovery Findings
Recovery data was generated from 10 samples fortified at the LOQ. The mean percentage recoveries were within the guideline requirements of 70-110% and the results are presented in Table 4.1.2-5 below.

Linearity
Linearity of detector response was demonstrated using six standard solutions. The correlation coefficient (r) was determined to be 0.9995 (slope = 1.65×10^5, intercept = 1.17×10^3).

Specificity
No interferences at or above the LOQ were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.1 mg/kg.

Repeatability
Repeatability data was generated from 10 samples fortified at the LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-5 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-5: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Wheat whole plant	0.1	76.0 – 102.8 (10)	85.1	9.6
Wheat grain	0.1	88.8 – 110.0 (10)	97.3	7.3
Wheat straw	0.1	78.1 – 95.5 (10)	88.0	6.5
Grape	0.1	78.2 – 101.0 (10)	89.8	8.8
Tomato	0.1	76.0 – 103.0 (12)	90.3	9.0
Dried peas	0.1	94.4 – 105.0 (10)	98.9	3.6
Soybean	0.1	91.6 – 108.8 (16)	99.8	5.0
Overall	0.1	76.0 – 110.0 (78)	93.2	9.0

Report:	CA 4.1.2/6 Fleischer G., 2014a Study on the residue behaviour of BAS 560 F (Metrafenone) in grapes (wine and table) after treatment with BAS 560 02 F under field conditions in Germany, France (North and South), Greece, Italy and Spain, 2013 2014/1161029
Guidelines:	EEC 7029/VI/95 rev. 5 Appendix B, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 6.3.1/1

Principle of the Method (Method No. 535/2)
Samples are extracted with a mixture of methanol, water and hydrochloric acid. Aliquots of the extracts are centrifuged and partitioned in alkaline conditions with cyclohexane. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a Kinetix C18 100A column (50 mm x 4.6 mm, 2.6 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411 > 229 is used for quantification and the ion transition m/z 411 > 209 is used for confirmation.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-6 below.

Linearity
Linearity of detector response was demonstrated using six standard solutions across the concentration range 0.05 ng/mL to 5.0 ng/mL. The correlation coefficient (r) was determined to be 0.9998 (slope = 292629.0584, intercept = 12078.0398).

Specificity
HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences were observed at or above the LOQ at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-6 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-6: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Wine grapes	0.01	63 – 106 (16)	83.9	15
	0.1	76 – 94 (16)	87.8	6
	0.5	66, 78 (2)	72.0	-
	1.0	82, 83 (2)	82.5	-
Overall		63 – 106 (36)	84.9	12
Table grapes	0.01	102 – 121 (4)	109.3	8
	0.1	87 – 94 (4)	89.5	4
Overall		87 – 121 (8)	99.4	12

Report:	CA 4.1.2/7 Oxspring S., 2014a Study on the residue behaviour of BAS 560 F (Metrafenone) in grapes (wine and table) after treatment with BAS 560 02 F under field conditions in Germany, France (North and South), Italy and Spain during 2014 2014/1161529
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5, OECD 509 Crop Field Trial (2009), EEC 7524/VI/95 rev. 2, SANCO/3029/99 rev. 4
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Cross reference to M-CA Section 6.3.1/2

Principle of the Method (Method No. 535/2)
Samples are extracted with a mixture of methanol, water and hydrochloric acid. Aliquots of the extracts are centrifuged and partitioned in alkaline conditions against cyclohexane. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a BetasilC18 column (100 mm x 2.1 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411 > 209 is used for quantification and the ion transition m/z 411 > 229 is used for confirmation.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The mean percentage recoveries obtained are presented in Table 4.1.2-7 below.

Linearity
Linearity of detector response was demonstrated using six standard solutions across the concentration range 0.05 ng/mL to 5.0 ng/mL. The correlation coefficient (r) was determined to be 1.0000 (slope = 948219, intercept = 17069).

Specificity
HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences were observed at or above the LOQ at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The relative standard deviations (RSD) obtained are presented in Table 4.1.2-7 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-7: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Grapes	0.01	97 – 118 (4*)	106	8
	1	65 – 105 (3)	91	25
	10	98, 93 (2)	96	-
Overall		65 – 118 (9)	99	14.7

* One sample identified as an outlier and removed from statistical calculation

Report:	CA 4.1.2/8 Raunft E. et al., 2004a Study on the residue behaviour of BAS 560 F in cereals after application of BAS 560 00 F under field conditions in Germany, Denmark, France (N) and United Kingdom, 2002 2003/1001354
Guidelines:	EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, 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
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 6.3.2/22

Principle of the Method (Method No. 993/0)
Samples are extracted with methanol/water (80:20) and filtered. Aliquots are taken and the methanol evaporated. The residue is partitioned with dichloromethane and cleaned up through a strong anion exchange cartridge. The samples are analysed by high performance liquid chromatography with mass specific detection (HPLC-MS).

Recovery Findings
Recovery data was generated from samples fortified at the LOQ, 10 x LOQ and 100 x LOQ for each matrix. The overall percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-8 below.

Linearity
Linearity of detector response was demonstrated using six external standard solutions. The correlation coefficient (r) was determined to be 0.9994 (slope = 24204.8068, intercept = 1027.2910)

Specificity
No interferences were observed at or above the LOQ at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ, 10 x LOQ and 100 x LOQ for each matrix. The overall relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-8 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-8: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Wheat plant, straw, ears, grains	0.01	70.8 – 98.8 (4)	79.1	9
	0.1	78.2 – 92.7 (4)	85.8	8
	1.0	75.2 – 93.9 (3)	87.2	12
Barley culms, grains	0.01	92.3, 98.8 (2)	95.6	5
	0.1	103.8, 104.9 (2)	104.4	1
	1.0	92.1	92.1	-
Overall		70.8 – 104.9 (16)	88.3	11.5

Report:	CA 4.1.2/9 Raunft E. et al., 2004b Study on the residue behaviour of Fenpropimorph and BAS 560 F in cereals after application of BAS 421 12 F, BAS 560 00 F and BAS 564 AF F under field conditions in France, Germany, Denmark, United Kingdom, Italy and Spain, 2003 2004/1010542
Guidelines:	EEC 91/414 (1607/VI/97), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 6.3.2/23

Principle of the Method (Method No. 535/0)
See CA 4.1.2/1 above.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-9 below.

Linearity
Linearity of detector response was demonstrated using six standard solutions. The correlation coefficient (r) was determined to be 0.9990 (slope = 34361.2301, intercept = 1235.1178).

Specificity
HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences were observed at or above the LOQ at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-9 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-9: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Grain, plant, culms, ears, straw	0.01	78.6 – 115.4 (10)	94.6	11
	0.1	72.3 – 113.3 (8)	91.6	15
	1.0	65.7 – 97.0 (8)	85.6	13
	10.0	77.5, 85.6 (2)	81.6	7
Overall		65.7 – 115.4 (28)	90.2	13.0

Report:	CA 4.1.2/10 White M.T.,Stewart J., 2006a Residue of Metrafenone (BAS 560 F) Fenpropimorph, (BAS 421 F) Epoxiconazol (BAS 480 F) in formulation bridging on wheat, barley after BAS 565 00 F BAS 562 00 F BAS 560 00 F BAS 421 12 F BAS 480 31 F field, in DE, DK, N-FR, S-FR, SE, UK 2005 2005/7004267
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 6.3.2/24

Principle of the Method (Method No. 535/0)
See CA 4.1.2/1 above.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-10 below.

Linearity
Linearity of detector response was demonstrated using external standard solutions. The correlation coefficient (r) was determined to be 0.9995 (slope = 93131.3419, intercept = 563.2365).

Specificity
HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences were observed at or above the LOQ the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-10 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-10: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Ear	0.01	83 – 118 (8)	99.4	11.1
	0.05	94 – 117 (3)	106.3	10.9
	0.1	87 – 121 (3)	99.3	19.0
	2	86 (1)	86	-
	10	115 (1)	115	-
Grain	0.01	77 – 124 (9)	96.9	17.0
	0.10	80 – 121 (7)	103.7	14.8
	20	96 (1)	96	-
Straw	0.01	90 – 124 (15)	106.9	10.6
	0.05	84 – 104 (5)	94.2	8.1
	0.10	85 – 111 (11)	98.6	9.7
	1	100 – 112 (3)	106.3	5.7
Whole plant	0.01	83 – 110 (7)	100.4	9.1
	0.05	75 – 104 (3)	86.3	18.0
	10	91 – 115 (4)	99.8	10.6
Overall		75 – 124 (81)	101	12

Report:	CA 4.1.2/11 Pollmann B., 2002a Determination of residues of BAS 560 F in field samples and processed goods after application of BAS 560 00 F in summer wheat at 4 sites in Germany in 2001 2002/1006302
Guidelines:	IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, BBA IV 3-3, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 91/414 (1607/IV/97 Rev. 1)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Cross reference to M-CA Section 6.5.3/8

Principle of the Method (Method No. RLA 12619.03V also known as 993/0)
Samples are extracted with methanol/water (80:20) and filtered. Aliquots are taken and the methanol evaporated. The residue is partitioned with dichloromethane and cleaned up through a strong anion exchange cartridge. The samples are analysed by high performance liquid chromatography with mass specific detection (HPLC-MS).

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and higher fortification levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-11 below.

Linearity
Linearity of detector response was demonstrated using external standard solutions across the concentration range 0.2 to 20 ng/mL. The slope was determined to be 2720.4559, intercept = -159.6635.

Specificity
No interferences at or above the LOQ were observed at the retention times of interest in control matrix samples. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg, apart from plants without ears (0.1 mg/kg) and ears (1.0 mg/kg).

Repeatability
Repeatability data was generated from samples fortified at the LOQ and higher fortification levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-11 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-11: Accuracy and Precision Data

Matrix	Fortification Level (mg/kg)	Recoveries (%) (n)	Mean Recovery (%)	RSD (%)
Whole grain bread, coarse bran, wholemeal flour, fine bran, flour 550, grain	0.01	76.7 – 107.2 (6)	90.7	13.2
Whole grain bread, coarse bran, wholemeal flour, fine bran, flour 550, plants, grain	0.1	72.8 – 101.5 (7)	86.0	11.5
Ears, plants	1.0	101.9, 91.0 (2)	96.5	8.0
Ears, plants	5.0	93.8, 86.7 (2)	90.3	5.6
Overall		76.0 – 107.2 (17)	89.4	11.1

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

Method number APL0500/03 and an additional method (HPLC-UV at 220 nm) have been used in a large number of the aquatic ecotoxicology studies included in MCA Section 8. Validation data for these methods, generated in the individual studies, is presented below. Although each of the individual studies may not contain sufficient validation data to satisfy the requirements of SANCO/3029/99 rev. 4, the total volume of data generated is considered sufficient to demonstrate the validity of these two methods.

Report:	CA 4.1.2/12 [redacted] 2012a BAS 560 F (Metrafenone) - Acute toxicity study in fathead minnow (Pimephales promelas) 2011/1281328
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 (1992), EPA 540/9-82-024, EPA 850.1075, EPA 72-1
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.1/3

Principle of the Method (APL0500/03)
Samples of mixing water are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with a molecular mass of m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from four samples fortified at the LOQ and 500 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-12 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.002 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9994 (slope = 5.27×10^{-6}, intercept = -0.0311).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.001 mg/L metrafenone.

Repeatability
Repeatability data was generated from four samples fortified at the LOQ and 500 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-12 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-12: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.00095	96.8, 97.9, 105.3, 103.2	100.8	4.1
	0.473	96.6, 96.6, 98.7, 97.9	97.5	1.1

Report:	CA 4.1.2/13 [redacted] 2005a BAS 560 F: A 96-hour flow-through acute toxicity test with the sheepshead minnow (Cyprinodon variegatus) 2005/7003439
Guidelines:	EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.1/4

Principle of the Method
Samples are diluted with filtered saltwater and analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 220 nm, using an YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-13 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.4000 mg/L. The coefficient of determination (R^2) was determined to be 0.9987 (slope = 467.9515, intercept = -3.1055).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and from samples fortified at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-13 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-13: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	107, 107, 104	106.0	1.6
	0.250	103, 102, 100	101.7	1.5
	1.00	69.0, 68.6, 69.4	69.0	0.6
Overall			92.2	19.0

Report:	CA 4.1.2/14 [redacted] 2012b BAS 560 F (Metrafenone) - Early life-stage toxicity test on the fathead minnow (Pimephales promelas) in a flow through system 2012/1009601
Guidelines:	OECD 210, EPA 540/9-86-138, EPA 72-4 (a), EPA 850.1400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.2.1/2

Principle of the Method (APL0500/03)
Samples are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with a molecular mass of m/z 411 was monitored.

Recovery Findings
Recovery data was generated from four samples fortified at the LOQ and 6 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-14 below.

Linearity
Linearity of detector response was demonstrated using external standard solutions across the concentration range of 0.0025 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9990 (slope = 1.590×10^{-5}, intercept = -0.0121).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.074 mg/L.

Repeatability
Repeatability data was generated from four samples fortified at the LOQ and 6 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-14 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-14: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.074	101.4, 101.4, 101.4, 100.0	101.1	0.7
	0.463	100.2, 100.4, 95.5, 95.2	97.8	2.9

Report:	CA 4.1.2/15 Palmer S.J. et al., 2005b BAS 560 F: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) 2005/7003442
Guidelines:	EPA 850.1025
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.4.2/1

Principle of the Method
Samples are diluted with filtered saltwater and analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 220 nm, using an YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-15 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.4000 mg/L. The coefficient of determination (R^2) was determined to be 0.9997 (slope = 459.7391, intercept = -1.1277).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-15 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-15: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	97.9, 106, 99.7	101.2	4.2
	0.300	97.1, 95.4, 94.2	95.6	1.5
	1.00	81.1, 75.3, 75.7	77.4	4.2
Overall			91.4	12.2

Report:	CA 4.1.2/16 Claude M.B. et al., 2011a BAS 560 F - A 96-hour flow-through acute toxicity test with the saltwater mysid (Americamysis bahia) 2011/7000913
Guidelines:	EPA 850.1035
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.4.2/2

Principle of the Method
Samples are diluted with filtered saltwater and analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 220 nm, using an YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-16 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0300 to 0.3000 mg/L. The coefficient of determination (R^2) was determined to be 0.99897 (slope = 464.4594, intercept = 0.0725).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-16 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-16: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	99.2, 102, 98.5	99.9	1.9
	0.200	103, 98.2, 100	100.4	2.4
	1.00	83.6, 86.6, 83.8	84.7	2.0
Overall			95.0	8.36

Report:	CA 4.1.2/17 Janson G.-M., 2012a Chronic toxicity of BAS 560 F (Metrafenone) to Daphnia magna STRAUS in a 21 day semi-static test 2011/1260868
Guidelines:	OECD 211, EPA 850.1300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.5.1/2

Principle of the Method (APL0500/03)
Samples are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from four samples fortified at the LOQ and 10 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-17 below.

Linearity
Linearity of detector response was demonstrated using external standard solutions across the concentration range of 0.002 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9992 (slope = 4.18×10^{-6}, intercept = -0.0479).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.05 mg/L.

Repeatability
Repeatability data was generated from four samples fortified at the LOQ and 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-17 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-17: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.050	110.6, 112.0	104.2	7.9
	0.051	97.1, 97.1		
	0.511	99.4, 100.2, 102.9, 102.0	101.1	1.6

Report:	CA 4.1.2/18 Cafarella M.A., 2007a BAS 560 F - Life-cycle toxicity test with mysids (Americamysis bahia) 2007/7009454
Guidelines:	EPA 850.1350, FIFRA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.5.2/1

Principle of the Method
Samples are diluted with acetonitrile for a final composition of 20/80 acetonitrile/water and analysed by high performance liquid chromatography with ultra violet detection (HPLC-UV) at 225 nm, using an Agilent SB-C18 column (75 mm x 4.6 mm, 3.5 µm particle size) and gradient elution with mobile phases of 0.05% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-18 below.

Linearity
Linearity of detector response was demonstrated using six external standard solutions across the concentration range of 0.002 to 0.05 mg/L. The coefficient of determination (R^2) was determined to be 0.9999 (slope = 1336.605, intercept = -0.5424).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.003 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-18 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-18: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.003	108, 99.1, 118	108.4	8.7
	0.05	93.8, 92.9, 93.6	93.4	0.5
	0.25	94.4, 96.2, 102	97.5	4.1
Overall			99.8	8.58

Report:	CA 4.1.2/19 Pupp A.,Weltje L., 2007a Chronic toxicity of Metrafenone (BAS 560 F, Reg. No. 4037710) to the non-biting midge Chironomus riparius exposed via spiked water 2007/1018942
Guidelines:	OECD 219
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.5.3/1

No validation data is provided for the water analysis in this study. However the method used, APL0500/02, is the previous version of the method APL0500/03, which is used in a number of the other aquatic ecotoxicology studies included in this section and can therefore be considered to have been validated.

Principle of the Method (APL0542/01 - sediment)
Samples are extracted with methanol/water, diluted and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a Thermo Betasil C18 column (100 mm x 2.1 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411 > 209 is used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-19 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.05 to 1 µg/L. The correlation coefficient (r) was determined to be 0.9997 (slope = 1.13×10^5, intercept = 1.93×10^3).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 20 µg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-19 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-19: Accuracy and Precision Data

Analyte	Fortification Level (µg/kg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	19.9	99.0, 101.0	100.0	1.4
	19.6	102.6, 103.6	103.1	0.7
	777	101.0, 100.5	100.8	0.4
	720	92.6, 98.6	95.6	4.4

Report:	CA 4.1.2/20 Backfisch K.,Weltje L., 2011a Chronic toxicity of Reg.No. 4037710 (BAS 560 F; Metrafenone) to the non-biting midge Chironomus riparius - A spiked sediment study 2010/1145509
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.5.4/2

Principle of the Method – M4-Medium (APL0500/03)
Samples are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Principle of the Method – Sediment (APL0616/01)
Samples are extracted with acetonitrile/water/trimethylamine-mixture (90:9.5:0.5, v/v/v), diluted with methanol/water (1:1, v/v) and analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a Phenomenex Luna C18 column (50 mm x 2 mm, 5 µm particle size), a Phenomenex C18 RP guard column (4 mm x 3 mm) and gradient elution with mobile phases of 0.1% acetic acid in water and 0.1% acetic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantification and the ion transition m/z 409 > 227 is used for confirmation.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-21 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.5 to 20 µg/L for water samples and 5 external standard solutions across the concentration range of 0.01 to 1 µg/L for sediment samples. The results are presented in Table 4.1.2-20 below.

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0189 mg/kg for sediment and 0.0501 mg/L for M4-Medium.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-21 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-20: Linearity Data

Matrix	Concentration range (µg/L)	Correlation coefficient/ Coefficient of determination (r/R^2)	Slope	Intercept
M4-Medium	0.5 – 20	$R^2 = 0.9999$	1.86×10^{-6}	-0.000645
Sediment	0.01 – 1	R = 0.9998	7.74×10^{5}	1.56×10^{3}

Table 4.1.2-21: Accuracy and Precision Data

Analyte	Matrix	Fortification Level	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	Sediment	0.0189 mg/kg	106.3, 99.5	102.9	4.7
		18.9 mg/kg	98.9, 103.2	101.1	3.0
		303 mg/kg	100, 102.6	101.3	1.8
	M4-Medium	0.0501 mg/L	94.8, 94.2	94.5	0.4
		0.801 mg/L	99.1, 96.5	97.8	1.9
		0.571 mg/L	93.9, 92.6	93.3	1.0

Report:	CA 4.1.2/21 Thomas S.T. et al., 2011a BAS 560 F: A 10-day survival toxicity test with the marine amphipod (Leptocheirus plumulosus) using spiked sediment 2011/7000373
Guidelines:	EPA 850.1740
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.5.4/3

Principle of the Method – Saltwater
Samples are diluted with saltwater and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC PACK ODS AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Principle of the Method - Sediment
Samples are extracted with acetonitrile/water, 50/50, v/v, diluted with the same solvent mix and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC PACK ODS AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid in water and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and 2 x LOQ. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-22 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0600 to 0.600 mg/L. The coefficient of determination (R^2) was determined to be 0.99744 (slope = 481.7190, intercept = 1.9399).

Specificity
No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 1.0 mg/kg.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and 2 x LOQ. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-22 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-22: Accuracy and Precision Data

Analyte	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	1.00	96.5	96.5	-
	2.00	96.5		

Report:	CA 4.1.2/22 Hoffmann F., 2012a Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the green alga Pseudokirchneriella subcapitata 2011/1254828
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.6.1/2

Principle of the Method (APL0500/03)
Samples of OECD medium are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and 100 x LOQ. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-23 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.001 to 0.01 mg/L. The coefficient of determination (R^2) was determined to be 0.9989 (slope = 4.527×10^{-6}, intercept = -0.02223).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0050 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and 100 x LOQ. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-23 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-23: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0050	104.0, 104.0, 108.0, 108.0	106.0	2.2
	0.496	94.6, 93.5, 98.8, 98.2	96.3	2.7

Report:	CA 4.1.2/23 Desjardins D. et al., 2005a BAS 560 F: A 96-hour toxicity test with the freshwater alga (Anabaena flos-aquae) 2005/7003441
Guidelines:	OECD 201, EPA 850.5400, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.6.2/1

Principle of the Method
Samples are diluted with freshwater algal medium and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC-PACK ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-24 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.400 mg/L. The coefficient of determination (R^2) was determined to be 0.9998 (slope = 455.0429, intercept = -0.9916).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-24 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-24: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	103, 101	102.0	1.4
	0.300	100, 98.7	99.4	0.9
	1.00	86.4, 85.7	86.1	0.6
Overall			95.8	8.02

Report:	CA 4.1.2/24 Hoffmann F., 2012b Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of blue-green alga Anabaena flos-aquae 2011/1254829
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.6.2/2

Principle of the Method (APL0500/03)
Samples are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and 20 x LOQ. The mean percentage recoveries obtained were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-25 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.002 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9990 (slope =1.653 x 10^{-5}, intercept = -0.0114).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.025 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and 20 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-25 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-25: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.025	97.2, 97.2, 100.0, 100.0, 108.0, 107.2	101.6	4.7
	0.50	98.4, 98.0, 91.4, 91.4, 95.9, 95.5	95.1	3.2

Report:	CA 4.1.2/25 Desjardins D. et al., 2005b BAS 560 F: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa) 2005/7003436
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.6.2/3

Principle of the Method
Samples of algal media are diluted with freshwater algal medium and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC-PACK ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-26 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.400 mg/L. The coefficient of determination (R^2) was determined to be 0.9998 (slope = 455.0429, intercept = -0.9916).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0600 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-26 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-26: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	103, 102	102.5	0.7
	0.300	100, 98.1	99.1	1.4
	1.00	81.1, 82.7	81.9	1.4
Overall			94.5	10.5

Report:	CA 4.1.2/26 Hoffmann F., 2012c Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the freshwater diatom Navicula pelliculosa 2011/1254831
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.6.2/4

Principle of the Method (APL0500/03)
Samples of OECD media are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-27 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.009 to 0.07 mg/L. The coefficient of determination (R^2) was determined to be 0.9999 (slope = 1.9235×10^{-5}, intercept = -0.01699).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0275 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-27 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-27: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0275	104.0, 104.0, 104.1, 105.5	104.4	0.7
	0.403	95.3, 94.3, 101.0, 100.7	97.8	3.6

Report:	CA 4.1.2/27 Desjardins D. et al., 2005c BAS 560 F: A 96-hour toxicity test with the marine diatom (Skeletonema costatum) 2005/7003443
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.6.2/5

Principle of the Method
Samples are diluted with saltwater medium and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC-PACK ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-28 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.400 mg/L. The coefficient of determination (R^2) was determined to be 0.9996 (slope = 453.5065, intercept = -2.6959).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-28 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-28: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	102, 102	102.0	-
	0.300	101, 91.7	96.4	6.8
	1.00	75.1, 67.0	71.1	8.1
Overall			89.8	17.0

Report:	CA 4.1.2/28 Hoffmann F., 2012d Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the marine diatom Skeletonema costatum 2011/1254830
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.6.2/6

Principle of the Method (APL0500/03)
Samples are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-29 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.002 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9998 (slope = 1.413×10^{-5}, intercept = -0.0054).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.0264 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-29 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-29: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0264	86.7, 86.4	86.6	0.2
	0.529	91.5, 92.4	92.0	0.7

Report:	CA 4.1.2/29 Desjardins D. et al., 2005d BAS 560 F: A 7-day static-renewal toxicity test with duckweed (Lemna gibba G3) 2005/7003440
Guidelines:	EPA 850.4400, OECD 221
GLP:	yes (certified by United States Environmental Protection Agency)

Cross reference to M-CA Section 8.2.7/1

Principle of the Method
Samples are diluted with 20X AAP medium and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 220nm, using an YMC-PACK ODS-AM column (150 mm x 4.6 mm, 3 µm particle size) and gradient elution with mobile phases of 0.1% phosphoric acid in water and acetonitrile. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recovery obtained was within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-30 below.

Linearity
Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.0400 to 0.400 mg/L. The coefficient of determination (R^2) was determined to be 0.9996 (slope = 496.6161, intercept = -3.3079).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.06 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviation (RSD) obtained was within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-30 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-30: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.0600	106, 103, 94.8	101.3	5.7
	0.300	97, 92.8, 94.3	94.7	2.3
	1.00	82.2, 83.9, 82.8	83.0	1.0
Overall			93.0	9.26

Report:	CA 4.1.2/30 Hoffmann F., 2012e Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of Lemna gibba 2011/1254832
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Cross reference to M-CA Section 8.2.7/2

Principle of the Method (APL0500/03)
Samples of 20X AAP media are diluted with acetonitrile/water, acidified with formic acid and analysed by high performance liquid chromatography with mass specific detection (HPLC-MS) in positive ionisation mode, using an YMC Pro C18 column (50 mm x 3 mm, 3 µm particle size), a YMC Pro C18 guard column (10 mm x 3 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The metrafenone ion with m/z 411 was used for quantification.

Recovery Findings
Recovery data was generated from samples fortified at the LOQ and at higher concentration levels. The mean percentage recoveries obtained were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-31 below.

Linearity
Linearity of detector response was demonstrated using four external standard solutions across the concentration range of 0.002 to 0.02 mg/L. The coefficient of determination (R^2) was determined to be 0.9991 (slope = 4.76×10^{-6}, intercept = -0.0302).

Specificity
No interferences were observed at the retention time of interest in the control matrix samples, demonstrating the specificity of the methods.
Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.018 mg/L.

Repeatability
Repeatability data was generated from samples fortified at the LOQ and at higher concentration levels. The relative standard deviations (RSD) obtained were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-31 below.

Reproducibility
No data requirement.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-31: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.029	120.3, 119.7, 114.1, 113.4	116.9	3.1
	0.392	99.0, 98.7, 101.5, 100.8	100.0	1.4
	0.018	101.7, 101.1	101.4	0.4
	0.509	89.8, 89.6	89.7	0.2

Report:	CA 4.1.2/31 Eckert J., 2015a BAS 560 02 F and BAS 560 AA F blank formulation - Honey bee (Apis mellifera L.) larval toxicity test (repeated feeding exposure) 2014/1093921
Guidelines:	OECD Draft Test Guideline on Honey bee (Apis mellifera) Larval Toxicity Test Repeated Exposure (February 2014), OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Cross reference to M-CA Section 8.3.1.3/2

Principle of the Methods
Samples are diluted with acetonitrile/water, 1/1, v/v and analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 287 nm, using an Agilent Poroshell 120 EC-C18 column (50 x 4.6 mm, 2.7 µm particle size) and gradient elution with mobile phases of acetonitrile, water and 0.3% phosphoric acid in water. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from five samples fortified at the LOQ and from five samples fortified at 15 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-32 below.

Linearity
Linearity of detector response was demonstrated using nine external standard solutions across the concentration range of 0.8 to 30 mg/L. The coefficient of determination (R^2) was determined to be 0.9999 (slope = 0.1858, intercept = 0.0075).

Specificity
No interferences at >30% of the LOQ were present at the retention time of interest in the control matrix samples, demonstrating the specificity of the method.
Analyte identity was confirmed by retention time and UV spectra match with an analytical standard.

Limit of Quantification
The limit of quantiification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 1274 mg/L metrafenone (3000 mg/L formulated product).
The limit of detection (LOD) was determined to be 382 mg/L metrafenone.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and from five samples fortified at 15 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-32 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-32: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recovery (%)	Mean Recovery (%)	RSD (%)
Metrafenone	1274	102, 102, 102, 101, 98	101	2
	19118	107, 111, 100, 126, 106	110	9

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

Report:	CA 4.1.2/32 Cowlyn N., 2015a Metrafenone (pure grade) - Physico-chemical properties 2014/1036915
Guidelines:	EU Regulation 1107/2009 with Regulation 283/2013
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Cross reference to M-CA Section 2.5

Principle of the Methods
Samples (2.5 mL) are diluted to 5 mL with acetonitrile and are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 285 nm, using a Supelcosil LC-8-DB column (250 mm x 4.6 mm, 5 µm particle size) and isocratic elution with a mobile phase of acetonitrile/water, 80:20, v/v. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated form five samples fortified at 30% of the level determined in the definitive test and from five samples fortified at 70% of the level determined in the definitive test. The mean percentage recovery values obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-33 below.

Linearity
The linearity of detector response was demonstrated using nine external standard solutions across the concentration range of 0.05 to 20 mg/L. The correlation coefficient (r) was determined to be 1.0000 (slope = 12.81, intercept = -0.08562)

Specificity
No interfering peaks were observed at the retention time of interest, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable accuracy and precision data has been obtained, has been determined to be 0.08 mg/L.

Repeatability
Repeatability data was generated from five samples fortified at 30% of the level determined in the definitive test and from five samples fortified at 70% of the level determined in the definitive test. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-33 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-33: Accuracy and Precision Data

Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Metrafenone	0.08	121.7*, 99.8, 100.8, 100.1, 104.1	101	1.9
	0.18	103.4, 92.9, 91.3, 93.7, 116.5	99.6	10.6

*Value excluded from the mean based on Dixons outlier test

Report:	CA 4.1.2/33 Cowlyn N., 2015b Metrafenone (technical grade) - Organic solvent solubility 2014/1036914
Guidelines:	European Commission Regulation No 283/2013, EEC Method A6
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Cross reference to M-CA Section 2.6

Principle of the Methods
Samples are filtered through PTFE filters (0.2 µm). The n-heptane filtrates are evaporated to dryness at approximately 40°C under a steady stream of nitrogen, re-dissolved and diluted with acetonitrile/water, 1:1, v/v. The xylene, methanol and ethyl acetate filtrates are diluted with acetonitrile and further diluted with acetonitrile/water, 1:1, v/v. All samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 285 nm, using a Supelcosil LC-8-DB column (250 mm x 4.6mm, 5 µm particle size) and isocratic elution with a mobile phase of acetonitrile/water, 80:20, v/v. Quantification is performed using external standards.

Recovery Findings
Recovery data was generated from five samples fortified at the LOQ and from five samples fortified at 70% of the level determined in the definitive test. The mean percentage recovery values obtained for each fortification level were within the guideline requirements of 70 – 110% and the results are presented in Table 4.1.2-34 below.

Linearity
Linearity of detector response was demonstrated using eight external standard solutions across the concentration range of 0.9850 to 49.25 mg/L. The correlation coefficient (r) was determined to be 1.0000 (slope = 12.42, intercept = -0.2963)

Specificity
No interfering peaks were observed at the retention time of interest, demonstrating the specificity of the method. Analyte identity was confirmed by retention time match with an analytical standard.

Limit of Quantification
The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 4 mg/L for n-heptane, 2 mg/L for xylene, 1.2 mg/L for methanol and 2.5 mg/L for ethyl acetate.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and from five samples fortified at 70% of the level determined in the definitive test. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and the results are presented in Table 4.1.2-34 below.

Reproducibility
Not required by SANCO/3029/99 rev. 4.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision and LOQ in accordance with the requirements of SANCO/3029/99 rev. 4.

Table 4.1.2-34: Accuracy and Precision Data

Analyte	Solvent	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Metrafenone	n-heptane	4	94.5, 81.2, 93.8, 100.0, 99.6	93.8	8.1
		27	95.9, 92.8, 92.9, 90.2, 134.5*	93.0	2.5
	Xylene	2	97.0, 95.1, 99.2, 93.9, 93.6	95.8	2.5
		13	92.7, 96.7, 93.9, 93.6, 94.4	94.3	1.6
	Methanol	1.2	98.6, 98.4, 98.9, 100.5, 102.0	99.7	1.5
		9	102.1, 101.9, 103.4, 101.9, 98.8	101.6	1.7
	Ethyl acetate	2.5	109.2, 103.6, 105.8, 107.1, 103.9	105.9	2.2
		19	116.8, 113.1, 118.2, 112.9, 119.3	116.1	2.5

*Value excluded from the mean based on Dixons outlier test

CA 4.2 Methods for post-approval control and monitoring purposes

(a) Methods for the analysis in food and feed of plant and animal origin

Report:	CA 4.2/1 Meyer M., 2011a Metrafenone (BAS 560 F): Validation of the multi-residue enforcement method QuEChERS for the determination of residues in plant matrices using LC/MS/MS (including amendment no. 1) 2011/7007816
Guidelines:	SANCO/825/00 rev. 7 (17 March 2004), EPA 860.1360
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Principle of the methods

Samples of lemon and cucumber (10 g), beans, wheat forage, wheat grain and oilseed rape (5 g) and samples of wheat straw and hops (2 g) are weighed into centrifuge tubes (50 mL). Water is added to the samples with a water contents below 80% to adjust the total water content to approximately 10g. Acetonitrile (10 mL) is added and the samples are shaken vigorously for 1 minute. Magnesium sulfate, sodium chloride and citrate salts (DSPE citrate extraction tube) are added and the samples are shaken vigorously for 1 minute before being centrifuged at 3000 rpm for 5 minutes. An aliquot (8 mL) of the oilseed rape sample is transferred to a freezer overnight to freeze out the fatty components. Aliquots (1 mL) of the sample extracts are transferred into DSPE PSA clean up tubes containing magnesium sulphate (150 mg) and PSA (25 mg), and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (100 µL) is transferred to an autosampler vial and acetonitrile/water/formic acid, 50/50/0.1; v/v/v (900 µL) is added. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ion mode, using an Agilent Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantitation and the ion transition for 409 > 227 is used for confirmation except for hops (dried cones) where the ion transition m/z 411 > 209 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. Recovery data was also generated for additional fortification levels for various plant matrices. The mean percentage recoveries obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-2 to Table 4.2-9 below.

Linearity

Linearity of detector response was demonstrated using at least seven external standard solutions across the concentration range of 0.03108 to 1.989 ng/mL. The results are presented in Table 4.2-1 below. As a significant matrix effect was observed for hops (dried cones), matrix matched standards were used for this matrix.

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg for metrafenone in plant matrices.

Repeatability

Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. Repeatability data was also generated for additional fortification levels for various plant matrices. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-2 to Table 4.2-9 below.

Reproducibility

This is not a data requirement.

Stability

The stability of metrafenone was demonstrated by analysing old stock solutions against freshly prepared stock solutions. A recovery value of 97.5 % was obtained, following storage for 26 days demonstrating the stability of metrafenone stock solutions.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-1: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Wheat Forage	409 > 209	0.04973 – 1.591	0.99820	57589985.94	-6489
	409 > 227		0.99888	38896823.67	2623
Wheat Straw	409 > 209	0.04973 – 1.591	0.99970	66588094.74	-1854
	409 > 227		0.99969	44341335.52	-1330
Wheat Grain	409 > 209	0.04973 – 1.591	0.99982	56909839.65	1438
	409 > 227		0.99976	37935181.9	1504
Cucumber	409 > 209	0.04973 – 1.591	0.99990	56038915.78	2572
	409 > 227		0.99994	37198337.1	1820
Lemon	409 > 209	0.04973 – 1.591	0.99912	81969743.73	11785
	409 > 227		0.99933	54820174.7	7657
Beans	409 > 209	0.04973 – 1.591	0.99948	64446673.28	2684
	409 > 227		0.99948	42934977	1323
Oilseed Rape	409 > 209	0.04973 – 1.591	0.99994	85786052.73	16348
	409 > 227		0.99994	57419786.62	3832
Hops	409 > 209	0.03108 – 1.989	0.99987	18756393.72	3347
	411 > 209		0.99998	19538708.01	949.2

Table 4.2-2: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat forage	Ion transition m/z 409 > 209 (quantification)					
	0.01	102, 101, 96, 100, 101	100	60 – 120	2.5	30
	0.10	102, 102, 105, 108, 104	104	70 – 120	2.5	20
	5.0	99, 105, 111, 113, 114	108	70 – 110	6.1	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	101, 102, 97, 100, 100	102	60 – 120	4.8	30
	0.1	99, 100, 103, 106, 101	102	70 – 120	2.8	20
	5.0	95, 103, 108, 110, 113	106	70 – 110	6.5	10

Table 4.2-3: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat Straw	Ion transition m/z 409 > 209 (quantification)					
	0.01	98, 98, 100, 105, 96	100	60 – 120	3.4	30
	0.10	103, 105, 100, 98, 99	101	70 – 120	2.6	20
	5.0	98, 99, 94, 98, 91	96	70 – 110	3.8	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	103, 100, 105, 111, 101	104	60 – 120	4.2	30
	0.1	103, 104, 100, 101, 100	102	70 – 120	2.0	20
	5.0	98, 98, 93, 100, 92	96	70 – 110	3.6	10

Table 4.2-4: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat grain	Ion transition m/z 409 > 209 (quantification)					
	0.01	104, 105, 103, 103, 102	104	60 – 120	1.1	30
	0.10	101, 106, 102, 103, 103	103	70 – 120	1.7	20
	0.5	103, 104, 101, 102, 103	103	70 – 110	0.9	15
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	104, 103, 103, 101, 101	103	60 – 120	1.5	30
	0.1	99, 105, 101, 102, 102	102	70 – 120	2.0	20
	0.5	102, 103, 100, 101, 103	102	70 – 110	1.4	15

Table 4.2-5: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Cucumber	Ion transition m/z 409 > 209 (quantification)					
	0.01	103, 105, 102, 103, 105	104	60 – 120	1.1	30
	0.10	103, 107, 104, 101, 102	103	70 – 120	2.3	20
	5.0	104, 113, 113, 113, 103	109	70 – 110	5.0	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	104, 104, 101, 103, 105	103	60 – 120	1.2	30
	0.1	103, 107, 106, 101, 103	104	70 – 120	2.5	20
	5.0	103, 113, 115, 99, 103	107	70 – 110	6.6	10

Table 4.2-6: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Lemon	Ion transition m/z 409 > 209 (quantification)					
	0.01	103, 106, 108, 106, 96	104	60 – 120	4.4	30
	0.10	108, 110, 111, 111, 113	111	70 – 120	1.5	20
	5.0	116, 105, 110, 110, 108	110	70 – 110	3.7	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	104, 107, 108, 107, 96	104	60 – 120	4.5	30
	0.1	108, 109, 111, 111, 112	110	70 – 120	1.4	20
	5.0	114, 105, 110, 110, 109	109	70 – 110	3.0	10

Table 4.2-7: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Beans	Ion transition m/z 409 > 209 (quantification)					
	0.01	85, 84, 79, 91, 90	86	60 – 120	5.7	30
	0.10	89, 87, 92, 93, 92	91	70 – 120	2.8	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	85, 85, 80, 90, 90	86	60 – 120	4.9	30
	0.1	88, 87, 93, 93, 92	91	70 – 120	3.2	20

Table 4.2-8: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%))	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Oilseed rape	Ion transition m/z 409 > 209 (quantification)					
	0.01	96, 99, 98, 97, 94	97	60 – 120	2.0	30
	0.10	95, 94, 95, 91, 95	94	70 – 120	1.8	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	97, 99, 98, 97, 94	97	60 – 120	1.9	30
	0.1	96, 95, 96, 92, 96	95	70 – 120	1.6	20

Table 4.2-9: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Hops	Ion transition m/z 409 > 209 (quantification)					
	0.01	99, 97, 101, 97, 101	99	60 – 120	1.8	30
	0.10	96, 98, 96, 96, 97	97	70 – 120	0.9	20
	20	99, 101, 102, 101, 100	100	70 – 110	1.1	10
	Ion transition m/z 411 > 209 (confirmation)					
	0.01	98, 103, 103, 106, 110	104	60 – 120	4.3	30
	0.1	96, 96, 97, 94, 95	96	70 – 120	1.1	20
	20	98, 100, 102, 100, 99	100	70 – 110	1.7	10

Report:	CA 4.2/2 Weber H., 2011a Independent laboratory validation of multi-method QuEChERS for the determination of Metrafenone (BAS 560 F) in foodstuffs of plant origin 2011/1124162
Guidelines:	EEC 91/414, EEC 96/46 4.2.1, SANCO/825/00 rev. 7 (17 March 2004), BBA Guideline Residue Analytical Methods for Post-Registration Control Purposes of July 21 1998, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany)

Report:	CA 4.2/3 Weber H., 2011b Independent laboratory validation of multi-method QuEChERS for the determination of Metrafenone (BAS 560 F) in foodstuffs of plant origin 2011/1043486
Guidelines:	EEC 91/414, EEC 96/46 4.2.1, SANCO/825/00 rev. 7 (17 March 2004), BBA Guideline Residue Analytical Methods for Post-Registration Control Purposes of July 21 1998, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany)

This is a report amendment for document 2011/1124162. An amendment was issued to correct a typing error. The typing error has no impact on the validity of the study.

Report:	CA 4.2/4 Weber H., 2011c Independent laboratory validation of multi-method QuEChERS for the determination of Metrafenone (BAS 560 F) in foodstuffs of plant origin 2011/1285048
Guidelines:	EEC 91/414, EEC 96/46 4.2.1, SANCO/825/00 rev. 7 (17 March 2004), BBA Guideline Residue Analytical Methods for Post-Registration Control Purposes of July 21 1998, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany)

This is a report amendment for document 2011/1124162. An amendment was issued to correct a typing error. The typing error has no impact on the validity of the study.

Principle of the methods

Samples of lemon and cucumber (10 g), beans, wheat forage, wheat grain and oilseed rape (5 g) and samples of wheat straw and hops (2 g) are weighed into centrifuge tubes (50 mL). Acetonitrile (10 mL) is added along with water (wheat forage: 7 mL, wheat straw and dried beans: 9 mL, wheat grain, oilseed rape and hops: 10 mL) and the samples shaken vigorously for 1 minute. Magnesium sulphate (4.0 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g) and disodium hydrogen citrate (0.5 g) are added, the samples are shaken for 1 minute and centrifuged for 5 minutes at 3000 rpm. An aliquot (8 mL) of the oilseed rape sample is transferred to a freezer overnight to freeze out the fatty components. Aliquots (1.5 mL) of the sample extracts are transferred to Eppendorf tubes (2 mL) containing PSA (40 mg) and magnesium sulphate (225 mg). The samples are shaken for 30 seconds and centrifuged for 2 minutes at 3000 rpm. Aliquots of the samples (0.1 mL) are transferred to test tubes and diluted to 1 mL with acetonitrile/water/formic acid, 50/50/0.1, v/v/v (0.9 mL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive ionisation mode, using a Phenomenex Luna C18 column (150 mm x 2 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantification and the ion transition m/z 409 > 227 is used for confirmation except for hops where the ion transition m/z 411 > 209 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. Recovery data was also generated for additional fortification levels for various plant matrices. The mean percentage recoveries obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-11 to Table 4.2-18 below.

Linearity

Linearity of detector response was demonstrated using at least five external standard solutions across the concentration range of 0.050 to 10 ng/mL. The results are presented in Table 4.2-10 below. As a significant matrix effect was observed for hops, matrix matched standards were used for this matrix.

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.01 mg/kg for metrafenone in plant matrices.

Repeatability

Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. Repeatability data was also generated for additional fortification levels for various plant matrices. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-11 to Table 4.2-18 below.

Reproducibility

This is not a data requirement.

Stability

The stability of metrafenone was demonstrated by analysing final volume and extract solutions against freshly prepared solutions. Recovery values of 70-120 % were obtained, following refrigerated storage (3 – 8°C) for up to 7 days demonstrating the stability of metrafenone solutions.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-10: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Coefficient of Determination (R^2)	Slope	Intercept
Wheat Forage	409 > 209	0.050 – 10.0	0.9986	10976	-350.24
	409 > 227		0.998	6703.619	-461.161
Wheat Straw	409 > 209	0.050 – 10.0	0.9957	14973	-377.9
	409 > 227		0.999	8473	175.99
Wheat Grain	409 > 209	0.050 – 10.0	0.997	13855	-584.89
	409 > 227		0.997	7694.047	-114.223
Cucumber	409 > 209	0.050 – 10.0	0.995	13145	-1055.6
	409 > 227		0.998	7082.617	87.834
Lemon	409 > 209	0.050 – 10.0	0.9989	18559	151.66
	409 > 227		1.000	10303.857	441.412
Beans	409 > 209	0.050 – 10.0	0.999	16905.872	16.040
	409 > 227		0.995	10076.250	-582.290
Oilseed Rape	409 > 209	0.050 – 10.0	0.994	13899	1630
	409 > 227		0.994	8109.284	719.020
Hops	409 > 209	0.050 – 10.0	0.9965	7378.1	566.44
	411 > 209		0.9998	21021	1891.4

Table 4.2-11: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat forage	Ion transition m/z 409 > 209 (quantification)					
	0.01	86, 85, 93, 87, 94	89	60 – 120	4.7	30
	0.10	89, 101, 98, 97, 87	94	70 – 120	6.4	20
	5.0	76, 77, 77, 72, 26*	76	70 – 110	3.2	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	95, 89, 96, 84, 90	91	60 – 120	5.4	30
	0.1	98, 102, 88, 85, 81	91	70 – 120	9.8	20
	5.0	73, 81, 76, 79, 19*	77	70 – 110	4.5	10

*Sample identified as an outlier according to Grubbs test

Table 4.2-12: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat Straw	Ion transition m/z 409 > 209 (quantification)					
	0.01	92, 90, 95, 99, 92	94	60 – 120	3.7	30
	0.10	89, 97, 82, 85, 92	89	70 – 120	6.6	20
	5.0	85, 80, 86, 98, 78	85	70 – 110	9.1	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	100, 84, 99, 101, 92	95	60 – 120	7.6	30
	0.1	100, 97, 80, 97, 88	92	70 – 120	8.9	20
	5.0	85, 78, 71, 94, 78	81	70 – 110	11	10

Table 4.2-13: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat grain	Ion transition m/z 409 > 209 (quantification)					
	0.01	83, 80, 89, 82, 92	85	60 – 120	6.0	30
	0.10	91, 88, 84, 84, 82	86	70 – 120	4.2	20
	0.5	82, 86, 92, 88, 76	85	70 – 110	7.2	15
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	91, 82, 96, 84, 92	89	60 – 120	6.6	30
	0.1	102, 90, 85, 86, 84	89	70 – 120	8.3	20
	0.5	80, 82, 78, 90, 80	82	70 – 110	5.7	15

Table 4.2-14: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Cucumber	Ion transition m/z 409 > 209 (quantification)					
	0.01	85, 80, 87, 94, 97	89	60 – 120	7.8	30
	0.10	87, 94, 92, 92, 87	90	70 – 120	3.6	20
	5.0	92, 86, 77, 92, 75	84	70 – 110	9.6	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	79, 73, 80, 89, 92	83	60 – 120	9.4	30
	0.1	85, 87, 93, 86, 80	86	70 – 120	5.4	20
	5.0	89, 83, 76, 85, 71	81	70 – 110	8.9	10

Table 4.2-15: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Lemon	Ion transition m/z 409 > 209 (quantification)					
	0.01	94, 92, 97, 93, 94	94	60 – 120	2.0	30
	0.10	100, 98, 94, 86, 94	94	70 – 120	5.7	20
	5.0	101, 86, 79, 94, 95	91	70 – 110	9.4	10
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	95, 91, 98, 93, 92	94	60 – 120	3.0	30
	0.1	96, 94, 98, 81, 82	90	70 – 120	9.0	20
	5.0	102, 85, 81, 94, 86	90	70 – 110	9.4	10

Table 4.2-16: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Beans	Ion transition m/z 409 > 209 (quantification)					
	0.01	94, 10189, 95, 89	94	60 – 120	5.3	30
	0.10	94, 86, 90, 94, 85	90	70 – 120	4.8	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	99, 96, 98, 94, 90	95	60 – 120	3.8	30
	0.1	94, 93, 89, 97, 86	92	70 – 120	5	20

Table 4.2-17: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Oilseed rape	Ion transition m/z 409 > 209 (quantification)					
	0.01	71, 71, 71, 65, 70	70	60 – 120	3.7	30
	0.10	76, 31*, 71, 71, 66	71	70 – 120	5.7	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	77, 79, 70, 67, 65	72	60 – 120	8.6	30
	0.1	81, 27*, 70, 71, 62	71	70 – 120	11	20

*Sample identified as an outlier according to Grubbs test

Table 4.2-18: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Hops	Ion transition m/z 409 > 209 (quantification)					
	0.01	87, 73, 81, 99, 87	85	60 – 120	11	30
	0.10	84, 78, 96, 95, 59	82	70 – 120	18	20
	20	92, 84, 79, 83, 80	84	70 – 110	6.1	10
	Ion transition m/z 411 > 209 (confirmation)					
	0.01	88, 82, 107, 91, 109	95	60 – 120	13	30
	0.1	80, 78, 89, 99, 70	83	70 – 120	13	20
	20	92, 84, 83, 82, 80	84	70 – 110	5.5	10

Report:	CA 4.2/5 Kuhn T., 2014a Development and validation of an analytical method for the determination of the Metrafenone in foodstuffs of animal origin 2014/1181105
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

Samples of whole milk (10 g), eggs, bovine meat and liver (5.0 g) and samples of fat (2.5 g) are weighed into 50mL screw-capped centrifuge vials. The fat sample is warmed/ melted in a water bath at 40°C. Water (6 mL for eggs, meat and liver) is added along with acetonitrile (10 mL) and the samples are shaken vigorously for 1 minute. Water (10 mL) is added to the fat sample along with acetonitrile (10 mL) and the samples are warmed in a water bath set at 40°C before being shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute before being centrifuged at 3000 rpm for 5 minutes. The samples are transferred to a freezer for 30 minutes and then centrifuged for 1 minute at 4000 rpm. The raw extract (6 mL) is then transferred to Dispersive SPE (dSPE) Clean Up Tube 1 (Supelco 55228-U). Two tips of a spatula of Bakerbond C_{18} material is added to the samples and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (0.50 mL) is transferred to an autosampler vial and acidified with 5 % formic acid in acetonitrile (5 µL). The samples are diluted by a factor of 2 using acetonitrile/water (1/1, v/v). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Aqua C_{18} column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of 0.5 % formic acid in water and 0.5 % formic acid in acetonitrile. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantitation and the ion transition for 409 > 227 is used for confirmation.

Recovery findings

Recovery data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.2-20 to Table 4.2-24 below.

Linearity

Linearity of detector response was demonstrated using six external standard solutions across the working range of 0.250 to 60.0 ng/mL. The results are presented in Table 4.2-19 below. As matrix effects were observed, matrix matched standards were used.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for metrafenone in foodstuffs of animal origin.

Repeatability

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.2-20 to Table 4.2-24 below.

Reproducibility

This is not a data requirement.

Stability

Stability of metrafenone in stock and calibration solutions was proven for five days with consistent recovery results following frozen storage. The stability of the extracts was demonstrated by acceptable recoveries within 60 – 120% for samples fortified at 0.01 mg/kg and acceptable recoveries within 70 – 120% for samples fortified at 0.1 mg/kg.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-19: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Milk	409 > 209	1.0 – 60	0.9993	1.61×10^4	-652
	409 > 227	1.0 – 60	0.9990	7.76×10^3	190
Meat	409 > 209	0.5 – 30	0.9994	1.45×10^4	600
	409 > 227	0.5 – 30	0.9992	7.06×10^3	404
Egg	409 > 209	0.5 – 30	0.9996	2.19×10^4	1.38×10^3
	409 > 227	0.5 – 30	0.9993	1.05×10^4	415
Liver	409 > 209	0.5 – 30	0.9996	1.37×10^4	2.45×10^3
	409 > 227	0.5 – 30	0.9995	6.35×10^3	1.26×10^3
Fat	409 > 209	0.25 – 15.0	0.9998	1.56×10^4	60.6
	409 > 227	0.25 – 15.0	0.9998	7.34×10^3	180

Table 4.2-20: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Milk	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	99.6, 97.2, 99.8, 101, 101	99.7	1.5	30	60 – 120
	0.1	82.3, 92.8, 88.0, 95.6, 78.0	87.3	8.3	20	70 – 120
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	98.1, 94.8, 101, 97.4, 102	98.7	2.9	30	60 – 120
	0.1	80.6, 91.8, 86.4, 95.0, 75.8	85.9	9.2	20	70 – 120

Table 4.2-21: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Meat	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	94.8, 97.6, 97.2, 96.0, 92.4	95.6	2.2	30	60 – 120
	0.1	89.2, 101, 103, 98.8, 105	99.3	6.1	20	70 – 120
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	92.0, 95.6, 94.8, 96.0, 92.0	94.1	2.1	30	60 – 120
	0.1	87.4, 100, 102, 98.4, 104	98.3	6.6	20	70 – 120

Table 4.2-22: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Egg	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	117, 110, 110, 108, 102	109	4.9	30	60 – 120
	0.1	97.4, 99.6, 100, 96.4, 94.4	97.6	2.4	20	70 – 120
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	117, 110, 106, 108, 98.8	108	6.2	30	60 – 120
	0.1	94.8, 96.8, 99.6, 95.6, 93.2	96.0	2.5	20	70 – 120

Table 4.2-23: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Liver	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	115, 83.6, 118, 116, 113	109	13	30	60 – 120
	0.1	111, 105, 108, 114, 113	110	3.4	20	70 – 120
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	115, 85.6, 115, 117, 114	109	12	30	60 – 120
	0.1	114, 108, 109, 118, 115	113	3.9	20	70 – 120

Table 4.2-24: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)	Acceptable RSD (%)	Acceptable Recovery (%)
Fat	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	95.6, 98.4, 96.8, 95.2, 95.2	96.2	1.4	30	60 – 120
	0.1	92.8, 94.4, 90.4, 88.8, 92.8	91.8	2.4	20	70 – 120
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	95.2, 96.8, 98.4, 94.4, 88.0	94.6	4.2	30	60 – 120
	0.1	94.0, 95.2, 91.2, 89.6, 93.6	92.7	2.4	20	70 – 120

Report:	CA 4.2/6 Austin R.,Turner R., 2015a Independent laboratory validation for the determination of BAS 560 F (Metrafonone) in animal matrices 2014/1181106
Guidelines:	EU Regulation 1107/2009 with Regulation 283/2013, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Samples of whole milk (10 g), eggs, bovine meat, liver (5.0 g) and samples of fat (2.5 g) are weighed into 50mL screw-capped centrifuge vials. The fat sample is warmed/ melted in a water bath at 40°C. Water (6 mL for eggs, meat and liver) is added along with acetonitrile (10 mL) and the samples are shaken vigorously for 1 minute. Water (10 mL) is added to the fat sample along with acetonitrile (10 mL) and the samples are warmed in a water bath set at 40°C before being shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples. The samples are shaken vigorously for 1 minute and centrifuged at 3000 rpm for 5 minutes. The samples are transferred to a freezer for 30 minutes, and then centrifuged for 1 minute at 4000 rpm. The raw extracts (6 mL) are transferred to Dispersive SPE (dSPE) Clean Up Tube 1 (Supelco 55228-U). Bakerbond C_{18} material (20 mg) is added to the samples and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. Aliquots of the extracts (0.50 mL) are transferred to autosampler vials and acidified with 5 % formic acid in acetonitrile (5 µL). The samples are then diluted by a factor of 2 using water. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using an Aqua C_{18} column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of 0.5 % formic acid in water and 0.5 % formic acid in acetonitrile. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantitation and the ion transition for 409 > 227 is used for confirmation.

Recovery findings

Recovery data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.2-26 to Table 4.2-30 below.

Linearity

Linearity of detector response was demonstrated using six external standard solutions across the working range of 0.250 to 15.0 ng/mL. The results are presented in Table 4.2-25 below. No significant matrix effects were observed, however matrix matched standards were used.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for Metrafenone in foodstuffs of animal origin.

Repeatability

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.2-26 to Table 4.2-30 below.

Reproducibility

This is not a data requirement.

Stability

The stability of metrafenone was addressed in the initial study. See KCA 4.2/5 2014/1181105 above.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-25: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Bovine Meat	409 > 209	0.5 – 30	0.9999	1.92×10^4	-313
	409 > 227		0.9999	2.65×10^4	-693
Bovine Liver	409 > 209	0.5 – 30	1.0000	1.89×10^4	250
	409 > 227		0.9996	2.56×10^4	720
Whole Milk	409 > 209	1 – 60	0.9995	1.57×10^4	5.81×10^3
	409 > 227		0.9993	2.16×10^4	7.59×10^3
Eggs	409 > 209	0.5 – 30	0.9954	1.64×10^4	2.49×10^3
	409 > 227		0.9964	2.25×10^4	3.2×10^3
Fat	409 > 209	0.25 – 15	0.9999	1.85×10^4	330
	409 > 227		0.9999	2.5×10^4	810

Table 4.2-26: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Meat	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	108, 122, 105, 110, 112	111	60 – 120	5.8	30
	0.1	101, 104, 100, 98, 100	101	70 – 120	2.2	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	106, 123, 110, 111, 114	113	60 – 120	5.7	30
	0.1	101, 101, 98, 98, 100	100	70 – 120	1.5	20

Table 4.2-27: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Liver	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	113, 115, 111, 123, 115	115	60 – 120	4.0	30
	0.1	110, 110, 109, 112, 110	110	70 – 120	1.0	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	113, 115, 112, 121, 115	115	60 – 120	3.0	30
	0.1	111, 115, 112, 113, 110	112	70 – 120	1.7	20

Table 4.2-28: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Whole Milk	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	108, 112, 105, 103, 104	106	60 – 120	3.4	30
	0.1	105, 105, 104, 103, 105	104	70 – 120	0.9	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	107, 112, 106, 102, 102	106	60 – 120	3.9	30
	0.1	103, 103, 104, 103, 104	103	70 – 120	0.5	20

Table 4.2-29: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Eggs	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	100, 94, 90, 85, 86	91	60 – 120	6.8	30
	0.1	92, 86, 84, 86, 85	87	70 – 120	3.6	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	98, 94, 91, 86, 87	91	60 – 120	5.4	30
	0.1	92, 85, 85, 87, 86	87	70 – 120	3.4	20

Table 4.2-30: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Fat	**Ion transition m/z 409 > 209 (quantification)**					
	0.01	85, 89, 98, 94, 96	92	60 – 120	5.5	30
	0.1	91, 90, 82, 87, 85	87	70 – 120	4.2	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.01	90, 91, 94, 95, 94	93	60 – 120	2.3	30
	0.1	94, 90, 82, 89, 85	88	70 – 120	5.3	20

(b) Methods for the analysis in soil and water

Report:	CA 4.2/7 Austin R., 2015a Method validation study for the Determination of residues of BAS 560 F (Metrafenone) in Soil 2014/1181107
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 1107/2009 with Regulation 283/2013
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Samples (5 g) are weighed into extraction bottles (60 mL) and trimethylamine/acetonitrile/water, 0.5:90:9.5, v/v/v, (25 mL) is added. The samples are shaken for 60 minutes and centrifuged for 5 minutes at 4000 rpm. An aliquot (5 mL) of the extraction solution is transferred to a centrifuge tube (15 mL), diluted with 0.5% acetic acid in water (5 mL) and shaken by hand to mix. The samples are cleaned up using preconditioned Agilent Bond Elut C18 SPE cartridges (1g/ 6mL). The analytes are eluted from the SPE cartridge with methanol/dichloromethane, 10:90, v/v, (5 mL) and the collected eluates are evaporated to dryness with a stream of nitrogen (60°C). Methanol/dichloromethane, 10:90, v/v, (10 mL) is added and the samples are evaporated to dryness again. Methanol (2 mL) is added to the samples, the samples are capped and vortex mixed for 10 seconds. The samples are placed on a sample concentrator for 15 minutes to ensure they are fully dissolved before being vortex mixed again for 10 seconds. Water (2 mL) is added and the samples, the samples are vortex mixed again for 10 seconds and an aliquot (0.1 mL) of the sample is diluted with methanol/water, 50:50, v/v, (0.9 mL) for analysis. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2.1 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 > 227is used for quantitation and the ion transition for 409 > 209 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-32 below.

Linearity

Linearity of detector response was demonstrated using seven external standard solutions across the concentration range of 0.02 to 2.5 ng/mL. The results are presented in Table 4.2-31 below. As no significant matrix effects were observed, matrix matched standards were not used.

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.005 mg/kg for metrafenone in soil. The limit of detection (LOD) was determined to be 0.0008 mg/kg.

Repeatability

Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-32 below.

Reproducibility

This is not a data requirement

Stability

The stability of metrafenone was demonstrated following refrigerated storage (4°C) for up to 63 days for sample extracts, up to 89 days for stock solutions and up to 69 days for calibration standards with recovery values in the acceptable range of 70 – 120%.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-31: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Soil	409 > 227	0.02 – 2.5	0.9994	1.05×10^6	1.84×10^4
	409 > 209		0.9994	2.16×10^6	3.86×10^4

Table 4.2-32: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Soil	**Ion transition m/z 409 > 227 (quantification)**					
	0.005	116, 105, 115, 107, 114	111	70 – 120	4.5	20
	0.05	114, 111, 105, 111, 110	110	70 – 120	3.0	20
	Ion transition m/z 409 > 209 (confirmation)					
	0.005	115, 106, 116, 110, 117	113	70 – 120	4.1	20
	0.05	110, 110, 105, 110, 110	109	70 – 120	2.1	20

Report:	CA 4.2/8 Austin R., 2014a Method validation study for the determination of residues of BAS 560 F (Metrafenone) in surface and drinking water 2014/1181109
Guidelines:	EU Regulation 1107/2009 with Regulation 283/2013, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Samples (20 mL) are weighed into glass tubes and dichloromethane (20 mL) is added. The samples are shaken for 30 minutes on a mechanical shaker. An aliquot (2.5 mL) of the dichloromethane layer is transferred to a glass vial and evaporated to dryness under a gentle stream of nitrogen at 40°C. Methanol/water, 50/50, v/v, (1 mL) is added and the sample is sonicated for 30 seconds and vortex mixed for 10 seconds. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2.1 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 > 227 is used for quantitation and the ion transition for 409 > 209 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. The mean percentage recoveries obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-34 to Table 4.2-35 below.

Linearity

Linearity of detector response was demonstrated using six external standard solutions across the concentration range of 0.025 to 5 ng/mL. The results are presented in Table 4.2-33 below. Although no significant matrix effects were observed, matrix matched standards were used.

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been determined to be 0.05 µg/L for metrafenone in surface and drinking water.

The limit of detection (LOD) was determined to be 0.01 µg/L

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-34 to Table 4.2-35 below.

Reproducibility
This is not a data requirement.

Stability
The stability of metrafenone was demonstrated following refrigerated storage (4°C) for up to 15 days for sample extracts and fortification solutions, up to 89 days for stock solutions and up to 13 days for calibration standards with recovery values in the acceptable range of 70 – 120%.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-33: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Surface Water	409 > 227	0.025 – 5	0.9997	3.59 x 105	9 x 103
	409 > 209		0.9991	8.42 x 105	2.56 x 104
Drinking Water	409 > 227	0.025 – 5	0.9995	4.48 x 105	1.93 x 103
	409 > 209		0.9989	1.08 x 106	7.47 x 103

Table 4.2-34: Precision and Accuracy Data – Surface water

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Surface Water	**Ion transition m/z 409 > 227 (quantification)**					
	0.05	102, 96.8, 103, 101, 94.4	99.4	70 – 120	3.7	20
	0.5	96.8, 95.2, 99.2, 98.4, 102	98.3	70 – 120	2.6	20
	Ion transition m/z 409 > 209 (confirmation)					
	0.05	107, 102, 105, 103, 95.2	102	70 – 120	4.4	20
	0.5	101, 98.4, 103, 100, 104	101	70 – 120	2.2	20

Table 4.2-35: Precision and Accuracy Data – Drinking water

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Drinking Water	**Ion transition m/z 409 > 227 (quantification)**					
	0.05	102, 99.2, 105, 104, 109	104	70 – 120	3.5	20
	0.5	104, 106, 102, 108, 98.4	104	70 – 120	3.6	20
	Ion transition m/z 409 > 209 (confirmation)					
	0.05	106, 103, 108, 109, 116	108	70 – 120	4.5	20
	0.5	106, 109, 103, 110, 101	106	70 – 120	3.6	20

Report:	CA 4.2/9 Richter S., 2015a Independent laboratory validation (ILV) of an analytical method for the determination of BAS 560 F (Metrafenone) in drinking water 2014/1181108
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

Samples (20 mL) are transferred into jars, dichloromethane (20 mL) is added and the samples are shaken for 30 minutes. An aliquot (2.5 mL) of the dichloromethane phase is evaporated to dryness using a gentle stream of nitrogen at 40°C. The residue is dissolved in methanol/water, 50:50, v/v, (1 mL) using sonication for 30 seconds and vortex mixing for 10 seconds. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Thermo Betasil C18 column (100 mm x 2.1 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 > 209 is used for quantification and the ion transition m/z 409 > 227 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and from five samples fortified at 10 x LOQ. The mean percentage recovery values obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-37 below.

Linearity

Linearity of detector response was demonstrated using six external standard solutions across the concentration range of 0.025 to 5.0 ng/mL. The results are presented in Table 4.2-36 below. As significant matrix effects were observed, matrix matched standards were used.

Specificity

HPLC-MS/MS monitoring two ion transitions is considered to be a highly specific technique. No interferences at >30% of the LOQ were observed at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data have been obtained, has been determined to be 0.05 µg/L.

Repeatability

Repeatability data was generated from five samples fortified at the LOQ and from five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-37 below.

Reproducibility

This is not a data requirement.

Stability
The stability of metrafenone was addressed in the initial study. See KCA 4.2/8 2014/1181109 above.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision and LOQ in accordance with the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-36: Linearity Data

Analyte	Ion (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Metrafenone	409 > 209	0.025 – 5.0	0.9993	4.94×10^6	2.77×10^4
	409 > 227		0.9996	3.1×10^6	1.71×10^4

Table 4.2-37: Accuracy and precision Data

Matrix	Fortification Level (µg/L)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Drinking Water	**Ion transition m/z 409 > 209 (quantification)**					
	0.05	98.4, 90.4, 86.4, 92.8, 86.4	90.9	70 – 120	5.5	20
	0.5	97.2, 94.4, 90.4, 98.4, 95.6	93.2	70 – 120	5.6	20
	Ion transition m/z 409 > 227 (confirmation)					
	0.05	97.6, 89.6, 85.6, 92.8, 86.4	90.4	70 – 120	5.5	20
	0.5	96.0, 93.6, 89.6, 96.8, 84.0	92.0	70 – 120	5.7	20

(c) Methods for the analysis in air

Report:	CA 4.2/10 Austin R., 2015b Method validation study for the determination of BAS 560 F (Metrafenone) in air 2014/1181110
Guidelines:	EU Regulation 1107/2009 with Regulation 283/2013, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Amberlite® XAD®-2 (20/40), 100/50 mg sorbent tubes fortified with metrafenone are flushed with air (35°C, 80% relative humidity) at 1 L/min for 6 hours. The analyte is extracted using acetone (100 mL) and sonication for 5 minutes. An aliquot (0.1 mL) is transferred to a volumetric flask (20 mL) and diluted to volume with methanol/water, 1:1, v/v. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2.1 mm, 5 µm particle size) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 > 227 is used for quantitation and the ion transition for 409 > 209 is used for confirmation.

Recovery findings

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-39 below.

Linearity

Linearity of detector response was demonstrated using seven external standard solutions across the concentration range of 0.01 to 2.5 µg/mL. The results are presented in Table 4.2-38 below. No significant matrix effects were observed.

Specificity

HPLC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples, demonstrating the specificity of the method.

Limit of Quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.03 mg/m^3.

The limit of detection was determined to be 0.007 mg/m^3.

Repeatability
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and the results are presented in Table 4.2-39 below.

Reproducibility
This is not a data requirement

Breakthrough
No significant breakthrough of metrafenone was observed on the rear bed, demonstrating acceptable retention capacity.

Stability
The stability of metrafenone was demonstrated following refrigerated storage (4°C) for up to 39 days for sample extracts, up to 64 days for stock solutions and up to 64 days for calibration standards with recovery values in the acceptable range of 70 – 120%.

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Table 4.2-38: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (µg/mL)	Correlation Coefficient (r)	Slope	Intercept
Air	409 > 227	0.01 – 2.5	0.9991	1.35×10^6	8.83×10^4
	409 > 209		0.9991	2.78×10^6	1.75×10^5

Table 4.2-39: Precision and Accuracy Data

Matrix	Fortification Level (mg/tube)	Recovery (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Air	**Ion transition m/z 409 > 227 (quantification)**					
	0.0108	78.1, 72.8, 72.6, 76.5, 90.2	78.0	70 – 120	9.2	20
	0.108	106, 106, 96.1, 102, 109	104	70 – 120	4.8	20
	Ion transition m/z 409 > 209 (confirmation)					
	0.0108	78.5, 73.3, 73.7, 76.9, 91.5	78.8	70 – 120	9.4	20
	0.108	106, 106, 96.7, 103, 110	104	70 – 120	4.7	20

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

Not required, as metrafenone is not classified as toxic or very toxic, and is not classified according to GHS as acute toxicity (cat. 1-3), CMR (cat. 1) or STOT (cat. 1).

We create chemistry

Metrafenone

Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

Amended by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
19/Oct/2015	Original Submission	BASF DocID 2015/1170522
04/12/2015	M-CA Section 5 New Contact Person as of Nov. 2015	BASF DocID 2015/1253616
04/12/2015	C.A.5.3.2 Oral 90 Day-Study – Detailed summaries studies added	BASF DocID 2015/1253616

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

Table of Contents

CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Introduction

Metrafenone is a benzophenone fungicide for the control of powdery mildew fungi. The biochemical mode of action is not yet known. However, observations indicate that it inhibits growth of mycelium on the leaf surface, leaf penetration, and formation of haustoria and sporulation. In addition, metrafenone has been shown to inhibit sporulation in the fungi and may interfere with a dimorphic switch that allows the fungus to reproduce asexually versus sexually.

Metrafenone was first approved by Commission Directive 2007/6/EC (entry into force: 01 February 2007). This document is submitted to support the renewal of approval of metrafenone and complies with the Table of Contents described in the Annex to Regulation (EU) No 283/2013. It reviews the toxicology studies performed with the active substance.

- For studies performed since the first inclusion of metrafenone in Annex I to Directive 91/414/EEC, the study reports are submitted (See document K-CA) and full study summaries are provided.
- For studies submitted and assessed for the first EU review of metrafenone and that are still considered relevant to support renewal of approval, only the critical endpoints and a brief summary are included in this dossier. When the conclusion of the RMS differed from that of the Applicant, this is mentioned. For a more detailed assessment, reference can be made either to the summary dossier (Document M-II, Section 6, BASF SE, 2002) submitted for the first EU evaluation or to the Draft Assessment Report (DAR) for metrafenone (UK, 2005). The study reports are not submitted in document K-CA but can be found in the dossier submitted for the first EU Review.

Most of the studies with non-radiolabelled metrafenone submitted and assessed for the first EU review of metrafenone were performed with batch AC 12053-29, purity 95.86%. The impurity profile of the test batch is presented in Document J-CA of this dossier. The test batch is considered to be representative of technical metrafenone as currently manufactured.

The 13-week dietary toxicity study in albino rats ([redacted] 2001b) was performed with batch AC 11238-111A, purity 97.1 %. This study was used to establish the current AOEL for metrafenone and no change to the AOEL is proposed within this submission. Although the impurity profile was not determined for batch AC 11238-111A, this batch has a higher purity than batch AC 12053-29 (95.86%) used for the majority of toxicology studies and is slightly less pure than the current technical specification (minimum purity 98%). Given that none of the potential impurities of technical metrafenone are considered to be of toxicological relevance, batch AC 11238-111A is also considered to be representative of technical metrafenone as currently manufactured.

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

The absorption, distribution, metabolism and excretion, as well as the toxicokinetics of metrafenone (BAS 560 F), have been investigated in Sprague-Dawley rats in a GLP study ([redacted], 2002). Data were submitted and evaluated under the previous EU review of metrafenone.

According to the data requirements for active substances set out in Commission Regulation (EU) No 283/2013, "Comparative *in vitro* metabolism studies shall be performed on animal species to be used in pivotal studies and on human material (microsomes or intact cell systems) in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy". However, at the time of preparing this dossier, there is no agreed test guideline for conducting such studies and therefore, as provided by SANCO/10181/2013– rev. 2.1, 13 May 2013, Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013", it is proposed that this requirement can be waived for metrafenone.

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

Report: CA 5.1.1/1
[redacted] 2002a
BAS 560 F (AC 375839): Absorption, distribution, metabolism and excretion study in the rat
2002/7005208
Guidelines: US 40 CFR paragraph 158 340, EEC 91/414 Annex II 5.1, EEC 94/79, JMAFF 59 NohSan No 4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

Radiolabelled metrafenone, either as trimethoxphenyl-U-^{14}C metrafenone or bromophenyl-6-^{14}C metrafenone, was administered as single oral doses of 10 mg/kg or 1000 mg/kg as suspensions in 0.5% carboxymethylcellulose to groups of male and female Sprague Dawley rats. An additional group of rats received 14 daily oral doses of non-radiolabelled metrafenone followed by a single oral dose of radiolabelled metrafenone. With the exception of the single low dose experiments, a single label position (i.e. trimethoxphenyl-U-^{14}C) was used in metabolism experiments because there was evidence that the bond between the bromophenyl ring and trimethoxyphenyl ring remains intact.

Recoveries of radioactivity were satisfactory in all experiments ranging from 90.88 to 100.38%. Absorption is rapid and complete (>88%) at a low dose of 10 mg/kg bw, but limited to 15-20% at the high dose of 1000 mg/kg bw suggesting saturation of the absorption processes. Metrafenone is widely distributed in the body, with highest residue levels mainly found in the gastro-intestinal (GI) tract, liver and fat. There is no evidence of accumulation. The labelled material is excreted relatively rapidly into the GI tract via the bile (85-90%) resulting in extensive excretion via feces. Excretion via urine is relatively low (5-6% depending on radiolabel position), and even lower at the high dose level (*ca.* 1%).

In the toxicokinetic experiments, following the administration of a single oral dose of metrafenone, carbon-14 residues were observed in blood within 15 minutes and reached maximum concentrations (C_{max}) of 1.22–1.25 µg/g at 10 mg/kg bw, and 12.74–16.14 µg/g at 1000 mg/kg bw, within 15 hours (8.5 to 15 hours). A slightly longer time (14 to 15 hours) was needed to reach a maximum ^{14}C-blood level at the high dose rate as compared with the low dose rate suggesting a prolonged absorption throughout the GI tract. The differences between male and female were insignificant. The radioactive residues in the blood declined quickly with an elimination half-life of approximately 40 hours for the low dose groups and 50 hours for the high dose groups. The $AUC_{0-\infty}$ for the high dose was only ca. 16-fold higher than the low dose, despite there being a 100-fold difference between dose levels.

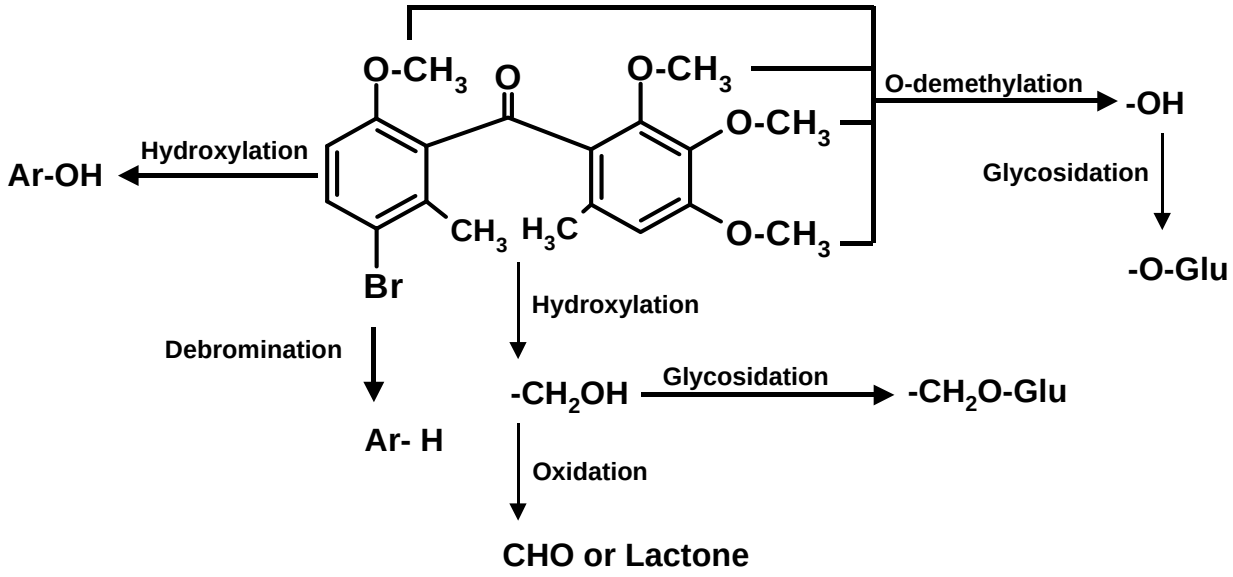
Metrafenone is extensively metabolized, as the radiolabel was mostly excreted as glucuronic acid conjugates in bile and urine. Five different possible sites of conjugation of glucuronic acid were identified, resulting from O-demethylation at one or two locations within the molecule. Residues in feces consisted primarily of parent compound and the aglycones of bile and urine conjugates. The transformation steps include:

- O-Demethylation of the aromatic methoxy-group(s) followed by mono-*O*-glycosilation
- Hydroxylation of the bromophenyl ring
- Hydroxylation of the methyl substituent to hydroxymethyl followed by *O*-glycosilation or further oxidation to aldehyde or lactone.

The bond between the bromophenyl ring and trimethoxyphenyl ring remains intact.

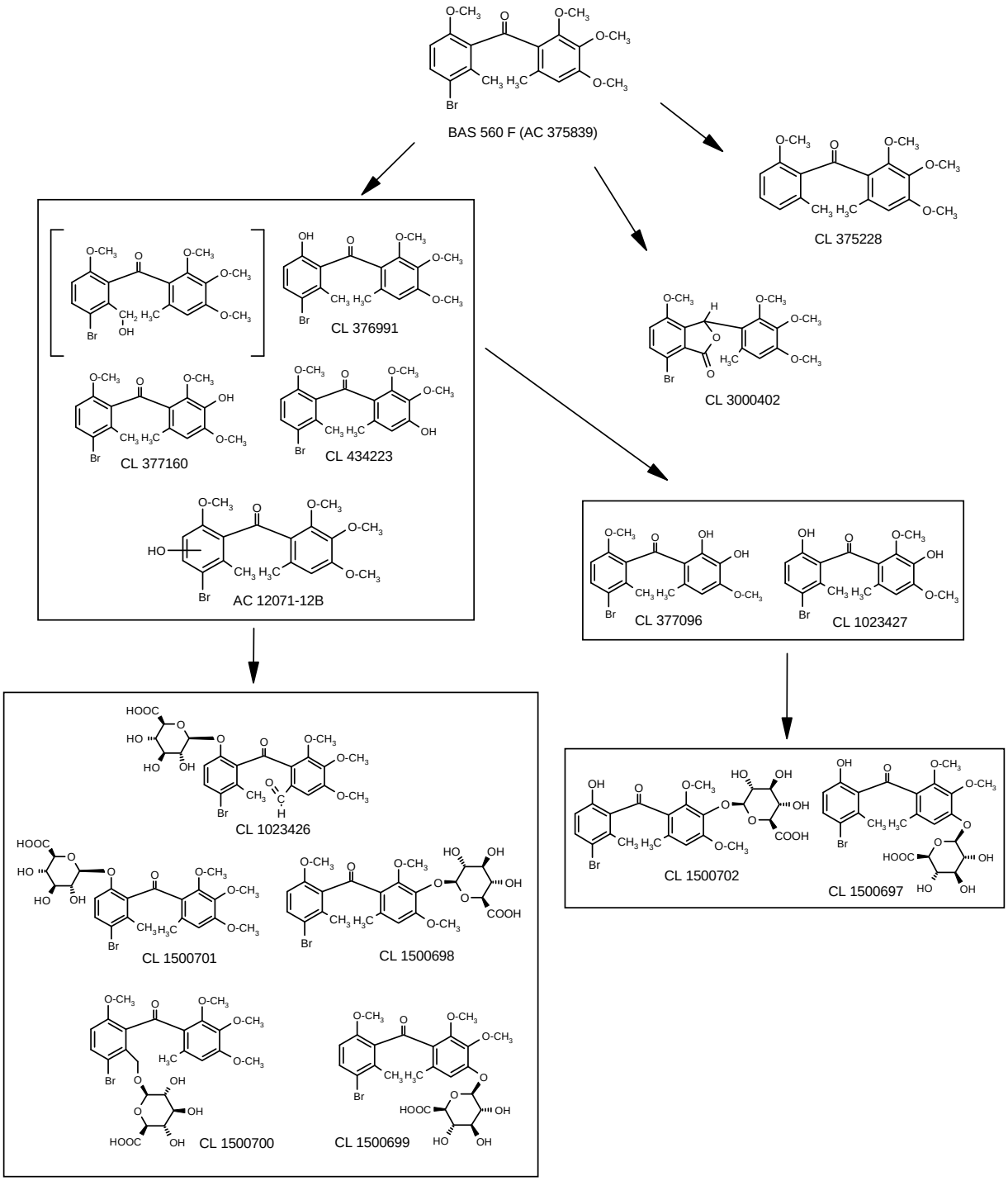
The site of metabolism is shown in Figure 5.1.1-1.

Figure 5.1.1-1: Site of metabolism of metrafenone



The proposed metabolic pathway in rats is presented in Figure 5.1.1-2.

Figure 5.1.1-2: Proposed metabolic pathway of metrafenone in the rat



Absorption, distribution, metabolism and excretion by other routes

An *in vivo* dermal absorption study, performed with the formulated product BAS 560 02F, is available and presented under point 7.3 of document M-CP, Section 7.

CA 5.2 Acute Toxicity

Acute toxicity data were submitted and evaluated for the first EU review of metrafenone. The acute toxicity of metrafenone is low: the oral and dermal LD_{50} in rat are >5000 mg/kg bw, the inhalatory LC_{50} is >5 mg/L. It is not a skin or an eye irritant, nor a skin sensitizer. No further acute toxicity data are required. The existing data package also includes an *in vivo* phototoxicity study which shows that metrafenone is not phototoxic *in vivo*. For completeness, an *in vitro* phototoxicity study pre-dating the adoption of the official OECD guideline 432, together with a document re-assessing the results of this study, are also provided. Table 5.2-1 summarizes the agreed endpoints for acute toxicity.

Table 5.2-1: Acute toxicity data for metrafenone (adapted from DAR UK dated July 2005)

Study type	Species (or system) / doses	Comments	Results	Classification	Reference
Acute oral toxicity	Sprague-Dawley Rat 5,000 mg/kg bw	No mortalities No signs of systemic toxicity	LD_{50} > 5,000 mg/kg bw	Not classified	CA 5.2.1/1 BASF Doc. ID 1999/7000303
Acute dermal toxicity	Sprague-Dawley Rat 5,000 mg/kg bw	No mortalities No signs of systemic toxicity	LD_{50} > 5,000 mg/kg bw	Not classified	CA 5.2.2/1 BASF Doc. ID 1999/7000301
Acute inhalation toxicity	Sprague-Dawley Rat 5 mg/L (4 h)	No mortality at 5 mg/L	LC_{50} > 5 mg/L	Not classified	CA 5.2.3/1 BASF Doc. ID 2000/7000119
Dermal irritation	New Zealand Rabbit 0.5 g/application site	No signs of irritation observed	Not irritating	Not classified	CA 5.2.4/1 BASF Doc. ID 1999/7000295
Eye irritation	New Zealand Rabbit	Slight reversible conjunctival irritation	Not irritating	Not classified	CA 5.2.5/1 BASF Doc. ID 1999/7000298
Skin sensitization Maximization test	Guinea pig	No reaction to challenge exposure	Not sensitizing	Not classified	CA 5.2.6/1 BASF Doc. ID 1999/7000304
In vivo phototoxicity	Guinea pig	Skin reaction for photoirritation very similar to other irradiated groups Results for treated animals similar to vehicle or untreated controls	No photoirritation No photoallergenic potential	Not classified	CA 5.2.7/1 BASF Doc. ID 2002/1005062
In vitro phototoxicity	BALB/C3T3 cells 100 µg/mL		Positive		CA 5.2.7/2 BASF Doc. ID 2001/1019677
Re-evaluation of the *In vitro* phototoxicity	Not applicable		Positive *in vitro*		CA 5.2.7/3 BASF Doc. ID 2013/1140594

CA 5.2.1 Oral

Report: CA 5.2.1/1
[redacted] 1999a
Oral LD50 study in albino rats with AC 375839
BASF Doc. ID 1999/7000303

Guidelines: EPA 870.1100, JMAFF 59 NohSan No 4200, OECD 401, EEC 92/69 B 1 No. L 383 A/110-112

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

Executive Summary

In an acute oral toxicity study, groups of 5/sex Sprague-Dawley rats received an oral dose of 5000 mg/kg bw of metrafenone (purity 95.86%). The test material (solid) had been ground to a fine powder and then mixed with 0.5% aqueous carboxymethylcellulose.
There were no deaths, no notable clinical signs of toxicity and bodyweight gains were normal. There were no gross necropsy findings. The LD_{50} following oral exposure is >5000 mg/kg and the test material is not classifiable for acute oral toxicity. Refer to the DAR (UK, 2005), Point B.6.2.1 for a more detailed assessment of the study.

CA 5.2.2 Dermal

Report: CA 5.2.2/1
[redacted] 1999a
Dermal LD50 study in albino rats with AC 375839
BASF Doc. ID 1999/7000301

Guidelines: EPA 870.1200, JMAFF 59 NohSan No 4200, OECD 402, EEC 92/69 B 3 No. L 383 A/121-123

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

Executive Summary

In an acute dermal toxicity study, groups of 5/sex Sprague-Dawley rats were treated with metrafenone (purity 95.86%) via dermal exposure at a limit dose of 5000 mg/kg bw. The test material (solid) was ground to a fine powder and administered as a paste in 0.5 mL of tap water to ≈10% of body surface area under an occlusive dressing for 24 hours. Following exposure, any remaining test material was removed by irrigating the test site with tap water. There were no deaths, no notable clinical signs of toxicity and bodyweight gains were normal. No skin irritation was noted. There were no gross necropsy findings. The LD_{50} following dermal exposure is >5000 mg/kg and the test material is not classifiable for acute dermal toxicity. Refer to the DAR (UK, 2005), Point B.6.2.2 for a more detailed assessment of the study.

CA 5.2.3 Inhalation

Report:	CA 5.2.3/1 [redacted] 2000a Acute inhalation toxicity study with AC 375839 in rats via nose-only exposure BASF Doc. ID 2000/7000119
Guidelines:	EPA 870.1300, OECD 403, JMAFF 59 NohSan No 4200, EEC 92/69 B 2, EEC 94/79 A II 5.2.3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In an acute inhalation toxicity study, groups of 5/sex Sprague-Dawley rats were exposed to metrafenone (purity 95.86%) for 4 hours via nose-only inhalation exposure at a test atmosphere concentration of 5.0 mg/L. The test material (solid) was jet-milled to be applied in the test atmosphere in the form of a dust.

The hourly analyses of test atmosphere concentration (gravimetric) ranged from 3.4-6.7 mg/L, with a mean value of 5.0 mg/L and an SD of ±1.35. The MMAD was 3.10 µm with GSD 1.95 (4.67% of particles ≤0.1 µm, 65.16% of particles ≤4.0 µm and 95.8% of particles ≤10 µm). There were no deaths, and bodyweight gains were normal except for a single female which lost weight (3 grams) during the second week of the study. Clinical signs recorded during exposure were labored breathing during the last three hours of exposure. Immediately following exposure, signs recorded were red nasal discharge, excessive salivation, chromodacryorrhea, dried red material on the facial area, labored breathing and moist rales. Labored breathing and moist rales continued in some animals until day 3 (48 hours after exposure). Red nasal discharge was recorded sporadically in 1 or 2 males and females over the remainder of the 14 day observation period, persisting in a single female at termination (though this female had not shown this sign throughout – only on days 2, 7, 10, 14 and 15). There were no notable necropsy findings at termination. The acute inhalation LC_{50} is >5 mg/L and the test material is not classifiable for acute inhalation toxicity. The clinical signs recorded following the completion of the exposure period are considered to be a non-specific effect related to animals breathing a highly dusty atmosphere (generated artificially for the purpose of conducting this study) and are not considered to be an appropriate basis for applying classification for respiratory irritation. Refer to the DAR (UK, 2005), Point B.6.2.3 for a more detailed assessment of the study.

CA 5.2.4 Skin irritation

Report: CA 5.2.4/1
[redacted] 1999a
Primary dermal irritation study in albino rabbits with AC 375839
BASF Doc. ID 1999/7000295

Guidelines: OECD 404, EEC 92/69 B 4 No. L 383 A/124-127, JMAFF 59 NohSan No 4200, EPA 870.2500

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

Executive Summary

The hair of six New Zealand White male rabbits was closely clipped to expose the dorsal area. Metrafenone (purity 95.86%) was ground into a fine powder and 500 mg was placed on a 6 cm^2 area of a 4-ply gauze pad and then moistened with 0.5 mL of distilled water. The gauze pad was then applied directly to one intact test site per animal. The gauze pads were secured with hypoallergenic tape, and the trunk was wrapped with a semi-occlusive wrap consisting of a non-allergenic adhesive tape and a filter cloth for a four-hour exposure period. After the four-hour exposure period, the wraps were removed and the test sites were irrigated with tap water to remove any residual test substance. The test sites were then patted dry with gauze pads. There were no skin reactions seen in any animal up to three days after exposure. No classification for skin irritation is therefore required. Refer to the DAR (UK, 2005), Point B.6.2.5 for a more detailed assessment of the study.

CA 5.2.5 Eye irritation

Report: CA 5.2.5/1
[redacted] 1999b
Primary eye irritation study in albino rabbits with AC 375839
BASF Doc. ID 1999/7000298

Guidelines: OECD 405, EEC 92/69 B 5 No. L 383 A/127-130, JMAFF 59 NohSan No 4200, EPA 870.2400

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

Executive Summary

Six New Zealand White male albino rabbits were dosed with a weight equivalent of a 0.1 mL volume of metrafenone (purity 95.86%), as received. The weight equivalent of this 0.1 mL volume was 0.049 g. The test substance was ground into a fine powder and instilled into the conjunctival sac of the left eye. The right eye served as the untreated control. At the end of the 24-hour exposure period, the treated eyes were rinsed with tap water to remove any residual test substance. The eyes were examined pre-treatment (the day prior to test substance administration), and at 1 hour, 24 hours, 48 hours and 72 hours after dosing. At each interval, the cornea, iris and conjunctivae of the treated eyes were examined using a hand held light and the naked eye. Fluorescein dye, with the aid of an ultraviolet light, was used to confirm the presence or absence of corneal effects in the treated eyes. There were no corneal or iridial effects. Minimal conjunctival redness (grade 1), accompanied by minimal to moderate discharge, was recorded after 1 hour in all six animals, but this had resolved to minimal redness only in 2 animals at 24 hours and there were no signs of irritation at 48 or 72 hours. No chemosis was observed in any animal. Mean scores (24-72 hours) for conjunctival redness were <1.0 in all animals. On the basis of these results, no classification for eye irritancy is required. Results are summarized in Table 5.2.5-1

Table 5.2.5-1: Summary of eye irritation (mean readings and symptoms at24, 48 and 72 hours)

Animal No.	Opacity	Iris	Conjunctiva		Symptoms
			Redness	Chemosis	
138 M	0.0	0.0	0.0	0.0	
146 M	0.0	0.0	0.0	0.0	
134 M	0.0	0.0	0.0	0.0	
125 M		0.0	0.3	0.0	
143 M	0.0	0.0	0.0	0.0	
133 M	0.0	0.0	0.3	0.0	Vocalization
Mean	0.0	0.0	0.1	0.0	

M = male

Refer to the DAR (UK, 2005), Point B.6.2.6 for a more detailed assessment of the study.

CA 5.2.6 Skin sensitisation

Report: CA 5.2.6/1
[redacted] 1999a
Dermal sensitization study of AC 375839 in guinea pigs - maximization test (EC guidelines)
BASF Doc. ID 1999/7000304
Guidelines: EPA 870.2600, EEC 92/69 B 6, OECD 406, JMAFF
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

Metrafenone (purity 95.86%) was tested for its skin sensitizing properties in Crl:(HA)BR guinea pigs using the Maximization Test based on the method of Magnusson and Kligman. Twenty male animals were used for the test group and ten male animals in the control group. Based on pre-tests for irritation, the following concentrations were chosen: 1% w/v in carboxymethylcellulose/Tween 80 (CMC/Tween - 0.5%/0.1%) or in Freund's complete adjuvant / sterile water (1:1) for the intradermal induction; 25% w/v in CMC/Tween 80 for the percutaneous induction, and 10% w/v in CMC/Tween 80 for the challenge. At the induction sites, mild to moderate erythema and edema with scab formation were recorded (in both test animals and controls). Following the challenge application, no signs of skin reaction were recorded in either test animals or control animals at either 24 or 48 hours. On the basis of these results, the test material is not classifiable as a skin sensitizer. Refer to the DAR (UK, 2005), Point B.6.2.7 for a more detailed assessment of the study.

CA 5.2.7 Phototoxicity

Report: CA 5.2.7/1
[redacted] 2002a
Phototoxic and photoallergenic potential by cutaneous route in guinea pigs
2002/1005062
Guidelines: none
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

The test was performed in groups of 5 or 10 Dunkin Hartley guinea pigs. Animals were treated via the skin with metrafenone (purity 95.86%) as a 30% w/w suspension in 0.5% aqueous methylcellulose or vehicle with and without UVA and UVB irradiation and skin reactions were recorded, as shown in the table below.

Group	Number of Animals	Induction Phase (6 applications - Days 1 to 8)		Treatment Phase (Day 29)		
		Treatment	Irradiation	Treatment	Irradiation	Scoring
1	5	None	UVA+UVB	None	UVA or UVB	1, 4, 24, 48 h
2	5	Test item	None	Test item	None	1, 4, 24, 48 h
3	10	Test item	UVA+UVB	Test item	UVA or UVB	1, 4, 24, 48 h
4	5	Vehicle	None	Test item	None	1, 4, 24, 48 h
5	5	Vehicle	UVA+UVB	Vehicle	UVA or UVB	1, 4, 24, 48 h

The study followed many of the principles of the draft OECD Guideline for a dermal photoirritation screening test (1995 draft). The aims of the study were:

1) to assess skin reactions for up to 24 hours after the first treatment/irradiation in order to determine phototoxic potential (photoirritation),

2) to assess photoallergenic potential by performing five further topical applications over an 8 day induction period, then after a 20 day rest period perform a challenge topical application and/or irradiation to areas of skin not previously treated and assess skin reactions for up to 48 hours.

The concentration of test material used was determined in a screening study. Test material was applied to clipped and shaved skin and gently massaged into the skin. Animals were restrained to prevent ingestion.

Details of the UV irradiation were as follows: UVA, 365 nm at a dose of 9 Joules/cm^2; UVB, 312 nm at a dose of 0.1 Joule/cm^2. These dose rates are compliant with the draft OECD Guideline. The irradiation dose was described as being 'infra-erythematogenic' – aiming to give rise to 'questionable' erythema (score 0.5 by comparison with the Draize scale). For the challenge phase, separate sites were treated with either UVA or UVB. The criteria for positive results were skin reactions clearly different from the appropriate control animals. The test was considered 'doubtful' if positive reactions occurred in only one or two animals.

Metrafenone did not show photoirritant or photoallergenic potential when tested under the conditions of this study. The results of positive control tests were provided. For photoirritation, a test with 8-methoxypsoralen gave a positive reaction in 3/3 animals tested. For photoallergenic potential, a test with chlorpromazine gave a positive reaction in 4/10 animals tested. Though this test method is not a validated test method (no validated test method exists), the results are reassuring. Refer to the DAR (UK, 2005), Point B.6.8.3 for a more detailed assessment of the study.

Report:	CA 5.2.7/2 Honarvar N., 2001a Cytotoxicity assay in vitro with BALB/C3T3 cells: Neutral red (NR) assay with BAS 560 F at simultaneous irradiation with artificial sunlight 2001/1019677
Guidelines:	Draft OECD In vitro 3T3 NRU Phototoxicity test, EEC 2000/33 B.41
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Report:	CA 5.2.7/3 Honarvar N., 2002a 1. Amendment: Cytotoxicity assay in vitro with BALB/C3T3 cells: Neutral red (NR) assay with BAS 560 F at simultaneous irradiation with artificial sunlight 2002/1004346
Guidelines:	Draft OECD In vitro 3T3 NRU Phototoxicity test, EEC 2000/33 B.41
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Executive Summary

Metrafenone was assessed *in vitro* for its cytotoxic potential to Balb/c3T3 cells in the presence and absence of artificial sunlight, using the Neutral Red (NR) uptake assay, according to the draft version of OECD 432 guideline. After 1 hour pre-incubation with 8 concentrations of the test substance (up to 100 µg/mL) or the positive control, the cells were irradiated with artificial sunlight for 50 minutes with 1.7 mW/cm^2 UVA, resulting in a radiation dose of 5 J/cm^2 UVA. Parallel cultures were kept in the dark for 50 minutes. Cells were then lysed and the extracted neutral red was quantified photometrically at 540 nm. The cytotoxic response curves of the test groups were compared. The EC_{50} values were determined and compared to calculate a Photo-Irritancy factor (PIF) and to measure possible phototoxicity.

A dose-dependent cytotoxicity was observed after treatment of cells with metrafenone in the presence of irradiation with artificial sunlight, with a calculated EC_{50} value of 22.85 µg/mL under irradiation. In the absence of artificial sunlight, metrafenone did not induce any strong cytotoxic effect (i.e. reduced neutral red uptake below 50% of negative control) up to and including the highest tested concentration and therefore an EC_{50} could not be calculated and 100 µg/mL was used as the C_{max}. The >PIF was 4.4.

Under the experimental conditions applied, a phototoxic potential was observed *in vitro* after treatment of Balb/c3T3 cells with metrafenone.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
   Description: Beige solid
   Batch/purity #: AC 12053-29: 95.86%
   Stability of test compound: stable in water and acetone for 4 hours (analytically determined – Analytical report CA/99/01/0535)

2. **Vehicle and/or positive control:** concurrent negative (Earle's Balanced Salt Solution – EBSS), solvent (0.5% acetone v/v) and positive (chlorpromazine at 6.25-200 µg/mL without irradiation and 0.125-4 µg/mL with irradiation) were used

3. **Test system:** Balb/c3T3, subclone 31 cells, obtained from LMP (Technical University of Darmstadt) or Dr. Steiling (Henkel KGaA).

B. STUDY DESIGN AND METHODS

1. Dates of work: 09/11/2001 00:00:00 - 11/12/2001 00:00:00

Twenty-four hours before the start of treatment, 1-2 x 10^4 cells per well were seeded in 100 µg/mL culture medium. Six wells per concentration were seeded with cells. Cultures were then treated with the test substance as follows:

- the cultures were washed with EBSS
- then 8 dilutions each with the solved test substance were spread on two 96-well plates
- both plates were pre-incubated for 1 h in the dark
- afterwards, one 90-well plate was irradiated through the lid at 1.7 mW/cm^2 (5 J/cm^2) for 50 min at room temperature, the other plate was stored for 50 min at room temperature in the dark
- after irradiation the test substance was removed and both plates were washed twice with EBSS
- fresh culture medium was added and the cells were incubated overnight at 37ºC and 4.5% CO_2.

Irradiation was performed with a Dr Hönle Sol 500 solar simulators which emits a continuum spectrum of artificial sunlight. A filter H1 was used to keep the UVB radiation as low as possible. The wavelength of the solar simulator produced with the filter was >320 nm. The exposure rates were determined with a UV-meter using a detector for UVA.

The following day the medium was removed and 0.1 mL medium containing 50 µg NR/mL were added to each well. The plate was returned to the incubator for another 3 hours to allow uptake of the vital dye into the lysosomes of viable cells. Thereafter, the medium was removed completely and the cells were washed with EBSS. Then 0.1 mL of a solution of 49% (v/v) deionized water, 50% (v/v/) ethanol and 1% (v/v/) acetic acid were added to each well to extract the dye. After an additional 10 minutes (approx.) at room temperature and a brief agitation, the plate was transferred to a microplate reader equipped with a 540 nm filter to read the absorbance of the extracted dye against the well containing no cells set as zero.

EC_{50} values were determined and the PIF was calculated as follows:

$$PIF = EC_{50}\ (-UV) / EC_{50}\ (+UV)$$

A PIF $\geq$ 5 predicts phototoxic potential. As the test substance was only cytotoxic +UV, the PIF cannot be calculated and therefore a ">PIF" was calculated using the –UV value at the highest concentration tested (C_{max}) as follows:

$$>PIF = C_{max}\ (-UV) /\ EC_{50}\ (+UV)$$

Any calculated >PIF value greater than 1 predicts phototoxic potential.

II. RESULTS AND DISCUSSION

A dose-dependent cytotoxicity was observed after treatment of cells with metrafenone in the presence of irradiation with artificial sunlight, with a calculated EC_{50} value of 22.85 µg/mL under irradiation. In the absence of artificial sunlight metrafenone did not induce any strong cytotoxic effect (i.e. reduced neutral red uptake below 50% of negative control) up to and including the highest tested concentration and therefore an EC_{50} could not be calculated and 100 µg/mL was used as the C_{max}. The >PIF was 4.4.

The positive control chlorpromazine induced phototoxicity in the expected range after irradiation with artificial sunlight, with EC_{50} values of 9.55 µg/mL and 0.5 µg/mL, respectively, in the absence and presence of radiation. The resulting PIF was 19.2.

III. CONCLUSION

Under the experimental conditions applied, a phototoxic potential was observed *in vitro* after treatment of Balb/c3T3 cells with metrafenone.

Report: CA 5.2.7/4
Honarvar N., 2013c
Metrofenone (BAS 560 F): Reassessment of the in vitro photocytotoxicity assay
2013/1140594
Guidelines: none
GLP: no

Executive Summary

The performance of the *in vitro* phototoxicity study (CA 5.2.7/2) was re-evaluated according to the current valid OECD 432 (adopted 13 April 2013). The study was in coherence with the respective guideline in practically all aspects except the following points:

1) For the cell lysis, a volume of 100 µL desorb solution was used instead of the 150 µL as described in the guideline.
2) The $O.D._{450nm}$ of the vehicle controls were not ≥ 0.4, which is a validity criterion of a study. The obtained $O.D._{450nm}$ values were 0.358±0.057 and 0.323±0.074 for the vehicle control cultures in the presence and absence of UV irradiation, respectively.
3) In the guideline, a >PIF is not calculated. Instead the mean photo effect (MPE) is used for the analysis of phototoxic potential.

The lower volume of the desorb solution used to lyse the cells and dissolve the retained neutral red is most probably the cause of the obtained low O.D. values. Although this shortcoming would invalidate the study under the current guideline, the difference between the UV light exposed and non-exposed cultures is so clear, that the phototoxic potential of the compound cannot be disputed. It is hardly likely that the higher vehicle control O.D. values could have had a significant impact on the outcome of the results.

Since the term ">PIF" is not used in the current guideline, the obtained values were used in the OECD program to calculate the MPE retrospectively. This resulted in a value of MPE = **0.175**, which is above the phototoxicity threshold of 0.15.

Although the *in vitro* study methodology has some deficiencies, the data still indicate, that under the test conditions BAS 560 F demonstrated a phototoxic potential in this *in vitro* assay.

CA 5.3 Short-Term Toxicity

The short-term toxicity of metrafenone (duration from 4 weeks to one year) was evaluated in rats, mice and dogs. All the studies were GLP-compliant and performed according to internationally accepted guidelines, with the exception of the range-finding studies.

The target organ in all species was the liver (increased weight and histopathological findings). The lowest NOAEL was obtained in the rat 90-day study (43 mg/kg bw/day). The battery of short-term studies available is sufficient to permit the identification of effects following repeated exposure. Since Annex I inclusion of metrafenone, a 28-day dermal toxicity study in rats has been performed, showing no evidence of either systemic or local toxicity following dermal exposure. This study is now submitted, and a complete summary is provided within this section.
A summary of short-term studies is presented in Table 5.3-1.

Table 5.3-1: Summary of short-term toxicity of metrafenone

Study	NOAEL	LOAEL	Findings at LOAEL	Reference
4-week rat (diet) 0, 1000, 5000, 10000 and 20000 ppm	1000 ppm (106-118 mg/kg bw/day)	5000 ppm (528-586 mg/kg bw/day)	↑ liver weights and histopathological findings in the liver	CA 5.3.1/1
4-week dog (capsule) 0, 12.5, 25, 250 and 500 mg/kg bw/day	From the DAR: 250 mg/kg bw/day	From the DAR: 500 mg/kg bw/day	From the DAR: ↑ liver weights, transient bodyweight loss	CA 5.3.1/2
	According to the Applicant: 500 mg/kg bw/day	-	The observed body weight effects were transient and minor (<10%) and the liver weight increases were not accompanied by concomitant microscopic effects	
13-week rat (diet) 0, 1000, 5000, 10000 and 20000 ppm	-	1000 ppm (79-94 mg/kg bw/day)	↑ cholesterol, ↑ liver weights, histopathological findings in the liver and kidneys [Unclear toxicological significance] More marked effects at 5000 ppm (404-486 mg/kg bw/day)	CA 5.3.2/1
13-week rat (diet) 0, 250 and 500 ppm	NOEL: 500 ppm (43-48 mg/kg bw/day)	-	No effects identified at the highest level tested	CA 5.3.2/2
13-week mouse (diet) 0, 1000, 3500 and 7000 ppm	1000 ppm (163-216 mg/kg bw/day)	3500 ppm (622-788 mg/kg bw/day)	↑ liver weights and histopathological findings in the liver (centrilobular hepatocellular hypertrophy)	CA 5.3.2/3
13-week mouse (diet) 0, 250 and 500 ppm	NOEL: 500 ppm (84-113 mg/kg bw/day)	-	No effects identified at the highest level tested	CA 5.3.2/4
13-week dog (capsule) 0, 50, 100 and 500 mg/kg bw/day	From the DAR: 50 mg/kg bw/day	From the DAR: 100 mg/kg bw/day	From the DAR: ↑ cholesterol and ↑ relative liver weights	CA 5.3.2/5
	According to the Applicant: 500 mg/kg bw/day	-	The increased liver weights were not associated with any other histopathological or clinical chemistry liver effects	

Study	NOAEL	LOAEL	Findings at LOAEL	Reference
52-week dog (capsule) 0, 50, 150 and 500 mg/kg bw/day	From the DAR: -	From the DAR: 50 mg/kg bw/day	↑ relative liver weights	CA 5.3.2/6
	According to the Applicant: 500 mg/kg bw/day	-	The increased alkaline phosphatase levels and absolute and relative liver weights were not accompanied by any histopathological changes in the liver	
4-week rat (dermal) 0, 100, 300 and 1000 mg/kg bw/day	1000 mg/kg bw/day	-	No local or systemic effects identified at the highest level tested	

CA 5.3.1 Oral 28-day study

Report: CA 5.3.1/1
[redacted] 2001a
28-Day dietary toxicity study in albino rats with BAS 560 F
2001/7000267
Guidelines: OECD 407, EEC 67/548 Part B No. L383A/136-139
GLP: no

Executive Summary

In a 4-week range-finding study, groups of 5/sex Sprague-Dawley rats were exposed to metrafenone (purity >99.9%) for 28 days via the diet at concentrations of 0, 1000, 5000, 10000 and 20000 ppm (mean substance intakes: 0, 106, 528, 1127 and 2245 mg/kg/day in males and 0, 118, 586, 1151 and 2294 mg/kg/day in females) as a preliminary study to a 90-day study. Due to a sexing error, the top dose group consisted of 4 males and 6 females instead of 5/sex. Investigations included clinical signs, bodyweights, food consumption, hematology and clinical chemistry at termination, organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and thymus), gross necropsy and histopathological examination (full range of tissues from control and high dose group, plus lungs, liver, heart, kidneys and gross lesions from remaining groups). Hematology results were considered to be unreliable due to the limited number of data available for the control groups and the high-dose group males.

There were no deaths and no clinical signs of toxicity were observed throughout the study. There was no effect of treatment on food consumption or bodyweight gain. There were no obvious hematology effects. Clinical chemistry showed increased cholesterol values in female rat groups, although without a clear dose-response relationship. The slightly increased mean cholesterol value observed in females at 1000 ppm was not considered to be adverse because only 2 of 5 females showed slightly elevated values, and there was no correlation to any microscopic change. The only significant organ weight effects were recorded in the liver. Absolute and/or relative liver weights were statistically significantly increased in both sexes at ≥5000 ppm, the increases being more marked in females. Liver weights at 1000 ppm were not increased compared with controls. At necropsy, discolored liver was noted in 3/5 females at 10000 ppm and 2/6 females at 20000 ppm. There were no other notable gross necropsy findings. Significant microscopic findings were recorded in the liver and consisted of periportal cytoplasmic vacuolation of hepatocytes, recorded at a higher incidence in high dose males and in females at ≥5000 ppm. This finding was described by the pathologist as being consistent with the accumulation of lipid (hematoxylin and eosin staining performed only). Severity in females ranged from minimal to moderate and severity increased in a dose-related pattern. Severity in males was minimal in all cases. Scattered cytoplasmic vacuolation (not limited to periportal localization) was also recorded in some animals but the pattern of incidence did not suggest a treatment-related effect. It was noted by the pathologist that the cytoplasmic vacuolation was not accompanied by necrosis, and was therefore considered to be an adaptive rather than a toxic effect. The gross findings of discolored liver correlated with periportal cytoplasmic vacuolation in each case.

In summary, treatment with metrafenone was associated only with effects on the liver. The clinical chemistry findings were suggestive of effects on the liver, and this was confirmed at termination with increased liver weights, discolored livers and an increased incidence of periportal cytoplasmatic vacuolation being recorded. There were no other significant effects of treatment identified. Increased group mean liver weights and increased incidence of hepatocyte vacuolation were recorded at ≥5000 ppm, hence 5000 ppm is a clear effect level. Group mean liver weights were not increased at 1000 ppm and the incidence of vacuolation at 1000 ppm was no higher than controls. However, increased group mean cholesterol was recorded in females at 1000 ppm. The applicant notes that only 2 out of the 5 females in this group showed higher cholesterol levels, the remaining 3 animals being similar to controls, and the 2 females with raised cholesterol did not show hepatocyte vacuolation. Therefore, 1000 ppm can be considered a NOAEL.

It is concluded that the NOAEL in this study was 1000 ppm (equal to 106-118 mg/kg/day), based on effects on the liver at ≥5000 ppm (≥528-586 mg/kg/day). The same NOAEL was proposed by the applicant.

Report: CA 5.3.1/2
[redacted] 1999a
28-day oral toxicity study with AC 375839 in purebred beagle dogs via capsule administration
1999/7000325
Guidelines: none
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 4-week range-finding study, groups of 2 male and 2 female Beagle dogs received metrafenone (purity 97.1%) in their diets at concentrations of 0, 500, 1000, 10000 and 20000 ppm. As poor food consumption with a corresponding decrease in body weight was observed at 10000 and 20000 ppm, the dietary administration was terminated after 4 days. All animals were returned to control diet until body weights and food consumption values were comparable to control levels. The study was reinitiated and metrafenone was administered via oral capsule (7 days/week), to the same dogs at dose levels of 12.5, 25, 250, and 500 mg/kg/day for a period of at least 28 days. Control animals received empty gelatin capsules. The dogs were observed daily for signs of overt toxicity, morbidity, and mortality. Ophthalmological examinations were conducted prior to treatment and at termination. Detailed clinical observations were recorded weekly during the 28-day study period. Individual body weights and food consumption data were recorded weekly during the study period. Doses were adjusted weekly to compensate for changes in body weight. Samples for hematological, clinical chemistry and urinalysis examinations were collected from all surviving dogs pre-treatment and at termination. At termination, all surviving animals were subjected to a gross necropsy and selected organs were weighed. Samples of selected tissues were processed for histopathological evaluation from all surviving test animals.

There were no deaths, no notable ophthalmoscopy findings and no clinical signs of toxicity were observed. Transient bodyweight loss (ranging from 1-10% of bodyweight) was recorded in both sexes during the first week of treatment at 500 mg/kg/day, but bodyweights subsequently recovered. There was no effect on bodyweights at lower dose levels and no effect on food consumption at any dose level. There were no effects of treatment on hematology, clinical chemistry or urinalysis parameters. The only notable organ weight findings were increased liver weights at 500 mg/kg/day (relative liver weights (mean of two animals) were 30% higher (males) or 22% higher (females) than control means at 500 mg/kg/day). There were no notable gross necropsy or microscopic findings in any group.

Based on the transient bodyweight effects and increased liver weights at 500 mg/kg/day, the NOAEL was 250 mg/kg/day. The same NOAEL was proposed by the applicant.

CA 5.3.2 Oral 90-day study

Report: CA 5.3.2/1
[redacted] 2001b
13-week dietary toxicity and 28-day recovery study in albino rats with BAS 560 F
2001/7000270
Guidelines: EPA 82-1, OECD 408, EEC 67/548 Part B No. L133/8-11
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 90-day toxicity study, groups of 10/sex Sprague-Dawley rats were exposed to metrafenone (purity 97.1%) for 13 weeks via the diet at concentrations of 0, 1000, 5000, 10000 and 20000 ppm (mean substance intakes: 0, 79, 404, 800 and 1663 mg/kg/day in males and 0, 94, 486, 967 and 1938 mg/kg/day in females). Five additional animals/sex were added to the control and high dose groups to be maintained for a 4-week recovery period after the exposure period. Investigations included clinical signs, bodyweights, food consumption, hematology, clinical chemistry and urinalysis (at termination of exposure and recovery periods), ophthalmoscopy (pre-exposure and day 87), organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid gland), gross necropsy and histopathological examination (full range of tissues from control and high dose groups (treatment and recovery phases), plus lungs, liver, kidneys and gross lesions from remaining groups).

There were 2 accidental deaths (mechanical or blood sampling) but no treatment-related mortalities. There were no treatment-related clinical signs. Ophthalmoscopy, urinalysis and hematology did not reveal treatment-related findings. There were no toxicologically significant effects on food consumption. Weekly body weights and weight gains for treated males and females receiving 1000 ppm were generally comparable to or in excess of those of the untreated controls at most measurement intervals. Treatment-related decreases in weekly body weights and weight gains were observed for females at 5000, 10000, and 20000 ppm at most measurement intervals. Although a statistically significant decrease in overall body weight gain (0-13 weeks) was observed in female rats at 5000 (14.6%), 10000 (14.6%) and 20000 ppm (13.2%), there was no evidence of a dose-response relationship. Body weights and weight gains (weekly and overall) for males and females previously treated at 20000 ppm for 13 weeks were generally comparable to or in excess of those of the untreated controls at most measurement intervals during the recovery phase of the study. Clinical chemistry investigations at termination revealed increases in cholesterol levels in both males and females, statistically significant at 5000 ppm and above, and more marked and more clearly dose-related in females. Cholesterol levels were also slightly higher in males and females at 1000 ppm, without attaining statistical significance. Total protein was significantly higher in treated animals, with females more markedly affected. Additionally, there was a slight but statistically significant increase in albumin values for 20000 ppm females. Following the 4 week recovery period, cholesterol, total protein and albumin values for both sexes previously treated at 20000 ppm returned to control levels with no statistically significant differences. Statistically significant increases in absolute and relative (to body weight) liver weights were noted for males and females at 5000 and above when compared to controls.

Absolute and relative (to body weight) liver weights for males and females at 1000 ppm were only very slightly increased over control liver weights with the relative weights for 1000 ppm males being statistically significant. As there were no correlating histopathological changes, the liver weight changes at 1000 ppm were not considered to be adverse. At the end of the 28-day recovery phase, liver weights for rats previously treated at 20000 ppm were generally comparable to control weights. There were no notable gross necropsy findings in any group. Significant microscopic findings were recorded in the liver. An increased incidence of periportal cytoplasmic vacuolation, consistent with the accumulation of lipid, was recorded in males at 20000 ppm and in females at ≥5000 ppm. The incidence and severity of this finding in females did not show clear dosage-related trends. These findings were shown to be reversible in males and largely reversible in females.

In summary, treatment with metrafenone was associated with reduced bodyweight gains (females), increased serum cholesterol, increased serum protein (both sexes) and increased urinary protein (males). There were marked increases in liver weights associated with periportal cytoplasmic vacuolation. Increased kidney weights were also recorded along with microscopic findings described as typical of early chronic nephropathy. Most findings showed evidence of reversibility. There were clear effects of treatment recorded at ≥5000 ppm. The toxicological significance of some findings at 1000 ppm was uncertain (increased cholesterol and total protein levels in serum, marginally increased liver weights, microscopic kidney findings, scattered cytoplasmic vacuolation in hepatocytes). A clear NOAEL was not identified in this study and hence a precautionary LOAEL of 1000 ppm is proposed. The applicant did not consider the effects recorded at 1000 ppm to be adverse (since the liver effects appeared to be reversible they were considered to be adaptive, and the urinary protein effects were not considered to be treatment-related) and proposed 1000 ppm as the NOAEL.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
   Description: White powder
   Batch/purity #: AC 11238-111A: 97.1%
   Stability of test compound: Stable for the duration of the study (expiry date in 2001)

2. **Vehicle and/or positive control:** Ground PMI Certified Rodent Diet #5002

3. Test animals:

Species: Rat
Strain: Sprague-Dawley (Crl:CD®(SD) BR)
Age: 4-5 weeks old at dosing
Weight: At start of dosing: Males: 113-150 g; females: 88-114 g
Source: Charles River Breeding Laboratories Inc., New York, USA
Acclimation period: 10 days
Diet: Ground PMI Certified Rodent Diet #5002, *ad libitum*
Water: Filtered drinking water from an automatic watering system, *ad libitum*
Housing: 2 per cage during the acclimatization period. Individually in stainless-steel, suspended, screen-bottomed cages held on racks, with absorbent paper to collect urine and feces

4. Environmental Conditions

Temperature: 66-77ºF (approx. 19-25 ºC)
Humidity: 50±20%
Air changes: At least 10 per hour
Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. Dates of work: 13/04/1998 00:00:00 – 11/08/1998 00:00:00

2. Animal assignment and treatment

On study day 0, the 120 animals for the study were selected from a larger batch on the basis of body weight and randomly divided into 5 treatment groups using a computerized randomization procedure. All animals received the appropriate diets for 13 consecutive weeks. Five additional animals of each sex from the control and high dose groups received the untreated diet for an additional 4-week recovery period after the exposure period.

3. Dose preparation and analysis

The test diets were prepared at approximately 7-day intervals during the study. Concentrations were adjusted to correct for the purity of the test substance. For each concentration, metrafenone was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose groups, were added to this premix in order to obtain the desired concentration. Mixing was carried out for about 10 min in a laboratory mixer.

Prior to the beginning of the experimental period, test batches of diets for the low and high concentrations were mixed to check the suitability of mixing procedures, and to determine stability of the test substance in the diet. The results indicated that the test diets were homogenous and the test substance was stable in the diet for at least 14 days in the animal room or in bulk storage food containers used in the study. Concentration control analyses were performed with samples taken at each weekly diet preparation, and stored frozen until analysis.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Standard one-way of variance (ANOVA) was used to analyze the following data for each sex: body weight and body weight gains, food consumption, organ weight and organ to body weight percentages. When data tested by analysis of variance yielded a significant F value, the data was subtested by Dunnett's method ($p<0.05$). The hematology data were tested for normality of distribution by the Kolmogorf-Smirnov test with Lilliefor's correction, and for equality of variances by the Levene median test. If the data met these parametric assumptions, the data were then tested by analysis of variance. When data tested by analysis of variance yielded a significant F value, the data was subtested by Dunnett's method ($p<0.05$). Non-parametric data were evaluated using the Kruskal-Wallace one way ANOVA on ranks. When significant, the data was subtested by Dunnett's method ($p<0.05$). For the histopathological evaluation, statistical analysis (e.g. Fisher's Exact Test) was performed for selected lesions for all animals on study.

C. METHODS

1. Clinical observations

All animals were observed for overt signs of toxicity daily. Observations for mortality and morbidity were made twice daily. Detailed physical examinations outside the home cage were conducted weekly during both the treatment and recovery phases.

2. Bodyweight and food consumption

Individual body weight and food consumption were recorded weekly.

3. Ophthalmological examinations

Ophthalmological examinations were performed on all animals during the acclimation period (day -4) and on all surviving animals during the last week (day 87) of the treatment phase of the study.

4. Clinical pathology

Hematological, clinical chemistry and urinalysis parameters were evaluated for all surviving rats at termination of treatment (days 92-94) and recovery (day 120) phases. Blood samples from overnight fasted animals were obtained under carbon dioxide anesthesia from the retro-orbital sinus. The following parameters were measured:
Hematology: hematocrit, hemoglobin, MCV, MCH, MCHC, erythrocyte count, platelet count, total and differential leukocyte count.
Clinical chemistry: albumin, alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), chloride, cholesterol, creatinine, gamma glutamyl transferase (GGT), glucose, phosphorus, potassium, sodium, triglycerides, total bilirubin, total protein.
Urinalysis: volume, color, appearance, pH, specific gravity, urobilinogen, ketones, occult blood, protein, glucose, bilirubin, microscopic examination of sediment.

5. Anatomical pathology

At termination of the treatment and recovery phases, all surviving animals were anesthetized with carbon dioxide, blood samples were collected as required and the animals exsanguinated. A complete necropsy was performed.

Organ weight: the following organs were trimmed of fat and other contiguous tissues and weighed: adrenal glands (combined weight), brain, hearth, kidney (combined weight), liver, ovaries/oviducts (combined weight), spleen, testes (combined weight), thymus, thyroid gland.

Tissue preservation: the following tissues from all animals were fixed in 10% neutral phosphate buffered formalin solution: adrenal glands, aorta (thoracic), urinary bladder, bone and marrow (sternum and femur), brain (cerebral cortex, cerebellar cortex, pons/medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, heart, seminal vesicles, ileum, jejunum, kidneys, liver, lungs (with mainstem bronchi), lymph nodes (mesenteric and submaxillary), mammary gland (females), skeletal muscle, sciatic nerve, ovaries, oviduct, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, skin (mammary area), spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes, thymus, thyroid gland, trachea, uterus (both horns, corpus and cervix), any other tissue(s) with gross lesions.

Histopathology: samples were processed and examined by light microscopy at Experimental Pathology Laboratories Inc. (ELP), USA. The following were examined: a complete set of tissues from all animals from the control and high-dose groups at termination of the treatment or recovery phases; a complete set of tissues from all animals sacrificed *in extremis* or found dead during the study; all gross lesions from all animals in all groups; lungs, liver, and kidneys from the low-dose and two mid-dose groups.

II. RESULTS AND DISCUSSION

Analysis confirmed test-diet concentrations to be acceptable (100% of nominal values at 1000, 5000 and 10000 ppm, 99% at 20000 ppm, with coefficients of variation between 4 and 6).

Table 5.3.2-1: Test substance intake

Dietary Dose Level (ppm)	Average Test Substance Intake (mg/kg bw/day)	
	Males	Females
1000	79	94
5000	404	486
10000	800	967
20000	1663	1938

There were 2 incidental deaths during the study. One male receiving 20000 ppm died on day 12 because its head was shut in the cage during routine maintenance and one control female dead on day 92 during the collection of terminal blood sample. No treatment-related clinical signs or signs of overt toxicity were observed during the treatment or recovery phases of the study.

No treatment-related ophthalmological findings were noted during the study.

Food consumption values for male rats at all dietary concentrations were generally comparable to or in excess for those of the control rats. Food consumption values for female rats showed slight, non-dose-related decreases at the 20000, 10000, 5000 and 1000 ppm levels of 3.1%, 2.9%, 3.5%, and 4.2%, respectively, during the 13-week treatment phase of the study. Food consumption values for male and female treated rats during the 28-day recovery phase of the study were generally comparable to those of the untreated controls.

Weekly body weights and weight gains for males at 1000, 5000, 10000 and 20000 ppm and females in the 1000 ppm group were generally comparable to or in excess of those of the untreated controls at most measurement intervals. Treatment-related decreases in weekly body weights and weight gains were observed for females at 5000, 10000, and 20000 ppm at most measurement intervals. Although a statistically significant decrease in overall body weight gain (0-13 weeks) was observed in female rats at 5000 (14.6%), 10000 (14.6%) and 20000 ppm (13.2%), there was no evidence of a dose-response relationship.

Body weights and weight gains (weekly and overall) for males and females previously treated with 20000 ppm of metrafenone for 13 weeks were generally comparable to or in excess of those of the untreated controls at most measurement intervals during the recovery phase of the study.

Table 5.3.2-2: Body weight gain data (from the DAR, slightly modified)

Parameter	0 ppm	1000 ppm	5000 ppm	10000 ppm	20000 ppm
Males					
Total gain Weeks 0-13 (g)	368.6	399.7	375.8	351.7	389.9
% of control	-	108	102	95	106
Gain during the 4-week recovery phase (g)	30.4				26.0
Females					
Total gain Weeks 0-13 (g)	183.5	169.2	156.7*	156.7	159.1*
% of control	-	92	85	85	87
Gain during the 4-week recovery phase (g)	16.3				20.0

* $p<0.05$ (ANOVA – Dunnett's test)

No treatment-related hematological changes were noted in either sex at the 1000, 5000, 10000, or 20000 ppm concentrations at termination of the treatment phase (13 weeks) or at the end of the 28-day recovery period.

Slight but statistically significant increases in cholesterol and total protein values were observed in both sexes at 20000, 10000, and 5000 ppm at termination of the treatment phase (13-weeks) of the study. Additionally, albumin values for 20000 ppm females were also slightly but statistically significantly increased which is related to the increase in total protein observed at this dose. The increases in these parameters appeared to be treatment-related.

Clinical chemistry values measured at the end of the 28-day recovery phase showed cholesterol, total protein and albumin values for both sexes previously treated with 20000 ppm of metrafenone generally returning to control levels with no statistically significant differences. This return to normal levels for cholesterol, total protein, and albumin, which paralleled the general absence of liver pathology following the 28-day recovery period in these animals, may indicate that these elevated clinical chemistry parameters during the treatment phase were an adaptive response of the liver to toxic insult.

Table 5.3.2-3: Selected clinical chemistry findings (from the DAR, slightly modified)

Dose level (ppm)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Males – treatment phase				
0	53.7	59.7	6.6	4.6
1000	61.6	66.5	7.0	4.6
5000	72.1*	53.3	7.3*	4.9
10000	83.2*	44.9	7.3*	4.7
20000	77.0*	64.2	7.2*	4.8
Males – recovery phase				
0	54.2	89.8	7.1	3.9
20000	60.0	88.2	7.3	4.1
Females – treatment phase				
0	68.1	39.3	7.2	5.3
1000	85.6	31.0	7.6*	5.6
5000	97.6*	34.5	8.2*	5.7
10000	108.8*	34.9	8.2*	5.6
20000	124.0*	46.5	8.4*	5.8*
Females – recovery phase				
0	70.8	41.3	7.6	4.9
20000	71.0	42.6	7.8	4.7

* $p<0.05$ (ANOVA – Dunnett's test)

No adverse effects of treatment with metrafenone were evident from the urinalysis data collected at the end of the 13-week treatment phase or following the 28-day recovery phase of the study. The summary in the DAR mentions that significantly increased (and highly variable) urinary protein was recorded in males receiving 10000 and 20000 ppm (females were not affected); urinary protein was still higher than controls in males previously receiving 20000 ppm at the end of the recovery period.

Table 5.3.2-4: Urinary protein data (from the DAR, slightly modified)

Dose level (ppm)	Urinary protein (mg/dL)	
	Treatment phase	Recovery phase
Males		
0	30.0 ± 0.0	44.0 ± 31.3
1000	65.0 ± 36.9	
5000	58.0 ± 36.1	
10000	152.0* ± 130.2	
20000	165.7* ± 123.8	112.0 ± 110.8
Females		
0	19.0 ± 6.9	30.0 ± 0.0
1000	15.0 ± 0.0	
5000	28.0 ± 26.3	
10000	19.5 ± 7.2	
20000	28.7 ± 21.1	30.0 ± 0.0

* $p<0.05$ (ANOVA – Dunnett's test); ± standard deviation

Statistically significant increases in absolute and relative (to body weight) liver weights were noted for males and females at 5000, 10000 and 20000 ppm when compared to controls. Absolute and relative (to body weight) liver weights for males and females at 1000 ppm were only very slightly increased over control liver weights with the relative weights for 1000 ppm males being statistically significant. The increases in absolute and relative liver weights noted in females at 5000 ppm and 10000 ppm, and both sexes at 20000 ppm were considered treatment-related because they correlated with the microscopic hepatic changes of increased periportal cytoplasmic vacuolation noted for animals in these same treatment groups. A similar correlation to liver pathology for male rats at 5000 ppm and 10000 ppm was not evident. The very slight increases in absolute and relative liver weights observed for both sexes at 1000 ppm were not considered to be adverse because there was no correlating histopathological change. The increases in liver weight and microscopic changes observed in the treated female rats at 5000 ppm and above and males at 20000 ppm, most likely represent an adaptive response to the test substance which was reversible. No other treatment-related organ weight changes were observed at the end of the 13-week treatment period.

At the end of the 28-day recovery phase, absolute and relative (to body weight) liver weights for rats previously treated with 20000 ppm of metrafenone were generally comparable to control weights. No other treatment-related organ weight changes were observed at the end of the 28-day recovery period.

Table 5.3.2-5: Absolute and relative liver and kidney weights (from the DAR, slightly modified)

Parameter	0 ppm	1000 ppm	5000 ppm	10000 ppm	20000 ppm
Males – treatment phase					
Liver weight (g)	13.28	15.35	16.19*	16.16*	18.97*
% of control	-	116	122	122	143
Relative liver weight (% bw)	2.82	3.07*	3.39*	3.54*	3.76*
% of control	-	109	120	126	133
Kidney weight (g)	3.49	3.83	3.84	4.06*	4.59*
% of control	-	110	110	116	132
Relative kidney weight (% bw)	0.74	0.77	0.81	0.89*	0.91*
% of control	-	104	109	120	123
Males – recovery phase					
Liver weight (g)	13.84				14.77
% of control	-				107
Relative liver weight (% bw)	2.87				2.98
% of control	-				104
Kidney weight (g)	3.63				4.18
% of control	-				115
Relative kidney weight (% bw)	0.76				0.84
% of control	-				112
Females – treatment phase					
Liver weight (g)	7.68	8.18	9.94*	11.06*	11.54*
% of control	-	107	129	144	150
Relative liver weight (% bw)	2.87	3.21	4.11*	4.60*	4.89*
% of control	-	112	143	160	170
Kidney weight (g)	2.15	2.15	2.29	2.32	2.43
% of control	-	100	107	108	113
Relative kidney weight (% bw)	0.80	0.85	0.94*	0.96*	1.02*
% of control	-	106	118	120	128
Females – recovery phase					
Liver weight (g)	7.77				8.10
% of control	-				104
Relative liver weight (% bw)	2.75				3.15
% of control	-				115
Kidney weight (g)	2.13				2.32
% of control	-				109
Relative kidney weight (% bw)	0.75				0.90*
% of control	-				120

* $p<0.05$ (ANOVA – Dunnett's test)

No treatment-related macroscopic pathological changes were noted for treated rats when compared to controls at either the end of the 13-week treatment phase of the study or the end of the 28-day recovery phase.

Dietary administration of metrafenone at dietary concentrations up to 20000 ppm for 13-weeks to albino rats resulted in histomorphologic changes attributable to the test substance in the livers of females receiving 5000, 10000, or 20000 ppm and equivocal effects in the livers of males receiving 20000 ppm. Specifically, in the livers of females at 5000 ppm and above and for males at 20000 ppm, periportal cytoplasmic vacuolation, consistent with the accumulation of lipid, was increased in incidence as compared to the untreated controls. No dose-response relationship was evident for either incidence or severity of the alteration in female rats. No other treatment-related tissue changes were observed at the end of the 13-week treatment phase of the study.

After 13-weeks of treatment with 20000 ppm of metrafenone followed by a 28-day recovery period, no males showed periportal cytoplasmic vacuolation in the liver and only 2 of 5 female rats at the highest concentration had minimal to slight/mild periportal cytoplasmic vacuolation in the liver, which is indicative of adaptation and reversibility. No histomorphologic tissue alterations attributable to treatment with metrafenone were present in any of the other tissues examined at the end of the 28-day recovery phase.

Table 5.3.2-6: Histopathological findings (from the DAR, slightly modified)

Parameter	0 ppm	1000 ppm	5000 ppm	10000 ppm	20000 ppm
Males – treatment phase					
Liver – No. examined	10	10	10	10	10
Cytoplasmic vacuolation, periportal (total)	1	1	0	0	3
minimal	1				
slight/mild		1			2
moderate					1
Cytoplasmic vacuolation, scattered	1	1	1	4	3
Kidney - No. examined	10	10	10	10	10
Cast(s) - proteinaceous	1	5	2	3	4
Mononuclear cell infiltrate	3	4	7	4	3
Regenerative tubular epithelium	4	8	8	9	7
Males – recovery phase					
Liver – No. examined	5				5
Cytoplasmic vacuolation, periportal (total)	0				0
minimal					
slight/mild					
moderate					
Cytoplasmic vacuolation, scattered	3				3
Kidney - No. examined	5				5
Cast(s) - proteinaceous	0				2
Mononuclear cell infiltrate	3				3
Regenerative tubular epithelium	4				4

Parameter	0 ppm	1000 ppm	5000 ppm	10000 ppm	20000 ppm
Females – treatment phase					
Liver – No. examined	10	10	10	10	10
Cytoplasmic vacuolation, periportal (total)	1	0	7	8	5
minimal	1		3	6	5
slight/mild			2	2	
moderate			2		
Cytoplasmic vacuolation, scattered	4	8	3	4	5
Kidney - No. examined	10	10	10	10	10
Cast(s) - proteinaceous	1	0	0	0	1
Mononuclear cell infiltrate	0	3	2	3	3
Regenerative tubular epithelium	0	2	0	0	3
Females – recovery phase					
Liver – No. examined	5				5
Cytoplasmic vacuolation, periportal (total)	0				2
minimal					1
slight/mild					1
moderate					
Cytoplasmic vacuolation, scattered	4				3
Kidney - No. examined	5				5
Cast(s) - proteinaceous	0				0
Mononuclear cell infiltrate	0				1
Regenerative tubular epithelium	1				1

III. CONCLUSION

The Applicant concluded that the results from this 13-week dietary toxicity study support a No-Observable-Adverse-Effect-Level (NOAEL) of 1000 ppm, based on increases in total protein and cholesterol values in both sexes at 5000 ppm, and increased incidences of microscopic periportal cytoplasmic vacuolation in the liver and decreased weight gain in female rats at 5000 ppm, the next highest concentration tested. A non-adverse increase in absolute and relative (to body weight) liver weight was observed at 1000 ppm in both sexes. A concentration of 1000 ppm is equivalent to an average daily intake of 79 mg/kg bw/day for males and 94 mg/kg bw/day for females, calculated from food consumption data.

According to the DAR, treatment with metrafenone was associated with reduced bodyweight gains (females), increased serum cholesterol, increased serum protein (both sexes) and increased urinary protein (males). There were marked increases in liver weights associated with periportal cytoplasmic vacuolation. Increased kidney weights were also recorded along with microscopic findings described as typical of early chronic nephropathy. Most findings showed evidence of reversibility. There were clear effects of treatment recorded at ≥5000 ppm. The toxicological significance of some findings at 1000 ppm was uncertain (increased cholesterol and total protein levels in serum, marginally increased liver weights, microscopic kidney findings, scattered cytoplasmic vacuolation in hepatocytes). A clear NOAEL was not identified in this study and hence a precautionary LOAEL of 1000 ppm is proposed. The applicant did not consider the effects recorded at 1000 ppm to be adverse (since the liver effects appeared to be reversible they were considered to be adaptive, and the urinary protein effects were not considered to be treatment-related) and proposed 1000 ppm as the NOAEL.

Report: CA 5.3.2/2
[redacted] 2001c
13-week dietary toxicity study in albino rats with BAS 560 F
2001/7000272
Guidelines: EPA 82-1, OECD 408, EEC 67/548 Part B No. L133/8-11
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a subsequent 13-week study to determine a clear NOEL for liver effects, groups of 10/sex Sprague-Dawley rats received metrafenone (purity: 97.1%) in their diets at 0, 250 or 500 ppm (0, 21 and 43 mg/kg/day in males and 0, 24 and 48 mg/kg/day in females). Investigations included clinical signs, body weights and food consumption measurements, hematology, clinical chemistry and urinalysis (at the end of the study), organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid gland), gross necropsy and histopathological examination (full range of tissues from control and high dose groups, plus lungs, liver, kidneys and gross lesions from the intermediate dose group).

There was one accidental death but no treatment-related mortalities and no clinical signs of toxicity in any group. There were no effects on food consumption, body weight gains or hematology parameters in any group. Clinical chemistry investigations at termination did not reveal any treatment-related effects, and no changes were observed in cholesterol levels in any group. There were also no effects on urinalysis parameters. Absolute liver and spleen weights were significantly higher in males treated at 250 ppm however, relative weights were not significantly higher and there was no similar effect at the higher dosage (500 ppm). Relative heart weights were 12% lower than controls in 500 ppm females (a statistically significant reduction) but in the absence of any correlating histopathological findings in the heart (or a similar effect in high dose males or animals treated at higher dose levels in the previous study), the change was considered to be a spontaneous finding and not toxicologically significant. There were no notable gross necropsy findings in any group, and histopathological examination did not reveal any lesions considered to be treatment-related.

In summary, there were no effects of treatment identified in this study. None of the effects recorded at higher dose levels in the previous 13-week rat study were identified at the lower dose levels used in this study. The NOAEL was 500 ppm (equal to 43-48 mg/kg/day), the highest dose tested. The same NOAEL was proposed by the applicant.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metrafenone
Description: White powder
Batch/purity #: AC 11238-111A: 97.1%
Stability of test compound: Stable for the duration of the study (expiry date in 2001)

2. Vehicle and/or positive control: Ground PMI Certified Rodent Diet #5002

3. Test animals:
Species: Rat
Strain: Sprague-Dawley (Crl:CD®(SD) BR)
Age: 4 weeks old at dosing
Weight: At start of dosing: Males: 75-91 g; females: 74-89 g
Source: Charles River Breeding Laboratories Inc., New York, USA
Acclimation period: 7 days
Diet: Ground PMI Certified Rodent Diet #5002, *ad libitum*
Water: Filtered drinking water, from an automatic watering system, *ad libitum*
Housing: 2 per cage during the acclimatization period. Individually in stainless-steel, suspended, screen-bottomed cages held on racks, with absorbent paper to collect urine and feces

4. Environmental Conditions
Temperature: 66-77ºF (approx. 19-25 ºC)
Humidity: 50±20%
Air changes: At least 10 per hour
Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. Dates of work: 06/08/1998 00:00:00 - 10/11/1998 00:00:00

2. Animal assignment and treatment
On study day 0, the 60 animals for the study were selected from a larger batch on the basis of body weight and randomly divided into 3 treatment groups using a computerized randomization procedure. All animals received the appropriate diets for 13 consecutive weeks.

3. Dose preparation and analysis

The test diets were prepared at approx. 7-day intervals during the study. Concentrations were adjusted to correct for the purity of the test substance. For each concentration, metrafenone was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose groups, were added to this premix in order to obtain the desired concentration. Mixing was carried out for about 10 min in a laboratory mixer.
Samples were collected from the initial mix of diet from the low-dose level to determine the homogeneity of mixing and stability of the test substance in the diet. The results indicated that the test diets were homogenous and the test substance was stable in the diet for at least 14 days in the animal room or in bulk storage food containers used in the study. Concentration control analyses were performed with samples taken at each weekly diet preparation, and stored frozen until analysis.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Standard one-way of variance (ANOVA) was used to analyze the following data for each sex: body weight and body weight gains, food consumption, organ weight and organ to body weight percentages. When data tested by analysis of variance yielded a significant F value, the data was subtested by Dunnett's method ($p<0.05$). The hematology data were tested for normality of distribution by the Kolmogorf-Smirnov test with Lilliefor's correction, and for equality of variances by the Levene median test. If the data met these parametric assumptions, the data were then tested by analysis of variance. When data tested by analysis of variance yielded a significant F value, the data was subtested by Dunnett's method ($p<0.05$). Non-parametric data were evaluated using the Kruskal-Wallace one way ANOVA on ranks. When significant, the data was subtested by Dunnett's method ($p<0.05$). For the histopathological evaluation, statistical analysis (e.g. Fisher's Exact Test) was performed for selected lesions for all animals on study.

C. METHODS

1. Clinical observations

All animals were observed for overt signs of toxicity daily. Observations for mortality and morbidity were made twice daily. Detailed physical examinations outside the home cage were conducted weekly during both the treatment and recovery phases.

2. Bodyweight and food consumption

Individual body weight and food consumption were recorded weekly.

3. Clinical pathology

Hematological, clinical chemistry and urinalysis parameters were evaluated for all surviving rats at termination of treatment (days 95 and 96). Blood samples from overnight fasted animals were obtained under carbon dioxide anesthesia from the retro-orbital sinus. The following parameters were measured:

Hematology: hematocrit, hemoglobin, MCV, MCH, MCHC, erythrocyte count, platelet count, total and differential leukocyte count.
Clinical chemistry: albumin, alkaline phosphatase, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), chloride, cholesterol, creatinine, gamma glutamyl transferase (GGT), glucose, phosphorus, potassium, sodium, triglycerides, total bilirubin, total protein.
Urinalysis: volume, color, appearance, pH, specific gravity, urobilinogen, ketones, occult blood, protein, glucose, bilirubin, microscopic examination of sediment.

4. Anatomical pathology

At termination of the treatment and recovery phases, all surviving animals were anesthetized with carbon dioxide, blood samples were collected as required and the animals exsanguinated. A complete necropsy was performed.
Organ weight: the following organs were trimmed of fat and other contiguous tissues and weighed: adrenal glands (combined weight), brain, hearth, kidney (combined weight), liver, ovaries/oviducts (combined weight), spleen, testes (combined weight), thymus, thyroid/parathyroid gland (combined weight).
Tissue preservation: the following tissues from all animals were fixed in 10% neutral phosphate buffered formalin solution: adrenal glands, aorta (thoracic), urinary bladder, bone and marrow (sternum and femur), brain (cerebral cortex, cerebellar cortex, pons/medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, heart, seminal vesicles, ileum, jejunum, kidneys, liver, lungs (with mainstem bronchi), lymph nodes (mesenteric and submaxillary), mammary gland (females), vagina, skeletal muscle, sciatic nerve, ovaries, oviduct, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, skin (mammary area), spinal cord (cervical, thoracic and lumbar), seminal vesicles, spleen, stomach, testes, thymus, thyroid gland, trachea, uterus (both horns, corpus and cervix), any other tissue(s) with gross lesions.
Histopathology: samples were processed and examined by light microscopy at Experimental Pathology Laboratories Inc. (ELP), USA. The following were examined: the complete set of tissues from all animals from the control and high-dose groups; lungs, liver, and kidneys from the low-dose group.

II. RESULTS AND DISCUSSION

Analysis confirmed test-diet concentrations to be acceptable (97% of nominal values, with coefficients of variation between 2 and 4).

Table 5.3.2-7: Test substance intake

Dietary Dose Level (ppm)	Average Test Substance Intake (mg/kg bw/day)	
	Males	Females
250	21	24
500	43	48

There was a single incidental death during the study. One female receiving 250 ppm was found dead on day 14 because its head was shut in the cage during routine maintenance. No treatment-related clinical signs or signs of overt toxicity were observed.

There were no effects on food consumption, body weight gains or hematology parameters in any group.

Clinical chemistry investigations at termination did not reveal any treatment-related effects, and no changes were observed in cholesterol levels in any group.

No treatment-related effects on urinalysis parameters were noted.

Absolute liver and spleen weights were significantly higher in males treated at 250 ppm however, relative weights were not significantly higher and there was no similar effect at the higher dosage (500 ppm). Relative heart weights were 12% lower than controls in 500 ppm females (a statistically significant reduction) but in the absence of any correlating histopathological findings in the heart (or a similar effect in high dose males or animals treated at higher dose levels in the previous study), the change was considered to be a spontaneous finding and not toxicologically significant.

There were no notable gross necropsy findings in any group, and histopathological examination did not reveal any lesions considered to be treatment-related.

Principal effects recorded in the study are listed in **Table 5.3.2-8**.

Table 5.3.2-8: Principal findings

Dose level (ppm)	**0 (control)**	**250**	**500**
Clinical Chemistry			
Cholesterol (mg/dL)	49.7 (m) 72.2 (f)	59.8 (m) 85.3 (f)	53.9 (m) 72.1 (f)
Organ weights			
Liver – absolute (g)	13.793 (m) 7.862 (f)	15.986* (m) 8.463 (f)	14.189 (m) 7.941 (f)
Liver – relative (% bw)	2.833 (m) 2.989 (f)	3.027 (m) 3.103 (f)	2.905 (m) 2.978 (f)
Microscopic Changes: Liver – Periportal cytoplasmic vacuolation			
Incidence	1/10 (m) 0/10 (f)	0/10 (m) 0/10 (f)	0/10 (m) 0/10 (f)
Severity	Minimal		

* - Significantly different from control ($p<0.05$); (m) = Male; (f) = Female

III. CONCLUSION

There were no effects of treatment identified in this study. None of the effects recorded at higher dose levels in the previous 13-week rat study were identified at the lower dose levels used in this study. The NOAEL was 500 ppm (equal to 43-48 mg/kg/day), the highest dose tested. The same NOAEL was proposed by the applicant.

Report: CA 5.3.2/3
[redacted] 2001d
13-week dietary toxicity study in albino mice with BAS 560 F
2001/7000273
Guidelines: EPA 82-1, OECD 408, EEC 67/548 Part B No. L133/8-11
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 90-day toxicity study, groups of 10 male and 10 female CD-1 mice received metrafenone (purity 97.1%) in their diet at concentrations of 0, 1000, 3500 or 7000 ppm (mean substance intakes: 163, 622 and 1206 mg/kg/day for males and 216, 788 and 1663 mg/kg/day for females) for 13 weeks. Investigations included clinical signs, body weights, food consumption, hematology and clinical chemistry at termination, organ weights (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and thymus), gross necropsy and histopathological examination (full range of tissues from control and high dose group, plus lungs, liver, kidneys and gross lesions from remaining groups).

There were no deaths and no clinical signs of toxicity. There were no effects of treatment on food consumption. Reduced body weight gain was recorded in males at 7000 ppm from week 5 onwards such that overall body weight gains in this group were approximately 20% lower than controls, although not attaining statistical significance and not affecting mean body weight. Body weight gains in the other male groups and in all female groups were similar to controls. Treatment had no effect on hematological parameters. Clinical chemistry showed treatment-related changes in hepatic parameters i.e. increases in total bilirubin for both male and female mice at 3500 and 7000 ppm (attaining statistical significance in males only), slight increases in cholesterol values at 7000 ppm (attaining statistical significance in females only), and slight non statistically significant increases in triglycerides levels in females at 3500 and 7000 ppm. The only significant organ weight effects were increased liver weights in males and females treated at 3500 and 7000 ppm. There were no treatment-related macroscopic findings. Histopathology revealed treatment-related findings in the liver of animals of both sexes treated at 3500 and 7000 ppm, where hepatocellular hypertrophy (principally in the centrilobular area but frequently blending into the midlobular areas) was noted. The affected hepatocytes were filled with eosinophilic cytoplasm with a ground glass appearance. No vacuolation of the cytoplasm was evident. The incidence and severity of this finding showed a dose-related pattern. There were no liver findings in animals receiving 1000 ppm, and no treatment-related effects in any other organs or tissues.

In summary, treatment with metrafenone was associated with effects on the liver (increased weight and centrilobular hepatocellular hypertrophy) in both sexes at ≥3500 ppm, with accompanying clinical chemistry effects (increases in cholesterol, triglycerides and total bilirubin). The small increases in cholesterol levels in females at 1000 ppm are not considered to be significant in the absence of any supporting findings. Slightly reduced bodyweight gains were also recorded in males only at 7000 ppm.

The NOAEL was 1000 ppm (equal to 163-216 mg/kg/day). The same NOAEL was proposed by the applicant.

Report:	CA 5.3.2/4 [redacted] 2001e 13-week dietary toxicity study in albino mice with BAS 560 F 2001/7001312
Guidelines:	EPA 82-1, OECD 408, EEC 67/548 Part B No. L133/8-11
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a subsequent 13-week study intended to show a clear NOEL for liver effects, groups of 10 male and 10 female CD-1 mice received metrafenone (purity 97.1%) in their diets at concentrations of 0, 250 or 500 ppm (0, 42 and 84 mg/kg/day in males and 0, 55 and 113 mg/kg/day in females). Investigations were the same as in the previous study.

There were no mortalities, no clinical signs of toxicity and no effects of treatment on body weights or food consumption in any group. There were no treatment-related hematology effects. Statistically significant increased cholesterol levels were recorded at 500 ppm in males (125.9 vs. 98.5 mg/dL). In females the increase in triglyceride levels was statistically significant increased at 500 ppm (118.8 vs. 90.1 mg/dL) . There were no statistically significant or toxicologically relevant organ weight changes and gross necropsy or histopathological findings in any group.

The effects on cholesterol levels and the minimal increases in liver weights without accompanying histopathological findings are not considered to be toxicologically significant. The NOAEL was therefore 500 ppm (equal to 84-113 mg/kg/day), the highest dose tested. The same NOAEL was proposed by the applicant.

Report:	CA 5.3.2/5 [redacted] 2001b 90-day oral toxicity study with AC 375839 in purebred beagle dogs via capsule administration 2001/7000276
Guidelines:	EPA 870.3150, OECD 409, EEC 67/548 Part B No. L133/12-15, JMAFF 59 NohSan No 4200
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 90-d toxicity study, groups of 4 male and 4 female Beagle dogs received metrafenone (purity 95.86%) via oral capsule at 0, 50, 100, and 500 mg/kg/day for 13 weeks. Hematology, blood biochemistry evaluations and urinalysis were performed for all animals prior to initiation and at monthly intervals. Gross necropsy and histopathology evaluations were performed for all animals at termination; organ weights were recorded for all animals.

All animals survived to study termination. Clinical observations, body weights, food consumption, opthalmoscopic examinations, hematology, clinical chemistry, and urinalysis evaluations, macroscopic and microscopic examinations did not reveal any adverse effects of treatment with metrafenone. Absolute and relative (to body weight and brain weight) liver weights were increased in male and female dogs which received 500 mg/kg/day. However, the relative (to body weight) liver weight remained below 4%, and the finding was not associated with any microscopic findings in the liver.

The liver was identified as the target organ in this study, with effects on cholesterol levels, increased liver weights and increased severity of histopathological findings in the liver being recorded in treated animals. The NOAEL for the study is 50 mg/kg/day, based on increased cholesterol and increased relative liver weight at 100 mg/kg/day in females, and in both sexes at 500 mg/kg/day. It is notable that the histopathological findings were only recorded in males even though other liver-related effects were recorded at lower dose levels in females. The applicant considered the NOAEL in this study to be 500 mg/kg/day since they did not consider the cholesterol levels and histopathological findings to be treatment-related effects, and did not consider increases in liver weights to be an adverse effect in the absence of other findings. The same conclusion was reached by the Joint Meeting on Pesticides Residues (JMPR) in the recent toxicology evaluation[1].

[1] Pesticides residues in food 2014. Joint FAO/WHO Meeting on Pesticides Residues. FAO Plant Production and Protection Paper 221.

Report:	CA 5.3.2/6 [redacted] 2001c One-year oral toxicity study with BAS 560 F in purebred beagle dogs via capsule administration 2001/7001049
Guidelines:	EPA 870.4100, OECD 452, EEC 94/79 A II 5.5, JMAFF 59 NohSan No 4200
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 1 year toxicity study, groups of 4 male and 4 female Beagle dogs received metrafenone (purity 95.86%) via oral capsule at 0, 50, 150 and 500 mg/kg/day. Ophthalmological examinations were performed on all animals pre-test and at termination. Hematology, blood biochemistry and urinalysis evaluations were conducted for all animals prior to treatment initiation and at weeks 13, 26 and 52. Gross necropsy and histopathology evaluations were performed for all animals at study termination; organ weights were recorded for all animals at termination.

Administration of metrafenone resulted in mild increases in mean alkaline phosphatase in males dosed at 500 mg/kg/day after 9 and 12 months of dosing. Both absolute and relative liver weights were statistically significantly increased for 500 mg/kg/day females, and relative liver weights were statistically significantly increased for 500 mg/kg/day males. However, the recorded increases in liver weights did not worsened even with the longer-term duration (compared to the 90-day study), relative liver weight did not exceed 4% of body weight, and there were no associated macroscopic or microscopic findings. Therefore, the differences in liver weights and alkaline phosphatase (males) were not considered to be of toxicological significance.

The only clear effect of treatment in this study was markedly increased liver weight. There were no accompanying macroscopic or microscopic findings in the liver, but the magnitude of the liver weight increases was sufficient to be considered an adverse effect. The only supporting evidence was possible increases in cholesterol and alkaline phosphatase levels though these increases in many cases were not clearly treatment-related and would not be considered toxicologically significant in isolation. It is concluded that a NOAEL was not established in this study, based on markedly increased relative liver weights in all treated female groups (50 mg/kg/day and above). Liver weight increases were recorded in males at ≥150 mg/kg/day. The applicant did not consider any of the effects seen to be toxicologically significant and proposed that the NOAEL should be 500 mg/kg bw/day (the highest dose tested). The same conclusion as the Applicant was derived during the recent JMPR toxicology evaluation of metrafenone.

CA 5.3.3 Other routes

Report: CA 5.3.3/1
[redacted] 2003a
BAS 560 F - Repeated dose dermal toxicity study in Wistar rats - Administration for 4 weeks
2003/1020325
Guidelines: EEC 92/69, OECD 410, EPA 870.3200
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a dermal 28-day study, groups of 10 male and 10 female Wistar rats were treated dermally with metrafenone formulated as a suspension in 0.5% carboxymethyl cellulose, at dose levels of 0, 100, 300 and 1000 mg/kg/day for 4 weeks (5 days per week). The volume of application was 4 mL/kg and duration of treatment was 6 hours daily. Observations included body weight and food consumption measurements, clinical observations and detailed weekly clinical observations outside the cage, ophthalmological examinations (before treatment and at week 4) and hematology, clinical chemistry and urinalysis at termination. At the end of the study the animals were sacrificed and examined for gross pathology. Absolute and relative organ weights were calculated. After necropsy, designed organs and tissues of all animals were fixed and preserved; samples from all animals from the control and highest dose groups as well as all gross lesions in any rat were examined microscopically.

There were neither systemic nor local effects at any dose level. The NOEL of the study was 1000 mg/kg/day, the highest dose level tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: Beige solid
Batch/purity #: AC 12053-29: 94.2%
Stability of test compound: Proven by reanalysis after the in-life phase of the study (94.2% - Study 170488_1, CoA included in the report)

2. **Vehicle and/or positive control:** aqueous solution of 0.5% carboxymethyl cellulose / no positive control required

3. Test animals:

Species: Rat
Strain: Wistar CrlGIxBrlHan:WI
Age: 62 ± 1 day at start of dosing
Weight: At start of dosing (group mean values) Males: 239.6-242.8 g; females: 167.3-168.5 g
Source: Charles River, Germany
Acclimation period: 7 days
Diet: Kliba maintenance diet mouse/rat GLP *ad libitum*
Water: Drinking water, from water bottles) *ad libitum*
Housing: Individually in type DK III stainless steel wire mesh cages

4. Environmental Conditions

Temperature: 20-24ºC
Humidity: 30-70%
Air changes: Not indicated
Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. Dates of work: 02/11/2003 00:00:00 - 09/26/2003 00:00:00

2. Animal assignment and treatment

Four days prior to the administration period the animals with clipped skin areas were distributed according to weight among the individual test group, separated by sex, using a randomization list compiled with a computer. The fur of the animals allocated for the study was clipped one day before the administration of the test substance, thereafter when necessary but at least once a week. The test substance was administered uniformly to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk; at least 10% of the body surface) using 3 mL syringes, for about 4 weeks (5 days per week). The administration volume was 4 mL/kg bw, based upon the latest individual body weight determination. The skin was covered for 6 hours after administration using a semi-occlusive dressing, consisting of 4 layers of porous gauze dressing and an elastic dressing. After removal of the dressing, the treated skin was washed with lukewarm water. Control animals received the vehicle only.

3. Dose preparation and analysis

Appropriate amounts of metrafenone were weighed, depending on the desired concentration, and then the vehicle (0.5% carboxymethylcellulose aqueous solution) was filled up to the desired volume and subsequently mixed using a high speed homogenizer. During administration the test substance preparations were kept homogeneous using a magnetic stirrer. The test substance preparations were prepared twice a week and kept cold in a refrigerator during this time. Stability over a period of 4 days at room temperature was previously confirmed (Project No. 15S0437/01092). Homogeneity and concentration were verified from all dose levels in samples prepared before the start of the administration period and at the end of the administration period.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Food consumption, body weight, body weight change and food efficiency of each group were compared to the control group using Dunnett's test (two-sided) for the hypothesis of equal means. Clinical pathology parameters (except reticulocytes and differential blood count) and organ weight data were subjected to 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. Urinalysis (except volume, color, turbidity and specific gravity) was subjected to pair-wise comparison with the control group using Fisher's exact test for the hypothesis of equal proportions.

C. METHODS

1. Clinical observations

All animals were observed for overt signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further clinical examinations – including detailed examination of the skin – were carried out daily just before treatment. Detailed clinical examinations outside the home cage were conducted prior to initiation of treatment, and weekly thereafter (usually in the morning prior to test substance administration). The animals were transferred to a standard arena and the findings were ranked using scoring systems defined in the animal house software.

2. Bodyweight

Body weights were measured before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on the day of initiation of treatment (day 0) and thereafter at weekly intervals.

3. Food and water consumption and food efficiency

Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. Food efficiency (group means) was calculated based upon individual values for body weight and food consumption**.**

4. Ophthalmoscopic examination

Examinations were carried out on all animals before initiation of treatment (day -4), and on day 27 on animals of the control and high dose groups.

5. Clinical pathology

Blood was taken from the retroorbital venous plexus in the morning from all animals, fasted, and under anesthesia with isoflurane. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. For urinalysis the individual animals were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight. The urine samples were evaluated in a randomized sequence.

Hematology
The following parameters were determined in blood with EDTA-K_3 as anticoagulant using a particle counter: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, and reticulocytes. Differential blood smears were also prepared and stained according to Wright but were not evaluated. The clotting analyses were performed using a ball coagulometer; the prothrombin time was determined (Hepato Quick's test).

Clinical Chemistry
An automatic analyzer was used to determine the following parameters: total protein, albumin, globulins, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, cholesterol, triglycerides, glucose, creatinine, urea, sodium, potassium, chloride, calcium, inorganic phosphorus, magnesium.

Urinalysis
With the exception of volume (graduated tubes), color, turbidity (by visual evaluation), sediment examination (evaluated microscopically) and the specific gravity (determined using a urine refractometer), all the urine constituents were determined semi-quantitatively using test strips. The following examinations were carried out: volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment.

6. Sacrifice and pathology
All animals were sacrificed by decapitation under CO_2-anesthesia. The exsanguinated animals were subjected to macroscopic examination.

Organ weights
The following organ weights were recorded in all animals: anesthetized animal, liver, kidneys, adrenals, brain, epididymides, heart, ovaries, spleen, testes, thymus, and uterus.

Histology
The following organs and tissues were retained at necropsy and preserved in neutral-buffered 4% formalin: all gross lesions, salivary glands (mandibular gland, sublingual gland), esophagus, stomach (fore- and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord (cervical, thoracic and lumbar cord), eyes, adrenal glands, thyroid glands, parathyroid glands, trachea, lungs, pharynx, larynx, nose, nasal cavity, aorta, heart, bone marrow (femur), lymph nodes (mesenteric and mandibular lymph node), spleen, thymus, kidneys, urinary bladder, testes, ovaries, oviduct, uterus, vagina, epididymides, prostate, seminal vesicle, female mammary gland, skin, treated skin, skeletal muscle, sternum with marrow, femur with knee joint, extraorbital lacrimal glands. Microscopic examination of slides stained with hematoxylin and eosin was performed on all animals of the control and high dose groups; gross lesions from all animals were also examined.

II. RESULTS AND DISCUSSION

At all concentrations, analysis demonstrated acceptable concentrations (90.3-96.7%) and homogeneity. The stability of metrafenone in an aqueous solution of 0.5% carboxymethylcellulose over a period of 4 days at room temperature was proven in a previous study.

No animals died during the study and there were neither clinical signs of systemic toxicity nor signs of local irritation at any dose level.

Food and water consumption, body weights and food efficiency were not affected by treatment.

No substance-related effects were noted at ophthalmic examination; all findings were spontaneous in nature and equally distributed between treated and control animals.

Clinical pathology investigations revealed no changes in hematology parameters and urinalysis. Statistically significant decreases in alanine and aspartate aminotransferase activities were recorded in the high dose females; these were considered not to be of toxicological importance because not accompanied by any other change, including liver histopathology.

All gross lesions occurred singly. All mean absolute and relative organ weight data did not show any statistically significant difference compared to the control group. All findings noted at microscopic examination were either single observations or they were equally distributed between treated and control animals. All of them were considered to be incidental or spontaneous in origin and without any relation to treatment.

III. CONCLUSION

There were neither systemic nor local effects at any dose level. The NOEL of the study was 1000 mg/kg/day, the highest dose level tested.

CA 5.4 Genotoxicity Testing

The potential genotoxicity of BAS 560 F was investigated in a series of both *in vitro* and *in vivo* studies. All standard end points for genetic damage (point mutations and chromosome damage) were assessed. In addition, since Annex I inclusion of metrafenone an *ex vivo* UDS assay in rat was performed, showing no evidence of DNA damage repair in hepatocytes from treated rats. This study is now submitted and summarized within this section.

In all studies both *in vitro* and *in vivo*, metrafenone gave clear negative results. It is concluded that metrafenone did not show genotoxic potential when tested in an adequate battery of genotoxicity assays. A summary of genotoxicity studies with metrafenone is presented in Table 5.4-1.

Table 5.4-1: Summary of genotoxicity studies

Test system	Test conditions	Result	Reference
Bacterial point mutation assay (Ames test): *S. typhimurium* TA98, TA100, TA1535 & TA1537 *E. coli* WP2 *uvrA*	With and without S9 25-5000 µg/plate	Negative (with and without S9)	CA 5.4.1/1 BASF Doc. ID 1999/7000326
Mammalian cell gene mutation assay: CHO cells, HGPRT locus	With and without S9 17-5000 µg/mL	Negative (with and without S9)	CA 5.4.1/2 BASF Doc. ID 2001/7000288
Clastogenicity in CHO cells	Without S9 2.5-100 µg/mL With S9 10-250 µg/mL	Negative (with and without S9)	CA 5.4.1/3 BASF Doc. ID 2001/7000340
Mouse bone marrow micronucleus assay (CD-1 mice)	0, 500, 1000 or 2000 mg/kg bw	Negative	CA 5.4.2/1 BASF Doc. ID 2001/7000268
Unscheduled DNA synthesis (UDS) (Wistar male rats)	0, 1000 or 2000 mg/kg bw	Negative	CA 5.4.2/2 BASF Doc. ID 2006/1025861

CA 5.4.1 In vitro studies

Report: CA 5.4.1/1
Wagner V.O.,Sly J.E., 1999a
Bacterial reverse mutation assay with AC 375839
1999/7000326

Guidelines: EEC 94/79 A II 5.4.1, OECD 471, EPA 870.5100, JMAFF 59 NohSan No 4200

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

Executive Summary

In a bacterial reversal mutation assay (Ames test), metrafenone (purity 95.86%) in DMSO was tested in *Salmonella typhimurium* (strains TA100, TA1535, TA1537 and TA98) and *Escherichia coli* (WP2 *urvA*) up to 5000 µg/plate with and without S9 from SD rats pre-treated with Aroclor 1254. The confirmatory assay was repeated using the same method as per the first experiment i.e. plate incorporation method. Precipitate was observed at ≥1000 µg per plate, but no cytotoxicity was observed up to 5000 µg/plate. The positive controls gave acceptable results. There were no increases in mutation frequency induced by the test material either with or without S9 for any strain in either experiment. Metrafenone was concluded to be non-mutagenic in this assay. Refer to the DAR (UK, 2005), Point B.6.4.1 for a more detailed assessment of the study.

Report:	CA 5.4.1/2 Pant K.J., 2001a BAS 560 F: Test for chemical induction of gene mutation at the HGPRT locus in cultured chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay 2001/7000288
Guidelines:	EEC 94/79 A II 5.4.1, OECD 476, EPA 870.5300
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a mammalian cell gene mutation assay (HGPRT locus), cultured Chinese hamster ovary (CHO) cells were treated with metrafenone (purity 95.86%) in DMSO at concentrations ranging from 0.1 to 5000 µg/mL with and without S9 mix prepared from Aroclor 1254 pre-treated SD rats. Dosages for the mutation experiments (17, 50, 167, 500, 1667, and 5000 µg/mL) were based on results of a range-finding toxicity screen, which showed Relative Cloning Efficiencies (RCEs) ranging from 100% to 59% in the non-activated system, and from 76% to 31% in the activated system.

In the initial mutation assay RCEs were 105% to 56% and 96% to 60% with and without metabolic activation, respectively. The number of mutants in the treated cultures was very similar to the number in the solvent controls, both with and without metabolic activation. Occasional instances of increases over controls were less than two-fold, which was the test laboratory criteria to consider a possible positive result. The positive controls (EMS and DMBA) gave the expected results (242-335 mutants per $1x10^6$ surviving cells). In the confirmatory assay, RCEs were 108% to 70% and 111% to 76% with and without metabolic activation, respectively. The number of mutants was again very similar between solvent control and treated cultures. The positive controls gave acceptable results. Metrafenone gave negative results in this assay. Refer to the DAR (UK, 2005), Point B.6.4.1 for a more detailed assessment of the study.

Report:	CA 5.4.1/3 Xu J., 2001a BAS 560 F: Test for chemical induction of chromosome aberration in cultured chinese hamster ovary (CHO) cells with and without metabolic activation 2001/7000340
Guidelines:	EEC 94/79 A II 5.4.1, OECD 473, EPA 870.5375, JMAFF 59 NohSan No 4200
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

To investigate clastogenic potential, metrafenone (purity 95.86%) in DMSO was tested on CHO cells in the absence and in the presence of metabolic activation, at concentrations of up to 100 and 250 µg/mL respectively. Tested dose levels were based on a range finding test (3 hours exposure, harvest after 18 hours) in which reductions in mitotic index were recorded at 25-250 µg/mL without S9 and at 250 µg/mL with S9. Treatment with metrafenone was associated with a reduction in relative mitotic index of >50% at ≥25 µg/mL without S9 and at ≥125 µg/mL with S9 in the initial assay, and at ≥25 µg/mL in the confirmatory assay. There was no increase in the percentage of cells with aberrations at any of the concentrations tested, either with or without metabolic activation, in either assay. No more than 1 or 2 aberrations were recorded in any culture (treated or control), most contained no aberrations except for the occasional chromatid gap. The percentages of endoreduplicated and polyploid cells were also recorded separately, but the results were not significant. The positive controls gave the expected results. Metrafenone gave negative results in this assay. Refer to the DAR (UK, 2005), Point B.6.4.1 for a more detailed assessment of the study.

CA 5.4.2 In vivo studies in somatic cells

Report: CA 5.4.2/1
[redacted] 2001b
AC 375839: In vivo test for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells
2001/7000268
Guidelines: OECD 474
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

Groups of CD-1 mice (5/sex/group) received a single oral dose of metrafenone (purity 95.86%) in DMSO at dose levels of 500, 1000 and 2000 mg/kg (dosing volume 4 mL/kg) in order to assess the potential to induce micronuclei in bone marrow cells. The test animals were sacrificed at approximately 24, 48, and 72 hours after dosing. The positive control group received a single oral gavage dose of cyclophosphamide at 80 mg/kg. Positive controls were only included in the 24-hour harvest. Metrafenone did not induce any increase in the incidence of micronuclei in male or female mice at any sampling time. The results of the ADME study showed distribution of orally administered material to the bone marrow. Refer to the DAR (UK, 2005), Point B.6.4.2 for a more detailed assessment of the study.

Report: CA 5.4.2/2
[redacted] 2006b
In vivo unscheduled DNA synthesis (UDS) assay with BAS 560 F (Metrafenone) in rat hepatocytes single oral administration
2006/1025861
Guidelines: OECD 486, EEC 2000/32 B.39
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an *in vivo* study, metrafenone was tested for its ability to induce DNA repair synthesis (unscheduled DNA synthesis – UDS) in rat hepatocytes. Groups of 3 male Wistar rats received a single oral dose of metrafenone (purity 99.4%) in corn oil at 1000 or 2000 mg/kg. Negative control rats received corn oil only (10 mL/kg) and the positive control rats received 2-acetylaminofluorene at 50 mg/kg. Hepatocytes were harvested 3 and 14 hours after administration.

Metrafenone did not lead to an increase in the mean number of net nuclear grain count. Recorded values at both dosages and time points were comparable to vehicle control values, and within the historical control range. The induction of DNA repair by the positive control confirmed the sensitivity of test method and procedures.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metrafenone
Description: Beige solid
Batch/purity #: COD-000610: 99.4%
Stability of test compound: Stable until 1 July 2007

2. Vehicle and/or positive control: Corn oil / 2-acetylaminofluorene (2-AAF) at the dose of 50 mg/kg bw

3. Test animals:
Species: Rat
Strain: Wistar [Crl:WI (Han)]
Age: 8-12 weeks
Weight at dosing: Males: approx. 250 g
Source: Charles River Laboratories, Germany
Number of animals per dose: 3 males

Animal husbandry: Animals were housed in Makrolon cages type III. They were acclimated to laboratory conditions for at least 5 days. Drinking water from bottles and standard pelleted feed (Maus/Ratte Haltung "GLP", from Provimi Klibe, SA, Switzerland) were provided *ad libitum*. Food was withdrawn for at least 6 hours before administration.

Environmental Conditions

Temperature:	20-24ºC
Humidity:	30-70%
Air changes:	not indicated
Photoperiod:	12 hours light/12 hours dark

4. Dose Levels

(a) Range-finding toxicity test:	2000 mg/kg bw (10 mL/kg bw)
(b) UDS assay:	2000 mg/kg bw and 1000 mg/kg bw (10 mL/kg bw)

B. STUDY DESIGN AND METHODS

1. Dates of work: 05/05/2006 00:00:00 - 07/18/2006 00:00:00

2. Test procedure

Animals were treated once, by gavage, after a fasting period of at least 6 hours. Three hours or 14 hours after dosing, groups of 3 rats were anesthetized by intraperitoneal injection of a mixture of ketamine/xylazine and their livers were perfused to provide a primary culture of hepatocytes. Viability of hepatocyte was determined by trypan blue staining and animals with at least 70% viable cells were used. It was also examined whether significant morphological changes of the cells or a reduction of the cell material occurred after test substance treatment. Isolated hepatocytes were seeded on coverslips on 1.9 cm^2 well containing attached medium (about 4 x 10^5 cells per well, 6 wells per animal). Cultures were then treated with [^{3}H] thymidine and slides of the cultures were prepared for analysis by autoradiographic methods. The quantification of UDS was performed microscopically using 2-3 slides per animal. 25-50 cells in good morphological conditions were randomly selected per slide and examined to achieve a total number of 100 cells/animal and 3 animals per group. For each cell, the following counts were performed with an automatic image analyzer:

- Nuclear grain (NG) count (= number of silver grains overlying the nucleus)
- Cytoplasmic grain (CG) count (= number of grains in two or three nucleus-equivalent areas adjacent to the nucleus)

The following parameters were evaluated:

- Net nuclear grain (NNG) count of each cell (= NG count - CG count)
- Mean NG count
- Mean CG count
- Percentage of cells in repair (= cells showing NNG count ≥0)
- Percentage of cells in repair (= cells showing NNG count ≥5)

Slides were coded before microscopic evaluation.

3. Statistics
No statistical analysis was performed because of the clearly negative results obtained.

4. Acceptance criteria
The study is considered valid if the following criteria are met:
- Clearly negative results in the vehicle controls (negative controls) in the range of the historical control data.
- Clearly positive results in the positive control group (≥30% of cells in repair, mean value taken from 3 animals) in the range of historical control data or above.
- Viability (trypan blue staining) of at least 70% in liver cells from vehicle control animals.

5. Evaluation criteria
The test article was considered positive if a dose-related increase is demonstrated in both of the following:
- The mean number of NNG counts, which must exceed zero at one of the test points.
- The percentage of cells in repair (NNG ≥5) is ≥20%.

A dose-related increase of the cells in repair (≥5), which is outside the values for both the concurrent negative control and the historical control data base (≥5 <20) and a dose-related increase in the mean number of NNG counts near to but without exceeding zero is considered to be an indication for a marginal response which needs to be confirmed / clarified in a further experiment.

A test substance is considered negative if the following criteria are met:
- In all dose groups both the NNG counts and % cells in repair are close to the values of the concurrent vehicle control in the range of historical negative control data.

II. RESULTS AND DISCUSSION

The stability of the test substance in corn oil over a period of 96 hours at room temperature was verified analytically. The homogeneity of the test preparations was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 6 individual samples of each concentration. The concentration control analyses revealed acceptable values (104.8 and 101.1% of nominal values, respectively, for the 100 and 200 mg/mL dosing formulations).

No increase in mean number of NNG counts was observed. Cell viability was not influenced by treatment. No morphological changes of the cells, and no reduction of cell material were observed.

Results are summarized in Table 5.4.2-1, Table 5.4.2-2, Table 5.4.2-3 and Table 5.4.2-4.

Table 5.4.2-1: DNA repair activity – perfusion 3 hours after treatment

Test group	Vehicle control (corn oil)			1000 mg/kg bw			2000 mg/kg bw			Positive control (2-AAF, 50 mg/kg bw)		
No. of cells	100	100	100	100	100	100	100	100	100	100	100	100
NG counts*	2.99 ± 2.48	2.79 ± 2.16	1.63 ± 1.46	1.92 ± 1.57	3.06 ± 2.23	2.13 ± 2.11	2.79 ± 2.25	1.38 ± 1.38	2.95 ± 2.25	17.51 ± 7.83	13.42 ± 5.12	20.27 ± 7.94
CG counts*	8.51 ± 3.66	7.35 ± 2.53	5.83 ± 1.80	7.29 ± 2.09	8.91 ± 3.27	6.97 ± 2.67	9.48 ± 3.67	4.93 ± 2.19	6.77 ± 2.51	9.49 ± 4.49	7.62 ± 3.01	9.72 ± 4.03
NNG counts*	-5.52 ± 2.96	-4.56 ± 2.44	-4.20 ± 2.01	-5.37 ± 2.03	-5.85 ± 3.17	-4.84 ± 2.50	-6.69 ± 3.40	-3.55 ± 2.28	-3.82 ± 2.80	8.03 ± 6.91	5.81 ± 4.77	10.56 ± 7.54
% cells in repair NNG ≥ 0	0	3	4	0	6	3	3	4	9	93	90	96
% cells in repair NNG ≥ 5	0	0	0	0	0	0	0	0	1	62	55	77
NG counts**	2.47 ± 0.73			2.37 ± 0.61			2.37 ± 0.86			17.07 ± 3.45		
CG counts**	7.23 ± 1.34			7.72 ± 1.04			7.06 ± 2.29			8.94 ± 1.15		
NNG counts**	-4.76 ± 0.68			-5.35 ± 0.51			-4.69 ± 1.74			8.13 ± 2.38		
% cells in repair NNG ≥ 0**	2.33 ± 2.08			3.00 ± 3.00			5.33 ± 3.21			93.00 ± 3.00		
% cells in repair NNG ≥ 5**	0.00 ± 0.00			0.00 ± 0.00			0.33 ± 0.58			64.67 ± 11.24		

*: mean ± SD per animal (mean of 100 cells); **: mean ± SD per group (mean of 3 animals)

Table 5.4.2-2: DNA repair activity – perfusion 14 hours after treatment

Test group	Vehicle control (corn oil)			1000 mg/kg bw			2000 mg/kg bw			Positive control (2-AAF, 50 mg/kg bw)		
No. of cells	100	100	100	100	100	100	100	100	100	100	100	100
NG counts*	3.19 ± 2.30	1.65 ± 1.23	3.02 ± 1.92	5.00 ± 2.77	2.74 ± 1.88	2.16 ± 1.86	2.28 ± 2.17	4.66 ± 3.27	2.25 ± 2.05	10.68 ± 3.95	15.87 ± 5.65	13.28 ± 4.91
CG counts*	8.25 ± 3.56	6.23 ± 1.42	6.64 ± 2.57	9.54 ± 3.17	6.75 ± 2.89	5.41 ± 2.29	6.60 ± 3.03	9.58 ± 3.89	5.82 ± 2.29	5.29 ± 2.00	9.44 ± 3.61	6.87 ± 3.26
NNG counts*	-5.06 ± 3.63	-4.58 ± 1.49	-3.62 ± 2.75	-4.54 ± 2.67	-4.01 ± 3.02	-3.25 ± 2.14	-4.32 ± 2.72	-4.92 ± 3.30	-3.57 ± 2.13	5.39 ± 3.91	6.43 ± 4.98	6.42 ± 4.74
% cells in repair NNG ≥ 0	8	0	10	4	9	6	7	8	4	95	91	93
% cells in repair NNG ≥ 5	0	0	0	1	0	0	0	0	0	50	62	61
NG counts**	2.62 ± 0.84			3.30 ± 1.50			3.06 ± 1.38			13.28 ± 2.60		
CG counts**	7.04 ± 1.07			7.23 ± 2.11			7.33 ± 1.98			7.20 ± 2.09		
NNG counts**	-4.42 ± 0.73			-3.93 ± 0.65			-4.27 ± 0.68			6.08 ± 0.60		
% cells in repair NNG ≥ 0**	6.00 ± 5.29			6.33 ± 2.52			6.33 ± 2.08			93.00 ± 2.00		
% cells in repair NNG ≥ 5**	0.00 ± 0.00			0.33 ± 0.58			0.00 ± 0.00			57.67 ± 6.66		

*: mean ± SD per animal (mean of 100 cells); **: mean ± SD per group (mean of 3 animals)

Table 5.4.2-3: Cytotoxicity – perfusion 3 hours after treatment

Test group	Viability (%)		
	Individual values	Mean ± SD	% of control
Vehicle control Corn oil 10 mL/kg bw	92.9	88.6 ± 3.9	100.0
	85.2		
	87.7		
1000 mg/kg bw	92.5	90.4 ± 1.8	102.1
	89.7		
	89.1		
2000 mg/kg bw	89.1	87.0 ± 4.9	98.2
	81.4		
	90.6		
Positive control 2-AAF 50 mg/kg bw	85.7	86.2 ± 1.4	97.3
	87.8		
	85.2		

Table 5.4.2-4: Cytotoxicity – perfusion 14 hours after treatment

Test group	Viability (%)		
	Individual values	Mean ± SD	% of control
Vehicle control Corn oil 10 mL/kg bw	85.1 90.4 90.9	88.8 ± 3.2	100.0
1000 mg/kg bw	89.3 89.6 39.0	90.6 ± 2.1	102.1
2000 mg/kg bw	90.6 86.0 85.0	87.2 ± 3.0	98.2
Positive control 2-AAF 50 mg/kg bw	92.9 88.5 88.9	90.1 ± 2.4	101.5

III. CONCLUSION

Metrafenone did not lead to an increase in the mean number of net nuclear grain count. Recorded values at both 1000 and 2000 mg/kg bw and time points (3 and 14 hours after administration) were comparable to vehicle control values, and within the historical control range. The induction of DNA repair by the positive control confirmed the sensitivity of test method and procedures.

CA 5.4.3 In vivo studies in germ cells

No study available, not required.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Long-term toxicity and carcinogenicity potential of metrafenone were evaluated in mice and rats. All the studies were certified to comply with GLP and performed according to internationally accepted guidelines.

Table 5.5-1: Summary of chronic toxicity and carcinogenicity studies (adapted from the DAR, UK, 2005)

Study	NOAEL	LOAEL	Effects at LOAEL	Reference
104 week rat (diet) 0, 500, 5000 and 20000 ppm (Top dose females reduced to 10000 ppm from Week 69)	500 ppm 25-30 mg/kg bw/day Neoplastic effects: 500 ppm 25-30 mg/kg bw/day	5000 ppm 260-320 mg/kg bw/day Neoplastic effects: 5000 ppm 260-320 mg/kg bw/day	↓ bodyweight gains, ↑ relative liver weights, ↑ incidence of histopathological findings in the liver, ↑ kidney weights, ↑ incidence and severity of chronic nephropathy ↑ incidence of hepatocellular adenomas	CA 5.5/1 BASF Doc. ID 2002/ 7004381
18 month mouse (diet) 0, 250, 1000 and 7000 ppm	250 ppm 39-53 mg/kg bw/day Neoplastic effects: 1000 ppm 156-223 mg/kg bw/day	1000 ppm 156-223 mg/kg bw/day Neoplastic effects: 7000 ppm 1109-1493 mg/kg bw/day	↑ liver weights, increased incidence and severity of hepatocellular hypertrophy and chronic nephropathy ↑ incidence of liver neoplasms	CA 5.5/2 BASF Doc. ID 2002/ 7004484

The mechanism underlying the formation of liver tumours is considered under point 5.8.2 (supplementary studies with the active substance). Clear NOAELs for liver and kidney effects, and for increased incidence of liver tumours, were established.

Report: CA 5.5/1
[redacted] 2002a
A 24-month dietary toxicity and oncogenicity study with BAS 560 F in rats
2002/7004381
Guidelines: EPA 870.4300, OECD 453, EEC 94/79 A II 5.5, JMAFF 59 NohSan No 4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a combined chronic toxicity/carcinogenicity study in rats, groups of 75 male and 75 female Sprague-Dawley rats (65/sex/main groups, 10/sex/satellite groups) received metrafenone (purity 95.86%) in their diets at concentrations of 0, 500, 5000 and 20000 ppm for 104 weeks. Due to a marked decrease in body weight gain (>40%) and microscopic pathology findings in the liver observed at the 12-month interim sacrifice [increased incidences of hepatocellular centrilobular necrosis (7/11 and 5/10 for females at 5000 and 20000 ppm, respectively, as compared to 1/12 in controls) and hepatocellular polyploidy (2/11 and 8/10 for females at 5000 and 20000 ppm, respectively, as compared to 0/12 in controls)], the dose concentration for the high-dose females was reduced from 20000 ppm to 10000 ppm beginning with the first day of study week 69. Mean substance intakes were 0, 24.9, 260 and 1068.5 mg/kg day for males and 0, 30.4, 320.2, 1418.6 (up to the end of wk 68) and/or 592.8 mg/kg/day (wks 72 to 104) for females. Viability checks were performed twice daily and physical examinations were performed twice prior to treatment and weekly during the study period. Body weight and food consumption measurements were recorded pre-test, once per week for the first 16 weeks of treatment and once every 4 weeks thereafter. Feed efficiency was calculated for the first 16 weeks of the study. Ophthalmological examinations were performed on all animals pre-test, on 10 animals/sex/group during month 12 and on all surviving animals at termination. Clinical pathology studies (hematology, coagulation, clinical chemistry, and urinalysis) were conducted at 3, 6, 12 and 18 months, and at study termination. Neurobehavioral assessments (functional observation battery and motor activity measurement) were conducted on 10 animals/sex/group pre-test and at week 12. After 12 months of treatment 10 animals/sex/group were sacrificed, and after 24 month of treatment all surviving animals were sacrificed. Selected organs were weighed and organ/body weight and organ/brain weight ratios were calculated. Complete macroscopic examinations and histopathological evaluation of selected tissues were conducted on all animals dying during the study and all control and high dose animals. Histopathology was limited to lungs, liver, kidneys and gross lesions for animals of the low and mid-dose groups.

Survival at termination was increased in a treatment-related pattern in females (presumably as a result of lower body weights in treated females). Survival at termination in males was slightly lower in treated animals but there was no indication of a treatment-related effect and it was considered to be adequate for assessing carcinogenic potential in males since survival only fell below acceptable levels during the last month of the study. The timings of premature deaths did not indicate any effect of treatment. No treatment-related effects were observed in food consumption data, clinical observations, ophthalmoscopic examinations, neurobehavioral evaluations (including FOB and motor activity evaluations), and macroscopic examinations. Body weights and body weight gains were statistically significantly reduced compared to controls in males at 20000 ppm and in females at ≥5000 ppm. Body weight gains were significantly lower in males receiving 20000 ppm from the start of the study, achieving a decrease of 10% relative to controls after week 64. Body weight gains were significantly lower in females receiving 20000 ppm from the start of the study and were >10% lower than controls from week 2 onwards. A similar but less marked effect on body weight gains was seen in females at 5000 ppm, whose gains were consistently >10% lower than controls from approximately week 9. By week 68, body weights of top dose females were only 70% of controls and the top dose level was subsequently reduced by half to 10000 ppm, following which body weights did not decline any further relative to controls. Hematology and coagulation revealed decreases in mean hemoglobin, hematocrit and/or erythrocyte values of females administered 20000 ppm at 3, 6, and 12 months. These changes were accompanied by corresponding decreases in mean corpuscular volume, mean corpuscular hemoglobin, mean hemoglobin concentration and increased platelet counts. These differences progressed from marginal to moderate following one year of treatment. After 18 months of treatment, mild decreases in mean hemoglobin, hematocrit and erythrocyte count were observed in females administered 5000 and 10000 ppm. No differences from control were apparent on these parameters at termination of the study or at any time point in male animals. Prolongation of mean prothrombin time relative to controls was seen in males at 5000 and 20000 ppm after 3, 6 and 12 months and in males at 20000 ppm after 18 months (but not at termination). Mean prothrombin time was not increased in females. Mild prolongation of mean activated partial thromboplastin time was observed in males and females at 5000 and 20000 ppm after 3 months and in males at 20000 ppm after 6 and 12 months (but not at later time points). Treatment-related clinical chemistry changes were observed in animals treated at 5000 and 20000/10000 ppm when compared to concurrent control values. In males receiving 5000 and 20000 ppm, transient, mild increases in serum total protein and albumin was observed only at the 3-month sampling interval. After 12-month treatment, a mild increase in gamma glutamyl transpeptidase (GGT) and a mild decrease in total bilirubin in males dosed with 20000 ppm were observed. After 18 and/or 24 months of treatment, mild/moderate increases in total cholesterol and/or GGT as well as mild decreases in total bilirubin were seen in males administered 5000 and 20000 ppm. In females receiving 5000 and 20000 ppm, mild, transient increases in serum total protein albumin and calcium were observed after 3 and/or 6 months of treatment. Moderate dose-related increases in serum cholesterol were observed after 3, 6 and 12 months of treatment. Dose-related mild/moderate increases in GGT were seen after 6 and 12 months of treatment. Changes only seen in females dosed with 20000 ppm were mild decreases in serum albumin and bilirubin and a minimal increase in blood urea nitrogen (BUN) at the end of 12 months of treatment. After 18 and/or 24 months of treatment, mild/moderate increases in serum cholesterol, total protein, GGT and calcium were seen in females receiving 5000 and/or 20000/10000 ppm.

Table 5.5-2: Clinical chemistry data

Dose	Sex	Interval	Group mean value					
(ppm)		(months)	Cholesterol (mg/dL)	GGT (U/L)	Total protein (g/dL)	Total bilirubin (mg/dL)	BUN (mg/dL)	Ca^{++} (mg/dL)
0	Male	3	72	2	6.7	0.19	#	#
500			65	2	6.6	0.14*		
5000			76	1*	7.1*	0.13**		
20000			82	1*	7.4**	0.11**		
0	Female		73	2	6.8	0.19	15.5	10.6
500			77	3	6.9	0.18	14.0	10.3
5000			117**	1	7.8**	0.14*	14.7	11.1**
20000			146**	3	8.2**	0.13*	17.1	11.2**
0	Male	6	82	0	6.9	0.21	#	#
500			69	0	6.8	0.18		
5000			82	2	7.2	0.17		
20000			88	3	7.0	0.16		
0	Female		90	0	7.4	0.24	14.1	10.6
500			98	0	7.7	0.21	13.0	10.8
5000			140**	2**	8.3**	0.19	15.8	11.7**
20000			198**	5**	8.9**	0.17	15.9	11.4**
0	Male	12	97	2	6.9	0.18	#	#
500			105	1	7.1	0.17		
5000			105	3	7.1	0.16		
20000			110	4**	7.0	0.14**		
0	Female		96	0	7.9	0.21	13.2	11.3
500			99	1	7.7	0.20	12.4	11.0
5000			177**	2*	8.2	0.18	13.5	11.5
20000			314**	4**	8.2	0.15**	15.9**	11.2
0	Male	18	87	1	7.3	0.14	#	#
500			123	0	7.5	0.13		
5000			129*	2	7.3	0.12		
20000			145*	4**	7.4	0.11		
0	Female		97	1	8.1	0.15	12.7	11.4
500			106	0	8.2	0.16	12.9	11.7
5000			160*	2*	8.7**	0.14	14.1	12.3*
10000[a]			264**	2*	8.8**	0.13	16.3*	12.3*
0	Male	24	100	1	6.9	0.14	#	#
500			144	1	7.0	0.15		
5000			151	5**	6.8	0.11*		
20000			198**	8**	7.1	0.11**		
0	Female		98	1	7.7	0.26	13.2	11.1
500			98	0	8.0	0.16	14.0	11.2
5000			187**	2	8.0	0.13**	15.6	11.2
10000[a]			223**	4**	8.1	0.13**	25.3*	11.8*

[a] Dosage for high dose females reduced from 20000 ppm to 10000 ppm from Week 69
* p<0.05, ** p<0.01 (ANOVA – Dunnett's test)
# values similar to control values throughout – data omitted for clarity

Increased urinary protein levels were noted at urinalysis in high dose females from 6 months onwards. There was no similar effect in males. A dose-related increase in urinary volume in males treated at ≥5000 ppm was also noted at termination.

Effects on serum liver enzymes, cholesterol, and/or albumin in male and female animals treated at 5000 and 20000/10000 ppm correlated with dose-related increased liver weights, dose-related increased incidences of centrilobular hepatocellular hypertrophy and eosinophilic hepatocellular alteration, dose-related increased incidences of non-zonal vacuolation (females only) and basophilic hepatocellular alteration (females only) seen microscopically. The lesions in females were more severe than in males; in addition, the females had increased incidences of centrilobular necrosis and hepatocellular polyploidy. In addition to liver effects, males and females treated at 5000 and 20000/10000 ppm had increased kidney weights and microscopically had a dose-related increased incidence of subacute/chronic interstitial inflammation/chronic nephropathy of the kidney. Females treated at 20000/10000 ppm had the greatest severity, which correlated with increases in blood urea nitrogen and urinary protein levels following 12, 18, or 24 months of treatment.

Table 5.5-3: Organ weight data

Dose level	Absolute (g) / % of control		Relative (%) to bw / % of control	
	M	F	M	F
Liver – 12 mo				
0	17.77	10.30	2.57	2.65
500	19.29 / 109	11.25 / 109	2.56 / 100	2.74 / 103
5000	20.15 / 113	12.01 / 117	2.93 / 114	3.53** / 133
20000	22.92** / 129	15.14** / 147	3.43** / 133	5.65** / 213
Liver – 24 mo				
0	18.99	13.91	2.84	3.08
500	19.92 / 105	13.19 / 95	2.87 / 101	3.12 / 101
5000	24.57* / 129	16.03 / 115	3.74* / 132	4.36* / 142
20000/10000	27.62** / 145	19.32** / 139	4.53** / 160	5.98** / 194
Kidney – 12 mo				
0	4.47	2.78	0.66	0.72
500	4.86 / 109	2.86 / 103	0.65 / 98	0.70 / 97
5000	5.25* / 117	2.94 / 106	0.76 / 115	0.87* / 121
20000	5.65** / 126	3.29** / 118	0.85** / 129	1.23** / 171
Kidney – 24 mo				
0	5.69	3.57	0.87	0.82
500	6.02 / 106	3.55 / 99	0.87 / 100	0.85 / 104
5000	6.67* / 117	3.84 / 108	1.01 / 116	1.04** / 127
20000/10000	7.17** / 126	4.48** / 125	1.19** / 137	1.42** / 173

* $p < 0.05$; ** $p < 0.01$ (ANOVA – Dunnett's test)

Table 5.5-4: Summary of non-neoplastic microscopic findings in liver and kidney after 12 months (interim sacrifice and decedents)

Organ - histopathological finding	Incidence							
	Male				Female			
	0	500	5000	20000	0	500	5000	20000
No. examined - liver	14	12	12	10	12	10	11	10
- centrilobular hypertrophy	0	0	5	10	0	0	11	10
- centrilobular necrosis	0	1	0	0	1	0	7	5
- hepatocellular polyploidy	0	0	0	0	0	0	2	8
No. examined - kidneys	14	12	12	10	12	10	11	10
- subacute/chronic interstitial inflammation / chronic nephropathy	6	5	7	8	2	0	7	10

After 24 months

Organ - histopathological finding	Incidence at (mg/kg bw/day):							
	Male				Female			
	0	500	5000	20000	0	500	5000	20000[a]
Liver – number examined	75	75	75	73	75	75	75	75
Centrilobular hepatocellular hypertrophy	1	2	15*	39*	0	1	42*	51*
Hepatocellular polyploidy	0	1	0	4	0	2	26*	54*
Hepatocellular alteration – basophilic	24	18	14	23	30	20	39	47*
Hepatocellular alteration – eosinophilic	6	8	12	14	3	4	27*	41*
Hepatocellular necrosis (centrilobular)	2	2	1	0	3	2	7	8
Hepatocytes cytoplasm vesicular / vacuolated	14	8	1	6	3	1	10	13
Bile duct(s) epithelial hyperplasia	37	33	29	37	26	28	44*	53*
Bile duct(s) dilated/cyst(s)	2	7	3	3	7	3	9	17*
Biliary fibrosis	16	18	11	12	12	13	13	21
Kidney – number examined	75	75	75	74	75	75	75	75
Subacute /chronic interstitial inflammation / chronic progressive nephropathy / cysts	45	48	59*	71*	18	21	54*	71*
Cortex/medulla tubular epithelium and/or reticuloendothelial cells - brown pigment	9	7	19	15	8	10	28*	41*

* $p<0.05$ (Fisher's Exact test);
[a] Dosage for high dose females reduced from 20000 ppm to 10000 ppm from Week 69

A dose-related increase in the incidence of hepatocellular adenoma was recorded in females at 5000 and 20000/10000 ppm. One high dose female had both a hepatocellular adenoma and a carcinoma (there were no hepatocellular carcinomas in any other female groups in this study, or in 10 previous studies from the testing facility (1993-1998) cited as historical control data). Of the 12 hepatocellular adenomas identified in the high dose female group, 2 were identified at the interim sacrifice after 12 months. The increased incidence of benign hepatocellular adenoma in females at 20000/10000 ppm and 5000 ppm occurred at doses that exceeded the Maximum-Tolerated-Dose (MTD) as evidenced by a respective decrease of 42% and 26% in body weight gain, and an increased incidence of hepatic centrilobular necrosis already noted at the 12-month interim sacrifice at these same doses. Although there was a slightly increased incidence of benign hepatocellular adenoma for males treated at 20000 ppm (4/75 or 5.3%) which was outside of the historical control range of the test facility (max. 4.0%), this finding was considered to be incidental because 1) the slight increased incidence (5.3%) was not statistically different from control (1.3%) by Fishers Exact Test; 2) the incidence (5.3%) was well within the maximum incidence rates for 2 groups of Charles River Laboratories Sprague-Dawley male historical controls (18.2% - Lang, 1992 and 8.0% - Giknis and Clifford, 2001).

Table 5.5-5: Summary of neoplastic findings in liver (includes historical control data)

Dose level	0 ppm		500 ppm		5000 ppm		20000/10000 ppm	
	M	F	M	F	M	F	M	F
No. examined	75	75	75	75	75	75	75	73
Hepatocellular adenoma	1	1	0	0	1	6	4	12*
Hepatocellular carcinoma	1	0	3	0	1	0	1	1
Historical control data at the testing facility (1993 - 1998)								
	Overall incidence		Range per study		Range %			
Males								
Hepatocellular adenoma	4/500 / 0.8%		0/60		2/50	0.0%	4.0%	
Hepatocellular carcinoma	14/500 / 2.8%		0/15		3/50	0.0%	6.0%	
Females								
Hepatocellular adenoma	4/489 / 0.8%		0/60		3/57	0.0%	5.3%	
Hepatocellular carcinoma	0/489 / 0.0%		None in 10 studies					

Historical control data from the supplier (Charles River Laboratories) (19 control groups between 1986 and 1991 for Lang, 23 control groups between 1991 and 1997 for Giknis)						
	Lang (1992)			Giknis (2001)		
	Overall incidence	Range %		Overall incidence	Range %	
Males						
Hepatocellular adenoma	53/1258 / 4.21%	1.3%	18.2%	37/1531 / 2.42%	1.43%	8.00%
Hepatocellular carcinoma	33/1258 / 2.62%	1.1%	9.1%	32/1531 / 2.09%	0.77%	6.67%
Females						
Hepatocellular adenoma	28/1263 / 2.22%	1.0%	5.5%	35/1729 / 2.02%	0.77%	13.33 %
Hepatocellular carcinoma	5/1263 / 0.4%	1.0%	4.0%	7/1729 / 0.40%	0.77%	1.67%

* $p < 0.05$ (ANOVA – Dunnett's test)

Treatment with metrafenone for 24 months resulted in markedly reduced body weight gains in females at 5000 (26%) and 20000/10000 ppm (42%), indicating that the maximum tolerated dose had been exceeded in females at ≥5000 ppm. A lesser reduction in body weight gain over the whole study in males at 20000 ppm (14%) was also recorded, which indicates that the MTD was also marginally exceeded in high dose males. The liver and kidney were the clear target organs in this study. Increased kidney weights and increased incidence and severity of chronic nephropathy were recorded in both sexes at ≥5000 ppm. Increased relative liver weights were recorded in both sexes at ≥5000 ppm, along with increased incidences of histopathological findings in the liver (with females affected to a greater extent than males). The marked liver effects were accompanied by increased incidences of hepatocellular adenomas in females at ≥5000 ppm, and also marginally in males at 20000 ppm. The NOAEL for the study was 500 ppm (equal to 25-30 mg/kg bw/day), based on effects on bodyweight gain, liver and kidneys at ≥5000 ppm (≥260-320 mg/kg bw/day). The NOAEL for increased incidence of liver tumours was also 500 ppm. The same NOAEL was proposed by the applicant.

Report: CA 5.5/2
[redacted] 2002a
18-Month dietary oncogenicity study in albino mice with BAS 560 F (Report Amendment #1)
2002/7004484
Guidelines: EPA 870.4200, OECD 451, EEC 87/302 B No. L133/32-36, JMAFF 59 NohSan No 4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a carcinogenicity study, groups of 65 male and 65 female CD-1 mice received metrafenone (purity 95.86%) in their diets at concentrations of 0, 250, 1000 and 7000 ppm for 18 consecutive months (mean substance intakes: 0, 39, 156, 1109 mg/kg/day in males and 0, 53, 223 and 1492 mg/kg/day in females). The high dose male group had 66 animals due to an early death and subsequent replacement. Samples were taken from 10 animals/sex/group at week 52 and week 78 for hematology. At termination, all surviving animals were subjected to a gross necropsy and selected organs were weighed. Gross necropsies were performed for all animals that died prior to scheduled sacrifice (found dead or sacrificed moribund). Samples of selected tissues were processed for histopathological evaluation from all surviving test animals and from any unscheduled deaths, which occurred during the study. In the mid and low dose groups, histopathology was limited to lungs, liver, spleen, kidneys and gross lesions.

There was no adverse effect of treatment on survival. Survival at week 78 was 69%, 83%, 86% and 82% for males and 74%, 74%, 72% and 82% for females at 0, 250, 1000 and 7000 ppm, respectively. Food consumption values for both male and female mice at all dietary concentrations were generally comparable to or in excess for those of the control mice, and no adverse effects of treatment were evident from the body weight data. Body weights for males and females in all treated groups were generally similar to controls at most measurement intervals during the study. No treatment-related hematological changes were noted in either sex at either 12 months or at study termination (18 months). Statistically significant increases in absolute and relative (to body weight) liver weights were noted for males at 7000 ppm and for females at 1000 ppm and 7000 ppm (dose-related), when compared to controls. The only notable gross necropsy finding was an increased incidence of grossly visible liver masses in males treated at 7000 ppm.

Table 5.5-6: Organ weight data

Dose level	Absolute (g) / % of control		Relative (%) to bw / % of control	
	M	F	M	F
0	2.436	2.003	6.469	6.088
250	2.636 / 108	2.178 / 109	6.475 / 100	6.476 / 106
1000	2.543 / 104	2.326* / 116	6.394 / 99	7.085* / 116
7000	3.315* / 136	3.079* / 154	8.474* / 131	9.127* / 150

* $p < 0.05$ (ANOVA – Dunnett's test)

Non-neoplastic findings were noted in the liver, kidneys and spleen. In the liver, hepatocellular hypertrophy was recorded in males at ≥1000 ppm and in females at 7000 ppm. In males this finding ranged from centrilobular to diffuse with dose-related severity, but in females this finding was less severe and was mostly diffuse. Other liver histopathological findings were limited in number and did not show any treatment-related increases (in particular there were no increases in the incidences of findings such as necrosis or basophilic, clear cell or eosinophilic foci). In the kidneys, there was an increase in the incidence and severity of chronic nephropathy in males at ≥1000 ppm and in females at 7000 ppm. In the spleen there was an increased incidence of extramedullary hematopoiesis in females receiving 1000 and 7000 ppm, as compared to the controls, but no increase in extramedullary hematopoiesis was noted in the spleens of treated male mice.

A statistically significant increase in the incidence of hepatocellular neoplasms (adenomas and carcinomas) was observed in the livers of male mice receiving 7000 ppm (19/66, 29%), as compared to controls (6/65, 9%). In male mice that died prematurely, one control group mouse was found with a hepatocellular carcinoma and one mouse each at 1000 and 7000 ppm with hepatocellular adenoma. Thus, there was no indication of accelerated time-to-tumor formation in male mice. There was also a marginal increase in the incidence of hepatocellular neoplasms in females receiving 7000 ppm (4/65), as compared to the control mice (2/65), but the increase was very small, was not statistically significant and the time-to-tumor formation was not apparently shortened by treatment (based on the fact that no females dying prematurely had hepatocellular neoplasms). The incidence of 14/66 (21.2%) hepatocellular adenoma for males at 7000 ppm is slightly above the maximum range of historical control data at the testing laboratory (14.8%) and at Charles River Laboratories (19.2%). The incidence of 5/66 (7.6%) hepatocellular carcinoma in males at 7000 ppm is within the maximum range of historical control data at both the testing facility (9.2%) and Charles River Laboratories (11.5%). Although the number of hepatocellular neoplasms in males receiving 1000 ppm was also slightly increased (9/65, 13.8%) compared to the untreated controls (6/65, 9.2%), this slight increase was not statistically significant, was within the range of the historical controls (9.2-18.5%) at the testing laboratory and it should be considered that survival was higher than controls in this group (55/65 survivors at 1000 ppm compared with 45/65) and almost of these hepatocellular neoplasms were identified in animals at terminal sacrifice. Further statistical analyses of the liver tumours in male mice were performed, taking the differences in survival between the groups into account. The survival-adjusted methods used were Tarone's test (Tarone, 1975) and the Poly3 test (Bailer and Porter, 1988) which are survival-adjusted quantal-response procedures that modify the Cochran-Armitage linear trend test to take survival differences into account. When all male dose groups were analyzed (total incidence of hepatocellular adenomas and carcinomas), there was a statistically significant difference between the control and the high dose group (Fisher's Exact test) with significant linear trends by all three (Cochran Armitage, Tarone's, Poly3) trend tests ($p \leq 0.001$). When the high dose male group was dropped from the analysis, there were no significant differences and no significant trends across the groups to the 1000 ppm group. Therefore, based on these statistical analyses, 1000 ppm was considered to be the no statistical significance of trend (NOSTASOT) dose.

The RMS provided the following assessment of liver tumors in the DAR (UK, 2005).
The increased incidence of liver tumours in high dose level males is clearly treatment-related. It is agreed that the incidence of liver tumours in high dose females is of equivocal toxicological significance since the increase over controls is very small (two adenomas and two carcinomas at the high dose compared to just two adenomas in controls). Also if a progression from adenomas to carcinomas was taking place, the number of adenomas in the group would be expected to exceed the number of carcinomas. The pattern of incidence of liver tumours in high dose females is considered to be more likely to be spontaneous rather than treatment-related.
The historical control data supplied are considered to be only weak evidence on which to dismiss the increased tumor incidence seen in males at 1000 ppm. This is because data from only 3 other studies are available and no study details are available to accompany the data from the animal supplier (studies as old as 1991 would normally be considered too old to be relevant to the current study (performed 1999-2000)). However the effect of the higher survival in the 1000 ppm male group on tumor incidence also has to be taken into account. The results of the statistical analyses performed using tests which take survival differences into account are reassuring in this respect. On the basis that the total number of liver tumours at 1000 ppm is only slightly above the controls (9 versus 6), is broadly comparable with the (limited) historical control data and since the increase in this group is not statistically significant (including survival-adjusted statistical tests), it is considered that the increase in liver tumours in males at 1000 ppm was spontaneous and not treatment-related.

Table 5.5-7: Overview of liver microscopic findings (includes historical control data)

Dose level	0 ppm		250 ppm		1000 ppm		7000 ppm	
	M	F	M	F	M	F	M	F
Non-neoplastic								
No. examined	65	65	65	65	65	65	66	65
Centrilobular hepatocyte hypertrophy - TOTAL	0	0	0	0	15**	0	34**	5*
Minimal					13		8	5
Slight/mild					2		12	
Moderate							13	
Moderately severe							1	
Diffuse hepatocellular hypertrophy - TOTAL	0	0	0	0	6*	0	20**	24**
Minimal					1			14
Slight/mild					5		11	9
Moderate							9	1
Neoplastic								
Hepatocellular adenoma	4	2	2	1	8	1	14*	2
Hepatocellular carcinoma	2	0	1	0	1	0	5	2
TOTAL	6	2	3	1	9	1	19**	4
Historical control data at the BASF (Princeton, NJ) Toxicology Laboratory (1991 – 2000)								
	Study 1	Study 2	Study 3	Total			Range	
Males								
No. examined	54	65	65	184				
Hepatocellular adenoma	8 / 14.8%	0 / 0.0%	5 / 7.7%	13 / 7%			0.0%	14.8%
Hepatocellular carcinoma	2 / 3.7%	6 / 9.2%	2 / 3.0%	10 / 5.4%			3.0%	9.2%
Total adenoma/carcinoma	10 / 18.5%	6 / 9.2%	7 / 10.8%	23 / 12.5%			9.2%	18.5%

Dose level	0 ppm		250 ppm		1000 ppm		7000 ppm	
	M	F	M	F	M	F	M	F
Females								
No. examined	55	65	65	185				
Hepatocellular adenoma	0 / 0.0%	0 / 0.0%	2 / 3.0%	2 / 1.1%			0.0%	3.0%
Hepatocellular carcinoma	0 / 0.0%	1 / 1.5%	0 / 0.0%	1 / 0.5%			0.0%	1.5%
Total adenoma/carcinoma	0 / 0.0%	1 / 1.5%	2 / 3.0%	3 / 1.6%			0.0%	3.0%
Historical control data from the supplier (26 studies for males, 27 studies for females initiated between 1987 and 2000 in 11 different laboratories). Charles River Laboratories production sites: Raleigh, NC; Stone Ridge, NY; Kingston, NY and Portage MI								
Males	Total		Range per study		Range %			
No. examined	1371		47	68				
Hepatocellular adenoma	130		2 / 50	12 / 50	4%	24%		
Hepatocellular carcinoma	55		1 / 60	6 / 47	1.7%	12.8%		
Females								
No. examined	1392		47	85				
Hepatocellular adenoma	6		1 / 60	1 / 49	1.7%	2%		
Hepatocellular carcinoma	8		1 / 70	2 / 60	1.4%	3.3%		

* $p < 0.05$; ** $p < 0.01$ (ANOVA – Dunnett's test)

No adverse effects on food consumption, bodyweights or hematology parameters were recorded in any group (it is noted that the highest dose level used was close to the OECD recommended limit dose (1000 mg/kg bw/day), so the issue of achieving the maximum tolerated dose (MTD) is not relevant). Absolute and relative liver weights were increased in females at ≥1000 ppm and in males at 7000 ppm only. Increased incidence and severity of hepatocellular hypertrophy (dose-related) was recorded in males at ≥1000 ppm, and in females (less severe than males) at 7000 ppm only. The other treatment-related histopathological findings were increased incidence and severity of chronic nephropathy in males at ≥1000 ppm and in females at 7000 ppm, and an increased incidence of extramedullary hematopoiesis in the spleen in females at 7000 ppm.
There was a clear treatment-related increase in hepatocellular adenomas and carcinomas in males at 7000 ppm (and also a slight increase of equivocal significance in females at 7000 ppm). The slightly increased incidence of hepatocellular neoplasms in males at 1000 ppm was not considered to be treatment-related.

The NOAEL for the study was 250 ppm (equal to 39-53 mg/kg bw/day), based on increased incidence and severity of hepatocellular hypertrophy and chronic nephropathy in males, and increased liver weights in females, at 1000 ppm (equal to 156-223 mg/kg bw/day). The NOAEL for increased incidence of liver tumours was 1000 ppm, based on increased incidence of liver neoplasms in males at 7000 ppm. The applicant proposed the same NOAELs.

Report: CA 5.5/3
Anonymous, 2002a
Historical control data Sprague Dawley rat - Neoplasms after 24 months
2002/7006021
Guidelines: none
GLP: no

Executive Summary

This document presents historical control data for liver neoplasms in Charles River Sprague-Dawley rats, from 10 previous studies (1993-1998) conducted at the same the testing facility where the metrafenone study was performed.

These data are included in Table 5.5-5: Summary of neoplastic findings in liver (includes historical control data), above, as “Historical control data at the testing facility (1993 - 1998)”.

CA 5.6 Reproductive Toxicity

The reproductive and pre-natal developmental toxicity of metrafenone were investigated in rats and rabbits. All the studies were GLP compliant, with the applied experimental design often exceeding guidelines requirements, or anticipating requirements included in more recently adopted versions of the guidelines. A summary of reproductive and developmental toxicity of metrafenone is presented in **Table 5.6-1**

Table 5.6-1: Summary of reproductive and developmental toxicity of metrafenone

Study	NOAEL	LOAEL	Findings at LOAEL	Reference
Pilot reproductive study in rats (diet) 0, 500, 1000 and 10000 ppm	From the DAR: Parental animals and offspring: 500 ppm [M: 27 mg/kg bw/day and F: 43.8 mg/kg bw/day]	From the DAR: Parental animals and offspring: 1000 ppm [M: 55.1 mg/kg bw/day and F: 91.3 mg/kg bw/day]	From the DAR: Parental animals: ↑ liver weights, increased incidence and severity of hepatocellular hypertrophy Offspring: ↓ pup weights	CA 5.6.1/1 BASF Doc ID 2001/ 7000338
	According to the Applicant: Parental animals and offspring: 1000 ppm [M: 55.1 mg/kg bw/day and F: 91.3 mg/kg bw/day]	According to the Applicant: Parental animals and offspring: 10000 ppm [M: 582 mg/kg bw/day and F: 882 mg/kg bw/day	According to the Applicant: Effects were limited to a slight increase in absolute and/or relative (to body weight) liver weights in parent males and F1 male and female pups sacrificed at PND 21 or 29. Microscopically, 3 of 10 parental males exhibited hepatocellular hypertrophy, whereas the livers of F1 male and female pups sacrificed at PND 21 or 29 were unremarkable. Pups weights were reduced at 10000 ppm, but not at 1000 ppm.	
	Reproductive effects: 10000 ppm [M: 582 mg/kg bw/day and F: 882 mg/kg bw/day]	Reproductive effects: -	No effects on reproductive parameters at any dosage.	
Two generation reproductive study in rats (diet) 0, 500, 1000 and 10000 ppm	Parental animals: 500 ppm [39 mg/kg bw/day] Offspring: 1000 ppm [79 mg/kg bw/day] Reproductive effects: 1000 ppm [79 mg/kg bw/day]	Parental animals: 1000 ppm [79 mg/kg bw/day] Offspring: 10000 ppm [811 mg/kg bw/day] Reproductive effects: 10000 ppm [811 mg/kg bw/day]	Parental animals: ↓ bodyweight gains (F1 males) Offspring: ↓ pup weights, ↑ liver weights Reproductive effects: ↑ proportion of abnormal sperm (F1 males) No clear effects on reproductive performance at any dosage	CA 5.6.1/2 BASF Doc ID 2002/ 7004752
Developmental toxicity study in rats (gavage) 0, 50, 500 and 1000 mg/kg bw/day	1000 mg/kg bw/day	-	No effects recorded at the highest dose tested	CA 5.6.2/1 BASF Doc ID 2001/ 7001372

Study	NOAEL	LOAEL	Findings at LOAEL	Reference
Developmental toxicity study in rabbits (gavage) 0, 50, 350 and 700 mg/kg bw/day	Maternal toxicity: 50 Developmental toxicity: 50 Teratogenicity: 700	Maternal toxicity: 350 Developmental toxicity: 350 Teratogenicity: -	Maternal: ↓ bodyweights and food consumption, ↑ liver weights, histopathological effects in the liver Developmental: single incidence of premature delivery	CA 5.6.2/2 BASF Doc ID 2001/7001288

CA 5.6.1 Generational studies

Report: CA 5.6.1/1
[redacted] 2001a
A pilot dietary reproduction study with AC 375839 in rats
2001/7000338
Guidelines: none
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a dietary dose-range finding study for a two-generation study, groups of 10 male and 10 female Sprague-Dawley rats received metrafenone (purity 95.86%) in their diets at concentrations of 0, 500, 1000 and 10000 ppm for a 10-week pre-pairing period, a mating period of up to 14 days and then females were allowed to deliver and nurse litters over a 21 day lactation period. On day 4 of lactation litters were standardized to 8 pups (4/sex where possible), and following weaning 10 pups/sex/group were selected to continue on study for a 1 week period (F1 generation). Investigations included clinical examinations, body weights, food consumption, estrus cyclicity, reproductive parameters and litter parameters. At termination, a blood sample for hematology investigation was taken, followed by a gross necropsy, weighing of organs and microscopic examination of reproductive tissues, livers, spleens and gross lesions for the control and high-dose animals, liver and gross lesions only for the low- and mid-dose animals. Brain, liver, spleen and thymus weight were recorded for all F1 pups (post-natal day - PND 21 and PND 29), and liver and spleen (5/sex) from F1 pups were examined microscopically at PND 29.

There were no effects on any reproductive parameters (i.e., estrous cycles, number of animals mating, males siring or females becoming pregnant, mean number of days prior to mating, gestation length, number of pups per litter and liver birth index, sex ratio or pup survival) up to 10000 ppm (582-790 mg/kg/day in males, 882 mg/kg/day in females – pre-pairing period only). Effects following treatment at 1000 ppm (55.1-76.3 mg/kg/day in males, 91.3 mg/kg/day in females – pre-pairing period only) were limited to a slight increase in absolute and/or relative (to body weight) liver weights in parent males and F1 male and female pups sacrificed at PND 21 or 29. Microscopically, 3 of 10 parent males exhibited hepatocellular hypertrophy whereas the livers of F1 male and female pups sacrificed at PND 21 or 29 were unremarkable.

At 10000 ppm, parental females had lower body weights and gained less (decreased by 11%) weight during the pre-mating period, compared to controls. During gestation, body weights were statistically significantly lower than controls on days 0, 7 and 14 of gestation, but gains over the entire gestation period were comparable to controls. At termination, slight anemia (decreases in hemoglobin, hematocrit and RBC counts) was observed in the parent females. Absolute and relative liver weights were increased in both male and female parent animals. Microscopic examination of the livers from these animals revealed centrilobular hepatocellular hypertrophy. At 10000 ppm F1 pup body weights were decreased at birth and throughout lactation. At weaning, pup body weights were decreased by 21% compared to controls. At PND day 29, hematological effects consisting of slight decreases in hemoglobin (females only), hematocrit and RBC counts (both sexes) were observed in the F1 pups. Liver weights (absolute and/or relative to body or brain weight) were increased in the F1 pups at both PND 21 and 29. Microscopic examination of the livers from these animals showed generalized to diffuse hepatocellular hypertrophy.

The NOAEL for systemic toxicity was 1000 ppm (equal to 91.3 mg/kg bw/day in females during the pre-mating period, and 55.1-76.3 mg/kg bw/day in males). The same conclusion as the Applicant was derived during the recent JMPR toxicology evaluation of metrafenone, whereas in the DAR the NOAEL for systemic toxicity was considered to be 500 ppm (equal to 43.8 mg/kg bw/day in females during the premating period, and 27.2-38.4 mg/kg bw/day in males), based on liver weight effects. The NOAEL for reproductive toxicity and fertility was 10000 ppm (582-790 mg/kg/day in males, 882 mg/kg/day in females during the pre-mating period).

Report: CA 5.6.1/2
[redacted] 2002a
A two-generation reproduction study with BAS 560 F in rats
2002/7004752
Guidelines: EPA 870.3800, OECD 416, EEC 94/79 A II 5.6.1, JMAFF 59 NohSan No 4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 2-generation study on reproductive toxicity groups of 30 male and 30 female Sprague-Dawley rats received metrafenone (purity 95.86%) in their diets at concentrations of 0, 500, 1000 or 10000 ppm over the course of two generations (P and F1). P generation animals were treated for a 10-week pre-mating period prior to pairing. Animals were paired 1 male to 1 female for a mating period of a maximum of 14 days. Females delivered and nursed litters to weaning (litter size was standardized at 4/sex where possible on day 4 of lactation). At weaning of the F1 generation, 30 animals/sex/group were selected to form the F1 parent generation. The treatment scheme was the same applied to the P generation. F2 pups were reared to weaning. In-life investigations included clinical signs, body weights, food consumption, estrus cyclicity, reproductive parameters and litter parameters. Developmental landmarks (vaginal opening and preputial separation) were recorded in F1 animals and anogenital distance was recorded for F2 pups at birth. Hematology was conducted in all parent (P and F1) animals, and in 1/sex/litter F2 pup at weaning. Reproductive tissues, adrenals, liver, pituitary and gross lesions were examined microscopically for all P and F1 parent animals from the control and high dose groups; examination of liver was extended to all F1 parent animals of the low- and mid-dose groups. Sperm evaluations were conducted in all parent males, and detailed ovarian examination and primordial follicle count in females of the control and high-dose group for the P generation, and all F1 females. Metrafenone intakes, as average combined pre-mating values for P and F1 males and females, were 0, 39, 79 and 811 mg/kg/day.

In the P generation, there was a single mortality (one male treated at 500 ppm), unrelated to treatment. There were no deaths among P females and no notable clinical signs of toxicity in either sex. Body weight gains of females at 10000 ppm were lower than controls over the second half of the pre-mating period (the overall gain in the pre-mating period was ca. 18% lower than controls), with food consumption also reduced. During gestation, lower body weight gains were again recorded in females at 10000 ppm, accompanied by lower food consumption. In the lactation period, females at 10000 ppm initially (days 0-4) showed a body weight loss (compared to slight gains in controls) and body weight gain over the whole lactation period was statistically significantly lower than controls. Lower food consumption was also recorded over all recording intervals in the lactation period.

There was no effect of treatment on estrous cyclicity, mating, fertility or fecundity indices or the time to mating in any group. The number of females delivering litters was 28, 26, 29 and 30 at 0, 500, 1000 and 10000 ppm, respectively, with all animals identified as pregnant delivering live litters. There was no increase in the number of stillborn pups and litter size both at birth and throughout lactation were comparable to controls in all treated groups. Gestation length was 22.2-22.3 days at 0, 500 and 1000 ppm, and 21.9 days at 10000 ppm.

Hematology performed at termination of P animals revealed reduced erythrocyte counts, reduced hemoglobin and reduced hematocrit in animals at 10000 ppm (both sexes but more marked in females). There were no notable gross necropsy findings in P generation animals, but liver weights were found to be significantly increased in both sexes at 10000 ppm. Other organ weight changes at 10000 ppm included increased kidney weights (both sexes – relative weights 112-118% of controls), lower thymus weights (both sexes – relative weights 79-85% of controls), increased adrenal weights (females only – relative weights 125% of controls) and increased spleen weights (males only – relative weights 113% of controls). The changes in adrenal and spleen weights were not accompanied by any histopathological findings and were not considered to be toxicologically significant. Kidneys and thymus were not examined microscopically in these animals. In females at 500 and 1000 ppm, there were slight increases in liver weight and slight decreases in thymus weight. These findings were not considered to be toxicologically significant due to absence of correlating microscopic findings, absence of dose-response (i.e., relative thymus weights in all three groups were 83-86% of controls) and absence of similar findings in males. There were no effects of treatment on primordial follicle counts in the ovaries or any of the sperm parameters evaluated in any treated group.

At microscopic examination of P generation animals, minimal hepatocellular hypertrophy was identified in males and females at 10000 ppm. There were no notable microscopic findings in other tissues (including reproductive tissues) in any group.

F1 pup weights at 10000 ppm were lower than controls, both at birth and throughout lactation (ca. 8% lower at birth, increasing to ca. 31% lower at weaning). There was no effect of treatment on pup survival or on sex ratio. Body weights of pups at 1000 ppm were statistically significantly lower than controls at weaning, but the differences from control were <10%. Body weights of females at 1000 ppm during the post-weaning period were also lower than controls, but by <10% and not statistically significant.

Among F1 pups sacrificed at weaning there were no treatment-related macroscopic or microscopic findings (only gross lesions were examined microscopically). Increased relative liver weights were recorded at 10000 ppm. There were no other notable organ weight findings in F1 weanlings.

Pup weights at 10000 ppm only remained lower than controls in the immediate post-weaning period (PND28 and PND35). Body weights of males at 10000 ppm were statistically significantly lower than controls during the post-weaning period, but differences remained <10% and were no longer apparent by the start of the F1 parental pre-mating period. Sexual maturation, as indicated by vaginal opening, was delayed in females in this group (mean 34.7 days compared to 32.4 days – statistically significant), likely as a secondary effect to the reduced body weights recorded in these animals rather than a specific effect. There was no similar effect in females at lower dose levels and no effect on preputial separation in males.

In F1 parental animals, the death of one male treated at 10000 ppm on day 18 was attributed to treatment (microscopic examination identified severe atrophy of hepatocytes and hydronephrosis). One female at 500 ppm was killed *in extremis* and had an adenocarcinoma of the mammary tissue; in the absence of similar findings or mortality in females at higher dose levels this finding was considered incidental.

Body weights and body weight gains of males and females at 10000 ppm were lower throughout the pre-mating period. Body weight gains of males (but not females) at 1000 ppm were also significantly lower than controls (statistically significant from week 6 onwards), and overall (pre- and post-mating) body weight gains were approximately 12% lower than controls. There was no clear corresponding effect on food consumption. During gestation and lactation, body weights of females at 10000 ppm reflected the lower body weights recorded during pre-mating, but the same differences from control were maintained and body weight effects did not worsen during pregnancy and lactation, although food consumption over these periods was slightly reduced.

There was no effect of treatment on estrous cyclicity, mating, fertility or fecundity indices or the time to mating in any group. The number of females delivering litters was 24, 26, 23 and 24 at 0, 500, 1000 and 10000 ppm, respectively, and gestation length was comparable among groups. The number of live pups born per litter was slightly (but not statistically significantly) lower in the high dose group, but litter size post-cull remained similar to controls. There was no effect of treatment on sex ratio or anogenital distance at birth.

Hematology investigations performed at termination revealed mild but statistically significant reductions in erythrocyte counts, hemoglobin and hematocrit in females at 10000 ppm. Hematocrit only was slightly but statistically significantly lower in males at 10000 ppm. Other hematology findings recorded at the high dose level were lower total leukocyte counts (females) or lower neutrophil, monocyte and eosinophil (but not total leukocyte) counts (males) and increased platelet counts (females). There were no effects on hematology parameters at 1000 or 500 ppm.
There were no notable macroscopic findings in F1 parental animals, but relative liver weights and relative kidney weights were increased in animals receiving 10000 ppm. Lower relative thymus weights were also recorded (69-79% of controls).

Primordial follicle counts were lower than controls in treated females (attaining statistical significance at 1000 and 10000 ppm but without dose-response relationship). Group mean values were 110.0 ± 61.15, 89.4 ± 40.16, 74.7 ± 30.25 and 79.2 ± 34.23 at 0, 500, 1000 and 10000 ppm respectively. This decrease in follicle counts was considered to be spurious, and attributed to an abnormally high follicle count in the F1 controls. F1 control counts recorded in a recently completed reproductive study at the testing facility were very similar (83.9 ± 39.7) to the counts in treated animals in this study, and it was also noted that the control counts in the F1 control group were also notably higher than the follicle counts in the P generation controls (46.3).

At 10000 ppm, a lower proportion of progressively motile sperm (possibly secondary to the adverse body weight effects) and an increase in the proportion of abnormal sperm were observed. The predominant sperm abnormality seen in the 10000 ppm group was changes in the appearance of the sperm hook (either absence of hook or excessive hook). All other sperm parameters of the high-dose F1 group were comparable to controls. A relationship to treatment is equivocal since there were no corresponding effects in P males, or accompanying effects on reproductive performance or histopathological findings in the testes and epididymides.
At microscopic examination of F1 parent animals, minimal hepatocellular hypertrophy was identified in males and females at 10000 ppm (similar to the finding identified in the P generation). There were no notable microscopic findings in other tissues (including reproductive tissues) in any group.

F2 pup body weights at 10000 ppm were lower than controls at birth and throughout lactation (ca. 10% lower at birth, increasing to ca. 32% lower at weaning). Hematological findings in randomly selected F2 pups were restricted to longer activated partial thromboplastin times (males and females) and longer prothrombin time values (females only) at 10000 ppm. These changes were not considered to be treatment-related in the absence of similar findings in P and F1 parental animals.

Among F2 weanlings there were no treatment-related macroscopic or microscopic findings (only gross lesions were examined microscopically). Increased relative liver weights at 10000 ppm were attributed to treatment. Smaller spleen and thymus weights at 10000 ppm (relative weights 69-73% of controls for spleens and 82-84% of controls for thymus) were attributed to the smaller size of these animals. There were no other notable organ weight findings in F2 weanlings.

The NOAEL for parental systemic toxicity was 500 ppm (39 mg/kg/day), based on effects on body weight gain in F1 parent males at 1000 ppm (79 mg/kg/day). The NOAEL for effects on reproductive parameters was 1000 ppm based on an increased proportion of abnormal sperm in F1 males at 10000 ppm (811 mg/kg/day). There were no clear effects on reproductive performance at any dosage. The NOAEL for effects on pups was 1000 ppm based on adverse effects on pup weights and increased liver weights at 10000 ppm.

CA 5.6.2 Developmental toxicity studies

Report: CA 5.6.2/1
[redacted] 2001a
A definitive oral developmental toxicity (embryo-fetal toxicity/teratogenicity) study with BAS 560 F in rats
2001/7001372

Guidelines: EPA 870.3700, OECD 414, EEC 94/79 A II 5.6.2, JMAFF 59 NohSan No 4200

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

Executive Summary

In a study of prenatal developmental toxicity performed in line with OECD 414 adopted in 2001, groups of 25 presumed pregnant Sprague-Dawley rats were dosed with metrafenone (purity: 95.86%) at doses of 0, 50, 500 or 1000 mg/kg/day in 0.5% carboxymethylcellulose via oral gavage on days 6 to 20 of gestation (DG6 to DG20). In-life observations were clinical signs, body weights and food consumption. All animals were sacrificed on GD21 and a blood sample was taken at sacrifice for hematology analyses. A Caesarean section and gross necropsy were performed, with liver and gravid uterine weights recorded. Livers from control and high dose animals were fixed and examined microscopically. The following reproductive parameters were recorded: number and distribution of corpora lutea, pregnancy status, number and distribution of implantations, live and dead fetuses and early and late resorptions. Fetuses were weighed and examined for gross external alterations and sex. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by microdissection techniques. The heads of these fetuses were fixed and subsequently examined by free-hand sectioning. The remaining fetuses were examined for skeletal alterations after staining with Alizarin Red S. Dose levels were set following a pilot study (same dose levels, 8 rats/group, external examination of fetuses only) in which no effects of treatment were identified at dosages up to 1000 mg/kg/day.

There were no deaths and no notable clinical signs of toxicity. Body weight gains and food consumption were similar between treated and control groups. Relative liver weights were higher than controls at 500 and 1000 mg/kg/day, attaining statistical significance at 1000 mg/kg/day only, but the increases were lower that 10% and were not considered to be toxicologically significant (relative liver weights were 99%, 103% and 106% of controls at 50, 500 and 1000 mg/kg/day respectively). There were no effects of treatment on any hematology parameters. Histopathological examination of livers from control and high dose animals did not reveal any notable findings. At termination, the number of rats which were pregnant in each group was 21, 23, 22 and 24 at 0, 50, 500 and 1000 mg/kg/day respectively, and all pregnant animals had viable fetuses and there were no dead fetuses. The number of corpora lutea, implantation sites, live fetuses, early and late resorptions, percentage of resorbed concepti, percentage of male fetuses per litter and fetal body weights were all similar between treated animals and controls. The number of fetuses with alterations was similar between groups and there were no fetuses with alterations visible at gross external examination. The number and pattern of soft tissue alterations, skeletal alterations and ossification sites were very similar between all treated groups and the controls.

The NOAEL for both maternal toxicity and developmental toxicity was 1000 mg/kg/day, the highest dose tested. Metrafenone did not show teratogenic potential.

Report: CA 5.6.2/2
[redacted] 2001b
A definitive oral developmental toxicity (embryo-fetal toxicity/teratogenicity) study with BAS 560 F in rabbits
2001/7001288
Guidelines: EPA 870.3700, OECD 414, EEC 94/79 A II 5.6.2, JMAFF 59 NohSan No 4200
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a prenatal developmental toxicity study performed in line with OECD 414 adopted in 2001, groups of 25 presumed pregnant New Zealand White rabbits were dosed at 0, 50, 350 or 700 mg/kg/day with metrafenone (purity: 95.86%) in 0.5% carboxymethylcellulose via oral gavage on days 6 to 28 of gestation (DG6 to DG28). In-life observations were clinical signs, body weights and food consumption. All animals were sacrificed on GD29 and a blood sample was taken at sacrifice for hematology analyses. A Caesarean section and gross necropsy were performed, with liver and gravid uterine weights recorded. Livers from all animals were fixed and examined microscopically. The following reproductive parameters were recorded: number and distribution of corpora lutea, pregnancy status, number and distribution of implantations, live and dead fetuses and early and late resorptions. Fetuses were weighed, sexed and examined for gross external alterations. All fetuses were dissected to detect soft tissue alterations. The heads of one-half of the fetuses were processed for evaluation of soft tissue alterations by serial sectioning methods. The heads of the remaining fetuses were sectioned by a single mid-coronal incision for evaluation of the internal structure of the brain and then processed for evaluation of skeletal alterations. All fetuses were examined for skeletal alterations following staining with Alizarin Red S. Dose levels were set following a pilot study (0, 50, 500 and 1000 mg/kg/day, 5 rabbits/group, external examination of fetuses only) in which abortions, clinical signs and reduced body weight gains were recorded at 1000 mg/kg/day.

A number of animals died or were sacrificed prior to scheduled termination. These were 3 in the control group (one found dead on GD15, 12 minutes after dosing, and thus considered likely related to an intubation error; one sacrificed moribund on GD21 because of an intubation error; and one aborting on GD26), one treated at 350 mg/kg/day (which delivered on GD29), and 4 treated at 700 mg/kg/day (one found dead on GD10 because of an intubation error; one aborting on GD28; and 2 delivering on GD29). The abortion in the high dose group was attributed to treatment since this animal had shown reduced food consumption, body weight loss and clinical signs prior to the abortion. The early deliveries at 350 and 700 mg/kg/day were also attributed to treatment since the animals had each shown reduced food consumption (either from the start of dosing or late in the study) and body weight loss shortly before the premature delivery. Each litter from the premature deliveries consisted of a mixture of live fetuses, dead fetuses and late resorptions (this may have indicated a lower litter size if delivery had been normal). Other than clinical signs associated with abortions or intubation errors, the only clinical sign considered treatment-related was scant feces in animals at 700 mg/kg/day. Apart from findings associated with the intubation errors, there were no abnormalities detected at necropsy.

Lower maternal body weight gains were recorded at 350 and 700 mg/kg/day, with differences from control starting to become apparent around day 11 and with body weight losses recorded at 700 mg/kg/day in the last week of the study. Food consumption was significantly reduced at 350 and 700 mg/kg/day over the course of the study. Gravid uterine weights were also lower at 350 and 700 mg/kg/day.

Absolute and relative liver weights were significantly increased in a dose-related pattern at 350 and 700 mg/kg/day. The liver weight increases were accompanied by increased incidence and severity of periportal hepatocellular hypertrophy and diffuse hepatocellular cytoplasmic vacuolation at 350 and 700 mg/kg/day (dose-related patterns). Hematology investigations at termination revealed significantly increased prothrombin and activated partial thromboplastin times at 700 mg/kg/day.

The number of animals with litters at termination in each group was 20, 24, 23 and 19 at 0, 50, 350 and 700 mg/kg/day. There were no animals which had totally resorbed their litters and there were no dead fetuses in the treated groups. Data from the animals which had delivered prematurely on day 29 were excluded from the litter observations data. The number of corpora lutea, implantation sites, live fetuses, early and late resorptions, percentage of resorbed concepti and percentage of male fetuses per litter were all similar between treated animals and controls. Fetal body weights were slightly but statistically significantly reduced at 700 mg/kg/day. The number of fetuses with alterations was similar between groups. Gross external alterations were limited to one fetus in the controls (umbilical hernia) and one fetus at 700 mg/kg/day (short tail, with fused caudal vertebrae and only 14 caudal vertebrae present identified at skeletal examination). The other malformations identified (absent kidneys in a single fetus at 50 mg/kg/day and malformations in the thoracic vertebrae of single control fetus) did not indicate a treatment-related teratogenic effect. The pattern of soft tissue and skeletal variations did not suggest any effect of treatment since the fetal and litter incidences of findings were generally similar in treated and control groups (most findings occurred in only one or two fetuses/litters). The average numbers of ossification sites per fetus were similar between treated and control groups; the only statistically significant increase was in forelimb phalange ossification sites (13.96, 13.99*, 14.00** and 14.00** at 0, 50, 350 and 700 mg/kg/day respectively, where * $p<0.05$ and ** $p<0.01$) but the increases were minimal and within the historical control range for the testing facility (average (1998-2000) was 13.93 with a range 13.68-14.00) and therefore not considered to be toxicologically significant.

The NOAEL for both maternal toxicity and developmental toxicity was 50 mg/kg/day, based on lower body weight gains and food consumption, increased liver weights and histopathological effects in the liver at 350 and 700 mg/kg/day. These effects were accompanied by a single incidence of premature delivery at 350 mg/kg/day (the only adverse developmental effect at 350 mg/kg/day, in a single animal which was showing clear maternal toxicity), and incidences of premature delivery, abortion and lower fetal weights at 700 mg/kg/day.

CA 5.7 Neurotoxicity Studies

Although metrafenone has no structural similarity to substances which are capable of inducing delayed neurotoxicity, and no signs indicative of neurotoxic effects were observed in any of the toxicity studies, its neurotoxicity potential was investigated subsequent to the first EU assessment in an acute neurotoxicity study performed according to OPPTS 870.6200 guideline, and in a subacute neurotoxicity study performed according to OECD 424 guideline. These studies, which confirmed that metrafenone is not neurotoxic, are summarized below. No further study is considered necessary.

CA 5.7.1 Neurotoxicity studies in rodents

Report: CA 5.7.1/1
[redacted] 2003b
BAS 560 F - Acute oral neurotoxicity study in Wistar rats - Single administration by gavage
2003/1013528
Guidelines: EPA 870.6200
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute neurotoxicity study, groups of 5 male and 5 female Wistar rats received a single oral dose of metrafenone (purity: 94.2%) as suspensions in either double-distilled water or 0.5% aq. carboxymethylcellulose (CMC) at 0, 125, 500 or 2000 mg/kg bw. After unexpected low concentrations of metrafenone in double-distilled water, the vehicle was changed to 0.5% aq. CMC, and administered to further groups of 5 animals per sex, resulting in groups consisting of 10 animals per sex per dose. Doses were chosen on the basis of a peak range-finding study, where no effects were observed at 2000 mg/kg bw. The animals were observed for up to 14 days after treatment. General observations were performed daily; body weight determinations, functional observation batteries (FOBs) and motor activity assessments were carried out on days -7, 1 (12.5 hrs after treatment for FOBs, and 16.5 hrs for motor activity assessment, based on toxicokinetic data indicating C_{max} is attained at 14 and 15 hrs for males and females, respectively), 7 and 14. Five animals per sex and dose (those treated using 0.5% aq. carboxymethylcellulose as the vehicle) were fixed by *in situ* perfusion and subjected to neuropathological examinations. The remaining animals were sacrificed under CO_2 anesthesia without any further examination.

There were no mortality, no clinical signs, no treatment-related effects on body weights and no treatment-related effects at FOBs and motor activity assessments. Neuropathological investigations did not evidence any treatment-related findings. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose level tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: Beige solid
Batch/purity #: AC 12053-29: 94.2%
Stability of test compound: Proven by re-analysis after the in-life phase of the study (study 170488_1 – CoA included in the report)

2. **Vehicle and/or positive control:** double-distilled water and/or 0.5% carboxymethylcellulose / Historical and positive control data included in the study report.

3. Test animals:

Species: Rat
Strain: Wistar (CrlGIxBrlHan:WI)
Age: 7 weeks old at dosing
Weight: At dosing (group mean values) Males: 187.7-190.0 g; females: 133.0-135.8 g
Source: Charles River, Germany
Acclimation period: 13 days
Diet: Kliba maintenance diet mouse/rat GLP *ad libitum*
Water: Drinking water, from water bottles *ad libitum*
Housing: Individually in type DK III stainless steel wire mesh cages

4. Environmental Conditions

Temperature: 20-24ºC
Humidity: 30-70%
Air changes: Not indicated
Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. Dates of work: 09/10/2002 00:00:00 - 06/02/2003 00:00:00

2. Animal assignment and treatment

Prior to the 1st functional observational battery (FOB) on day -7, the animals were distributed according to the weight among the individual test groups, separated by sex, using a computer-generated randomization list. In order to balance the groups for FOBs and motor activity, the study was conducted with several subsets (Section A males and females = first 5 animals of each dose group; Section B males and females = second 5 animals of each dose group). All animals of Section A received a single oral dose of metrafenone as a suspension in double-distilled water. After unexpectedly low concentrations of metrafenone in double-distilled water, the vehicle was changed and the same dosages administered as a suspension in 0.5% carboxymethylcellulose to the animals of section B.

3. Dose preparation and analysis

To prepare the suspension in double-distilled water, appropriate amounts of metrafenone were weighed in an appropriate container, filled up to the desired volume with double-distilled water, and subsequently mixed using a magnetic stirrer. To prepare the suspension in 0.5% aq. carboxymethyl cellulose, metrafenone was powdered in a mortar; the appropriate amount was then weighed, depending on the desired concentration, and transferred in a small quantity of the test vehicle. The vehicle was then made up to the desired volume and mixed using a high speed homogenizer. Each of the test substance preparations was prepared once. During administration the test substance preparations were kept homogeneous using a magnetic stirrer. Stability in 0.5% aq. carboxymethylcellulose over a period of 4 days at room temperature was previously confirmed (Project No. 15S0437/01092), and this also implied stability in double-distilled water. The homogeneity of metrafenone in each vehicle was proven in samples of the high and low concentrations at the day of respective administration.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Body weight and body weight change of each group were compared to the control group using Dunnett's test (two-sided) for the hypothesis of equal means. Feces, rearing, grip strength (fore- and hindlimbs), landing footsplay test and motor activity data as well as brain weight data were subjected to 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.

C. METHODS

1. Clinical observations

The general state of health of the animals was checked twice a day from Mondays to Fridays and once a day on Saturdays, Sundays and public holidays. The animals were also examined carefully once each working day, except on the days when FOBs were carried out.

2. Bodyweight

Body weights were measured before the 1st FOB in order to randomize the animals. During the administration period the body weight was determined on the days when FOBs were carried out (days -7, 1, 7 and 14).

3. Functional observational battery (FOB)

FOBs were performed in all animals once before the administration (day -7) and on days 1, 7 and 14, each time starting at 10 am. The FOB started with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. In order to guarantee the blind status of the observer, the cages were randomly distributed in the rack at least 30 min prior to the examinations, and the cage labels (indicating the dose group) were turned. Only the animal number, but not the allocation of the animal to the different dose groups could be identified by the observer. In addition, the examinations were carried out in randomized order. The findings and values obtained were documented by another technician knowing the identification of the animals. Historical and positive control data are included in the study report.

4. Motor activity assessment

Motor activity was measured for all animals on the same days as FOB was performed. The measurement was performed in the dark using the Multi-Varimex-System with 4 infrared beams per cage. During the measurement the animals were kept in polycarbonate cages with absorbent material, cleaned prior to each use. The animals were put in the cages in a randomized order. The measurements started at about 2 pm and the number of beam interrupts were counted over 12 intervals, each lasting 5 minutes. Measurements did not commence at the same instant for all cages; the period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 min thereafter. During the measurements the animals received no food and no water.

5. Neuropathology

Necropsy

The five surviving animals per sex and test group selected for neuropathology (those treated using carboxymethylcellulose as the vehicle) were deeply anesthetized (Narcoren, approx. 4 mL/kg bw) at the end of the study and sacrificed by perfusion fixation. Sorensen's phosphate buffer served as the rinsing solution and the fixation solution according to Karnovsky served as fixative. The sacrificed animals were necropsied and the visible organs assessed by gross pathology as thoroughly as possible for perfused animals. The organs/tissue samples listed below were carefully removed.

Plastic embedding, sectioning, staining and storage

The histotechnical processing of the peripheral nervous system samples listed below was as follows: T: plastic embedding (epoxy resin), semi-thin sectioning and staining with Azure II-methylene blue-basic Fuchsin (AMbf); P: storage of fixed specimen in buffer solution; 5: all perfused animals per sex and per group

Organ sample	Dose level (mg/kg bw)			
	0	125	500	2000
Dorsal root ganglia (3 from C3–C6)	T5	P5	P5	T5
Dorsal root fiber (C3–C6)	T5	P5	P5	T5
Ventral root fiber (C3–C6)	T5	P5	P5	T5
Dorsal root ganglia (3 from L1-L4)	T5	P5	P5	T5
Dorsal root fiber (L1-L4)	T5	P5	P5	T5
Ventral root fiber (L1-L4)	T5	P5	P5	T5
Proximal sciatic nerve	T5	P5	P5	T5
Proximal tibial nerve (at knee)	T5	P5	P5	T5
Distal tibial nerve (at lower leg)	T5	P5	P5	T5

The semi-thin sections were examined by light microscopy and assessed.

Paraffin embedding, sectioning, staining and preservation
The histotechnical processing of the central and peripheral nervous system samples listed below was as follows: A: paraffin embedding (paraplast), sectioning and staining with hematoxylin-eosin (H&E); F: preservation in 4% formaldehyde; 5: all perfused animals per sex and per group;

Organ sample	Dose level (mg/kg bw)			
	0	125	500	2000
Central nervous system				
Brain (cross sections)		F5*	F5*	
Frontal lobe	A5			A5
Parietal lobe with diencephalon	A5			A5
Midbrain with occipital and temporal lobe	A5			A5
Pons	A5			A5
Cerebellum	A5			A5
Medulla oblungata	A5			A5
Brain-associated organs/tissues				
Eyes with retina and optical nerve	A5	F5	F5	A5
Spinal cord (cross sections)				
Cervical swelling (C3–C6)	A5	F5	F5	A5
Lumbar swelling (L1-L4)	A5	F5	F5	A5
Peripheral nervous system				
Gasserian ganglia with nerve	A5	F5	F5	A5
Gastrocnemius muscle	A5	F5	F5	A5

*: in toto

The H&E sections were examined by light microscopy and assessed.

II. RESULTS AND DISCUSSION

In double-distilled water, metrafenone was not homogenously distributed in the suspensions at the low and mid dose levels (1.25 and 5 g/100 mL). Moreover, the concentrations of those substance preparations were lower than expected. The preparation homogeneity as well as a correct concentration for the high dose group (20 g/100 mL) was demonstrated. Due to these results, the vehicle and the preparation procedure of the dosing suspensions were changed for treating Section B animals. Analytical results confirmed these suspensions were homogeneously distributed, and with acceptable mean concentrations at 92.6 to 97.1% of nominal concentrations. Given that no treatment-related findings were observed in the high dose animals during the entire study, and especially no neurotoxicity was demonstrated, the inhomogeneity and lower concentrations of the low and mid dose preparations used for treating 5 animals per sex did not affect the validity of the study.

No mortality and no clinical signs were noted. Body weight data and FOB data did not show any effect of administration. The overall motor activity was not affected by treatment. Few statistically significant decreases and/or increases were recorded comparing the single intervals (i.e., decreased values in females of the high dose at interval 9 on day -7, in females of the mid dose group at interval 11 on day 7, and in females of the high dose group at interval 11 on day 14; increased values in females of the mid and high dose groups at interval 1 on day 1, in females of the mid dose group at interval 7 on day 14). Due to single occurrences and the lack of a dose-response, these findings were considered incidental and not related to treatment. No abnormality was noted at necropsy. There were no treatment-related neuropathological findings. The finding "degeneration, vacuolar" in lumbar ganglia cells of one control as well as the single (grade 1) "axonal degeneration" in the peripheral nerves of one top dose male were regarded as spontaneous in nature and not treatment-related.

III. CONCLUSION

The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose level tested.

Report: CA 5.7.1/2
[redacted] 2003c
BAS 560 F - Subacute neurotoxicity study in Wistar rats; Administration in the diet for 4 weeks
2003/1016756
Guidelines: OECD 424
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a repeated-dose neurotoxicity study, groups of 10 male and 10 female Wistar rats received metrafenone (purity 94.2%) in their diets at 0, 1500, 5000 or 20000 ppm for 4 weeks. Food consumption was determined once a week. Body weights were recorded weekly, and on the days when functional observation batteries and motor activity assessments were carried out (days -1 and 27). Detailed clinical examinations in an open field were conducted prior to start of dosing, and weekly thereafter with the exception of the last week. At the end of the study the first 5 animals per sex and dose level were sacrificed by perfusion fixation and subjected to neuropathological examinations. The remaining animals were sacrificed under CO_2 anesthesia without any further examination.

Metrafenone intakes were 0, 143, 458.7 and 1370.9 mg/kg/day in males and 0, 151.9, 492.5 and 1371.1 mg/kg/day in females. No specific signs of systemic or neurotoxicity were noted. Piloerection was observed in 2 males and one female treated at 5000 ppm (by day 21), and in 3 males treated at 15000 ppm (by day 16, and also noted at FOB performed on day 27). In addition, red discolored urine were noted in one female treated at 5000 ppm (by day 15) and in 5 females treated at 15000 ppm (by day 16). Body weight, body weight gain and food consumption were affected by treatment at 15000 ppm. Mean body weights of males were significantly reduced at days 7 and 14 (up to -8.5%); body weight gains were significantly reduced in males at days 7, 14 and 21 and in females at days 14 and 28, and food consumption was significantly reduced in males at days 7 and 14 and in females throughout the entire study. Terminal body weights of animals treated at 15000 ppm were ca. 8% lower than controls for males, and ca. 7% lower than controls for females.

During the FOBs, several deviations from base-line were noted, but because most of these were equally distributed between the treated and control groups, were without dose-relationship, were observed in single animals, or were observed before the start of treatment, they were considered to be incidental and not related to treatment. The same applied to motor activity assessments, which showed no effect on the overall activity, but significantly decreases values at intervals 10 and 11 in females treated at 1500 ppm, and at intervals 1 and 3 in females treated at 15000 ppm, without any dose-response. Neuropathology investigations showed no differences between the treated and control groups. Axonal degeneration (grade 1) was observed in the peripheral nerves of one control male, and in one male and one female of the high dose group and was considered unrelated to treatment.

The NOAEL for systemic toxicity was 1500 ppm (458.7 mg/kg/day in males and 492.5 mg/kg/day in females), based on decreased body weights and food consumption at 15000 ppm.
The NOAEL for neurotoxicity was 15000 ppm (1370.9 mg/kg/day in males and 1371.1 mg/kg/day in females), the highest dose level tested, based on the absence of adverse effects.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
   Description: Beige solid
   Batch/purity #: AC 12053-29: 94.2%
   Stability of test compound: Proven by re-analysis after the in-life phase of the study (study 170488_1 – CoA included in the report)

2. **Vehicle and/or positive control:** ground Kliba maintenance diet mouse/rat GLP / positive control data included in the study report.

3. **Test animals:**
   Species: Rat
   Strain: Wistar (CrlGIxBrlHan:WI)
   Age: 6 weeks old at dosing
   Weight: At dosing (group mean values) Males: 149.0-151.6 g; females: 124.5-126.6 g
   Source: Charles River, Germany
   Acclimation period: 8 days
   Diet: Kliba maintenance diet mouse/rat GLP *ad libitum*
   Water: Drinking water, from water bottles *ad libitum*
   Housing: Individually in type DK III stainless steel wire mesh cages

4. **Environmental Conditions**
   Temperature: 20-24ºC
   Humidity: 30-70%
   Air changes: Not indicated
   Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. **Dates of work:** 11/05/2002 00:00:00 - 08/11/2003 00:00:00

2. Animal assignment and treatment

Prior to the 1st functional observational battery (FOB) on day -1, the animals were distributed according to weight amongst the test groups, separated by sex, using a computer-generated randomization list. In order to balance the groups for FOBs and motor activity, the study was conducted with several subsets (Section A males and females = first 5 animals of each dose group; Section B males and females = second 5 animals of each dose group). All animals received the appropriate diets for 28 consecutive days.

3. Dose preparation and analysis

For each concentration, metrafenone was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose groups, were added to this premix in order to obtain the desired concentration. Mixing was carried out for about 10 min in a laboratory mixer. The diet were prepared once before the study.

The stability of metrafenone in the diet over a period of 49 days at room temperature was proven with a comparable batch of the substance before the start of the study (study 08B0437/016038). The homogeneity of the test substance preparation was proven in samples of the low and high concentrations at the start of the administration period. Concentration control analyses were performed with samples taken at the start of the administration period.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Food and water consumption, body weight and body weight change and food efficiency of each group were compared to the control group using Dunnett's test (two-sided) for the hypothesis of equal means. Feces, rearing, grip strength (fore- and hindlimbs), landing footsplay test and motor activity data were subjected to 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. Wilcoxon-test is identical with Man-Whitney-U-test (two-sided) for the equal medians. Brain weight data were also subjected to 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 for the hypothesis of equal medians.

C. METHODS

1. Clinical observations

All animals were observed for overt signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further clinical examinations were carried out daily. Detailed clinical examinations outside the home cage were conducted on days 7, 14 and 21. The animals were transferred to a standard arena and the findings were ranked according to the degree of severity, if applicable.

2. Food consumption

Food consumption was determined weekly over a period of 7 days and calculated as food consumption in grams per animal and day.

3. Water consumption

Water consumption was observed daily by visual inspection of the water bottles for any over change in volume.

4. Bodyweight

Body weights were measured before the 1st FOB in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of the administration period) and at weekly intervals thereafter. Body weight was also determined on the days when FOBs were carried out.

5. Food efficiency

Food efficiency (group means) was calculated based upon individual values for body weight and food consumption.

$$\frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100 = \text{Food efficiency for day x}$$

BW_x = body weight on day x (g)
BW_y = body weight on day y (last weighing date before x) (g)
$FC_{y\ to\ x}$ = mean food consumption 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 (g)

6. Intake of test substance

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\frac{FC_x \times C}{BW_x} = \text{Substance intake for day x}$$

FC_x = mean daily food consumption on day x (g)
C = concentration in the diet on day x (mg/kg diet)
BW_x = body weight on day x (g)

7. Functional observational battery (FOB)

FOBs were performed in all animals once before the administration (day -1) and on day 27, each time starting at 10 am. The FOB started with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. In order to guarantee the blind status of the observer, the cages were randomly distributed in the rack at least 30 min prior to the examinations, and the cage labels (indicating the dose group) were turned. Only the animal number, but not the allocation of the animal to the different dose groups could be identified by the observer. In addition, the examinations were carried out in randomized order. The findings and values obtained were documented by another technician knowing the identification of the animals. Historical and positive control data are included in the study report.

8. Motor activity assessment

Motor activity was measured for all animals on the same days as FOB was performed. The measurement was performed in the dark using the Multi-Varimex-System with 4 infrared beams per cage. During the measurement the animals were kept in polycarbonate cages with absorbent material, cleaned prior to each use. The animals were put in the cages in a randomized order. The measurements started at about 2 pm and the number of beam interrupts were counted over 12 intervals, each lasting 5 minutes. Measurements did not commence at the same instant for all cages; the period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 min thereafter. During the measurements the animals received no food and no water.

9. Neuropathology

Necropsy

The five surviving animals per sex and test group selected for neuropathology were deeply anesthetized (Narcoren, approx. 4 mL/kg bw) at the end of the study and sacrificed by perfusion fixation. Sorensen's phosphate buffer served as rinsing solution and the fixation solution according to Karnovsky served as fixative. The sacrificed animals were necropsied and the visible organs assessed by gross pathology as thoroughly as possible for perfused animals. The organs/tissue samples listed below were carefully removed.

Weight parameters

Weight assessment of the brain (without olfactory bulb) was carried out on all perfused animals after removal of the brain but before any further preparation.

Organ / tissue preservation list

In addition to the organs/tissues listed below, the brain (remaining material after trimming), spinal cord (parts of cervical and lumbar cord) and all gross lesions were preserved in neutral buffered 4% formaldehyde. The remaining organ material and the animal body were also store in in neutral buffered 4% formaldehyde solution.

Plastic embedding, sectioning, staining and storage

The histotechnical processing of the peripheral nervous system samples listed below was as follows: T: plastic embedding (epoxy resin), semi-thin sectioning and staining with Azure II-methylene blue-basic Fuchsin (AMbf); P: storage of fixed specimen in buffer solution; 5: all perfused animals per sex and per group

Organ sample	Dose level (ppm)			
	0	1500	5000	15000
Dorsal root ganglia (3 from C3–C6)	T5	P5	P5	T5
Dorsal root fiber (C3–C6)	T5	P5	P5	T5
Ventral root fiber (C3–C6)	T5	P5	P5	T5
Dorsal root ganglia (3 from L1-L4)	T5	P5	P5	T5
Dorsal root fiber (L1-L4)	T5	P5	P5	T5
Ventral root fiber (L1-L4)	T5	P5	P5	T5
Proximal sciatic nerve	T5	P5	P5	T5
Proximal tibial nerve (at knee)	T5	P5	P5	T5
Distal tibial nerve (at lower leg)	T5	P5	P5	T5

The semi-thin sections were examined by light microscopy and assessed.

Paraffin embedding, sectioning, staining and preservation
The histotechnical processing of the central and peripheral nervous system samples listed below was as follows: A: paraffin embedding (paraplast), sectioning and staining with hematoxylin-eosin (H&E); F: preservation in 4% formaldehyde; 5: all perfused animals per sex and per group

Organ sample	Dose level (mg/kg bw)			
	0	125	500	2000
Central nervous system				
Brain (cross sections)		F5*	F5*	
Frontal lobe	A5			A5
Parietal lobe with diencephalon	A5			A5
Midbrain with occipital and temporal lobe	A5			A5
Pons	A5			A5
Cerebellum	A5			A5
Medulla oblungata	A5			A5
Brain-associated organs/tissues				
Eyes with retina and optical nerve	A5	F5	F5	A5
Spinal cord (cross sections)				
Cervical swelling (C3–C6)	A5	F5	F5	A5
Lumbar swelling (L1-L4)	A5	F5	F5	A5
Peripheral nervous system				
Gasserian ganglia with nerve	A5	F5	F5	A5
Gastrocnemius muscle	A5	F5	F5	A5

; *: in toto.

The H&E sections were examined by light microscopy and assessed.

II. RESULTS AND DISCUSSION

Analysis confirmed test-diet concentrations and homogeneity to be acceptable (ranging from 94.2 to 100.4% of nominal values, with standard deviations of 1.7 and 2.0 at 1500 and 15000 ppm, respectively).

No animals died during the study. Piloerection was noted in 3 males receiving 15000 ppm, and in 2 males and one female at 5000 ppm, predominantly toward the end of the study. In addition, red discoloration of the urine was noted in 3 females at 15000 ppm and one female at 5000 ppm.

Body weight, body weight gain, food consumption and food efficiency were affected by treatment at 15000 ppm, mainly in males. Mean body weight of males of this group was statistically significantly reduced on days 7 and 14 (up to –8.5%), while body weight change was statistically significantly reduced in males on days 7, 14 and 21 (-28.7, -22.3 and -16.8%, respectively), and in females on days 14 and 28 (-24.4 and -25.1%, respectively). Terminal body weights of animals treated at 15000 ppm were ca. 8% lower than controls for males, and ca. 7% lower than controls for females. These effects on body weight were accompanied by decreases in food consumption, which was statistically significantly reduced in males on days 7 and 14, and in females throughout the entire study. Consequently, food efficiency was also reduced in males, attaining a level of statistical significance on day 7.

The mean daily test substance intake over the entire study period is shown in Table 5.7.1-1.

Table 5.7.1-1: Intake of test substance

Concentration in the diet (ppm)	mg/kg bw/day	
	Males	Females
1500	143.0	151.9
5000	458.7	492.5
15000	1370.9	1371.1

During the FOBs, several deviations from base-line were noted, but because most of these were equally distributed between the treated and control groups, were without dose-relationship, were observed in single animals, or were observed before the start of treatment, they were considered to be incidental and not related to treatment. The same applied to motor activity assessments, which showed no effect on the overall activity, but significantly decreases values at intervals 10 and 11 in females treated at 1500 ppm, and at intervals 1 and 3 in females treated at 15000 ppm, without any dose-response.

Neuropathology investigations showed no differences between the treated and control groups. Axonal degeneration (grade 1) was observed in the peripheral nerves of one control male, and in one male and one female of the high dose group and was considered unrelated to treatment.

III. CONCLUSION

The NOAEL for systemic toxicity was 1500 ppm (458.7 mg/kg/day in males and 492.5 mg/kg/day in females), based on decreased body weights and food consumption at 15000 ppm.

The NOAEL for neurotoxicity was 15000 ppm (1370.9 mg/kg/day in males and 1371.1 mg/kg/day in females), the highest dose level tested, above the limit dose of 1000 mg/kg bw/day, based on the absence of any adverse effects.

CA 5.7.2 Delayed polyneuropathy studies

No study available or required.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

In silico and *in vitro* studies were performed with the crop metabolites CL 3000402 (also a rat metabolite) and CL 1500831, identified as lactone metabolites within the wheat metabolic pathway, and CL 1500834, identified within the wheat metabolic pathway and also observed in the grape metabolic pathway. Grouping of lactone metabolites into a single category was considered suitable and in addition, the parent substance metrafenone was considered a suitable reference substance for read-across to the group using the OECD QSAR Toolbox v3.3. Derek Nexus analysis showed that CL 1500834 shares similar alerts to the parent, with the addition of hepatotoxicity which is a known effect of the parent, whereas no specific alerts were identified for CL 3000402. As no alerts for genotoxicity were identified for either metabolite or for the parent, no further testing is considered necessary for CL 3000402 whereas, for completing the evaluation of its relevance, *in vitro* genotoxicity studies with CL 1500834 (i.e., the bacterial reverse mutation assay and the *in vitro* micronucleus test) are planned, in line with the conclusions of the EFSA Scientific Committee on genotoxicity testing strategy (EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. [68 pp.] doi:10.2903/j.efsa.2011.2379). Reports of these studies will be submitted as soon as the studies have been completed.

Report: CA 5.8.1/1
Thornber-Barwick A.,Silva M. Da, 2015a
Grouping and read-across assessment of Metrafenone Lactone metabolites
2015/1123831
Guidelines: OECD QSAR Toolbox v3.3
GLP: no

Executive Summary

The metabolites of metrafenone CL 3000402 (also a rat metabolite) and CL 1500831, identified as lactone metabolites, were profiled using the OECD QSAR Toolbox v3.3 to investigate the suitability of the grouping method and also the validity of using the parent substance, metrafenone, as a source substance in a surrogate based read-across approach. The identified metabolites and the parent were evaluated using the pre-defined empiric, mechanistic and endpoint specific profiles of toxicological relevance in the program. Predictions were made using pre-defined QSARs within the software for toxicological endpoints and also physico-chemical endpoints relevant to toxicological properties (e.g. those that affect absorption).

A clear correlation was evident between both of the metabolites profiled in the program, along with the parent substance. Based on the available information, the proposed category of lactone metabolites is considered suitable and the parent substance, metrafenone, is a suitable source substance to read-across to for this category.

Report: CA 5.8.1/2
Cotterill J.V., 2014a
In silico toxicity assessment of Metrafenone and two metabolites using Derek Nexus
2014/1289354
Guidelines: none
GLP: no

Executive Summary

The toxicological relevance of the metrafenone crop metabolites CL 1500834 (6-bromo-3-hydroxy-2-(2,3,4-trimethoxy-6-methylbenzoyl)-benzaldehyde) and CL 3000402 (1(3H)-isobenzo furanone, 7-bromo-4-methoxy-3-(2,3,4-trimethoxy-6-methylphenyl)-benzaldehyde), also a rat metabolite, has been investigated by DEREK (Nexus) analysis. The parent compound, metrafenone, has also been included in the analysis to allow a comparative evaluation.

Table 5.8.1-1: Alerts identified in metrafenone and two metabolites using DEREK (Nexus)

Metrafenone	Carcinogenicity[a] (Alert 707), Photoallergenicity[a] (Alert 456), Skin Sensitisation[a] (Alert 439)
CL 1500834	Carcinogenicity[a] (Alert 707), Photoallergenicity[a] (Alert 456), Skin Sensitisation[a] (Alert 439) and Hepatotoxicity[b] (Rapidprototype 019)
CL 3000402	No reported alerts

[a]: plausible [b]: equivocal

Results indicate that CL 1500834 shares similar alerts to the parent, with the addition of hepatotoxicity which is a known effect of the parent, whereas no specific alerts were identified for CL 3000402. Critically, no genotoxicity alerts were identified for either metabolite or for the parent.

CA 5.8.2 Supplementary studies on the active substance

For the first EU assessment of metrafenone, a Mode of Action (MoA) was proposed for the dose-related increase in the incidence of hepatocellular adenomas in female rats, and the increased incidence of hepatocellular adenomas and carcinomas in high dose male mice, applying the IPCS Conceptual Framework for Cancer Risk Assessment. The mode of action was also investigated through a number of mechanistic studies.

Overall, the mode-of action studies provided the following insights on the liver carcinogenicity potential of metrafenone:

1. Metrafenone does not have any initiating potential in the liver of rats, even at a very high (5000 mg/kg bw) oral dose.
2. Since metrafenone does not show initiation potential in the liver, and did not show genotoxic potential in a battery of genotoxicity studies, the mechanism underlying the liver tumours in the chronic rat and mouse studies must be a non-genotoxic mechanism.
3. Metrafenone is a cytochrome P450 enzyme inducer.
4. Metrafenone can induce an increase in cell proliferation in the liver; this increase is selective for the periportal zones, affects the intermediate zones to a lesser extent but does not occur in the centrilobular zone. The increase in cell proliferation is reversible following the cessation of treatment.

Applying the IPCS Conceptual Framework for Cancer Risk Assessment for addressing the dose-related increase in the incidence of hepatocellular adenomas in female rats, and the increased incidence of hepatocellular adenomas and carcinomas in high dose male mice, the Mode of Action proposed is as follows:

1. Metrafenone induces an increased rate of hepatocyte proliferation, which was shown to be reversible upon cessation of exposure
2. Metrafenone treatment leads to cytochrome P450 enzyme induction in mice and rats
3. Continuing high-dose exposure to metrafenone leads to chronic stimulation of proliferation
4. Chronic stimulation of proliferation is a known mechanism which can give rise to tumours in rodent species.

The **key event** in the mode of action is induction of an increased rate of hepatocyte proliferation. This has been demonstrated directly in rats in the S-Phase response study. Indirect evidence is the increased liver weights consistently demonstrated in animals treated with metrafenone. Cytochrome P450 enzyme induction (as demonstrated in rats) has not been investigated in mice but the histopathological evidence is consistent with enzyme induction.

In both rats and mice there is evidence of dose-response relationship.
In female rats treated on a chronic basis, there were dose-related increases in i) liver weights (NOAEL = 500 ppm), and ii) hepatocellular polyploidy, basophilic or eosinophilic alterations and hepatocellular necrosis (NOAEL = 500 ppm). The incidence of hepatocellular adenomas in female rats was dose-related (NOAEL = 500 ppm).

In male mice treated on a chronic basis, the only liver finding showing a dose-related response was centrilobular hepatocellular hypertrophy (NOAEL = 250 ppm). Significantly increased liver weight occurred only at the high dose level (7000 ppm). The incidence of hepatocellular adenoma in male mice was dose-related, but the toxicological significance of the apparent increase in adenomas at 1000 ppm is uncertain. Only the increased incidence in adenomas at 7000 ppm is clearly treatment-related.

The temporal association is clearly demonstrated in rats, and highly likely in mice.

In the chronic rat study, dose-related increases in liver weight, hepatocellular necrosis and hepatocellular polyploidy were recorded in females at the 12-month interim sacrifice. Also at the-12 month interim sacrifice, 2 out of the total 12 hepatocellular adenomas identified in the high dose female group were detected. There were no hepatocellular adenomas identified in control animals after 12 months.

As an interim sacrifice was not performed in the mouse chronic study, a similar comparison cannot be made. Increased liver weights and hepatocellular hypertrophy were evident in mice after 13 weeks of treatment in a sub-chronic study.

The strength of association between the effects identified and the adenomas in high dose female rats is high because the adenomas in rats are associated with histopathological lesions such as hepatocellular alterations, polyploidy and necrosis at the same dose levels giving rise to the adenomas. The less marked histopathology in males is not clearly associated with tumour formation. Increased cell proliferation has been demonstrated in a specific study in rats, with females showing the more marked effect and it is females rats in which the most marked increases in tumours have occurred. Cytochrome P450 enzyme induction has also been demonstrated in rat liver.

The strength of association in mice is less strong since mechanistic studies have not been performed. However, the liver tumours are associated with histopathological evidence consistent with cytochrome P450 enzyme induction and mice are known to be susceptible to liver tumour formation in response to chronic enzyme induction.

Increased liver weights have been consistently recorded in all tested species (rats, mice, rabbits and dogs). Hepatocellular hypertrophy has been observed in both rats and mice. This represents a relatively high degree of consistency between species.

Metrafenone has only been associated with tumours in the liver. The liver is also the only organ or tissue in which a proliferative-type effect or induction of enzymes has been identified (other organs or tissues have not been investigated in detail but the liver is the only organ to show clear evidence of this). Specificity of the tumour response to the key events has therefore been shown.

Metrafenone did not show genotoxic potential when tested in a battery of genotoxicity assays. Metrafenone also did not show initiating potential when tested in a specific study in rats.

The proposed mechanism of action (chronic induction of liver enzymes/liver cell proliferation) is a mechanism which is well accepted as giving rise to tumours in rodent species by non-genotoxic processes, and therefore has biological plausibility.

The overall coherence of the database regarding effects on the liver is high. In rats, it has been shown that the effects of metrafenone include the appropriate aspects of the key events within the proposed mechanism (i.e. increased cell proliferation and cytochrome P450 enzymes induction). Increased incidence of histopathological changes in the liver have been identified in rats at the same dose levels giving rise to increased incidence of adenomas, but no increase in adenomas is seen in groups not showing an increase in these histopathological changes. In mice, treatment-related liver tumours only occur in dose groups showing the most marked severity of centrilobular hepatocellular hypertrophy (graded moderate or moderately severe), but not in groups showing this finding as mild or less. Induction of cytochrome P450 enzymes has not been demonstrated directly in mice, but the centrilobular hypertrophy is consistent with this effect. The correlation between the most severe centrilobular hypertrophy and the liver tumours therefore supports the proposed mechanism of action.

All the main effects of metrafenone on the liver (increased weight, enzyme induction, cell proliferation and hypertrophy) would tend towards the formation of liver tumours in rodents.

No other obvious mechanisms to give rise to these liver tumours are apparent from the database. Metrafenone is not genotoxic, and has not been associated with an increased incidence of neoplastic findings in any other organ or tissue. No effects suggesting hormonal disturbance have been identified. Few effects other than those relating to the liver have been identified (the remaining effects being mostly confined to the kidneys), so there are no obvious mechanisms by which the tumours in the liver might be secondary to effects occurring in a different organ or tissue.

It can be concluded that there is good evidence that the liver tumours arise due to the proposed mechanism. The proposed mechanism is certainly biologically plausible, the key events have been demonstrated and the strength of association between the tumours and the effects seen is high. No other obvious mechanism is apparent from the database.

The treatment-related liver tumours identified in rats and mice were induced by the proposed mechanism, a mechanism which is non-genotoxic and which would be expected to show a threshold. Levels of exposure which do not give rise to induction of liver enzymes and liver cell proliferation would not be expected to cause liver tumours following chronic exposure. The level of confidence in the proposed mechanism of action is considered to be high.

No inconsistencies have been identified. There are no significant uncertainties or data gaps regarding rats. There is less information available in mice, since mechanistic studies have been performed only in rats. However, it is considered very likely that the centrilobular hepatocellular hypertrophy observed histopathologically in mice represents enzyme induction. These data gaps and uncertainties are not considered to be critical to support the proposed mechanism of action in mice because this mechanism is very well established for causing liver tumours in mice. There is a strong association between the more severe centrilobular hypertrophy identified and the increased incidence of liver tumours in mice. It is considered that no further supporting information is required for mice.

A brief summary of each of these mechanistic studies is provided below. The long-term studies and relevant mechanistic studies available are sufficient to permit the identification of effects following repeated exposure, and to conclude that the liver tumours are of limited significance to human health risk assessment. Carcinogenicity labeling was not agreed on by the experts, due to the non-relevance to humans, since they are never likely to be exposed to these extreme doses for such a prolonged period of time. No further studies are therefore considered necessary.

Since Annex I inclusion of metrafenone a specific GLP study investigating the immunotoxicity potential of metrafenone was performed in female Wistar rats, according to internationally accepted guidelines (OPPTS 870.7800). The study confirmed that metrafenone has no immunotoxic potential; the complete summary of this study is provided below.

Report: CA 5.8.2/1
[redacted] 2002a
BAS 560 F - Hepatic enzyme induction study in Sprague Dawley rats - Administration in the diet for 4 weeks
2002/1005176
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a study intended to determine whether metrafenone can induce liver enzyme activities or peroxisome proliferation, groups of 5 male and 5 female Sprague-Dawley rats received metrafenone (purity 95.86%) in their diet at 0 or 20000 ppm for 4 weeks. Investigations included clinical signs, bodyweights and food and water consumption. At the end of the study, animals were sacrificed and livers were perfused with 0.9% NaCl-solution via the portal vein. The following parameters were analysed:
a) Cyanide-insensitive Palmitoyl-CoA-oxidation (PALCoA) in liver homogenate
b) Glutathione concentration (GSH) in liver homogenate
c) Ethoxyresorufin-O-deethylase (EROD) activity in S9 fraction
d) Pentoxyresorufin-O-dependentylase (PROD) activity in S9 fraction

Intakes were 0 and 1526 mg/kg/day in males, and 0 and 1654 mg/kg/day in females.
There were no deaths and no clinical signs of toxicity. Water consumption was significantly increased in males at day 21, and was also increased but to a lesser extent (and not significantly) at day 28 (prior to day 21 water bottles had been observed visually only; due to obviously increased water consumption weighing of water bottles was commenced on day 21). Food consumption was significantly lower in treated females on days 7, 21 and 28. Decreased bodyweight gains were recorded in treated animals such that after 4 weeks bodyweight gains were 10% or 19% lower than controls in males and females respectively.

Table 5.8.2-1: Liver enzyme data following dietary administration with metrafenone for 4 weeks.

Enzyme	Male		Female	
	Control	20000 ppm	Control	20000 ppm
PALCoA	5.13 ± 0.57	4.64 ± 1.12	5.60 ± 0.72	4.69 ± 0.55
GSH	7.18 ± 0.57	7.34 ± 0.62	5.19 ± 0.50	6.03 ± 0.56
EROD	6.68 ± 4.49	115.50** ± 29.95	2.70 ± 0.38	27.60** ± 7.54
PROD	3.76 ± 3.02	2.69 ± 5.90	3.37 ± 0.89	6.11* ± 1.44

* $p<0.05$, ** $p<0.01$ (Wilcoxon test)

PROD was significantly increased in females, but the increase was less than two-fold. EROD was significantly increased in males (17-fold increase over controls) and females (10-fold increase over controls). There was no effect of treatment on PALCoA or GSH.

It can be concluded that metrafenone induces liver enzymes of the cytochrome P450 family in Sprague-Dawley rats following treatment at a high dose (>1000 mg/kg/day) via the diet for 4 weeks.

Report: CA 5.8.2/2
[redacted] 2002b
Phenobarbital sodium salt - Hepatic enzyme induction study in Sprague Dawley rats - Administration in the diet for 4 weeks
2002/1005177
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

To provide positive control data for the previous study, groups of 5 male and 5 female Sprague-Dawley rats received phenobarbital in their diet at 0 or 500 ppm for 4 weeks. Investigations included clinical signs, bodyweights and food and water consumption. At the end of the study, animals were sacrificed and livers were perfused with 0.9% NaCl-solution via the portal vein. The following parameters were analysed:
a) Cyanide-insensitive Palmitoyl-CoA-oxidation (PALCoA) in liver homogenate
b) Glutathione concentration (GSH) in liver homogenate
c) Cytochrome P450 (CYT.P450) microsomal content
d) Ethoxyresorufin-O-deethylase (EROD) activity in S9 fraction
e) Pentoxyresorufin-O-dependentylase (PROD) activity in S9 fraction
Intakes were 0 and 38.9 mg/kg/day in males, and 0 and 42.6 mg/kg/day in females.

There were no deaths and no clinical signs. Food consumption was slightly but significantly higher in males over the first 2 weeks of the study. Body weight gains of males and females were significantly higher in the 1st week, and remained higher in males (not significantly) over the remainder of the study.

Table 5.8.2-2: Liver enzyme data following dietary administration with phenobarbital for 4 weeks

Enzyme	Male		Female	
	Control	500 ppm	Control	500 ppm
PALCoA	5.23 ± 0.90	4.57 ± 0.92	5.21 ± 1.08	4.66 ± 0.58
GSH	7.47 ± 0.23	7.96 ± 0.82	6.72 ± 0.26	6.54 ± 0.37
CYT.P450	0.88 ± 0.13	1.66** ± 0.34	0.44 ± 0.08	0.73* 0.30
EROD	8.27 ± 3.45	23.51** ± 5.25	9.30 ± 2.08	13.03 ± 3.96
PROD	12.77 ± 4.58	225.65** ± 105.27	3.37 ± 0.60	83.73** ± 50.27

* $p<0.05$, ** $p<0.01$ (Wilcoxon test)

There were marked increases in cytochrome P450 content (89% in males and 66% in females) in the liver. PROD activity was statistically significantly increased in both sexes (18-fold increase in males and 25-fold increase in females). Additionally, EROD activity was slightly but significantly increased in males with corresponding females showing a slight non-statistical increase, which was considered to be biologically significant. No effects on PALCoA or GSH concentration were observed in either sex treated with phenobarbital. Administration of phenobarbital in the diet at 500 ppm for 4 weeks was associated with clear increases in cytochrome P450 content and EROD and PROD activities. The methodology used in the previous enzyme induction assay with metrafenone is therefore validated.

Report: CA 5.8.2/3
[redacted] 2002c
BAS 560 F - S-phase response study in the liver of Sprague Dawley rats - Administration in the diet for 1 and 4 weeks and recovery period of 2 weeks
2002/1006201
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The proliferation of liver cells following treatment with metrafenone was evaluated in rats. Groups of 8 male and female Sprague-Dawley rats received metrafenone (purity: 95.86%) in their diets at 0, 500 or 20000 ppm over a period of either 1 or 4 weeks. A further group received 20000 ppm for 4 weeks and was then maintained on control diet for a 2-week recovery period. In-life investigations included clinical signs, body weights and food and water consumption. Seven days prior to termination, osmotic minipumps containing bromodeoxyuridine (BrdU) were implanted subcutaneously. At termination, a gross necropsy was performed and livers were weighed and prepared for both histopathological examination (haematoxylin & eosin staining) and immunohistochemistry (BrdU staining for cell proliferation (DNA synthesis) and TUNEL stain for apoptotic cells). Intakes were 0, and approximately 30 and 1000 mg/kg/day.

There were no deaths considered to be treatment-related and no clinical signs of toxicity. Significantly increased water consumption and significantly decreased food consumption were recorded in animals treated at 20000 ppm. Significantly lower body weight gains were also recorded in these animals, but differences from control were less than 10%. There were no notable gross necropsy findings. Liver weights were significantly increased in both sexes at 20000 ppm. Relative (to bodyweight) liver weights were 17% higher (after 1 week treatment) or 15% higher (after 4 weeks treatment) than controls in males, and 48% higher (after 1 week) or 65% higher (after 4 weeks) than controls in females. After 1 weeks treatment, centrilobular hypertrophy of hepatocytes was identified in all males and in 5/7 females at 20000 ppm (minimal to slight). After 4 weeks treatment, centrilobular hypertrophy of hepatocytes was identified in all males and in 7/8 females at 20000 ppm (minimal to moderate). Two high dose females also showed minimally or moderately increased numbers of mitotic figures in the periportal region after 4 weeks of treatment. No hypertrophy or increased mitosis was identified in animals treated at 20000 ppm following the 2-week recovery period. There were no effects on liver weight or liver histopathology at 500 ppm.

Table 5.8.2-3: S-Phase liver response (absolute and relative) following treatment with metrafenone in the diet for 4 weeks

Treatment period	Test group	Zone 1		Zone 2		Zone 3		All zones	
		%	LI	%	LI	%	LI	%	LI
Males									
1 Week	Control	100	1.77	100	1.47	100	0.95	100	1.40
	500 ppm	108	1.92	97	1.43	61	0.58	94	1.31
	20000 ppm	645**	11.41	214	3.14	62	0.59	361**	5.05
4 Weeks	Control	100	0.55	100	1.87	100	0.10	100	0.84
	500 ppm	93	0.51	128	2.40	160	0.16	122	1.02
	20000 ppm	120	0.66	80	1.50	60	0.06	88	0.74
4 weeks with 2 weeks recovery	20000 ppm	33**[a]	0.18	111	2.07	40	0.04	91*	0.76
Females									
1 Week	Control	100	0.17	100	2.71	100	0.63	100	1.17
	500 ppm	394**	0.67	100	2.70	81	0.51	111	1.29
	20000 ppm	9559**	16.25	241**	6.52	22**	0.14	653**	7.64
4 Weeks	Control	100	2.70	100	1.69	100	1.54	100	1.98
	500 ppm	69*	1.87	71	1.20	47*	0.73	64	1.27
	20000 ppm	418**	11.29	111	1.88	117	1.80	252**	4.99
4 weeks with 2 weeks recovery	20000 ppm	7** a	0.19	8**	0.13	20**	0.31	11**	0.21

Significantly different from control p<=0.05 (*) or p<=0.01 (**)
LI = BrdU Labelling index
a = as compared with the 4-week controls
Zone 1 = periportal zone; Zone 2 = intermediate zone between zones 1 and 3; Zone 3 = centrilobular zone

After 1 week of treatment at 20000 ppm there were marked significant increases in cell proliferation (as measured by BrdU index) in both males (6.5-fold increase of zone 1-hepatocytes and in a 3.6-fold increase for all zones combined) and females (95.6-fold increase in zone 1 and a 2.4-fold increase in zone 2; 6.5-fold increase for all zones combined). Females were affected to a greater extent and most of the increased activity was located in the periportal zone. After 4 weeks of treatment there was no clear increase in BrdU index at either dose level in males. In females there were significant increases at 20000 ppm (again mostly in the periportal zone) but the increases were of much smaller magnitude than after 1 week of treatment.

In both males and females after the 2-week recovery period following the 4-week treatment at 20000 ppm, there was a significant decrease in BrdU index relative to the control activity recorded at 4 weeks. The decrease was more marked in females, and in both sexes the largest decrease occurred in the periportal zone (which previously had shown the largest increases). This decrease was attributed to the influence of down-regulating mechanisms which had been activated during the induction of cell proliferation by the test substance but which had not yet ceased to operate after induction of cell proliferation had suddenly ceased. The apparent significant increase in BrdU index relative to controls in females treated at 500 ppm after 1 week at 500 ppm was considered due to an unusually low control value in zone 1. Available historical control data from 12 studies in 20 groups of female Wistar rats aged between 11 and 19 weeks for BrdU index in zone 1 ranged between 0.47 and 12.44 with a mean of 3.94 (SD ±2.48) and a median of 3.65. Although no specific data for Sprague-Dawley female rats were available, the control value in the current study is lower than these historical controls at week 1, while it was within the historical control range at week 4. For this reason, the statistically significant increase was not considered to be of biological relevance, as was the slight, but significant decrease recorded in females treated at 500 ppm at week 4.

Very low numbers of positively labelled hepatocytes with TUNEL staining were recorded in control males after 1 week. By comparison with the control values after 4 weeks it can be concluded that there was no clear effect of treatment on the number of apoptotic cells in any male group. Comparison with the 4 week control values was considered acceptable since the values in control males after 4 weeks were similar to the control values in females and the female values did not show any differences between 1 and 4 weeks. Therefore, the 1 week male control values appeared to be atypical. Among the female groups, there was a clear significant increase after 4 weeks at 20000 ppm (mostly in zones 1 and 2), and a smaller increase was still apparent in zone 1 after the 2-week recovery period.

Table 5.8.2-4: Number of apoptotic cells (TUNEL assay) following treatment with metrafenone in the diet for 4 weeks

Treatment period	Test group	Number of positively labelled hepatocytes			
		Zone 1	Zone 2	Zone 3	Zone 4
Males					
1 Week	Control	0	1	0	1
	500 ppm	6	8*	0	14*
	20000 ppm	3	7	0	10*
4 Weeks	Control	7	20	1	28
	500 ppm	4	9	0	13*
	20000 ppm	3	9	0	12*
4 weeks with 2 weeks recovery	20000 ppm	9	19	1	29
Females					
1 Week	Control	4	6	3	13
	500 ppm	2	2	0	4
	20000 ppm	2	3	2	7
4 Weeks	Control	4	5	3	12
	500 ppm	3	4	3	10
	20000 ppm	38**	28**	13	79**
4 weeks with 2 weeks recovery	20000 ppm	16*	10	3	29

Significantly different from control p<=0.05 (*) or p<=0.01 (**) at Wilcoxon test

Treatment with metrafenone at 20000 ppm (approximately 1000 mg/kg/day) was associated with the following effects on the liver.

- Liver weights were increased after both 1 and 4 weeks of treatment. Females were affected to a greater extent than males, and in females only the increase after 4 weeks was larger than after 1 week. Liver weight increases were fully reversible after 2 weeks of recovery.
- Centrilobular hypertrophy after both 1 and 4 weeks of treatment. Males were affected to a greater extent than females in terms of incidence but not severity (all males affected after 1 week in contrast to females where incidence increased with increasing duration). This effect was also reversible following 2 weeks of recovery. In addition to centrilobular hypertrophy, minimally or moderately increased numbers of mitotic figures were identified in the periportal region of 2 females after 4 weeks of treatment. No such finding was identified after 2 weeks of recovery.
- Cell proliferation was significantly increased in both males and females after 1 week of treatment, but only in females after 4 weeks and the magnitude of the increase was smaller. Females were affected to a greater extent than males in terms of magnitude of effect after 1 week, and increased cell proliferation was identified mostly in the periportal region. After the 2-week recovery period there was a significant decrease recorded in cell proliferation.
- The number of apoptotic liver cells was increased after 4 weeks (but not after 1 week) in females (but not males), mostly in the periportal and intermediate zones.

Report: CA 5.8.2/4
[redacted] 2002d
BAS 560 F - Initiation study in Sprague Dawley rats
2002/1006202
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

To evaluate the initiation potential of metrafenone, groups of 12 male and 12 female partially hepatectomized (14 hours earlier) Sprague-Dawley rats received a single oral dose of metrafenone (purity: 95.86%) in double-distilled water by gavage at 5000 mg/kg bw. The positive control for initiation, nitrosomorpholine (NNM), was administered as a single dose by oral gavage at 50 mg/kg bw in doubly distilled water. After a 2-week recovery period, phenobarbitone (PB) was given as a known promoter at a dietary concentration of 500 ppm for 8 weeks. Negative control groups were included and consisted of untreated animals or animals treated with PB only. At termination a gross necropsy was performed and liver sections were prepared for histopathology with haematoxylin & eosin (H&E) staining and for staining for glutathione-S-transferase placental form (GST-P). GST-P positive foci, considered as preneoplastic lesions, were evaluated quantitatively (foci/cm^2 of live tissue).

Several animals from different groups died during or shortly after partial hepatectomy (no more than 1 or 2 from each group). There were no treatment-related deaths, no clinical signs and no effects of metrafenone treatment on bodyweights or food consumption (NNM adversely affected body weight gains and food consumption).

Altered foci were identified both by histopathology (H&E staining) and by GST-P immunohistochemistry staining. Serial sections (3) from each of the three major remaining lobes were stained as i) GST-P immunohistochemical stain, ii) negative control for the GST-P immunohistochemical stain, and iii) H&E staining. Thus, altered foci could be correlated between GST-P staining and H&E histopathological examination (phenotypically-altered foci). The number of GST-P positive foci was higher than the number of phenotypically-altered foci identified by histopathology. Most correlates for GST-P positive foci were eosinophilic, some were clear cell type. Basophilic foci were not identified in metrafenone-treated animals. Animals treated with NNM or NNM/PB showed a number of altered foci of all types (basophilic, eosinophilic or clear cell). By contrast, control and metrafenone-treated animals showed only single foci. In addition, PB-treated animals showed centrilobular hypertrophy (moderate or severe in males, slight or moderate in females) as expected.

Table 5.8.2-5: Number of altered foci - H&E staining

Group	Number of animals		Number of altered foci	
	M	F	M	F
Control	11	12	4	2
PB control	12	10	6	3
Metrafenone	12	11	4	2
Metrafenone+PB	12	12	4	4
NNM	11	11	11	11
NNM+PB	12	11	12	11

PB: phenobarbitone; NNM: nitrosomorpholine

Table 5.8.2-6: Number of GST-P positive foci

Group	Number per cm^2 of liver tissue		Foci area as a percentage of total liver area	
	M	F	M	F
Control	0.17	0.11	0.001	0.003
PB control	0.34	0.40	0.004	0.011
Metrafenone	0.08	0.30	0.006	0.006
Metrafenone+PB	0.46	0.54	0.006	0.010
NNM	8.77**	7.34**	0.167**	0.211**
NNM+PB	16.07**	19.64**	0.252**	0.525**

PB: phenobarbitone; NNM: nitrosomorpholine; ** $p<0.01$ at Wilcoxon test

The results of this study indicate that a very large single dose of metrafenone does not initiate the formation of foci of cellular alteration in rat liver. This includes both foci which could be identified by histopathology following H&E staining and GST-P positive foci identified by immunohistochemistry. No increase in foci was recorded in metrafenone-treated animals compared to appropriate controls. A positive control with known initiating capacity (NNM) showed significant increases in the number and area of foci compared to controls which validates the method for identifying initiating potential.

Report: CA 5.8.2/5
[redacted] 2010a
BAS 560 F (Metrafenone) - Immunotoxicity study in female Wistar rats - Administration via the diet for 4 weeks
2010/1197373
Guidelines: EPA 870.7800
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of metrafenone was investigated in female Wistar rats. The study was reported to comply with GLP and performed according to internationally accepted guidelines (OPPTS 870.7800). Groups of 8 female Wistar rats received metrafenone (purity: 94.2%) in their diets at 0, 1000, 4000 or 12000 ppm for 4 weeks. A similar sized positive control group was treated with cyclophosphamide monohydrate at 4.5 mg/kg/day by oral gavage. All animals were immunized 6 days before blood sampling and necropsy using 0.5 mL of sheep red blood cells ($4x10^8$ SRBC/mL) administered by intraperitoneal injection. Food consumption was determined once a week and body weight was determined twice weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to start of treatment and weekly thereafter. SRBC IgM antibody titers were measured (by enzyme-linked immunosorbent assay - ELISA) at study termination. In addition, animals were subjected to a gross examination with liver, spleen and thymus weights recorded. Intakes of metrafenone were 0, 80, 315 and 1086 mg/kg/day.

No mortality and no signs of toxicity were noted. Treatment with metrafenone up to and including 12000 ppm had no impact on body weight and food consumption, whereas that with the positive control (cyclophosphamide) caused a consistently reduced food intake (attaining statistical significance on day 28), significantly impaired body weights at days 14 (-5.18%) and 28 (-8.62%), and significantly reduced body weight gains (maximum -23.59% at day 28). No changes in the SRBC IgM titers were found in female rats treated with up to 12000 ppm of metrafenone, whereas the SRBC titers were lower in rats of the positive control group. At necropsy liver changes were observed only in females treated at 12000 ppm. Organ weight data showed increased absolute and relative liver weights in animals treated at 4000 and 12000 ppm, and significantly decreased absolute and relative spleen and thymus weights in the positive control animals.

Metrafenone did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to female Wistar rats. Thus, the NOAEL for immunotoxicity was 12000 ppm (1086 mg/kg/day), the highest dose level tested. The NOAEL for systemic toxicity was 1000 ppm (80 mg/kg/day), based on significantly increased absolute and relative liver weights observed at 4000 ppm (315 mg/kg/day). The sensitivity of the assay was confirmed by results obtained in animals treated with the positive control, cyclophosphamide, administered by oral gavage at 4.5 mg/kg/day.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: Beige solid
Batch/purity #: AC12053-29: 94.2%
Stability of test compound: the expiry date exceeded the date of the in-life phase of the study

2. **Vehicle and/or positive control:** ground Kliba maintenance diet mouse/rat GLP / Cyclophosphamide monohydrate (purity 100%), provided by Sigma-Aldrich, Taufkirchen, Germany, and administered as a solution in drinking water by gavage at 4.5 mg/kg/day was used as positive control

3. **Test animals:**
Species: Rat
Strain: Wistar (Crl:WI) - females
Age: 6 weeks old at start of dosing
Weight: At dosing (group mean values) Females: 130.60-132.58 g
Source: Charles River, Germany
Acclimation period: 8 days
Diet: Kliba maintenance diet mouse/rat GLP *ad libitum*
Water: Drinking water, from water bottles *ad libitum*
Housing: In groups of 4 animals per cage, in Tecniplast H-Temp (PSU) cages, with Lignocel PS 14 dust-free bedding and gnawing blocks (Typ NGM E-022) as environmental enrichment

4. **Environmental Conditions**
Temperature: 20-24ºC – no or only minimal deviations occurred during the study
Humidity: 30-70% – no or only minimal deviations occurred during the study
Air changes: 15 per hour
Photoperiod: 12-h light/dark cycle

B. STUDY DESIGN AND METHODS

1. **Dates of work:** 02/23/2010 00:00:00 - 09/09/2010 00:00:00

2. **Animal assignment and treatment**

Prior to the 1st detailed clinical observation on day -1, the animals were distributed according to the weight among the individual test groups using a computer-generated randomization list. Animals then received the test substance daily via the diet for 4 weeks. Control animals received ground diet only. Cyclophosphamide monohydrate (positive control substance) was administered daily by gavage for 4 weeks. The volume to be administered was 10 mL/kg bw/day (related to the body weight determined most recently in each case). All rats were immunized 6 days before blood sampling and necropsy using 0.5 mL sheep red blood cells (4×10^8 SRBC/mL), administered intraperitoneally. At the end of the administration period the mice were sacrificed after a fasting period (withdrawal of food) of at least 16-20 hours.

3. Dose preparation and analysis

For each concentration, metrafenone was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose groups, were added to this premix in order to obtain the desired concentration. Mixing was carried out for about 10 min in a laboratory mixer. The diets were prepared once before the start of the administration period.
Cyclophosphamide monohydrate (positive control substance) was administered as a solution. To prepare the solution, the appropriate amount of cyclophosphamide monohydrate was weighed out depending on the desired concentration. Then the vehicle (drinking water) was filled up to the desired volume, subsequently mixed using a magnetic stirrer. The positive control-substance preparations were prepared once, split into daily aliquots and kept frozen at -18°C. The mixtures were administered after reaching room temperature.

The stability of metrafenone in the diet over a period of 49 days at room temperature was proven with a comparable batch of the substance before the start of the study (study 08B0437/016038). The stability of cyclophosphamide monohydrate (positive control substance) in the vehicle (drinking water) for 7 days at room temperature and 32 days frozen was determined before the start of the administration period (Project No. 01Y0012/058064).

Homogeneity analyses of the test-substance preparations were performed in samples of all concentrations at the start of the administration period. These samples also served for concentration control analyses. Concentration control analyses of cyclophosphamide monohydrate were performed before the beginning of the study and at the end of the administration period. A homogeneity control analysis was not performed, because cyclophosphamide monohydrate was administered as a solution in drinking water.

4. Statistics

Means and standard deviations of each test group were calculated for several parameters.
Body weight and body weight change of the test substance treated groups were compared to the control group using Dunnett's test (two-sided) for the hypothesis of equal means. Body weight and body weight change of the positive control treated group was compared to the control group using the t-test (which is identical to the DUNNETT test in the case of 1 test group only) (two-sided) for the hypothesis of equal means. Clinical pathology parameters data were subjected to non-parametric one-way analysis using Kruskal-Wallis test. 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 for the equal medians. Organ weight data were also subjected to 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 for the hypothesis of equal medians.

C. METHODS

1. Clinical observations

A check for moribund and dead rats was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. All rats were checked daily for any clinically abnormal signs. Detailed clinical examinations outside the home cage were conducted in all rats prior to the administration period and thereafter at weekly intervals. The animals were transferred to a standard arena and the findings were ranked according to the degree of severity, if applicable.

2. Food consumption

Food consumption was determined weekly (as representative value over 1 day) for each cage. The average food consumption/cage was used to estimate the mean food consumption in grams per rat and day.

3. Water consumption

Water consumption was observed daily by visual inspection of the water bottles for any over change in volume.

4. Bodyweight

Body weight was determined before the start of the administration period in order to randomize the rats. During the administration period the body weight was determined on day 0 (start of the administration period) and thereafter twice weekly. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

5. Intake of test substance

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\frac{FC_x \times C}{BW_x} = \text{Substance intake for day x}$$

FC_x = mean daily food consumption on day x (g)
C = concentration in the diet on day x (mg/kg diet)
BW_x = body weight on day x (g)

6. Clinical pathology

In the morning blood was taken from the retroorbital venous plexus from fasted animals. The animals were anaesthetized using isoflurane. The blood sampling procedure and subsequent analysis of serum samples were carried out in a randomized sequence. 8 female animals per test group were examined for primary T-cell dependent antibody response (anti-SRBC IgM ELISA)

7. Terminal procedures

Necropsy

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

Organ weight

Weight assessment was carried out on all animals sacrificed at scheduled dates. The anesthetized animal, and its liver, spleen and thymus were weighed.

Organ / tissue fixation

All gross lesions, the liver, spleen and thymus were fixed in 4% buffered formaldehyde solution. From the liver, each one slices of the Lobus dexter medialis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast. No histopathological investigations were carried out.

II. RESULTS AND DISCUSSION

Analyses showed mean values in the range of 96.3-99.4% of the nominal concentration and thus demonstrated the correctness of the concentrations of metrafenone in Kliba lab diet mouse/rat GLP. The mean values of cyclophosphamide monohydrate in drinking water were found to be in the range of 94.0-105.2% of the nominal concentration, confirming the correctness of the concentrations of cyclophosphamide monohydrate in drinking water.

No mortality occurred during the study, and no signs of toxicity were observed either in metrafenone or in positive control-treated animals. No significant differences of body weight and body weight change values were observed for the metrafenone-treated groups. The body weights of rats treated with cyclophosphamide monohydrate were significantly impaired on study days 14 (-5.18%) and 28 (-8.62%). In addition, body weight change of female rats treated with cyclophosphamide monohydrate was reduced from day 7 until 14 and from day 21 until 28, with a maximum of -23.59% on day 28. These findings were assessed as being treatment-related and were accompanied by a consistent decrease in food consumption, which attained statistical significance on day 28 (-13.41%). No effect on water consumption was observed in any group.

The approximate, mean daily test-substance intake over the entire study period was 80, 315 and 1086 mg/kg bw/day, respectively, for groups receiving 1000, 4000 and 12000 ppm.

Six days after immunization, no changes in the SRBC IgM titers were found in female rats dosed with metrafenone up to 12000 ppm, whereas the SRBC titers were lower in rats of the positive control group.

Table 5.8.2-7: SRBC IgM titers (arbitrary lab units/mL), 6 days after immunization with SRBC (mean ± SD and median for 8 animals per group)

Positive control 4.5 mg/kg/day		Negative control 0 ppm		1000 ppm		4000 ppm		12000 ppm	
mean ± SD	median	mean ± SD	median	mean ± SD	median	mean ± SD	median	mean ± SD	median
878** ± 686	688	4794 ± 1560	4636	7876 ± 8438	4057	6675 ± 4567	5028	6070 ± 4321	3705

** $p < 0.01$ at Wlcoxon-test

When compared to the control group, mean absolute liver weights at 12000 ppm were increased by +39% (which was considered to be treatment-related). Only slight increases in absolute liver weights (namely +10% and +14%) were observed respectively at 1000 and 4000 ppm. The positive control group revealed a significant decrease of spleen and thymus weights, which were expected findings. Additionally, the liver weights were significantly lower (-9%). However, the latter finding was regarded as incidental and not treatment-related. When compared to the control group (set to 100%), the relative liver weights of test groups receiving 4000 and 12000 ppm were dose-dependently significantly increased, i.e. +16% and +44%, respectively. This finding was regarded as being related to treatment. The positive control group revealed a significant decrease of relative spleen and thymus weights, which were expected findings.

In animals treated at 12000 ppm the livers revealed a treatment-related dark brown discoloration (3 animals) and a slight to moderate enlargement (6 animals). Additionally, a prominent acinar pattern was visible in one animal.

III. CONCLUSION

Metrafenone did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to female Wistar rats. Thus, the NOAEL for immunotoxicity was 12000 ppm (1086 mg/kg/day), the highest dose level tested. The NOAEL for systemic toxicity was 1000 ppm (80 mg/kg/day), based on significantly increased absolute and relative liver weights observed at 4000 ppm (315 mg/kg/day). The sensitivity of the assay was confirmed by results obtained in animals treated with the positive control, cyclophosphamide, administered by oral gavage at 4.5 mg/kg/day.

CA 5.8.3 Endocrine disrupting properties

Overall, toxicological data do not indicate any relevant endocrine mode of action for metrafenone. In the two-generation rat study, an increased proportion of abnormal sperm was seen in F1 males only and only at the highest dose level of 10000 ppm (811 mg/kg/day). However, for males in the highest dose group, no effects on reproductive performance was observed, all other sperm parameters were comparable to controls and no effects were observed at histopathological examination of the testes and epididymides. Metrafenone is not classified as a carcinogen or reproductive toxin and does not therefore meet the interim EU criteria for an endocrine disruptor. Although final criteria for the definition of 'endocrine disruptor' have yet to be agreed, based on the above assessment, it is unlikely that metrafenone would be considered to be an endocrine disruptor in the context of Regulation (EC) No 1107/2009. Further specific testing for endocrine disruption is not considered necessary.

CA 5.9 Medical Data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Medical data on metrafenone is limited, but no reports of adverse effects were identified during routine monitoring of production plant workers and among personnel involved in the experimental biological testing or field trials. As there are no specific parameters available for monitoring the effects of metrafenone, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments. The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, and serum liver enzyme levels. Adverse health effects suspected to be related to metrafenone exposure have not been observed, and no associations of adverse health effects in the course of production, transportation, formulation and packaging of metrafenone have been reported or documented in the BASF-internal medical files.

CA 5.9.2 Data collected on humans

Neither data on exposure of the general public, nor epidemiologic studies are available to BASF SE, nor is BASF SE aware of any epidemiologic studies performed by third parties. There is no evidence or data available to support any findings in relation to poisoning with metrafenone. According to the Proposed Registration Decision of PMRA dated 26 April 2013, there were no incident reports from Canada or the USA as of 30 January 2013 for products containing metrafenone.

CA 5.9.3 CA 5.9.3 Direct observations

See point CA 5.9.2.

CA 5.9.4 Epidemiological studies

See point CA 5.9.2.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Since metrafenone is not classified as toxic or very toxic, validated methods for the determination of the active substance or its metabolites in biological fluids have not been developed. There are no specific signs of intoxication or no known clinical tests for poisoning in humans.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

The following information is included in the MSDS for metrafenone (ID no. 30211628/SDS_CPA_EU/EN), Date of print 08.07.2014:

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

On skin contact:
Wash thoroughly with soap and water

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

On ingestion:
Rinse mouth immediately and then drink plenty of water.

CA 5.9.7 Expected effects of poisoning

There are no known effects of poisoning from BAS 560 F technical material. Based on the relatively high oral LD_{50} value for metrafenone technical (i.e., > 5000 mg/kg bw), the likelihood of human poisoning is small.

Summary of mammalian toxicity and overall evaluation

Following gavage dosing in rats, absorption of metrafenone was rapid and complete (> 88%) at the low dose of 10 mg/kg bw but was limited to 15–20% at the high dose of 1000 mg/kg bw, suggesting saturation of the absorption processes. Metrafenone was widely distributed in the body, with highest residue levels found mainly in the gastrointestinal tract, liver and fat. There was no evidence of accumulation. The labelled material was excreted relatively rapidly into the gastrointestinal tract via the bile (85–90%), resulting in extensive excretion via feces. Excretion via urine was relatively low at 10 mg/kg bw (5–6%), and even lower at the high dose of 1000 mg/kg bw (~1%). Metrafenone was extensively metabolized, with most of the labelled products excreted as glucuronic acid conjugates in bile and urine. Five possible sites of conjugation with glucuronic acid were identified, following O-demethylation of the molecule. Residues in faeces consisted primarily of parent compound and the aglycones of bile and urine conjugates. The transformation steps included:

- O-demethylation of the aromatic methoxy group(s) followed by mono-O-glycosidation;
- hydroxylation of the bromophenyl ring; and
- hydroxylation of the methyl substituent to hydroxymethyl followed by O-glycosidation or further oxidation to aldehyde or lactone.

The bond between the bromophenyl ring and trimethoxyphenyl ring remained intact.

Metrafenone has low acute toxicity when administered orally, dermally and via inhalation to rats, and it is not a skin or an eye irritant, nor a skin sensitizer. Although a pre-guideline largely compliant *in vitro* study indicated that metrafenone has phototoxic potential, an *in vivo* phototoxicity study showed it is not phototoxic *in vivo.* Considering that *in vivo* data are more relevant for human exposure, it is concluded that phototoxicity is not a concern.

The short-term toxicity of metrafenone (duration from 4 weeks to one year) was evaluated in rats, mice and dogs. All studies were GLP-compliant and performed according to internationally accepted guidelines, with the exception of the range-finding studies. The target organ in all species was the liver (increased weight and histopathological findings). The lowest NOAEL was obtained in the rat 90-day study (43 mg/kg bw/day). No evidence of either systemic or local toxicity was observed following dermal exposure in a 28-day dermal toxicity study in rats. The battery of short-term studies available is sufficient to permit the identification of effects following repeated exposure.

In an adequate battery of studies of genotoxicity *in vitro* and *in vivo*, including a more recently performed *ex vivo* UDS assay showing no evidence of DNA damage repair in hepatocytes from treated rats, metrafenone consistently gave negative results and it can be concluded that metrafenone is non-genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study in mice, the only significant treatment-related effects were recorded in the liver and kidneys. Increased incidence and severity of chronic nephropathy were recorded in the kidneys (more severe in males). Liver effects included increased weights (more severe in females) and increased incidence and severity of centrilobular and diffuse hepatocellular hypertrophy (more severe in males). There was a treatment-related increase in hepatocellular adenomas and carcinomas in high dose males. The NOAEL for liver and kidney effects was determined at 250 ppm (39-53 mg/kg/day in male and female mice, respectively). A clear NOAEL for increased incidence of liver tumours was also established at 1000 ppm (156-223 mg/kg bw/day in male and female mice, respectively). Similarly, in the long-term study of toxicity and carcinogenicity in rats, the liver and kidney were target organs. Kidney effects included increased weights and increased incidence and severity of chronic nephropathy. Effects on the liver were generally more marked in females and included increased weights, histopathological effects consistent with liver enlargement such as centrilobular hypertrophy, and also polyploidy and necrosis. There was an increased incidence of hepatocellular adenoma at the intermediate and high dose levels in females, both dosages exceeding the MTD. A clear NOAEL for liver and kidney effects was established at 500 ppm (25-30 mg/kg/day in male and female rats, respectively). A clear NOAEL for increased incidence of liver tumours was established at 500 ppm (30 mg/kg/day in female rats).

Mechanistics studies in rats showed that the treatment-related liver tumours identified in rats and mice were induced by a mechanism which is non-genotoxic and has a threshold. The postulated mechanism is that metrafenone induces an increased rate of hepatocyte proliferation (cytochrome P450 enzyme induction in mice and rats as an associated marker); continuing exposure to metrafenone leads to chronic stimulation of proliferation. Chronic stimulation of proliferation is a known mechanism which can give rise to tumours in rodent species. Levels of exposure which do not give rise to induction of liver enzymes and liver cell proliferation would not be expected to cause liver tumours following chronic exposure. Available mechanistic data provided a high level of confidence in the proposed mechanism of action. Humans are unlikely to be exposed to metrafenone above that threshold on a chronic basis. In view of the absence of any genotoxic potential in *in vitro* and *in vivo* studies, metrafenone is unlikely to pose a carcinogenic risk to humans. This conclusion was derived during the first EU evaluation of metrafenone and in the JMPR review completed in 2014.

In a two-generation study of reproductive toxicity in rats, there were no clear effects on reproductive performance at any dosage. However, the NOAEL for effects on reproductive parameters was 1000 ppm (79 mg/kg/day), based on an increased proportion of abnormal sperm in F1 males at 10000 ppm (811 mg/kg/day). The parental NOAEL was 500 ppm (39 mg/kg/day), based on effects on body weight gain in F1 parent males at 1000 ppm. The NOAEL for effects on pups was 1000 ppm based on adverse effects on pup weights and increased liver weights at 10000 ppm. In a study of prenatal developmental toxicity in rats, there was no evidence of either maternal or developmental toxicity. The maternal NOAEL and the NOAEL for developmental toxicity was 1000 mg/kg/day, the highest dose tested. In a study of prenatal developmental toxicity in rabbits, the NOAEL for both maternal and developmental toxicity was 50 mg/kg/day, on the basis of lower body weight gains and food consumption, increased liver weights and histopathological effects in the liver of does at 350 and 700 mg/kg/day. These effects were accompanied by a single incidence of premature delivery at 350 mg/kg/day (the only adverse developmental effect at 350 mg/kg/day, in a single animal which was showing clear maternal toxicity), and incidences of premature delivery, abortion and lower fetal weights at 700 mg/kg/day. Metrafenone is not a developmental toxicant.

Although metrafenone has no structural similarity to substances which are capable of inducing delayed neurotoxicity and there was no evidence of neurotoxic effects in the other toxicity studies, neurotoxicity was specifically investigated in an acute neurotoxicity study which resulted in a NOAEL of 2000 mg/kg bw, and in a subacute 28-day neurotoxicity study. In this later study the NOAEL for systemic toxicity was 1500 ppm (458.7 mg/kg/day in males and 492.5 mg/kg/day in females), based on decreased body weights and food consumption at 15000 ppm. The NOAEL for neurotoxicity was 15000 ppm (1370.9 mg/kg/day in males and 1371.1 mg/kg/day in females), the highest dose level tested.

On the basis of *in silico* and *in vitro* studies, none of the metabolites found in crop or livestock are of toxicological concern.

In a 28-day immuntoxicity study, the NOAEL for immunotoxicity was 12000 ppm (1086 mg/kg/day), the highest dose level tested. The NOAEL for systemic toxicity was 1000 ppm (80 mg/kg/day), based on significantly increased absolute and relative liver weights observed at 4000 ppm (315 mg/kg/day).

In reports on manufacturing plant personnel, no adverse health effects were noted.

Effects having relevance to human and animal health arising from exposure to the active substance or to impurities in the active substance or to their transformation products

Most of the studies with non-radiolabelled metrafenone submitted and assessed for the first EU review of metrafenone were performed with test batch AC 12053-29, purity 95.86%. The impurity profile of the test batch is presented in Document J-CA of this dossier. The test batch is considered to be representative of technical metrafenone as currently manufactured. The 13-week dietary toxicity study in albino rats was performed with batch AC 11238-111A, purity 97.1 %. Although the impurity profile was not determined for batch AC 11238-111A, this batch is significantly less pure than the new proposed technical specification of 980 g/kg. It is therefore likely that any significant impurities present would be at higher levels than in the proposed specification.

Table 5.9.7-1: Overview of repeat-dose studies on metrafenone

Species	Study	Effect	NOAEL	LOAEL
Mouse	90-day toxicity[a]	Toxicity	1000 ppm, equal to 163 mg/kg bw per day	3500 ppm, equal to 622 mg/kg bw per day
	18-month carcinogenicity[a]	Toxicity	250 ppm, equal to 39 mg/kg bw per day	1000 ppm, equal to 159 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 159 mg/kg bw per day	7000 ppm, equal to 1109 mg/kg bw per day
Rat	28-day toxicity[a]	Toxicity	1000 ppm, equal to 106 mg/kg bw per day	5000 ppm, equal to 528 mg/kg bw per day
	28-day neurotoxicity[a]	Toxicity	1500 ppm, equal to 459 mg/kg bw per day	15000 ppm, equal to 1371 mg/kg bw per day
		Neurotoxicity	15000 ppm, equal to 1371 mg/kg bw per day[c]	-
	28-day immunotoxicity[a]	Toxicity	1000 ppm, equal to 80 mg/kg bw per day	4000 ppm, equal to 315 mg/kg bw per day
		Immunotoxicity	12000 ppm, equal to 1086 mg/kg bw per day[c]	-
Rat	90-day toxicity[a]	Toxicity	-	1000 ppm, equal to 79 mg/kg bw per day[d]
	90-day toxicity[a]	Toxicity	500 ppm, equal to 43 mg/kg bw/day[c]	-
	2-year toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 25 mg/kg bw per day	5000 ppm, equal to 260 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 30 mg/kg bw per day	5000 ppm, equal to 320 mg/kg bw per day
	Multigeneration reproductive toxicity[a]	Reproduction/fertility	1000 ppm, equal to ca. 79 mg/kg bw per day	10000 ppm, equal to ca. 811 mg/kg bw per day
		Parental toxicity	500 ppm, equivalent to ca. 39 mg/kg bw per day	1000 ppm, equal to ca. 79 mg/kg bw per day
		Offspring toxicity	1000 ppm, equivalent to ca. 79 mg/kg bw per day	10000 ppm, equal to ca. 811 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day[c]	-
		Embryo- and fetotoxicity	1000 mg/kg bw per day[c]	-
Rabbit	Developmental toxicity[b]	Maternal toxicity	50 mg/kg bw per day	350 mg/kg bw per day
		Embryo- and fetotoxicity	50 mg/kg bw per day	350 mg/kg bw per day
Dog	28-day toxicity[b]	Toxicity	250 mg/kg bw per day	500 mg/kg bw per day
	90-day toxicity[b]	Toxicity	500 mg/kg bw per day[c]	-
	1 year toxicity[b]	Toxicity	500 mg/kg bw per day[c]	-

[a] Dietary administration
[b] Gavage administration
[c] Highest dose tested
[d] Lowest dose tested

Acceptable Daily Intake (ADI)

The first EU evaluation of metrafenone established an ADI of 0.25 mg/kg bw/day, on the basis of the NOAEL of 25 mg/kg bw per day for liver and kidney effects in the 2-year dietary study in rats, using a safety factor of 100. This provides a margin of exposure of at least 1000 relative to the LOAEL for the induction of liver tumours in rats and mice. There is no additional data submitted in this renewal application that impacts on this value and hence no reason to amend the ADI at this time.

Acceptable Operator Exposure Limit (AOEL)

During the previous EU review of metrafenone, an AOEL of 0.43 mg/kg bw/day was established, on the basis of the NOAEL of 43 mg/kg bw/day in the 13-week study in Sprague Dawley rats. There is no additional data submitted in this renewal application that impacts on this value and hence no reason to amend the AOEL at this time.

Acute Reference Dose (ARfD)

In view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose, there was and there is no need to set an ARfD for metrafenone.

BASF
We create chemistry

Metrafenone

Document M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Introduction

Metrafenone is a benzophenone fungicide for the control of powdery mildew fungi. The biochemical mode of action is not yet known. However, observations indicate that it inhibits growth of mycelium on the leaf surface, leaf penetration, and formation of haustoria and sporulation.

Metrafenone was first approved by Commission Directive 2007/6/EC (entry into force: 01 February 2007). This document is submitted to support the renewal of approval of metrafenone and complies with the Table of Contents described in the Annex to Regulation (EU) No 283/2013. It reviews the residues studies performed with the active substance.

- For studies performed since the first inclusion of metrafenone in Annex I to Directive 91/414/EEC, the study reports are submitted (See document K-CA) and full study summaries are provided.
- For studies submitted and assessed for the first EU review of metrafenone and that are still considered relevant to support renewal of approval, only the critical endpoints and a brief summary are included in this supplementary dossier. For a more detailed assessment, reference can be made either to the summary dossier (Document M-II, Section 7, BASF SE, 2002) submitted for the first EU evaluation or to the Draft Assessment Report (DAR) for metrafenone (UK, 2005). The study reports are not submitted in document K-CA but can be found in the dossier submitted for the first EU Review.

CA 6.1 Storage stability of residues

The stability of metrafenone in grapes, grape products, cereal whole plant, grain and straw has been previously reported in the DAR (UK, July 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (*EFSA Scientific Report* (2006) 58, 1- 72) and are reproduced below:

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)	Residues in grapes and wine are stable for up to 18 months at < -20°C. Residues in cereal grain and straw are stable for up to 24 months at < -18°C.

This conclusion was subsequently confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498).

"The potential degradation of residues during storage of the residue trial samples was also assessed. In the framework of the peer review, storage stability of metrafenone was demonstrated at -20°C for a period of 18 months in acidic commodities (grapes) and 24 months in dry commodities (cereals) (EFSA, 2006). An additional study evaluated in the framework of the MRL application on table and wine grapes, demonstrated the storage stability of metrafenone in commodities with high water content (tomatoes) for a period of 15 months (EFSA, 2011). According to the RMS, all residue trial samples reported in the PROFile were stored in compliance with the storage conditions reported above. Degradation of residues during storage of the trial samples is therefore not expected."

Report:	CA 6.1/1 Class T., 2000a AC 375839 (CL 375839): Storage stability of AC 375839 residues at <-18°C in grapes and wine 2000/7000144
Guidelines:	EEC 96/68, EEC 7032/VI/95 rev. 5, EPA 860.1000, EPA 860.1380, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). The stability of metrafenone was investigated under frozen storage conditions in grapes and wine over a period of 18 months. Samples were taken at 0, 3, 6, 12 and 18 month intervals and analysed by high pressure liquid chromatography (HPLC) or gas chromatography (GC). No significant degradation (≤30%) of the metrafenone residues in grapes and wine was observed over the 18 month study period.

Report:	CA 6.1/2 Jordan J.M.,Kasiri A., 2006a Magnitude of BAS 560 F residues in grapes and grape processed fractions following applications of BAS 560 00 F (amended final report) 2006/7007012
Guidelines:	EPA 860.1500, EPA 860.1520, EPA 860.1380
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The storage stability of metrafenone residues in raisins and yeast was investigated. Residues were shown to be stable for at least 3 months following storage at ≤ -5°C.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone BAS 56000F
Description: Active substance
Lot/Batch #: 3013: 298.6 g/L
Purity: 99.7%
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels: 0.1 mg/kg

2. **Test Commodity:**
Crop: Grapes
Type: wine/raisin
Variety: Grape varieties, Catawba, Vidal Blanc, Ruby Seedless, Thompson, Centurian, Zinfandel, Merlot, White Riesling, Pinot Noir
Botanical name: *Vitis vinifera*
Crop parts(s) or processed commodity: raisin, yeast
Sample size: unspecified

B. STUDY DESIGN AND METHODS

1. Test procedure

Control raisins and yeast samples were fortified with 0.1 mg/kg metrafenone. Fortified and unfortified samples were placed in plastic bags and stored at ≤ - 5°C. Each sample set compromised one, control, two freshly-fortified samples and two stored-fortified samples. Analysis was conducted following 0, 1 and 3 months storage.

2. Description of analytical procedures

Samples were analysed using BASF analytical method 535/0 (HPLC/MS/MS). Procedural recoveries ranged from 83-117%.

II. RESULTS AND DISCUSSION

Procedural recovery results for metrafenone were all within the range of 70 to 120% following up to 3 months deep frozen storage.

Table 6.1-1: Storage stability of metrafenone in raisins and yeast

matrix	Storage interval (days)	Mean procedural recovery (%)	Recovered residues (mg/kg)	Recovery corrected for procedural recovery (%)
raisins	0	111	0.089, 0.094	81, 85
	32	89	0.078, 0.077	88, 87
	89	101	0.082, 0.073	81, 73
yeast	0	98	0.076, 0.077	77, 78
	32	101	0.078, 0.075	77, 74
	89	103	0.086, 0.081	84, 78

III. CONCLUSION

The storage stability of metrafenone residues in raisins and yeast was investigated. Residues were shown to be stable for at least 3 months following storage at < -5°C.

Report:	CA 6.1/3 Smalley R., 2002a BAS 560 F (AC 375839) and metabolites (CL 3000402, CL 434223 and CL 376991) - freezer storage stability in wheat (whole plant, straw and grain) 2002/7005049
Guidelines:	EEC 7032/VI/95 rev. 5
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). This was an interim report with the full results now reported at CA 6.1/4.

Report:	CA 6.1/4 Class T., 2002a BAS 560 F (AC 375839): Storage stability of BAS 560 F residues at less than or equal to 18°C in cereal grain and straw 2002/7004653
Guidelines:	EEC 7032/VI/95 rev. 5, EEC 96/68, BBA IV 3-3, IVA-Leitlinie Rueckstandsversuche Teil II Lagerstabilitaet von Rueckstandsproben (Frankfurt/Main 1990), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). The stability of metrafenone was investigated under frozen storage conditions in wheat grain over a period of 29 months and in wheat straw over a period of 24 months. Wheat grain samples were analyzed in triplicate at the 0, 12, 18, and 29-month time intervals. Wheat straw samples were analyzed in triplicate at 10139280, 11, 18 and 24-month time intervals by gas chromatography (GC). Residues of metrafenone in wheat grain remained unchanged over the 29-month storage period. No significant degradation (≤30%) of the metrafenone residues in wheat straw was observed over the 24 month storage period. The study was subsequently extended to 31 months of storage with the full results now reported at CA 6.1/5.

Report:	CA 6.1/5 Smalley R., 2003a AC 375839 and metabolites - Freezer storage stability in wheat (whole plant, straw and grain) 2003/1013928
Guidelines:	EEC 91/414 (7032/VI/95)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

Specimens of wheat whole plant, straw and grain which were fortified with a combined standard solution containing metrafenone, CL 3000402, CL 434223 and CL 376991 at 1.0 mg/kg were analysed after approximately 2, 7, 15, 19, 24 and 31 months storage at -20°C. All of the compounds tested, the parent and the three metabolites, were stable after 31 months storage in wheat grain, wheat straw and whole plant. Results greater than 70% of the initial concentration were obtained for every combination after 31 months of storage.

I. MATERIAL AND METHODS

A. MATERIALS

1.

Test Material:	Metrafenone; AC 375839 (BAS 560 F)
Description:	Active substance
Lot/Batch #:	AC 11957-109B
Purity:	99.7%
CAS#:	220899-03-6
Development code:	BAS 560 F
Spiking levels:	0.1-2.0 mg/kg

Test Material:	CL 3000402
Description:	Metabolite
Lot/Batch #:	AC 12387-91
Purity:	98%
CAS#:	none
Development code:	none
Spiking levels:	0.1-2.0 mg/kg

Test Material:	CL 434223
Description:	Metabolite
Lot/Batch #:	AC 12387-75
Purity:	94%
CAS#:	none
Development code:	none
Spiking levels:	0.1-2.0 mg/kg

Test Material:	CL 376991
Description:	Metabolite
Lot/Batch #:	AC 12387-78, 12820-61
Purity:	95%, 100%
CAS#:	none
Development code:	none
Spiking levels:	0.1-2.0 mg/kg

2. Test Commodity:

Crop:	Wheat
Type:	not specified
Variety:	not specified (not relevant)
Botanical name:	*Triticum spp.*
Crop parts(s) or processed commodity:	Whole plant, straw, grain
Sample size:	not specified

B. STUDY DESIGN AND METHODS

1. Test procedure

The objective of the study was to determine the stability, under deep frozen storage conditions, of metrafenone and its metabolites, CL 3000402, CL 434223 and CL 376991 in wheat. Stability of each compound was tested at 1.0 mg/kg in whole plant, straw and grain over a 31 month period.

2. Description of analytical procedures

Specimens were homogenised using a commercial food processor prior to analysis by HPLC using method RLA 12619V.

II. RESULTS AND DISCUSSION

Procedural recovery results for the parent compound and metabolites were all within the range of 70 to 120% following up to 31 months deep frozen storage.

Table 6.1-2: Storage stability of metrafenone wheat whole plant, grain and straw

Matrix	Storage Interval (Months)	metrafenone		
		Procedural Recovery (%)	Stored Recovery (%)[1]	Relative Recovery (%)[2]
Wheat Whole Plant	1	0.1mg/kg 102 1.0mg/kg 107	96, 102, 107 (102)	90, 95, 100 **(95)**
	7	0.1mg/kg 94 1.0mg/kg 103	105, 100, 96 (100)	102, 97, 94 **(98)**
	15	0.1mg/kg 102 1.0mg/kg 107	100, 104, 114 (106)	93, 97, 106 **(99)**
	19	0.1mg/kg 101 1.0mg/kg 99	96, 92, 109 (99)	97, 93, 110 **(100)**
	24	0.1mg/kg 102 1.0mg/kg 97	101, 93, 95 (96)	104, 96, 98 **(99)**
	31	0.1mg/kg 95 1.0mg/kg 92	81, 73, 94 (83)	89, 80, 103 **(91)**
Wheat Straw	3	0.1mg/kg 88 1.0mg/kg 81	101, 98, 101 (100)	125, 121, 124 **(123)**
	7	0.1mg/kg 92 1.0mg/kg 97	92, 99, 92 (94)	95, 102, 95 **(97)**
	15	0.1mg/kg 78 2.0mg/kg 75	82, 88, 91 (87)	109, 117, 122 **(116)**
	19	0.1mg/kg 90 1.0mg/kg 90	90, 94, 91 (92)	100, 104, 101 **(102)**
	24	0.1mg/kg 84 1.0mg/kg 93	97, 92, 85 (91)	104, 99, 91 **(98)**
	31	0.1mg/kg 71 1.0mf/kg 90	91, 86, 85 (87)	101, 96, 94 **(97)**
Wheat Grain	2	0.1mg/kg 82 1.0mg/kg 80	67, 73, 83 (74)	83, 91, 103 **(92)**
	7	0.1mg/kg 87 1.0mg/kg 80	76, 80, 82 (79)	95, 101, 102 **(99)**
	15	0.1mg/kg 85 1.0mg/kg 90	89, 86, 95 (90)	99, 96, 106 **(100)**
	19	0.1mg/kg 72 1.0mg/kg 80	75, 65, 78 (73)	94, 81, 98 **(91)**
	24	0.1mg/kg 86 1.0mg/kg 82	97, 92, 71 (87)	118, 112, 87 **(106)**
	31	0.1mg/kg 74 1.0mg/kg 71	76, 76, 69 (74)	107, 107, 97 **(104)**

[1] Average recoveries are given in brackets. Average Relative Recoveries are written in bold. Values in this table have been rounded for reporting purposes.

[2] Relative recovery = $\frac{\text{Stored Recovery}}{\text{High Procedural Recovery}} \times 100$

Table 6.1-3: Summary of Storage Stability Data for CL 3000402 in Wheat Matrices Frozen up to 31 Months

Matrix	Storage Interval (Months)	CL 3000402		
		Procedural Recovery (%)	Stored Recovery (%)[1]	Relative Recovery (%)[2]
Wheat Whole Plant	1	0.1mg/kg 100 1.0mg/kg 100	92, 95, 101 (95)	92, 95, 101 **(96)**
	7	0.1mg/kg 100 1.0mg/kg 103	105, 108, 101 (105)	101, 104, 98 **(101)**
	15	0.1mg/kg 99 1.0mg/kg 99	101, 99, 105 (102)	103, 100, 106 **(103)**
	19	0.1mg/kg 99 1.0mg/kg 97	98, 89, 101 (96)	101, 92, 104 **(99)**
	24	0.1mg/kg 99 1.0mg/kg 96	97, 96, 95 (96)	101, 100, 99 **(100)**
	31	0.1mg/kg 105 1.0mg/kg 94	79, 79, 95 (84)	84, 84, 101 **(90)**
Wheat Straw	3	0.1mg/kg 95 1.0mg/kg 96	100, 95, 96 (97)	104, 99, 100 **(101)**
	7	0.1mg/kg 88 1.0mg/kg 92	95, 98, 94 (96)	104, 107, 102 **(104)**
	15	0.1mg/kg 97 2.0mg/kg 83	92, 91, 95 (93)	111, 110, 115 **(112)**
	19	0.1mg/kg 88 1.0mg/kg 90	91, 95, 88 (91)	101, 106, 98 **(102)**
	24	0.1mg/kg 89 1.0mg/kg 95	93, 104, 85 (94)	98, 109, 89 **(99)**
	31	0.1mg/kg 73 1.0mg/kg 82	85, 81, 83 (83)	104, 99, 101 **(101)**
Wheat Grain	2	0.1mg/kg 85 1.0mg/kg 77	60, 74, 87 (74)	78, 96, 112 **(95)**
	7	0.1 mg/kg 70 1.0mg/kg 83	92, 102, 97 (97)	111, 123, 117 **(117)**
	15	0.1mg/kg 75 1.0mg/kg 84	89, 84, 92 (88)	106, 100, 109 **(105)**
	19	0.1mg/kg 85 1.0mg/kg 88	79, 80, 80 (80)	90, 91, 91 **(91)**
	24	0.1mg/kg 94 1.0mg/kg 95	103, 90, 105 (99)	108, 95, 111 **(105)**
	31	0.1mg/kg 80 1.0mg/kg 81	88, 79, 82 (83)	109, 98, 101 **(103)**

[1] Average recoveries are given in brackets. Average Relative Recoveries are written in bold. Values in this table have been rounded for reporting purposes.

[2] Relative recovery = $\frac{\text{Stored Recovery}}{\text{High Procedural Recovery}} \times 100$

Table 6.1-4: Summary of Storage Stability Data for CL 434223 in Wheat Matrices Frozen up to 31 Months

Matrix	Storage Interval (Months)	CL 434223		
		Procedural Recovery (%)	Stored Recovery (%)[1]	Relative Recovery (%)[2]
Wheat Whole Plant	1	0.1mg/kg 98 1.0mg/kg 98	90, 90, 100 (93)	92, 92, 102 **(95)**
	7	0.1mg/kg 97 1.0mg/kg 104	100, 106, 103 (103)	96, 102, 99 **(99)**
	15	0.1mg/kg 100 1.0mg/kg 101	99, 98, 102 (100)	98, 97, 101 **(99)**
	19	0.1mg/kg 96 1.0mg/kg 96	96, 86, 97 (93)	100, 90, 101 **(97)**
	24	0.1mg/kg 97 1.0mg/kg 95	95, 87, 96 (93)	100, 92, 101 **(98)**
	31	0.1mg/kg 99 1.0mg/kg 92	83, 77, 99 (86)	90, 84, 108 **(94)**
Wheat Straw	3	0.1mg/kg 90 1.0mg/kg 95	93, 91, 92 (92)	98, 95, 97 **(97)**
	7	0.1mg/kg 92 1.0mg/kg 92	97, 100, 93 (97)	106, 109, 102 **(106)**
	15	0.1mg/kg 97 2.0mg/kg 89	94, 92, 94 (93)	106, 103, 106 **(105)**
	19	0.1mg/kg 85 1.0mg/kg 90	88, 93, 89 (90)	98, 103, 99 **(100)**
	24	0.1mg/kg 80 1.0mg/kg 92	87, 99, 89 (92)	95, 108, 97 **(100)**
	31	0.1mg/kg 84 1.0mg/kg 89	99, 97, 93 (96)	111, 109, 104 **(108)**
Wheat Grain	2	0.1mg/kg 88 1.0mg/kg 90	65, 72, 88 (75)	72, 80, 98 **(83)**
	7	0.1mg/kg 96 1.0mg/kg 88	89, 97, 97 (94)	101, 110, 110 **(107)**
	15	0.1mg/kg 74 1.0mg/kg 80	86, 71, 94 (84)	107, 89, 118 **(105)**
	19	0.1mg/kg 96 1.0mg/kg 95	80, 85, 81 (82)	84, 89, 85 **(86)**
	24	0.1mg/kg 101 1.0mg/kg 84	107, 88, 101 (99)	127, 105, 120 **(117)**
	31	0.1mg/kg 79 1.0mg/kg 79	85, 83, 81 (83)	108, 105, 103 **(105)**

[1] Average recoveries are given in brackets. Average Relative Recoveries are written in bold. Values in this table have been rounded for reporting purposes.

[2] Relative recovery = $\frac{\text{Stored Recovery}}{\text{High Procedural Recovery}} \times 100$

Table 6.1-5: Summary of Storage Stability Data for CL 376991 in Wheat Matrices frozen up to 31 Months

Matrix	Storage Interval (Months)	CL 376991		
		Procedural Recovery (%)	Stored Recovery (%)[1]	Relative Recovery (%)[2]
Wheat Whole Plant	1	0.1mg/kg 102 1.0mg/kg 99	102, 96, 98 (99)	103, 97, 99 **(100)**
	7	0.1mg/kg 98 1.0mg/kg 103	102, 107, 99 (103)	99, 104, 97 **(100)**
	15	0.1mg/kg 103 1.0mg/kg 105	100, 100, 102 (101)	95, 95, 97 **(96)**
	19	0.1mg/kg 95 1.0mg/kg 97	95, 86, 98 (93)	98, 89, 101 **(96)**
	24	0.1mg/kg 105 1.0mg/kg 98	98, 87, 88 (91)	100, 89, 90 **(93)**
	31	0.1mg/kg 94 1.0mg/kg 92	75, 75, 105 (85)	82, 82, 114 **(93)**
Wheat Straw	3	0.1mg/kg 90 1.0mg/kg 91	99, 97, 99 (98)	109, 107, 109 **(108)**
	7	0.1mg/kg 91 1.0mg/kg 92	99, 105, 94 (99)	107, 114, 102 **(108)**
	15	0.1mg/kg 93 2.0mg/kg 86	86, 89, 91 (89)	101, 103, 106 **(103)**
	19	0.1mg/kg 92 1.0mg/kg 91	87, 94, 90 (90)	96, 103, 99 **(99)**
	24	0.1mg/kg 87 1.0mg/kg 90	89, 107, 85 (94)	99, 119, 94 **(104)**
	31	0.1mg/kg 79 1.0mg/kg 87	96, 88, 94 (93)	110, 101, 108 **(106)**
Wheat Grain	2	0.1mg/kg 84 1.0mg/kg 80	64, 75, 93 (77)	80, 93, 116 **(96)**
	7	0.1mg/kg 91 1.0mg/kg 87	88, 100, 99 (96)	102, 115, 114 **(110)**
	15	0.1mg/kg 80 1.0mg/kg 86	85, 76, 99 (87)	99, 88, 115 **(101)**
	19	0.1mg/kg 92 1.0mg/kg 93	79, 84, 82 (82)	85, 90, 88 **(88)**
	24	0.1mg/kg 103 1.0mg/kg 89	101, 79, 96 (92)	113, 89, 108 **(103)**
	31	0.1mg/kg 76 1.0mg/kg 78	84, 74, 72 (77)	108, 95, 92 **(98)**

[1] Average recoveries are given in brackets. Average Relative Recoveries are written in bold. Values in this table have been rounded for reporting purposes.

[2] Relative recovery = $\frac{\text{Stored Recovery}}{\text{High Procedural Recovery}} \times 100$

III. CONCLUSION

Results from the study showed that recoveries from samples stored deep frozen for up to 31 months were stable in terms of both parent material and its metabolites in wheat grain, wheat straw and whole plant.

Report:	CA 6.1/6 Lehmann A.,Mackenroth C., 2012a Investigation of the storage stability of BAS 560 F in plant matrices 2012/1166088
Guidelines:	EEC 7032/VI/97 rev. 5, 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), EPA 860.1380
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The deep freeze stability of metrafenone in plant matrices was investigated over a period of two years. Samples were spiked with the test item at concentration levels of 0.1 mg/kg. The spiked samples were stored under the usual storage conditions for field samples (polyethylene containers, about -20°C) and analysed after different intervals. Samples were analysed with BASF method no. L0076/01 (also referred to as 535/1) with a limit of quantitation of 0.01 mg/kg. Procedural recoveries averaged at 93%. After a storage time of about two years, metrafenone was shown to be stable in the matrices investigated wheat (whole plant, grain and straw), grape, tomato, dried pea and soyabean seed.

I. MATERIAL AND METHODS

A. MATERIALS

1.

Test Material:	Metrafenone
Description:	Active substance
Lot/Batch #:	01814-189
Purity:	99.7%
CAS#:	220899-03-6
Development code:	BAS 560 F
Spiking levels:	0.1 mg/kg

2.

Test Commodity:	
Crop:	Wheat, grapes, tomato, pea, soybean
Type:	not specified
Variety:	not specified (not relevant)
Botanical name:	*Triticum spp., Vitis vinifera, Solanum lycopersicum, Pisum sativum, Glycine max*
Crop parts(s) or processed commodity:	Wheat: whole plant, grain, straw Grape: fruit Tomato: fruit Pea: dried pea seed Soybean: soybean seed
Sample size:	5g (stored sample)

B. STUDY DESIGN AND METHODS

1. Test procedure

Plant materials were spiked with a concentration equivalent to 10 x of the limit of quantitation (LOQ = 0.01 mg/kg) and stored at about -20°C in the dark. According to the Guidelines, storage stability studies can either be performed with treated field samples or with control samples fortified with the compound of interest. Samples of respective materials with residue concentrations high enough to monitor a potential decline of the analyte were not available, therefore spiked samples were used in this study.

2. Description of analytical procedures

The specimens were analyzed using BASF method L0076/01 (Validation of BASF Method No. 535/1 in plant matrices; A Lehmann, Ch Mackenroth BASF Doc ID 2006/1039426) which had a limit of quantification of 0.01 mg/kg for metrafenone. Samples of each matrix were fortified at 0.1 mg/kg (10X LOQ). Mean procedural recoveries were 85.1, 97.3, 88.0, 89.8, 90.3, 98.9 and 99.8% for wheat (whole plant), wheat grain, wheat grain, wheat straw, grape, tomato, dried peas and soybean respectively. The overall mean procedural recovery for all matrices was 93.2% (RSD 9.0%).

II. RESULTS AND DISCUSSION

Results from the analysis of samples stored for two years showed recoveries of residues for all matrices investigated ranged from 72 to 107% compared to 77 to 107% for freshly spiked samples.

Table 6.1-6: Storage stability of metrafenone in range of plant samples

Mean recovery (%)														
Days	A: mean in stored samples, % nominal								B: mean procedural, in freshly spiked samples					
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Wheat wh. plant		Wheat grain		Wheat straw		Grape fruit		Tomato fruit		Dried pea seed		Soyabean seed	
0	86	83	107	107	91	89	89	88	89	85	104	99	98	100
28-32	73	78	78	91	90	90	77	81	88	90	90	96	72	96
55													78	93
84													89	106
85													82	102
174-178	83	86	83	97	84	89	97	99	101	101	96	104	60	99
358-360	80	80	85	102	91	95	106	96	75	97	90	100	72	100
455									76	77				
726-739	85	99	77	90	78	78	83	84	96	91	83	96	98	103

Table 6.1-7: Mean storage stability of metrafenone in range of plant samples (mean % recovery corrected for procedural recovery)

Months (approx.)	Wheat wh. plant	Wheat grain	Wheat straw	Grape fruit	Tomato fruit	Dried pea seed	Soybean seed
0	104	100	102	101	105	105	98
1	94	86	100	95	98	94	75
2							84
3							80-84
6	97	86	94	98	100	92	61
12	100	83	96	110	77	90	72
15					99		
24	86	86	100	99	105	86	95

As soyabean seed showed degradation near 70% after 28 days, additional samples were analysed at 55, 84 and 85 days. Stability was confirmed using these additional samples.

III. CONCLUSION

After storage of about two years metrafenone was shown to be stable in all matrices investigated, wheat (whole plant, grain and straw), grape, tomato, dried pea and soybean seeds.

Summary of storage stability of residues

Studies have been carried out assessing the stability of residues following storage under frozen conditions. Metrafenone residues were shown to be stable for at least 31 months in wheat green material, grain and straw (high starch content), at least 24 months in grapes and tomato (high water content), soyabean seed (high oil content) and dried pea seed (high protein content), at least 18 months in wine (processed product) and at least 3 months in raisins and yeast.

Residues of metrafenone metabolites CL 3000402, CL 434223 and CL 376991 were shown to be stable for at least 31 months in wheat green material, grain and straw (high starch content).

No studies assessing the stability of residues in products of animal origin were carried out since livestock feeding studies were not required.

CA 6.2 Metabolism, distribution and expression of residues

The metabolism of metrafenone in plants, livestock and rotational crops has been previously reported in the DAR (UK, July 2005). The general plant metabolism is understood from previous submissions, but it has been extended with submission of a new cucumber metabolism study which confirms the parent only residues definition.

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	Cereals and grapevine
Rotational crops	Lettuce, radish, canola (replant intervals 30, 60, 90, 365)
Plant residue definition for monitoring	Metrafenone
Plant residue definition for risk assessment	Metrafenone
Conversion factor (monitoring to risk assessment)	Not applicable

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	Goat, hen
Animal residue definition for monitoring	Not proposed by RMS
Animal residue definition for risk assessment	Not proposed by RMS[19]
Conversion factor (monitoring to risk assessment)	Not applicable
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Not applicable

[19] should be defined for ruminant liver and kidney

This conclusion was subsequently confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498).

"Parent metrafenone is the most important compound in the residue for all crops. The metabolites identified in treated plants (grape leaves only) were oxidation products of the parent compound. In several crop parts, a significant degradation to polar components was also observed and the available metabolism studies did not indicate any evidence of a molecular cleavage of the parent compound. It is concluded that similar metabolism of metrafenone can be assumed in the two crop categories investigated (cereals and fruits and fruiting vegetables).
Based on the above finding, EFSA already concluded that the residue definition for enforcement and risk assessment can be set as metrafenone only (EFSA, 2006). Validated analytical methods for enforcement of the proposed residue definition are available (see also section 1.1). However, the proposed residue definition is restricted to cereals and fruits and fruiting vegetables and no general residue definition for commodities of plant origin can be proposed. It is also noted that metrafenone is authorised for application on cultivated fungi. Although, according to the current guidance documents, the metabolism study on fruits and fruiting vegetables is considered acceptable for cultivated fungi, this extrapolation is uncertain from a scientific point of view. A metabolism study on a third crop group is therefore considered desirable (minor deficiency) as this would provide more certainty on the expectation that the metabolic pattern will not differ in fungi."

"The available metabolism studies on ruminants indicate that metrafenone residues are not expected to occur in significant amounts in muscle, fat and milk. However, an extensive metabolism of the parent compound was observed in liver and kidney where residue levels were higher. The several identified metabolites mainly result from glucoronide conjugation of the trimethoxyphenyl ring. Several hydroxylations on different positions of both rings also occur, leading to the presence of a wide range of similar metabolites. These metabolites are structurally similar to those found in the rat metabolism study. As observed in rodent, the bond between the bromophenyl ring and the trimethoxyphenyl ring remained intact, leading to the conclusion that no cleavage of the parent compound occurs in animals. Out of these metabolites, CL 1500698 and CL 1023363 constitute the main significant part of the residue in liver and kidney while no predominant compound could be identified in muscle, fat and milk (also due to insignificant residue levels in these matrices). Moreover, CL 1500698 and CL 1023363 were both observed in rats and their toxicity is considered to be covered by toxicity data for the parent compound. Hence the relevant residue for enforcement and risk assessment in ruminant commodities should be defined as the sum of CL 1500698 and CL 1023363, expressed as metrafenone.

It is noted that assuming the current dietary burden, the maximum expected residue levels, including all relevant metabolites, would be 0.007 mg/kg (liver), which is not significant (see also section 3.2.3). Consequently, in order to simplify the enforcement of residue levels in livestock commodities, risk managers may also decide to restrict the residue definition to parent compound only (by default). As no residue above 0.01 mg/kg are expected anyhow, this proposal would not change the outcome of the risk assessment. However, risk managers should take into consideration that a default residue definition including parent compound only would not allow identification of misuses or illegal uses of metrafenone on feed crops (e.g. cereals straw). Moreover, regardless of the option selected, validated analytical methods for enforcement of the residue are not available for all relevant matrices and are therefore still required (see also section 1.2).

The available metabolism study on hens did not allow EFSA to conclude on a potential residue definition in poultry. Nevertheless since the calculated dietary burden of poultry to metrafenone residues amounted to less than 0.1 mg/kg DM, there is no need to establish a residue definition in this livestock group. If in the future the use metrafenone would be extended resulting in significant dietary burden of livestock for poultry, a suitable residue definition for enforcement and risk assessment would be further discussed. In the framework of this review no further consideration on the metabolism in poultry is required.

Parent metrafenone is fat soluble (Log Po/w: 4) (EFSA, 2006). However, residues in animal commodities are mainly represented by metabolites CL 1500698 and CL 1023363 and parent compound is completely degraded. The relevant metabolites (conjugates) are expected to be less fat soluble than parent metrafenone. Moreover, they were only present in liver and kidney. Consequently, EFSA concluded that the proposed residue definition for commodities of animal origin (sum of CL 1500698 and CL 1023363, expressed as metrafenone) should not be considered as fat soluble."

It was subsequently agreed that it was appropriate for the residues definition for products of animal origin for both monitoring and risk assessment to be parent only based on the predicted dietary burden of metrafenone and the very low levels of these metabolites predicted to occur in liver and kidney (measured as total TRR for both compounds plus an additional unknown compound). Refer to proposal in SANCO/11973/2014.

CA 6.2.1 Metabolism, distribution and expression of residues in plants

Report: CA 6.2.1/1
Zulalian J., 2002a
BAS 560 F (AC 375839): Metabolism of carbon-14 labeled AC 375839 in wheat under field conditions
2002/7005253
Guidelines: EPA 860.1300, EEC 91/414 Annex II (Part A Section 6.1), EEC 96/68, EPA 40 CFR 158.240
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). Three applications of radiolabelled-metrafenone were made to wheat at the Zadoks growth stages 32, 55 and 70 at nominal dose rates of 300, 300 and 200 g a.s./ha with a spray interval of approximately 14 days, and a pre-harvest interval (PHI) of 35 days. The total application rate of 800 g a.s./ha, corresponds to a 2.4-2.5-fold exaggerated dose rate, based on the proposed good agricultural practice (GAP) of 2 x 150 g a.s./ha (or 300 g a.s./ha) for metrafenone in cereals. The key points from the study are tabulated below:

Table 6.2.1-1: Design of metrafenone application

	Treatment Groups	
Position of radiolabel	[Bromophenyl]	[Trimethoxyphenyl]
Number of applications	3	3
Nominal application rates (g as/ha)	300, 300, 200	300, 300, 200
Interval between applications (days)	13-14	13-14
Growth stage at application (Zadoks)	32, 55, and 70	32, 55, and 70
Actual applied total dose (g as/ha)	756	719.3
Comparison to the maximum recommended use rate (2 x 150 g as/ha)	2.5 x	2.4 x
PHI (pre-harvest interval, i.e., days after last treatment)	35 (grain, straw, chaff)	35 (grain, straw, chaff)

Table 6.2.1-2: Total Radioactive Residues (TRRs) in Wheat Samples Following Application of [Bromophenyl-6-^{14}C] metrafenone and [Trimethoxyphenyl-U-^{14}C] metrafenone

		Treatment Groups			
Position of radiolabel		**[Bromophenyl]**		**[Trimethoxyphenyl]**	
Actual Applied Total Dose (g as/ha)		**756**		**719.3**	
Number of Applications		**3**		**3**	
Sample Type	Sampling Time (DAT) [a]	Cumulative dose g as/ha	TRR mg/kg	Cumulative dose g as/ha	TRR mg/kg
Immature whole plant	0DAT1	293.7	7.275	270.9	9.890
Forage	3DAT1	293.7	8.167	270.9	5.265
Immature whole plant	0DAT2	560.4	5.959	533.1	8.416
Hay	14DAT2	560.4	7.784	533.1	8.498
Immature whole plant	0DAT3	756	6.997	719.3	5.382
Mature Straw	35DAT3	756	8.914	719.3	8.248
Mature Grain	35DAT3	756	0.209	719.3	0.399

[a] DAT denotes days after treatment; for example, 3DAT1 denotes three days after treatment one.

Table 6.2.1-3: Extraction of Wheat Commodity Derived from [^{14}C] metrafenone

Radiolabel		**[Bromophenyl-6-^{14}C]**				**[Trimethoxyphenyl-U-^{14}C]**			
Raw Agricultural Commodity		**Forage**	**Hay**	**Straw**	**Grain**	**Forage**	**Hay**	**Straw**	**Grain**
DAT (Days After Treatment)		**3DAT1**	**14DAT2**	**35DAT3**	**35DAT3**	**3DAT1**	**14DAT2**	**35DAT3**	**35DAT3**
TRR	%	100	100	100	100	100	100	100	100
	(mg/kg)	(8.167)	(7.784)	(8.914)	(0.209)	(5.265)	(8.498)	(8.248)	(0.399)
Main Extracts									
Hexane	%	NC	NC	NC	6.4	NC	NC	NC	3.5
	(mg/kg)				(0.013)				(0.014)
MeOH/H_2O	%	96.6	79.2	60.3	35.8	92.6	77.0	62.0	34.7
	(mg/kg)	(7.889)	(6.168)	(5.387)	(0.075)	(4.877)	(6.545)	(5.109)	(0.138)
MeOH/2% HCl	%	NC	NC	NC	8.0	NC	NC	NC	8.0
	(mg/kg)				(0.017)				(0.032)
ERR [b]	%	96.6	79.2	60.3	50.2	92.6	77.0	62.0	46.2
	(mg/kg)	(7.889)	(6.168)	(5.387)	(0.105)	(4.877)	(6.545)	(5.109)	(0.184)
RRR [c]	%	3.4	20.8	39.6	49.8	7.4	23.0	38.1	53.8
	(mg/kg)	(0.278)	(1.615)	(3.527)	(0.104)	(0.388)	(1.952)	(3.139)	(0.215)

[a] DAT denotes Days After Treatment; for example, 3DAT1 denotes three days after first treatment.
[b] ERR = Extractable Radioactive Residue (sum of all extractable fractions).
[c] RRR = Residual Radioactive Residue (non extractable, post-extracted solids, PES).
NC = Extraction not conducted.

Table 6.2.1-4: Summary of the Quantification and Identification of TRR in Wheat Forage, Hay, Straw and Grain Derived from [Bromophenyl-6-^{14}C]metrafenone

Commodity		% TRR and mg/kg equivalents			
		Forage	Hay	Straw	Grain
Residue Fractions	Sampling Time	3DAT1	14DAT2	35DAT3	35DAT3
	TRR	% mg/kg	% mg/kg	% mg/kg	% mg/kg
	TRR in crop sample	100 8.167	100 7.784	100 8.914	1000.209
	TRR in the extract	96.67.889	79.26.168	60.35.387	35.8 [a]0.075
I. MeOH:H_2O [a]	Components in extracts	% mg/kg	% mg/kg	% mg/kg	% mg/kg
	ROI 1 (Unknowns)[b]	4.3 0.355	15.01.165	8.3 0.740	15.20.045
	ROI 2 (Unknowns)[d]	3.9 0.320	11.50.896	10.50.934	<2.0<0.004
	ROI 3A (CL 1500837) + ROI 3B (CL 1500836)	4.2 0.342	3.7 0.291	4.6 0.412	<2.0<0.004
	ROI 4 (CL 3000402)	4.7 0.381	4.7 0.367	2.3 0.203	<2.0<0.004
	ROI 5 (CL 1500838), conjugate of ROI 8	1.1 0.091	4.2 0.328	3.2 0.289	<2.0<0.004
	ROI 6A (CL 1500839) + ROI 6B (CL 1500832)	1.6 0.132	3.7 0.289	1.5 0.132	<2.0<0.004
	ROI 7A1 (CL1500833) + ROI 7A2 (CL1500834) + ROI 7A3 (CL1500835) + ROI 7B (CL 377160) + ROI 7C (Unknown)	2.0 0.159	3.1 0.245	4.90.434	<2.0<0.004
	ROI 8 (CL 434223)	3.0 0.243	0.8 0.065	2.50.219	<2.0<0.004
	ROI 9 (CL 376991)	0.3 0.024	0.4 0.033	0.70.062	<2.0<0.004
	ROI 10 (CL 1500831)	1.4 0.114	1.5 0.119	0.70.066	<2.0<0.004
	ROI 11 (Unknowns)	0.8 0.067	1.4 0.107	0.20.020	<2.0<0.004
	ROI 12 (BAS 560 F)	64.45.261	26.02.021	13.61.215	7.70.016
II. α-Amylase	ROI 1 (Unknowns)[e]	Not analysed	Not analysed	Not analysed	NQ [c]
Total Identified		91.77.489	76 5.926	53.04.726	42.90.093
III. PES	Unknown	3.4 0.278	20.81.615	39.63.527	49.80.104

[a] For grain sample, combined extractable residues from methanol:water, hexane/acetonitrile partition, and 2% HCl/methanol.
[b] Consisting of at least seven minor unknown components (individually 0.001-0.02 mg/kg).
[c] NQ denotes Not Quantified. However, of the radioactivity released from α-amylase treatment of PES-2, 59.1% of the HPLC radioactivity is attributed to ROI 1, which is composed of multiple components. Parent and other ROIs were each detected at < 2.0% TRR (< 0.004 mg/kg).
[d] Consisting of at least six minor unknown components (individually 0.07-0.23 mg/kg).
[e] Consisting of at least five minor unknown components

Table 6.2.1-5: Summary of the Quantification and Identification of TRR in Wheat Forage, Hay, Straw and Grain Derived from [Trimethoxyphenyl-U-14C]BAS 560 F

Commodity		% TRR and ppm equivalents			
		Forage	Hay	Straw	Grain
Residue Fractions	Sampling Time	3DAT1	14DAT2	35DAT3	35DAT3
	TRR	% mg/kg	% mg/kg	% mg/kg	% mg/kg
	TRR in Crop Sample	100 5.265	100 8.498	100 8.248	1000.399
	TRR in the extract	92.64.877	77.06.545	62.05.109	38.20.152
I. MeOH:H_2O [a]	Components in extracts	% mg/kg	% mg/kg	% mg/kg	% mg/kg
	ROI 1 (Unknowns)[b]	3.9 0.203	17.41.478	9.1 0.751	20.30.081
	ROI 2 (Unknowns)[d]	3.1 0.163	12.5 1.061	10.60.876	<2.0<0.004
	ROI 3A (CL 1500837) + ROI 3B (CL 1500836)	4.5 0.238	4.7 0.403	5.0 0.410	<2.0<0.004
	ROI 4 (CL 3000402)	4.2 0.219	6.6 0.56 4	3.9 0.319	<2.0<0.004
	ROI 5 (CL 1500838), conjugate of ROI 8	2.0 0.103	5.0 0.429	4.1 0.338	<2.0<0.004
	ROI 6A (CL 1500839) + ROI 6B (CL 1500832)	2.4 0.127	5.3 0.446	2.3 0.194	<2.0<0.004
	ROI 7A1 (CL1500833) + ROI 7A2 (CL1500834) + ROI 7A3 (CL1500835) + ROI 7B (CL 377160) + ROI 7C (Unknown)	2.6 0.136	3.2 0.275	6.5 0.540	<2.0<0.004
	ROI 8 (CL 434223)	3.4 0.177	1.2 0.105	2.3 0.189	<2.0<0.004
	ROI 9 (CL 376991)	0.2 0.011	0.4 0.033	0.2 0.016	<2.0<0.004
	ROI 10 (CL 1500831)	0.4 0.021	1.8 0.153	0.6 0.046	<2.0<0.004
	ROI 11 (Unknowns)	0.9 0.046	1.7 0.147	0.3 0.023	<2.0<0.004
	ROI 12 (BAS 560 F)	58.83.101	12.71.078	7.7 0.635	3.10.013
II. α-Amylase	ROI 1 (Unknowns)[b,e]	Not analysed	Not analysed	Not analysed	NQ
Total Identified		86.34.536	72.56.171	52.64.337	43.00.133
III. PES	Unknown	7.4 0.388	23.01.952	38.13.139	53.60.214

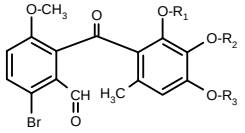
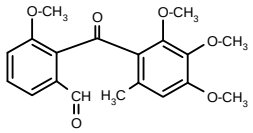
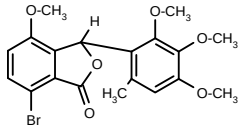
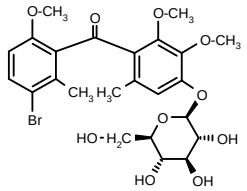
[a] For grain sample, combined extractable residues from methanol:water, hexane/acetonitrile partition, and 2% HCl/methanol.
[b] Consisting of at least six minor unknown components (individually 0.001-0.07 mg/kg)
[c] NQ denotes Not Quantified. However, of the radioactivity released from α-amylase treatment of PES-2), 74.0% of the HPLC radioactivity is attributed to ROI 1, which is composed of multiple components. Parent and other ROIs were each detected at < 2.0% TRR (< 0.004 mg/kg).
[d] Consisting of at least five minor unknown components (individually 0.03-0.12 mg/kg).
[e] Consisting of at least five minor unknown components

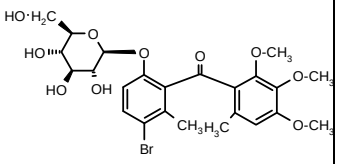
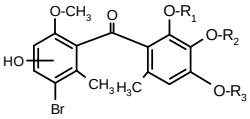
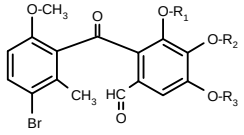
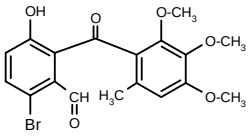
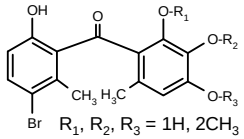
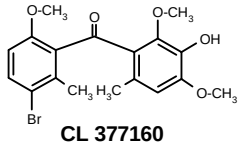
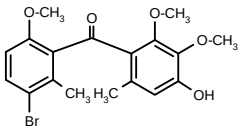
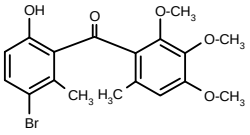
In the outdoor wheat metabolism study with [^{14}C] metrafenone, the highest residues were found in the straw (8.248 mg/kg - 8.914 mg/kg), with the lowest residues found in grain (0.209 - 0.399 mg/kg). The TRR in forage (3DAT1) amounted to 5.265 - 8.167 mg/kg. The TRR in hay (14DAT2) amounted to 7.784 - 8.498 mg/kg.

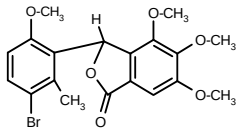
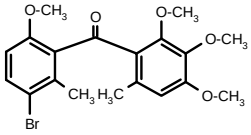
The major residue component in the forage, hay, straw and grain was identified as the parent metrafenone. The incurred total radioactive residues in straw included metrafenone, the lactone CL 3000402, and the mono-hydroxylated metabolites CL 434223 plus glucose conjugate, and CL 376991 plus glucose conjugates. Of the incurred residues in grain, metrafenone amounted for 0.013 - 0.016 mg/kg. ROI 1, which contained multiple, unknown components, accounted for 0.045 mg/kg - 0.081 mg/kg. All other individual components of the extractable residues in grain were <0.004 mg/kg.

In this study, the total application rate of 756 or 719.3 g as/ha, corresponds to a 2.5 or 2.4-fold exaggerated dose rate, based on the proposed good agricultural practice (GAP) of 2 x 150 g as./ha (or 300 g as./ha) for metrafenone in cereals. Thus, the potential maximal residue levels in each raw agricultural commodity under GAP could be calculated as shown below.

Table 6.2.1-6: Scaled Potential Maximum Residue Levels after Treatment of Wheat with 14C-BAS 560 F (AC 375839) for the Anticipated Maximum Use Rate of 2 x 150 g as/ha.

Residue Components	Chemical Structure	Maximal likely residue level (mg/kg) at 1x Maximum Proposed Use Rate (2 x 150g as/ha)			
		Forage	Hay	Straw	Grain
CL 1500837 + CL 1500836	 R_1, R_2, R_3 = 1H, $2CH_3$ **CL 1500837**  **CL 1500836**	0.143	0.168	0.172 (5-6 peaks comprising metabolites in the range of <0.01-0.07 mg/kg)	<0.004
CL 3000402		0.159	0.235	0.133	<0.004
CL 1500838, Glucose conjugate of CL 434223		0.043	0.179	0.141	<0.004

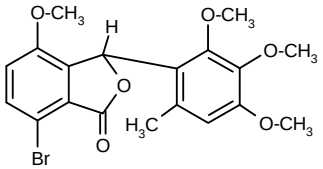
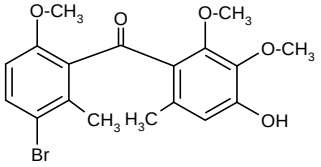
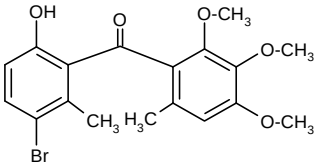
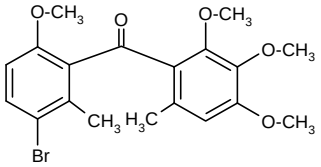
Residue Components	Chemical Structure	Maximal likely residue level (mg/kg) at 1x Maximum Proposed Use Rate (2 x 150g as/ha)			
		Forage	Hay	Straw	Grain
CL 1500839 + CL 1500832	 **CL 1500839**  R_1, R_2, R_3 = 1H, $2CH_3$ **CL 1500832**	0.055	0.186	0.081 (3 peaks comprising <0.01-0.04 mg/kg)	<0.004
CL 1500833 + CL 1500834 + CL 1500835 + CL 377160 + ROI 7C (Unknown)	 R_1, R_2, R_3 = 1H, $2CH_3$ **CL 1500833**  **CL 1500834**  R_1, R_2, R_3 = 1H, $2CH_3$ **CL 1500835**  **CL 377160**	0.066	0.115	0.225 (5 peaks comprising <0.01-0.09 mg/kg)	<0.004
CL 434223		0.101	0.044	0.091	<0.004
CL 376991		0.010	0.014	0.026	<0.004

Residue Components	Chemical Structure	Maximal likely residue level (mg/kg) at 1x Maximum Proposed Use Rate (2 x 150g as/ha)			
		Forage	Hay	Straw	Grain
CL 1500831		0.048	0.064	0.028	<0.004
metrafenone (AC 375839)	 BAS 560F (CL 375839)	2.192	0.842	0.506	0.007

Since the use of metrafenone is intended for cereals for grain production, no further consideration of metabolites in forage or hay are considered necessary (refer to the Minutes from the Pesticide Steering Committee, 19-20 June 2014;
http://www.efsa.europa.eu/sites/default/files/event/140619-m.pdf).

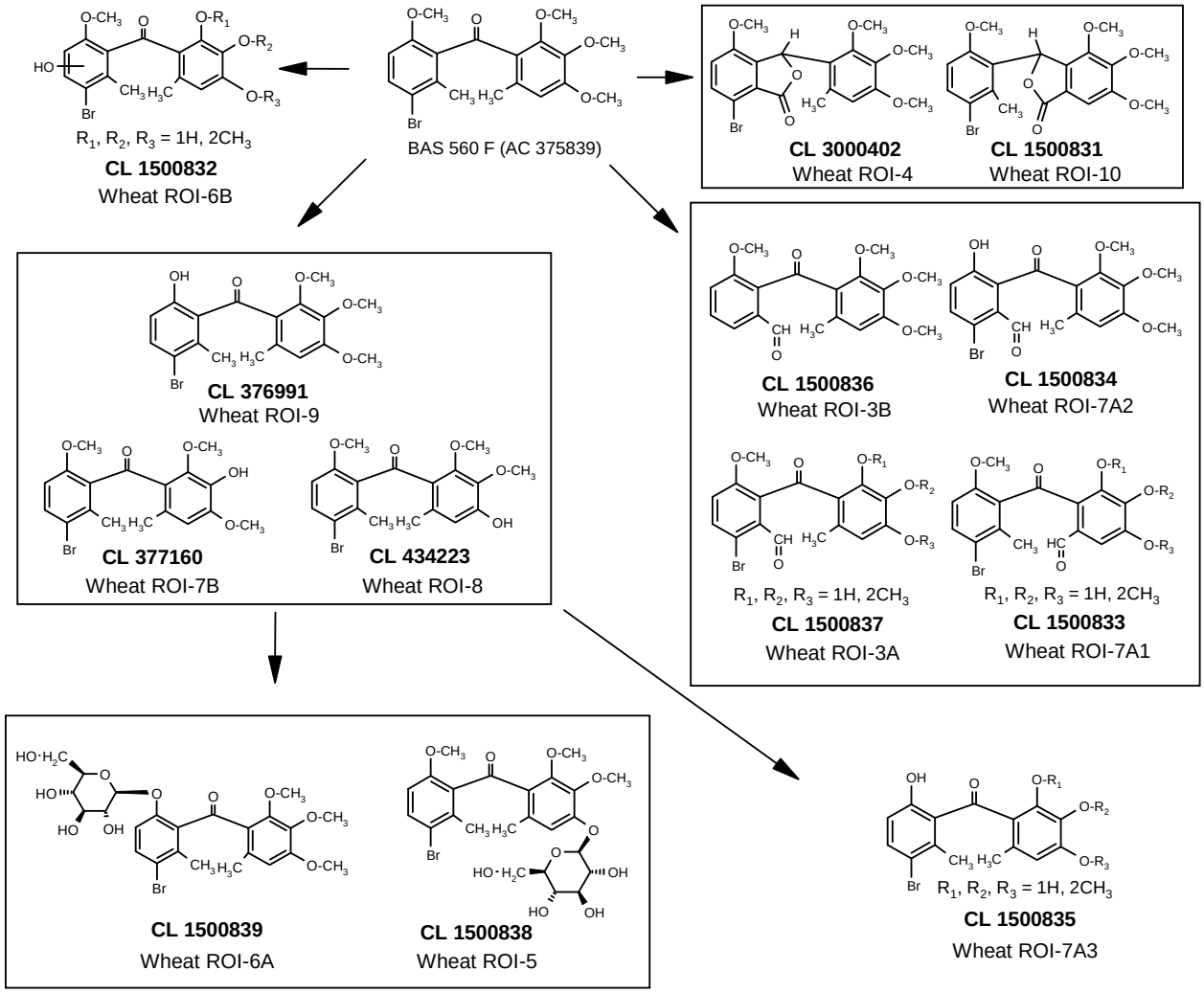
Metrafenone was the major component in all commodities. In grain, only the unknown ROI1 was present at >10% TRR. This unknown consisted of 6-7 compounds with one of these compounds being present at 0.03 mg/kg (1N rate equivalent); other compounds occurred at <<0.01 mg/kg. (1N rate equivalent). No other metabolites were detected. In straw, only the unknown ROI2 occurred at >10% TRR. This unknown consisted of 5-6 compounds with one of these compounds being present at 0.12 mg/kg (1N rate equivalent); other compounds occurred at 0.03-0.08 mg/kg. (1N rate equivalent). Both ROI1 and ROI2 were polar metabolites based on the chromatographic behaviour. Other metabolites were detected but only CL 3000402, CL 1500838 and CL 434223 occurred at levels ≥0.1 mg/kg at 1N rate *cf.* metrafenone which was detected at 0.506 mg/kg at 1N rate; metabolites were seen in rat metabolism (CL 3000402 was detected in the faeces, CL 434223, and its glucose conjugates (CL 1500838) were found in the bile and urine as glucuronides (CL 1500699) of their corresponding hydroxy-analogs.

Structures of Principal Components of Incurred Residues in Wheat

ROI	4 (CL 3000402)	8 (CL 434223)
Structure		
ROI	9 (CL 376991)	12 (BAS 560 F)
Structure		

In summary, based on the structures of metabolites that were identified and characterized in wheat plants, the metabolism of metrafenone in cereals first undergoes phase I biotransformations. These biotransformations proceed through a single oxidative de-methylation to form CL 434223, CL 376991, and CL 377160; followed by a second oxidative de-methylation to form CL 1500835. The lactones CL 3000402 and CL 1500831 are formed by oxidation at each benzylic carbon followed by cyclization. Oxidation of the methyl groups on either of the two phenyl rings gives rise to the aldehyde derivatives, CL 1500833, CL 1500834, and CL 1500837. Aromatic oxidation and oxidative de-methylations can produce CL 1500832. Additionally, CL 1500836 is formed by oxidation at the benzylic carbon of the bromophenyl ring followed by reductive de-bromination. The oxidations are proposed to be either enzymatic and/or chemical (photolysis) in nature. These phase I processes are then followed by phase II transformation reactions, i.e., enzymatic glucoside conjugation reactions to yield the conjugates CL 1500838 and CL 1500839 from their corresponding aglycones, CL 434223 and CL 376991. Thus, these results show that the benzophenone backbone of metrafenone remains intact without cleavage at the carbonyl group. Rather, the metabolites are derived from metrafenone through various reactions occurring on the ring systems. The proposed metabolic pathway of metrafenone in wheat is shown below.

Figure 6.2.1-1: Proposed Metabolic Pathway of [^{14}C] metrafenone in Wheat



Report: CA 6.2.1/2
Schlueter H.,Class T., 2001a
AC 375839: Metabolism of carbon-14 labeled AC 375839 in grapevines
2001/7000342
Guidelines: EEC 91/414 Annex II (Part A Section 6.1), EEC 96/68, EPA 860.1300
GLP: yes
(certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). An outdoor study on the metabolism of metrafenone in grapevines was conducted in Schwabenheim, Germany, during the 1998 growing season. The grapevines (variety: Kerner) were treated with five spray applications of [^{14}C]-metrafenone at a nominal dose rate of 200 g a.s/ha per application and a spray interval of 10-11 days. The total dosage corresponds to a *ca.* 2-fold exaggerated use rate in vines as compared with the proposed GAP of 3 x 160 g a.s./ha.

Table 6.2.1-7: Total Radioactive Residue (TRR) in Foliage and Bunches of Grapevines, expressed as mg active substance equivalents / kg sample material (mg/kg)

Sampling Date	Bromophenyl label		Trimethoxyphenyl label	
	Grapes	Leaves	Grapes	Leaves
0DAT1	0.431	30.67	0.552	21.92
11DAT1	1.065	25.90	0.471	20.41
22DAT1	0.386	42.58	0.326	37.43
32DAT1	0.241	59.22	2.102	52.41
42DAT1	0.768	39.82	0.604	42.33
61DAT1	0.314	59.11	0.150	55.48
77DAT1	0.442	38.13	0.275	24.74

DAT = Days After Treatment.

Total Radioactive Residue (TRR) in Grapes, expressed as mg active substance equivalents / kg sample material (mg/kg)

Table 6.2.1-8: A. Bromophenyl ring label

Sample	Juice		Extractable Residue		Nonextractable Residue	
	mg/kg [a]	% TRR	mg/kg	% TRR	mg/kg	% TRR
0DAT1	na	na	0.361	83.8	0.070	16.2
11 DAT1	0.031	2.9	0.967	90.7	0.068	6.4
22DAT1	0.024	6.3	0.317	82.1	0.045	11.6
32DAT1	0.021	8.7	0.178	73.8	0.042	17.4
42DAT1	0.054	7.1	0.690	89.9	0.023	3.0
61DAT1	0.060	19.2	0.201	63.9	0.053	16.9
77DAT1	0.080	18.0	0.277	62.6	0.086	19.4

Table 6.2.1-9: B. Trimethoxyphenyl ring label

Sample	Juice		Extractable Residue		Nonextractable Residue	
	mg/kg [a]	% TRR	mg/kg	% TRR	mg/kg	% TRR
0DAT1	na	na	0.550	99.7	0.002	0.3
11DAT1	0.012	2.6	0.408	86.7	0.050	10.7
22DAT1	0.019	5.8	0.264	81.1	0.043	13.1
32DAT1	0224	10.7	1.384	65.9	0.493	23.5
42DAT1	0.075	12.5	0.413	68.3	0.116	19.3
61DAT1	0.049	32.6	0.086	57.5	0.015	9.8
77DAT1	0.070	25.3	0.174	63.1	0.032	11.6

DAT = Days After Treatment. **a** = related to weight of grapes. **na** = not applicable.

TLC analysis of the grape extracts (42DAT1) showed that parent was the major metabolic product ((53.3% TRR (0.412 mg/kg) and 40.67 mg/kg TRR (0.246 mg/kg)) of radioactivity for the bromophenyl and trimethoxyphenyl labels, respectively. .

Due to the high sugar content and low radioactivity found in juice, direct chromatography was not possible. Radioactivity was partitioned sequentially with organic solvents (ethyl acetate and toluene) which extracted 54.7% (9.9% TRR, 0.044 mg/kg) of the juice radioactivity from the aqueous layer. No parent compound was detected in the organosoluble fraction. Radioactivity in the juice comprised multiple polar metabolites none of which exceeded 4% TRR (0.003 mg/kg) or 9% TRR (0.006 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. CL 197675 was tentatively identified at a maximum of 9% TRR (0.006 mg/kg) in the bromophenyl labelled samples.

Parent was the major radioactive residue detected in extracts of the marc from mature grapes (77DAT1) accounting for 24.7% TRR (0.109 mg/kg) and 22.8% TRR (0.063 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. Polar metabolites comprising multiple components were detected at 12% TRR ($\leq$0.05 mg/kg) 17% TRR ($\leq$ 0.046 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively.

Total Radioactive Residue (TRR) in Leaves, expressed as mg active substance equivalents / kg sample material (mg/kg)

Table 6.2.1-10: A. Bromophenyl ring label

Sample	Extractable Residue		Nonextractable Residue	
	mg/kg	% TRR	mg/kg	% TRR
0DAT1	30.64	99.9	0.024	0.1
11 DAT1	23.88	92.2	20.028	7.8
22DAT1	37.94	89.1	4.638	10.9
32DAT1	54.31	91.7	4.909	8.3
42DAT1	31.34	78.7	8.479	21.3
61DAT1	45.68	77.3	13.43	22.7
77DAT1	33.11	86.9	5.016	13.2

Table 6.2.1-11: B. Trimethoxyphenyl ring label

Sample	Extractable Residue		Nonextractable Residue	
	mg/kg	% TRR	mg/kg	% TRR
0DAT1	21.90	99.8	0.018	0.1
11 DAT1	19.31	94.5	1.107	5.4
22DAT1	33.94	90.7	3.485	9.3
32DAT1	49.14	93.7	3.272	6.2
42DAT1	35.66	84.3	6.664	15.7
61DAT1	43.59	78.6	11.89	21.4
77DAT1	20.17	81.6	4.566	18.5

DAT = Days After Treatment. **a** = related to weight of grapes. **na** = not applicable.

Table 6.2.1-12: Recovery of Unchanged Parent metrafenone in Grapevine Leaves, expressed as mg / kg sample material (mg/kg) and % TRR

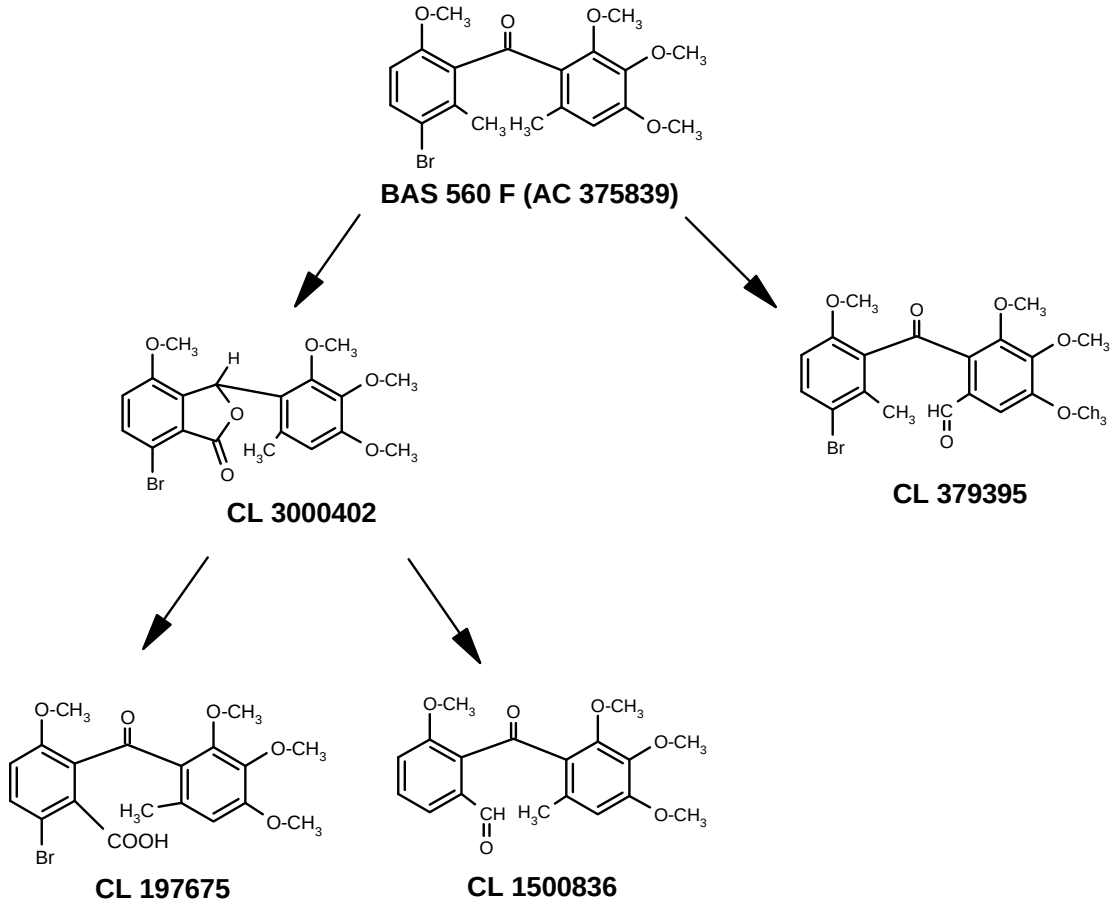
Sampling Date	Bromophenyl label		Trimethoxyphenyl label	
	mg/kg	% TRR	mg/kg	% TRR
0DAT1	26.6	96.9	20.3	92.4
11DAT1	9.6	36.9	10.6	52.2
22DAT1	15.6	36.6	13.9	37.2
32DAT1	20.0	33.7	17.1	32.7
42DAT1	14.2	35.6	14.2	33.5
61DAT1	8.4	14.2	9.8	17.6
77DAT1	5.8	15.2	2.7	11.0

DAT = Days After Treatment

Surface rinses and extracts of grape leaves were analysed by TLC showing parent was the major radioactive residue (86.9% TRR (26.6 mg/kg) or 92.4% TRR (20.3 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively, after the first application the level decreased to 33-37% TRR (ca. 14-20 mg/kg) and remained constant throughout the application period. Parent then decreased to 14% TRR TRR (8.4 mg/kg) and 17.6% TRR (9.8 mg/kg) at 61DAT1 and to 15.2% TRR (5.8 mg/kg) and 11% TRR (2.7 mg/kg) at harvest 77DAT1 from the bromophenyl and trimethoxyphenyl labels, respectively. CL 3000402, CL 1500836 and CL 379395 were also tentatively identified but were not accurately quantified.

Based on the structures of metabolites that were identified in grapevines, the metabolism of metrafenone in vines undergoes oxidative biotransformations. Metrafenone is metabolized readily by oxidation of either of the methyl groups on the bromophenyl and trimethoxyphenyl rings to yield the corresponding aldehydes. The aldehydic moiety on the bromophenyl ring can be further oxidized to the carboxylic acid or cyclized to form the lactone. A des-bromo aldehyde is formed by loss of the bromine. The proposed metabolic pathway for metrafenone in grapevines is presented below.

Figure 6.2.1-2: Proposed Metabolic Pathway of [^{14}C] metrafenone in Grapevines



Report:	CA 6.2.1/3 Grosshans F.,Ockert M., 2010a Metabolism of 14C-BAS 560 F in cucumber 2010/1054630
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), JMAFF 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)

Executive Summary

The metabolism of metrafenone was investigated in cucumber plants treated with U-^{14}C - trimethoxyphenyl-labelled metrafenone. The radiolabeled test item was applied in the BAS 560 02 F SC formulation to cucumbers (foliar) at an application rate of 2 x 200 g as/ha using a hand sprayer. The applications took place 17 and 3 days before harvest.

The highest levels of total radioactive residues (TRR) were found in cucumber "rest of plant" (17 DAT), at 8.807 mg/kg, followed by leaf (0 DAT) with 6.397 mg/kg. In fruit, considerably lower levels of radioactivity were found, accounting for 0.016 mg/kg at 14 DAT and for 0.051 mg/kg at 17 DAT. Residues in cucumber pulp and peel accounted for 0.013 and 0.263 mg/kg, respectively. The extractability of radioactive residues with methanol and water was very high (> 92 % TRR) for all cucumber matrices, except for fruit (14 DAT) where it was 89% TRR.

Because of the high extractability, the residual radioactive residues (RRR) after solvent extraction of cucumber matrices were generally very low (each below or equal to 11% TRR; fruit 14 DAT). Therefore, no further characterisation was carried out for the residual radioactive residues of the individual cucumber matrices.

Metrafenone was the only constituent identified in this metabolism study. The unchanged parent compound represented the main component in most cucumber matrices analysed. In the case of cucumber pulp (17 DAT) some highly polar components were detected at the same very low concentration range as the parent compound (each below or equal to 0.0016 mg/kg or 12.1 % TRR). In addition, further minor polar and medium polar metabolites at very low concentrations (each below or equal to 8.9% TRR or 0.0015 mg/kg) were characterized by their retention time behaviour in HPLC or by extractability with water.

In addition to methanol and water extraction used in the current metabolism investigation, all sampled cucumber matrices were extracted using a range of different solvents. Extractions with methanol/water 2N HCl and acetone/water released similar amounts of radioactive residues (>86 %). Extraction with n-heptane/acetone showed lower efficiencies for the matrices fruit (17 DAT), peel and rest of plant (17 DAT) and significantly lower efficiencies for the matrices fruit (14 DAT) and pulp (17 DAT). Lower extractability was observed in samples containing lower parent but higher polar fraction content. Due to the high solubility of metrafenone in non-polar solvents like acetone, a complete extraction of the parent compound would be expected for all matrices and solvents tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: parent
Lot/Batch #: 911-1101 (U-14C-trimethoxyphenyl): specific activity 6 MBq/mg
Purity: 99.7%
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels: not relevant

2. **Test Commodity:**
Crop: cucumber
Type: not relevant
Variety: Hokus
Botanical name: *cucumis sativus*
Crop parts(s) or processed commodity: whole cucumber, peel and pulp
Sample size: fruit or pulp: 300g; leaf: 30g; peel: 100g

3. **Soil:**

Peat plantation substrate

B. STUDY DESIGN AND METHODS

1. Test procedure

The cucumber metabolism study with ^{14}C-metrafenone was performed with one radiolabel (U-^{14}C -trimethoxyphenyl-label). The active substance was applied in the BAS 560 02 F SC formulation to cucumber (foliar) growing in 10 L pots at an application rate of 2 x 200 g a.s./ha using a hand sprayer.

Applications took place 17 and 3 days before harvest, respectively. Samples of cucumber leaf were taken directly after the first application (0 DAT). Additional samples of mature cucumber fruit were collected directly before the second application (14 DAT). Harvest of cucumber - rest of plant without roots and fruit, and the following separation of some cucumber fruits (2 per each plant) into peel and pulp was performed 17 days after the first application, corresponding to three days after the second application (17 DAT).

2. Description of analytical procedures

Solid subsamples were homogenised with dry ice and were combusted with measurement of radioactivity by LSC. Radioactivity in liquid samples was measured by LSC. Samples were extracted with methanol and water. Extracts were concentrated, partitioned into cyclohexane and subsequently ethyl acetate. The organic phases were dried over sodium sulfate and adjusted to volume with water or solvent. Extracts were analysed using HPLC (gradient elution on reversed-phase or normal phase columns) with UV and radiodetection.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The calculated total radioactive residues of cucumber leaf (0 DAT) sampled directly after the first application amounted to 6.397 mg/kg. In cucumber fruit collected directly before the second application (14 DAT), residues accounted for 0.016 mg/kg. In cucumber fruit sampled 17 days after the first application, corresponding to three days after the second application (17 DAT), residues amounted to 0.051 mg/kg, in pulp to 0.013 mg/kg, in peel to 0.263 mg/kg and in cucumber rest of plant to 8.807 mg/kg. The higher residue levels in cucumber leaf and rest of plant can be explained by the lower content of water in these matrices. These results also imply a low translocation through the plant.

B. EXTRACTION

1. Extractability

The extractability of the radioactive residues with methanol and water was very high in cucumber leaf (99.1 %), fruit (17 DAT: 94.3 %), pulp (92.2 %), peel (92.2%) and rest of plant (95.8%). The solvent extractability of radioactive residues was high in cucumber fruit (14 DAT: 89.0%). In all matrices, the major part of the radioactive residues was extracted with methanol, and only minor portions were extracted with water. Due to the high extractability, the residual radioactive residues after solvent extraction (ranging from 0.91 to 10.99% of the TRR in the different cucumber matrices) were not further characterized.

C. CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

Identification. Characterization and Quantification of Metabolites

In order to determine nature and quantities of the radioactive residues in the samples, aliquots of the combined or concentrated extracts and partition phases were analysed by means of HPLC. Identification of the parent compound was achieved by HPLC co-chromatography in cucumber leaf (0 DAT) and fruit (14 DAT) with the reference metrafenone. Peak assignment in the other samples was done by comparison of the retention times and the HPLC elution profiles with those of the extracts investigated by co-chromatography and of the reference item.

In cucumber leaf (0 DAT), the unchanged parent compound represented the main component (6.1 mg/kg or 95.0% TRR), in cucumber rest of plant (17 DAT) and in cucumber peel (17 DAT) the parent compound represented 7.084 mg/kg or 80.4% TRR and 0.161 mg/kg or 61.0% TRR, respectively. In cucumber fruit (17 DAT) and fruit (14 DAT), metrafenone was the main component, accounting for 0.0216 mg/kg or 42.4% TRR and 0.0021 mg/kg or 12.6% TRR, respectively. In cucumber pulp (17 DAT), the parent compound (0.0009 mg/kg or 6.5% TRR) and some highly polar components were detected in the same concentration range.

Metabolic Pathway

Metrafenone was found to be metabolised to a low extent. The unchanged parent compound represented the main component in most cucumber matrices analysed. In the case of cucumber pulp (17 DAT) some highly polar components were detected in the same very low concentration range as the parent compound (each below or equal to 0.0016 mg/kg or 12.1 % TRR). In addition, further minor polar and medium polar metabolites at very low concentrations were characterized by their retention time behaviour in HPLC or by extractability with water.

Storage Stability

All samples were stored at approximately -18°C during the course of the study. All extractions were performed within less than two months after sampling. Quantitative HPLC analyses were carried out within 36 days after extraction. Therefore, no storage stability investigations were necessary.

Extractability of residues according to analytical methods

Extractability with Other Solvents

Three further one-step extraction methods were investigated in all sampled cucumber matrices including an extraction procedure with methanol/water/2N HCl (70/25/5, v/v/v), an extraction procedure acetone/water (2/1, v/v) and an extraction procedure with n-heptane/acetone (80/20, v/v). Extraction of the different matrices produced similar results for the extraction with methanol/water/2N HCl and acetone/water (>86 %) compared to the method used in the current metabolism investigation. Extraction with n-heptane/acetone showed somewhat lower efficiencies for the matrices fruit (17 DAT), peel and rest of plant (17 DAT) and significantly lower efficiencies for the matrices fruit (14 DAT) and pulp (17 DAT). Lower extractability was observed in samples containing lower parent but higher polar fraction content. Due to the high solubility of metrafenone in non-polar solvents like acetone, a complete extraction of the parent compound can be expected for all matrices and solvents tested.

A summary of the relative extractability of various matrices in the different solvents and residual radioactivity levels and a summary of the identified and characterised residues is presented below.

Table 6.2.1-13: Extractability of Radioactive Residues in cucumber samples after foliar treatment with ^{14}C metrafenone

Matrix	DAT[1)]	TRR [2)]	Methanol extract		Aqueous extract		ERR [3)]		RRR [4)]	
		(mg/kg)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)
Leaf	0	3.397	6.319	98.78	0.020	0.31	6.339	99.09	0.058	0.91
Fruit	14	0.016	0.014	87.46	0.000	1.55	0.015	89.01	0.002	10.99
Fruit	17	0.051	0.047	92.86	0.001	1.42	0.048	94.27	0.003	5.73
Pulp	17	0.013	0.012	91.09	0.000	1.12	0.012	92.21	0.001	7.79
Peel	17	0.263	0.240	91.16	0.003	1.07	0.243	92.23	0.020	7.77
Rest of plant	17	8.807	8.151	92.56	0.282	3.20	8.433	95.76	0.374	4.24

[1)] DAT = Days after first treatment
[2)] TRR was calculated as the sum of ERR and RRR
[3)] ERR = Extractable Radioactive Residue
[4)] RRR = Residual Radioactive Residue

Table 6.2.1-14: Summary of Identified Components and Portions Characterised in Cucumber Matrices after foliar treatment with ^{14}C metrafenone

Matrix	DAT[1)]	Metrafenone		Total Characterised	
		(mg/kg)	(% TRR)	(mg/kg)	(% TRR)
Leaf	0	6.076	95.0	0.262	4.1
Fruit	14	0.002	12.6	0.011	67.7
Fruit	17	0.022	42.4	0.019	37.9
Pulp	17	0.0009	6.5	0.011	84.8
Peel	17	0.161	61.0	0.051	19.4
Rest of plant	17	7.084	80.4	1.301	14.8

[1)] DAT = Days after first treatment

III. CONCLUSION

The metabolism of metrafenone was investigated in cucumber plants treated with U-^{14}C-metrafenone. Radiolabelled metrafenone (Reg. No. 4037710) was applied as BAS 560 02 F SC formulation to cucumbers (foliar) growing in pots at an application rate of 2 x 200 g a.s./ha using a hand sprayer. The applications were made 17 and 3 days before harvest.

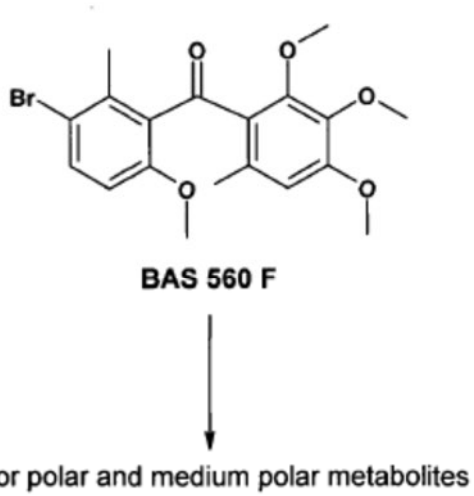
The highest levels of total radioactive residues (TRR) were found in cucumber rest of plant (17 DAT), amounting to 8.807 mg/kg, followed by leaf (0 DAT) with 6.397 mg/kg. In fruit, considerably lower residue levels were found, accounting for 0.016 mg/kg at 14 DAT and for 0.051 mg/kg at 17 DAT. Residues in cucumber pulp and peel accounted for 0.013 and 0.263 mg/kg, respectively.

The extractability of radioactive residues with methanol and water was > 92 % TRR for all cucumber matrices, except for fruit (14 DAT) where it was 89% TRR. Due to the high extractability, the residual radioactive residues (RRR) after solvent extraction of cucumber matrices were generally very low (each below or equal to 11% TRR; Fruit 14 DAT). Therefore, no further characterization was carried out for the residual radioactive residues of the individual cucumber matrices.

Metrafenone was the only constituent identified in this metabolism study. The unchanged parent compound represented the main component in most cucumber matrices analysed. In the case of cucumber pulp (17 DAT) some highly polar components were detected in the same very low concentration range as the parent compound (each below or equal to 0.0016 mg/kg or 12.1 % TRR). In addition, further minor polar and medium polar metabolites at very low concentrations (each below or equal to 8.9% TRR or 0.0015 mg/kg) were characterized by their retention time behaviour in HPLC or by extractability with water.

The proposed metabolic pathway is presented below.

Figure 6.2.1-3: Metabolic Pathway of metrafenone in Cucumber Plants



Summary of metabolism in plants

The metabolism of metrafenone was studied in wheat, grapes (fruit and leaves) and cucumbers. This covers cereals and fruiting crop groups but can also be considered suitable to cover leafy crops as grape leaves are considered to be a leafy crop. In all crops, there was no cleavage of metrafenone.

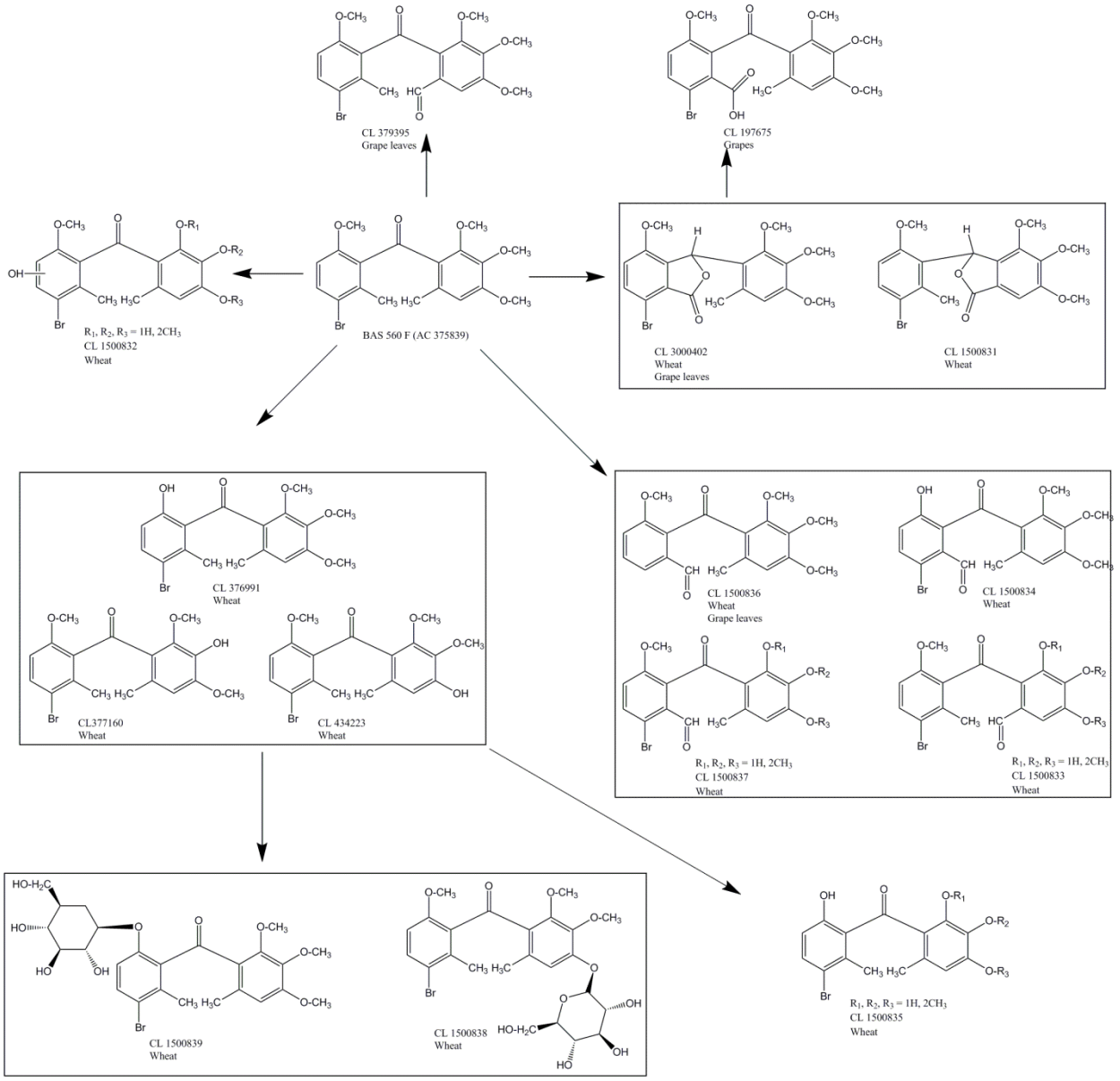
In wheat, the major metabolic product in forage, hay, straw and grain was metrafenone and would be likely to occur at detectable levels in all commodities. In grain, only the unknown ROI1 was present at >10% TRR. This unknown consisted of 6-7 compounds with one of these compounds being present at 0.03 mg/kg (1N rate equivalent); other compounds occurred at <<0.01 mg/kg. (1N rate equivalent). No other metabolites were detected. In straw, only the unknown ROI2 occurred at >10% TRR. This unknown consisted of 5-6 compounds with one of these compounds being present at 0.10 mg/kg (1N rate equivalent); other compounds occurred at 0.03-0.08 mg/kg. (1N rate equivalent). Both ROI1 and ROI2 were polar metabolites based on the chromatographic behaviour. Other metabolites were detected but only CL 3000402, CL 1500838 and CL 434223 occurred at levels >0.1 mg/kg at 1N rate *cf.* metrafenone which was detected at 0.506 mg/kg at 1N rate; metabolites were seen in rat metabolism (CL 3000402 was detected in the faeces, CL 434223, and its glucose conjugates (CL 1500838) were found in the bile and urine as glucuronides (CL 1500699) of their corresponding hydroxy-analogs. The metabolism of metrafenone in cereals first undergoes phase I biotransformations. These biotransformations proceed through a single oxidative de-methylation to form CL 434223, CL 376991, and CL 377160; followed by a second oxidative de-methylation to form CL 1500835. The lactones CL 3000402 and CL 1500831 are formed by oxidation at each benzylic carbon followed by cyclization. Oxidation of the methyl groups on either of the two phenyl rings gives rise to the aldehyde derivatives, CL 1500833, CL 1500834, and CL 1500837. Aromatic oxidation and oxidative de-methylations can produce CL 1500832. Additionally, CL 1500836 is formed by oxidation at the benzylic carbon of the bromophenyl ring followed by reductive de-bromination. The oxidations are proposed to be either enzymatic and/or chemical (photolysis) in nature. These phase I processes are then followed by phase II transformation reactions, i.e. enzymatic glucoside conjugation reactions to yield the conjugates CL 1500838 and CL 1500839 from their corresponding aglycones, CL 434223 and CL 376991.

Analysis of the grape extracts showed that parent was the major metabolic product at *ca*. 41-53% TRR. In juice, radioactivity was partitioned sequentially with organic solvents (ethyl acetate and toluene) which extracted 54.7% (9.9% TRR) of the juice radioactivity from the aqueous layer. No parent compound was detected in the organosoluble fraction. Radioactivity in the juice comprised multiple polar metabolites none of which exceeded 4% TRR (0.003 mg/kg) or 9% TRR (0.006 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. CL 197675 was tentatively identified at a maximum of 9% TRR (0.006 mg/kg) in the bromophenyl labelled samples. Parent was the major radioactive residue detected in extracts of the marc from mature grapes accounting for 24.7% TRR (0.109 mg/kg) and 22.8% TRR (0.063 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. Polar metabolites comprising multiple components were detected at 12% TRR (<0.05 mg/kg) 17% TRR (< 0.046 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. Surface rinses and extracts of grape leaves indicate parent was the major radioactive residue (86.9-92.4% TRR; 20.3-26.6 mg/kg) after the first application the level decreased to 33-37% TRR (*ca.* 14-20 mg/kg) and remained constant throughout the application period. Parent then decreased to 14-

17.6% TRR TRR (8.4-9.8 mg/kg) at 61DAT1 and to 11-15.2% TRR (2.7-5.8 mg/kg) at harvest (77DAT1). CL 3000402 (also detected in rat faeces), CL 1500836 and CL 379395 were also tentatively identified but were not accurately quantified. Based on the structures of metabolites that were identified in grapevines, the metabolism of metrafenone in vines undergoes oxidative biotransformations. Metrafenone is metabolized readily by oxidation of either of the methyl groups on the bromophenyl and trimethoxyphenyl rings to yield the corresponding aldehydes. The aldehydic moiety on the bromophenyl ring can be further oxidized to the carboxylic acid or cyclized to form the lactone. A des-bromo aldehyde is formed from dehalogenation by loss of the bromine.

The metabolism of metrafenone was investigated in cucumber plants. The highest levels of total radioactive residues (TRR) were found in cucumber "rest of plant" (17 DAT), at 8.8 mg/kg, followed by leaf (0 DAT) with 6.4 mg/kg. In fruit, considerably lower levels of radioactivity were found, accounting for 0.016 mg/kg at 14 DAT and for 0.051 mg/kg at 17 DAT. Residues in cucumber pulp and peel accounted for 0.013 and 0.26 mg/kg, respectively. The extractability of radioactive residues with methanol and water was very high (> 92 % TRR) for all cucumber matrices, except for fruit (14 DAT) where it was 89% TRR. Metrafenone was the only constituent identified in this metabolism study. The unchanged parent compound represented the main component in most cucumber matrices analysed. In the case of cucumber pulp (17 DAT) some highly polar components were detected at the same very low concentration range as the parent compound (each below or equal to 0.0016 mg/kg or 12.1 % TRR). In addition, further minor polar and medium polar metabolites at very low concentrations (each below or equal to 8.9% TRR or 0.0015 mg/kg) were characterized by their retention time behaviour in HPLC or by extractability with water.

Figure 6.2.1-4: Metabolic pathway of metrafenone in plants



CA 6.2.2 Poultry

Report: CA 6.2.2/1
[redacted] 2008a
BAS 560 F (AC 375839): Metabolism in laying hens
2005/1026047
Guidelines: EPA 860.1300, EEC 91/414 (7030(VI/95 Rev. 3)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

An interim version of this study was previously reported in the DAR (UK, July 2005).

Executive Summary

The metabolism of metrafenone was investigated in laying hens following repeated oral administration of ^{14}C-metrafenone. The test compound was ^{14}C or ^{13}C-labelled either on the trimethyoxyphenyl ring moiety (trimethoxyphenyl label) or on the bromophenyl ring moiety (bromophenyl label). After 12 consecutive daily oral administrations of ^{14}C-metrafenone at *ca.* 14 ppm diet to laying hens, there was rapid absorption and almost complete excretion within 24 hours. There was no indication of any accumulation in edible tissues or eggs. The total radioactive residues in edible tissues and organs were 0.099 mg/kg or 0.118 mg/kg in eggs, 10.214 mg/kg or 10.641 mg/kg in bile, 0.566 mg/kg or 0.346 mg/kg in the liver, 0.010 mg/kg or 0.013 mg/kg in muscle, and 0.070 mg/kg or 0.096 mg/kg in skin with adhering fat for the trimethoxyphenyl or bromophenyl label, respectively. The total identified and characterized radioactive residue accounted for 89.4% or 81.3% of the TRR for the trimethoxyphenyl or bromophenyl label, respectively, in eggs; 80.8% or 78.4% of TRR in excreta; 100% TRR in bile (both labels); 91.4% or 101.4% TRR in liver and 86.7% or 81.5% TRR in skin with fat, including residues released by protease digestion or characterize by hydrolysis.

The parent compound metrafenone was metabolized *via* three routes: hydroxylation of the active substance at the bromophenyl ring or the attached methyl group resulted in the formation of M560F05, which was demethylated and conjugated with glucuronic acid in bile to form the metabolite M560F07. Demethylation of the parent compound at the trimethoxyphenyl ring formed the metabolite M560F06 which was also conjugated with glucuronic acid in bile to form the metabolites M560F08 and M560F09. Full oxidation of the methyl group on the bromomethoxytoluene ring to the carboxylic acid (*via* the respective isomer of M560F05) led to the metabolite M560F03. M560F03 and M560F06 are possible intermediates for the formation of the oxidized and demethylated metabolites M560F01 and M560F02 which are further converted to the methyl ester M560F04.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: Active substance
Lot/Batch #: AC 12102-57 (Trimethoxyphenyl-U-^{14}C)
AC 12102-58 (Bromophenyl-6-^{14}C)
Purity: AC 12102-57: radiochemical purity: 99.2%
chemical purity: 108.8%
specific radioactivity 38.5 µCi/mg (1.42 MBq/mg)
AC 12102-58: radiochemical purity: 99.6%
chemical purity: 99.8%
specific radioactivity 39.8 µCi/mg (1.47 MBq/mg)
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels:

2. **Animals:** Hens
Species: white leghorn
Gender: female
Age: not specified (mature)
Weight at dosing: 1.0-2.5 kg
Number of animals: 16
Acclimation period: 7 days
Housing: stainless steel cages
Husbandry: 8 hens/group, eggs collected twice daily, excreta collected daily
Diet: layer crumble
Water: ad libitum
Environmental Conditions: room lights on continuously
Temperature: 70° ± 4°F
Humidity: 20 and 70%

B. STUDY DESIGN AND METHODS

1. Test procedure

Two groups of laying hens, eight hens per group, were treated daily for 12 consecutive days with gelatin capsules containing test substance at a nominal dose rate of 12 ppm in the feed based on an average feed consumption of 120 g per hen per day. The actual doses corrected for actual feed consumption were 14.19 ppm (trimethoxyphenyl label) and 13.87 ppm (bromophenyl label).

Excreta from each group were collected daily and composited per group. Eggs were collected daily during the dosing period with two collections (composited) per sampling interval (the afternoon collection of the previous day plus the morning collection of the sampling day). Approximately 22 hours after administration of the final dose, the animals were sacrificed. Tissue samples (skin adhering to fat, muscle and liver) and bile from the gall bladder were collected.

Details of the study design are summarised below:

Dose group	A Trimethoxyphenyl-U-^{14}C	B Bromophenyl-6-^{14}C
Number of animals	8	8
Specific Activity	30	30
Nominal dose (ppm feed)	12	12
Actual dose (ppm feed)	14.19	13.87
Actual dose (mg/animal/day)	1.55	1.56
Actual dose (mg/kg body weight)	1.00	0.95
Number of doses	12	12
Sacrifice time (hours post last dose)	22	22

2. Description of analytical procedures

All measurements of radioactivity in liquid samples were analysed directly by liquid scintillation counting (LSC). Solid samples were combusted prior to analysis by LSC. Extractions were performed using methanol and water.

II. RESULTS AND DISCUSSION

Absorption, Distribution and Excretion

Following oral administration of ^{14}C-metrafenone to laying hens, the overall recoveries of radioactivity were approximately 96 and 86% of the total applied dose for the trimethyoxyphenyl and bromophenyl labels, respectively. Radioactivity was rapidly eliminated within 24 hours with 95.1% (trimethyoxyphenyl label) and 85.9% (bromophenyl label) of the administered dosed recovered in the excreta. The percentage of radioactivity in eggs ranged from 0.236 to 0.251%, followed by liver from 0.063 to 0.088%. The lowest levels of radioactivity were found in skin with fat followed by muscle. Bile from the gall bladder contained 0.027 to 0.028% of the applied dose. There was no indication of accumulation of metrafenone related residues in eggs or tissues.

The material balance has been summarised below.

Table 6.2.2-1: Material Balance after administration of ^{14}C -metrafenone to laying hens

Matrix	Material balance in % of total applied dose	
	Dose Group A trimethyoxyphenyl label	Dose Group B bromophenyl label
Eggs	0.236	0.251
Organs and Tissues		
Muscle	0.003	0.003
Skin with fat	0.006	0.010
Liver	0.102	0.066
Bile	0.027	0.028
Excreta	95.1	85.9
Total	**95.5**	**86.3**

Total radioactive residues

Total radioactive residues (TRR) in all matrices are given below. Reported values are based either on direct analysis by liquid scintillation counting (LSC) or on extraction data. In the case of the latter, the TRR values are calculated as the sum of extractable radioactivity and residual radioactivity remaining in the post extraction solid.

Table 6.2.2-2: Total Radioactive Residues (TRRs) in Eggs, Tissue and Excreta

Matrix	Dose Group A trimethyoxyphenyl label	Dose Group B bromophenyl label
	[mg/kg]	[mg/kg]
Day 1	<0.001	<0.001
Day 2	0.016	0.018
Day 3	0.026	0039
Day 4	0.054	0.055
Day 5	0.072	0.064
Day 6	0.086	0.102
Day 7	0.095	0.094
Day 8	0.097	0.106
Day 9	0.107	0.115
Day 10	0.109	0.117
Day 11	0.106	0.118
Day 12	0.110	0.118
Eggs (Days 9-12 pooled)	0.099	0.118
Muscle	0.010	0.013
Skin with fat	0.070	0.096
Liver	0.566	0.346
Bile	10.214	10.641
Excreta (Days 6-12 pooled)	14.758	13.289

Extractability

Extraction of the incurred radioactivity in all matrices derived from each label are summarised below. All matrices were sequentially extracted with methanol (3x) and water (2x).

Table 6.2.2-3: Extractability of Residues of Metrafenone in Hen Matrices (Trimethyoxyphenyl Label and Bromophenyl Label)

Matrix	TRR[1)]	Methanol	Water	ERR [2)]	PES [3)]	TRR[4)] Recovery
	[mg/kg]	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] (% TRR)	[mg/kg] [%]
Trimethyoxyphenyl Label						
Eggs pooled (days 9-12)	0.108	0.043 _(39.7)	0.009 (8.3)	0.052 (48.0)	0.056 (52.0)	0.108 (100)
Muscle	0.010	0.003 (26.8)	0.0001 (1.0)	0.003 (27.8)	0.007 (72.2)	0.010 (100)
Skin with fat	0.070	0.034 (48.6)	n.a.	0.034 (48.6)	0.036 (51.4)	0.070 (100)
Liver	0.566	0.048 (8.5)	0.035 (6.2)	0.083 (14.7)	0.483 (85.3)	0.566 (100)
Excreta pool (days 6-12)	15.370	12.418 (80.8)	n.a.	12.418 (80.8)	2.952 (19.2)	15.370 (100)
Bromophenyl Label						
Eggs pooled (days 9-12)	0.117	0.043 (636.9)	0.009 (8.3)	0.53 (744.9)	0.065 (55.1)	0.117 (100)
Muscle	0.013	0.004 (30.7)	0.0001 (1.0)	0.004 (32.5)	0.009 (67.5)	0.013 (100)
Skin with fat	0.096	0.041 (42.9)	n.a.	0.041 (42.9)	0.055 (57.1)	0.096 (100)
Liver	0.346	0.037 (10.8)	0.029 (8.2)	0.066 (19.0)	0.280 (81.0)	0.346 (100)
Excreta pool (days 6-12)	14.089	11.051 (78.4)	n.a.	11.051 (78.4)	3.039 (21.6)	14.089 (100)

[1)] TRR values reported as sum of radioactivity on methanol and water extract measured directly by LSC and in post extraction solids after combustion
[2)] ERR – extractable radioactive residues (sum of methanol and water extracts)
[3)] PES – Post extraction solids remaining after extraction with methanol and water
[4)] Sum of all extracts and post extraction solids
n.a. = not applied

Excreta

Excreta were efficiently extracted with methanol releasing 75.0 to 79.0 (9.965 to 11.656 mg/kg). An additional 10.6 to 11.6% TRR (1.548 to 1.57 mg/kg) was recovered in water. In total 86.6 to 89.6% of the total residue was extracted. Unextractable residues accounted for 10.4 to 13.4% TRR.

Eggs

Extraction of pooled eggs (days 9-12) yielded 62.6 to 62.7% TRR (0.062 to 0.074 mg/kg) in the methanol extract and a further 16.9 to 17.2% (0.017 to 0.020 mg/kg) in water, giving a total of 79.7 to 79.8% TRR (0.079 to 0.094 mg/kg) as extractable residues. Unextractable residues accounted for 20.2 to 20.3% TRR.

Muscle

Low levels of radioactivity were found in muscle, with 0.010 mg/kg and 0.013 mg/kg from the trimethyoxyphenyl and bromophenyl labels, respectively. Extraction with methanol released 26.8 to 30.7% TRR, 1 to 1.8% TRR was extracted in water. Unextractable residues accounted for 67.5 to 72.2% TRR (0.007 to 0.009 mg/kg) in the PES.

Skin with adhering fat

Extraction of the skin lead to 58.0 to 60.6% TRR being extracted, with 39.4 to 42.0% remaining unextractable.

Liver

Extraction of liver samples yielded *ca* 11% in organic extracts and *ca.* 20% in the aqueous extracts. Overall extractability was around 30% with up to 70% remaining unextracted.

Characterisation and identification of metabolites

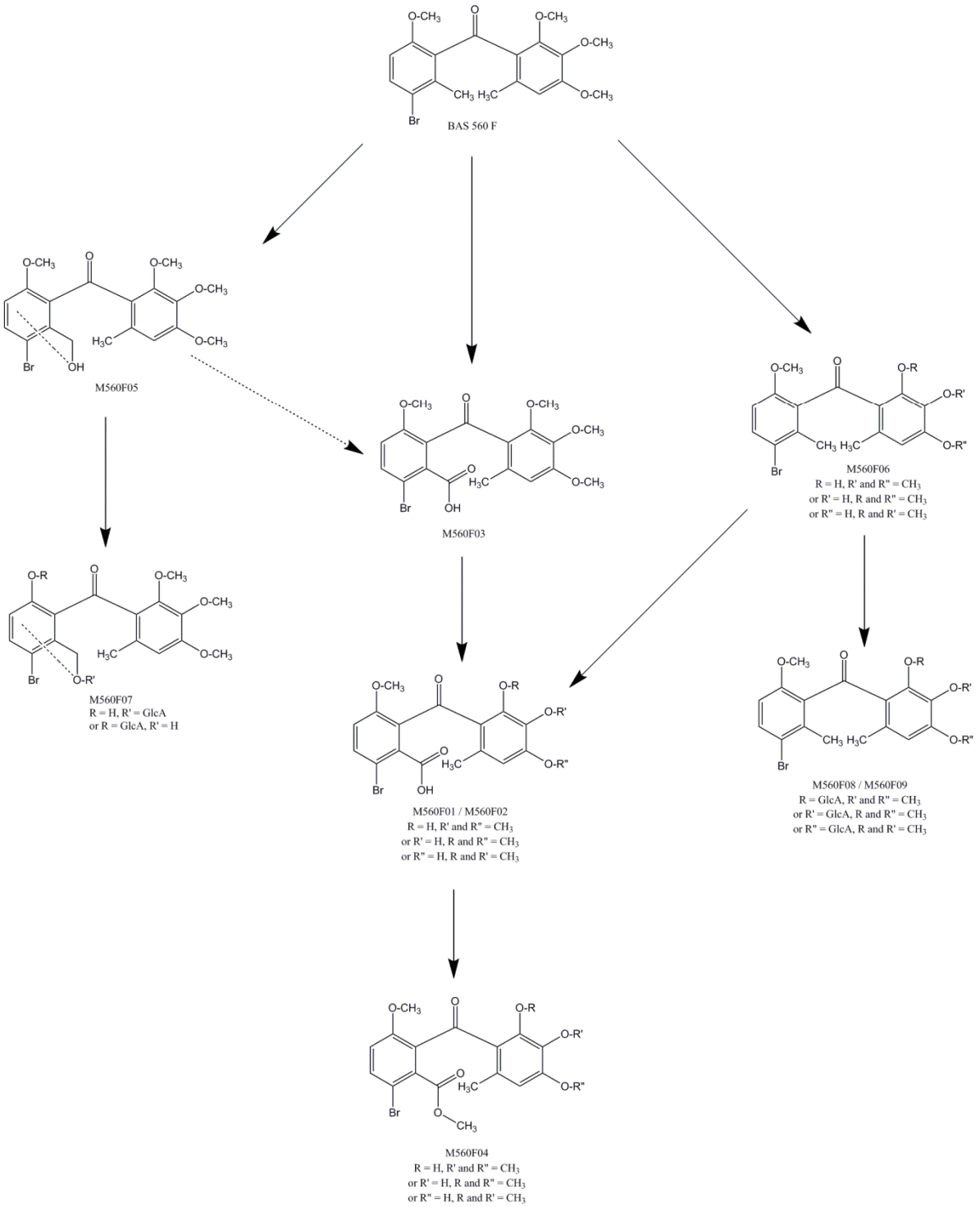
Characterisation of metabolites was performed by LC-MS and LC-MS/MS analysis. Metrafenone was found only in eggs (1.8–2.2% TRR) and in skin + fat (1.9% TRR). A comparison with the retention times and metabolite patterns for the components in the methanol extract of excreta (bromophenol-label), allowed the assignment of the metabolite M560F06 in the skin + fat extracts (at about 6–11% TRR) and tentatively in eggs (unquantifiable). With the exception of one unknown component in eggs (found at about 14% TRR and 0.015 mg eq./kg) all other components were below 10% TRR (< 0.01 mg eq./kg) in all tissues and eggs.

Table 6.2.2-4: Residues of metrafenone and other characterised components in hen matrices after 12 daily oral administrations of [14C]metrafenone.

Matrix	Metrafenone		Characterised (Methanol extract)		Characterised (Aqueous extract)	
	mg/kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
	Trimethoxyphenyl Label					
Eggs pooled (days 9–12)	0.002	2.2	0.04[a]	37.5	0.009[b]	8.4
Muscle	NA	NA	0.003	26.8	0.0001	1.0
Skin with fat	0.001	1.9	0.034[c]	48.6		
Liver			0.097[d]	17.3	0.07[e]	12.5
	mg/kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
	Bromophenyl Label					
Eggs pooled (days 9–12)	0.002	1.8	0.041[f]	35.1	0.01[g]	8.0
Muscle	NA	NA	0.004	30.7	0.0002	1.8
Skin with fat	<0.001	<0.001	0.042[h]	42.9		
Liver	<0.001	<0.001	0.096[i]	27.8	0.058[j]	16.6

[a] Includes nine peaks, each < 0.01 mg eq/kg or < 1.5% TRR, including 1 peak tentatively identified as M560F06
[b] Includes eight peaks, each < 0.01 mg eq/kg or < 3% TRR
[c] Includes 12 peaks, each < 7% TRR, and one peak identified as M560F06 (0.008 mg eq/kg, 11.4% TRR)
[d] Includes 10 peaks, each < 3.7% TRR
[e] Includes 12 peaks, each < 1.6% TRR
f Includes six peaks, each < 0.01 mg eq/kg or < 3% TRR, including one peak identified as M560F06
[g] Includes eight peaks, each < 0.01 mg eq/kg or < 2.7% TRR
[h] Includes 12 peaks, each < 9% TRR, and one peak identified as M560F06 (0.006 mg eq/kg (5.8% TRR)
[i] Includes six peaks, each < 4.5% TRR
[j] Includes five unknown peaks, each < 4.1% TRR

Figure 6.2.2-1: Proposed metabolic pathway of metrafenone in hens



III. CONCLUSION

After 12 consecutive daily oral administrations of ^{14}C-metrafenone F to laying hens, there was rapid absorption and almost complete excretion within 24 hours. Levels of TRR were low in all tissues. There was no indication of any accumulation in edible tissues or eggs. The parent compound metrafenone was metabolized *via* three routes: hydroxylation of the active substance at the bromophenyl ring or the attached methyl group resulted in the formation of M560F05, which was demethylated and conjugated with glucuronic acid in bile to form the metabolite M560F07. Demethylation of the parent compound at the trimethoxyphenyl ring formed the metabolite M560F06 which was also conjugated with glucuronic acid in bile to form the metabolites M560F08 and M560F09. Full oxidation of the methyl group on the bromomethoxytoluene ring to the carboxylic acid (*via* the respective isomer of M560F05) led to the metabolite M560F03. M560F03 and M560F06 are possible intermediates for the formation of the oxidized and demethylated metabolites M560F01 and M560F02 which are further converted to the methyl ester M560F04.

CA 6.2.3 Lactating ruminants

Report: CA 6.2.3/1
[redacted] 2002a
BAS 560 F (AC 375839) - Metabolism of 14C BAS 560 F in the lactating goat
2002/7005114
Guidelines: EPA 860.1300, EEC 96/68, EPA 40 CFR 158.240, EEC 91/414 Annex II (Part A Section 6.2)
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). The metabolism and distribution of ^{14}C-metrafenone was investigated in lactating goats following repeated oral administration over five consecutive days. Two radiolabels and two dose regimes were employed; thus dose levels were 0: 8 or 13 ppm diet for the low dose; and 60 or 87 ppm diet for the high dose, depending on the label. For all animals the dose was administered orally via gavage capsule dosing. The recovery of radioactivity in excreta and total radioactive residues in milk, liver, kidney, muscle, fat and excreta are below. In both dose groups, a high level of radioactivity 76-86% was eliminated via excreta. Liver, kidneys, adipose tissue and milk samples from the high dose treatment containing TRR at or greater than 0.01 mg/kg were further processed in order to identify residues.

Table 6.2.3-1: Recovery of Orally Administered Radioactivity in Excreta and Total Radioactive Residues in Goat Matrices Following Application of Metrafenone

	Treatment Groups			
Group ID	**B**	**D**	**C**	**E**
Radiolabel	**Trimethoxyphenyl-(U-^{14}C/3-^{13}C)**	**Bromophenyl-6 (^{14}C/^{13}C)**	**Trimethoxyphenyl-(U-^{14}C/3-^{13}C)**	**Bromophenyl-6 (^{14}C/^{13}C)**
Dose Group	**Low**	**Low**	**High**	**High**
Dose Rate (mg/kg)	**8**	**13**	**60**	**87**
Sample type	**Total Radioactive Residues (TRR mg/kg)**			
Milk (Day 5)	<0.005	<0.005	0.006	0.010
Liver	0.208	0.231	0.718	1.278
Kidneys	0.047	0.060	0.157	0.329
Muscle	<0.005	<0.005	0.006	0.008
Adipose tissue	<0.005	<0.005	0.022	0.015
Sample type	**Excretion (% total dose)**			
Urine + Faeces	85.7	84.0	82.2	75.7

Extraction of the adipose tissue samples with methanol released 112.8% and 116% of the applied radioactivity. Two sequential extractions of milk with acetone accounted for 98.8% of the TRR. A more extensive extraction scheme was needed to extract the radioactivity from the liver and kidneys, using a variety of organic solvents and enzyme treatment.

Table 6.2.3-2: Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Application of Metrafenone – Liver and Kidney

Sample Type	Liver				Kidney			
Dose Group (Rate in Diet)	**C (60 mg/kg)**		**E (87 mg/kg)**		**C (60 mg/kg)**		**E (87 mg/kg)**	
Radiolabel	**Trimethoxyphenyl**		**Bromophenyl**		**Trimethoxyphenyl**		**Bromophenyl**	
Fractions	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
TRR	**100**	**0.718**	**100**	**1.278**	**100**	**0.157**	**100**	**0.329**
Combined extracts	**96.8**	**0.695**	**94.6**	**1.21**	**97.7**	**0.153**	**100.6**	**0.331**
Distribution of Extractable Radioactive Residues								
ROI-1 (Unknown)	5.99	0.043	3.60	0.046	5.54	0.009	6.00	0.020
ROI-2 (Unknown)	3.76	0.027	2.97	0.038	0.61	0.001	1.23	0.004
ROI-3 (Unknown)	11.70	0.084	5.94	0.076	1.21	0.002	1.82	0.006
ROI-4 (Unknown)	4.18	0.030	4.77	0.061	3.12	0.005	7.39	0.024
ROI-5 (Unknown)	5.01	0.036	3.60	0.046	3.82	0.006	5.23	0.017
ROI-6 (Unknown)	2.64	0.019	3.68	0.047	4.97	0.008	4.16	0.014
ROI-7 (Unknown)	4.73	0.034	5.09	0.065	1.53	0.002	1.25	0.004
ROI-8 (Unknown)	2.92	0.021	2.97	0.038	4.33	0.007	3.80	0.013
ROI-9A (CL 1023361) + ROI-9B1 (CL 1023362) + ROI-9B2 (CL 1500702)	9.89	0.071	13.23	0.169	13.57	0.021	9.51	0.031
ROI-10 (Unknown)	2.65	0.019	2.35	0.030	2.80	0.004	4.80	0.016
ROI-11 (Unknown)	2.93	0.021	12.11	0.027	1.66	0.003	2.13	0.007
ROI-12 (Unknown)	2.23	0.016	1.96	0.025	5.67	0.009	3.10	0.010
ROI-13A1 (CL 1500698) + ROI-13A2 (CL 1023363) + Unknown	14.77	0.106	21.05	0.269	28.15	0.044	26.29	0.087
ROI-14 (Unknown)	3.35	0.024	2.90	0.037	4.14	0.007	4.32	0.014
ROI-15 (Unknown)	2.09	0.015	1.88	0.024	4.08	0.006	1.43	0.005
ROI-16A (CL 1500701) + ROI-16B (CL 1500699) + Unknown	6.41	0.046	7.12	0.091	1.46	0.002	4.19	0.014
ROI-17 (Unknown)	1.53	0.011	1.72	0.022	0.64	0.001	1.22	0.004
ROI-18 (Unknown)	1.53	0.011	1.25	0.016	0.63	0.001	1.49	0.005
ROI-19 (Unknown)	0.98	0.007	0.70	0.009	0.49	0.001	1.28	0.004
ROI-20 (Unknown)	1.81	0.013	1.80	0.023	1.21	0.002	2.16	0.007
ROI-21 (Unknown)	1.67	0.012	1.33	0.017	2.36	0.004	2.28	0.008
ROI-22 (Parent BAS 560 F)	3.49	0.025	2.74	0.035	3.25	0.005	4.35	0.014

Table 6.2.3-3: Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Application of Metrafenone – Milk and Adipose Tissues

Sample Type	Milk				Adipose Tissues			
Dose Group (Rate in Diet)	C (60 mg/kg)		E (87 mg/kg)		C (60 mg/kg)		E (87 mg/kg)	
Radiolabel	Trimethoxyphenyl		Bromophenyl		Trimethoxyphenyl		Bromophenyl	
Fractions	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	**100**	**0.006**	**100**	**0.010**	**100**	**0.022**	**100**	**0.015**
Combined extracts[1]	**NP**	**NP**	**98.9**	**~0.010**	**116**	**~0.026**	**112.6**	**0.020**
Distribution of Extractable Radioactive Residues								
ROI-1 (Unknown)	NP	NP	5.3	<0.005	8.0	<0.005	9.1	<0.005
ROI-2 (Unknown)	NP	NP	1.8	<0.005	ND	ND	ND	ND
ROI-3 (Unknown)	NP	NP	2.2	<0.005	ND	ND	ND	ND
ROI-4 (Unknown)	NP	NP	2.9	<0.005	ND	ND	ND	ND
ROI-5 (Unknown)	NP	NP	1.4	<0.005	ND	ND	ND	ND
ROI-6 (Unknown)	NP	NP	1.6	<0.005	ND	ND	ND	ND
ROI-7 (Unknown)	NP	NP	1.1	<0.005	ND	ND	ND	ND
ROI-8 (Unknown)	NP	NP	2.0	<0.005	ND	ND	ND	ND
ROI-9A (CL 1023361) + ROI-9B1 (CL 1023362) + ROI-9B2 (CL 1500702	NP	NP	2.8	<0.005	ND	ND	ND	ND
ROI-10 (Unknown)	NP	NP	1.0	<0.005	ND	ND	ND	ND
ROI-11 (Unknown)	NP	NP	1.2	<0.005	ND	ND	ND	ND
ROI-12 (Unknown)	NP	NP	1.3	<0.005	ND	ND	ND	ND
ROI-13A1 (CL 1500698) + ROI-13A2 (CL 1023363) + Unknown	NP	NP	10.7	<0.005	ND	ND	ND	ND
ROI-14 (Unknown)	NP	NP	2.8	<0.005	ND	ND	ND	ND
ROI-15 (Unknown)	NP	NP	7.2	<0.005	ND	ND	ND	ND
ROI-16A (CL 1500701) + ROI-16B (CL 1500699) + Unknown	NP	NP	3.8	<0.005	ND	ND	ND	ND
ROI-17 (Unknown)	NP	NP	2.6	<0.005	ND	ND	ND	ND
ROI-18 (Unknown)	NP	NP	5.0	<0.005	ND	ND	ND	ND
ROI-19 (Unknown)	NP	NP	4.3	<0.005	ND	ND	ND	ND
ROI-20 (Unknown)	NP	NP	6.7	<0.005	ND	ND	ND	ND
ROI-21 (Unknown)	NP	NP	5.5	<0.005	5.4	<0.005	5.3	<0.005
ROI-22 (Parent BAS 560 F)	NP	NP	24.1	<0.005	85.4	0.019	60.0	0.009

NP – not performed
[1] For milk, combined acetone extracts analysed by HPLC, for adipose tissues combined methanol extracts analysed by HPLC

The metabolism and distribution of ^{14}C- metrafenone were investigated in lactating goats following repeated oral administration over five consecutive days at a control, a low dose and a high dose level per label. For all animals the dose was administered orally via gavage capsule dosing.

Two labelled versions of the test substance were used, Trimethoxyphenyl-(U-^{14}C/3-^{13}C) and Bromophenyl-6 (^{14}C/^{13}C). One goat was also used as a control, receiving a placebo dose consisting of only lactose carrier (*ca* 1.5 g) in each capsule.

RESULTS AND DISCUSSION

The recovery of radioactivity in excreta and total radioactive residues in milk, liver, kidney, muscle, fat and excreta are shown below. For the low dose treatment groups, 84.0 and 85.7% of the cumulative dose was recovered in the excreta. Similarly, goats in the high dose groups eliminated 82.2 to 75.7% in the excreta.

Following five days of dosing with a low dose and a high dose of metrafenone) the maximum dietary burden of 1.42 mg/kg of feed equivalent, the incurred maximum TRR in milk was very low at or less than 0.01 mg/kg for both treatment levels and radiolabels. The TRRs were also <0.01 mg/kg in muscle irrespective of dose rate or label. Higher residues were detected in liver with 0.208 and 0.231 mg/kg for the low dose and 0.718 to 1.278 mg/kg for the high dose group. TRRs in kidney were approximately 4 to 5-fold lower than those found in liver, at 0.047 to 0.060 mg/kg and 0.157 to 0.329 mg/kg respectively for the low and high dose groups. Detectable TRR was also found in the fat at 0.022 and 0.015 mg/kg, respectively, for the trimethoxyphenyl-(U-^{14}C/3-^{13}C) and Bromophenyl-6 (^{14}C/^{13}C) labels. The TRR levels in the liver and kidneys were shown to be proportional to the dose rate.

Milk

Two regions of interest were observed. One co-eluted with parent (ROI-22) and the other (ROI-13), which following β-glucoronidase treatment co-eluted with CL 377160. Residue concentrations for each region were below the LOD (0.005 mg/kg).

Adipose tissue

Three regions of interest were observed. The majority of radioactivity was associated with parent in ROI-22, while the other two regions were at low levels and were below (0.005 mg/kg).

Liver

Profiles were similar for both radiolabels with up to 22 regions of interest. Following elucidation by LC/MSD/API-ES (positive mode), fragmentation patterns were consistent with CL 1023361, CL 1023362, CL 1500702, CL 1500698, CL 1023363, CL 1500701 and CL 1500699. The remaining 13 radiocomponents were each below 5% of the TRR or <0.05 mg/kg.

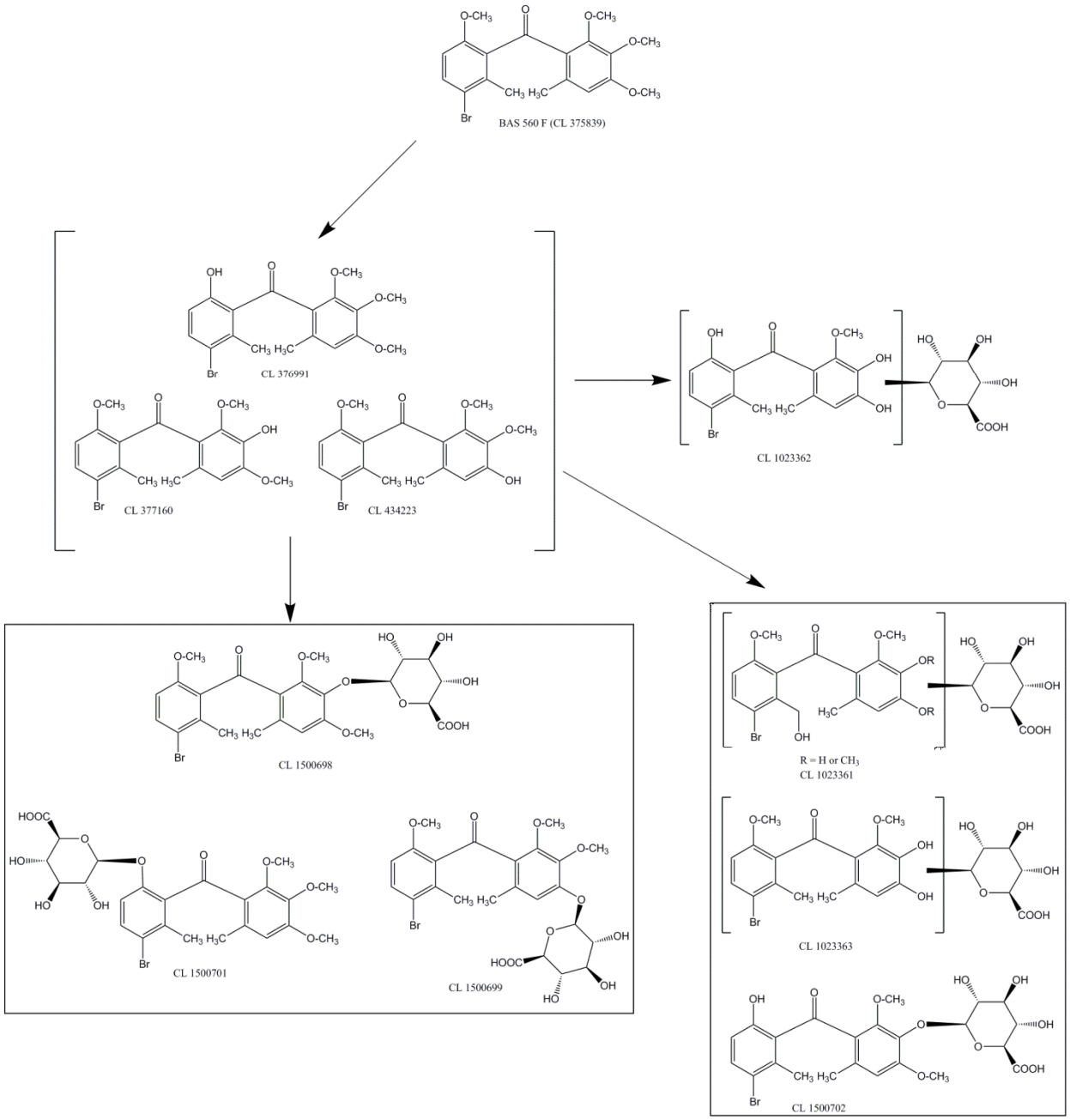
Kidney

Radio profile of extracts was similar to those seen for liver.

Metabolism of metrafenone in the lactating goat occurred at the methyl and methoxy groups. Hydroxylation of the methyl groups yielded hydroxymethyl derivatives; whereas O-demethylation yielded hydroxyphenyl derivatives. These hydroxylated metabolites underwent phase II glucoronidation to produce various mono-O-glucoronides. All of the prominent goat metabolites were identified as O-glucoronides, which is qualitatively very similar to the metabolism of metrafenone in the rat, where the main metabolites were also identified as glucoronides. However, some of the unconjugated hydroxyphenyl metabolites were also detected in the rat. Since the dosage rates were exaggerated based on the potential maximum dietary burden under good agricultural practise, metrafenone derived residues in edible tissues and milk would be below 0.01 mg/kg under normal dietary exposure at the 1X dose rate.

The proposed metabolic pathway of metrafenone in the lactating goat can be seen below.

Figure 6.2.3-1: Metabolic Pathway of BAS 560 F in the Lactating Goat



CA 6.2.4 Pigs

No metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly and intakes by pigs are not expected to be significantly higher than other livestock.

CA 6.2.5 Fish

In the draft working document on the nature of residues in fish (SANCO/11187/2013), there are two triggers for carrying out a fish metabolism study, log Pow >3 and dietary intake > 0.1 mg/kg total diet (dry matter). Although the log Po/w for metrafenone is >3, the dietary intake does not exceed 0.1 mg/kg total diet (dry matter). Therefore a fish metabolism study is not required. It should also be noted that a fish bioaccumulation study is available which confirms that residues of metrafenone or its metabolites would not occur in fish (please refer to the ecotoxicology section of MCA, point CA 8.2.2.3).

Summary of metabolism in livestock

Metabolism studies were carried out in hens (poultry) and goats (ruminants). Metabolism studies were not necessary in either pigs or fish.

In hens dosed for 12 days at *ca.* 14 ppm diet, there was rapid absorption and almost complete excretion within 24 hours. Levels of TRR were low in muscle (≤0.01 mg/kg) and skin with fat (0.06-0.08 mg/kg) but higher in liver (0.33-0.49 mg/kg). There was no indication of any accumulation in edible tissues or eggs (maximum daily TRR *ca.* 0.12 mg/kg). TRR plateaued in eggs around day 9. Little or no parent compound was detected in eggs or edible tissues. Any metabolites present were at very low levels (<0.01 mg/kg). Metrafenone was metabolized via three routes: hydroxylation of the active substance at the bromophenyl ring or the attached methyl group resulted in the formation of M560F05, which was demethylated and conjugated with glucuronic acid in bile to form the metabolite M560F07. Demethylation of the parent compound at the trimethoxyphenyl ring formed the metabolite M560F06 which was also conjugated with glucuronic acid in bile to form the metabolites M560F08 and M560F09. Full oxidation of the methyl group on the bromomethoxytoluene ring to the carboxylic acid (via the respective isomer of M560F05) led to the metabolite M560F03. M560F03 and M560F06 are possible intermediates for the formation of the oxidized and demethylated metabolites M560F01 and M560F02 which are further converted to the methyl ester M560F04.

In goats dosed for five days at 8-13 or 60-87 ppm metrafenone in the diet, the majority of radioactivity (76-86%) was excreted in faeces and urine. Radioactivity in milk reached a plateau after two days of dosing (*ca.* 0.01 mg/kg) in the high dose groups; it was not detectable (<0.005 mg/kg) in the low dose groups. The TRRs were also <0.01 mg/kg in muscle irrespective of dose rate or label. Higher residues were detected in liver with 0.21-0.23 mg/kg for the low dose and 0.72- 1.3 mg/kg for the high dose group. TRRs in kidney were approximately 4 to 5-fold lower than those found in liver, at 0.05- 0.06 mg/kg and 0.16-0.33 mg/kg. Detectable TRR was also found in the fat at ca. 0.02 mg/kg. The TRR levels in the liver and kidneys were shown to be proportional to the dose rate. In milk and fat, no parent or metabolites were detected above the LoD (0.005 mg/kg). Profiles of radioactivity were similar in liver and kidney with up to 22 "regions of interest" being separated. Fragmentation patterns suggested these metabolites were consistent with CL 1023361, CL 1023362, CL 1500702 (combined 0.02-0.03 mg/kg in kidney; 0.07-0.17 mg/kg in liver), CL 1500698, CL 1023363 (combined 0.04-0.09 mg/kg in kidney; 0.10-0.27 mg/kg in liver), CL 1500701 and CL 1500699 (combined ≤0.01 mg/kg; 0.05-0.09 mg/kg in liver). The remaining thirteen components were each below 5% of the TRR or <0.05 mg/kg.

CA 6.3 Magnitude of residues trials in plants

Residues of metrafenone in grapes and cereals have been previously reported in the DAR (UK, July 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Summary of critical residues data (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region	Trials results relevant to the critical GAP (a)	Recommendation /comments	MRL (mg/kg)	STMR (b) (mg/kg)
Wheat	Northern	6 x <0.01, 2 x 0.01, 2 x 0.03, 3 x 0.04	grain	0.05	0.01
Wheat	Northern	0.40, 0.58, 2 x 0.61, 0.67, 0.93, 0.98, 1.43, 1.72, 1.80, 1.85, 2.04, 2.32, 3.86	straw	-	1.43
Wheat	Mediterranean	10 x <0.01, 3 x 0.01, 0.03	grain	0.05	0.01
Wheat	Mediterranean	0.67, 0.88, 2 x 1.07, 1.08, 1.09, 1.10, 1.11, 1.25, 1.61, 1.64, 1.69, 1.70, 2.12	straw	-	1.11
Barley	Northern	0.01, 0.02, 0.03, 0.04, 0.06, 0.07, 0.09, 0.11, 0.14, 2 x 0.15, 0.16, 0.40	grain	0.50	0.09
Barley	Northern	0.54, 0.64, 0.78, 1.08, 1.10, 1.11, 1.12, 1.15, 1.28, 1.60, 1.70, 2.01	straw	-	1.11
Barley	Mediterranean	3 x 0.04, 2 x 0.05, 0.06, 0.08, 0.10, 2 x 0.12, 0.13, 0.23	grain	0.50	0.07
Barley	Mediterranean	0.41, 0.90, 0.96, 1.02, 1.22, 1.34, 1.56, 1.65, 1.94, 2.13, 4.03, 4.25.	straw	-	1.45
Grapevine	Northern	0.11, 0.12, 0.15, 0.18, 0.19, 0.20, 0.31, 0.36.	grape	0.50	0.18
Grapevine	Mediterranean	2 x 0.08, 0.11, 0.17, 0.20, 0.22, 0.23, 0.24, 0.30, 0.34, 0.38.	grape	0.50	0.22

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17
(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the critical GAP

These conclusions were essentially confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498). However, it should be noted that the GAP on grapes has changed since the previous review under Directive 91/414 was carried out (reduction in maximum number of applications from 8 to 3). New grape trials supporting the revised GAP are presented below.

CA 6.3.1 Crop 1 (Grapes)

Report: CA 6.3.1/1
Fleischer G., 2014a
Study on the residue behaviour of BAS 560 F (Metrafenone) in grapes (wine and table) after treatment with BAS 560 02 F under field conditions in Germany, France (North and South), Greece, Italy and Spain, 2013
2014/1161029

Guidelines: EEC 7029/VI/95 rev. 5 Appendix B, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009)

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

Executive Summary

A study was conducted to determine the magnitude of residues of metrafenone in grapes after three applications of BAS 560 02 F. During the 2013 growing season eight trials were conducted on grapes in both north and south Europe and in both wine and table varieties, to determine the residues of metrafenone.

Each trial consisted of two plots, one untreated control and one treated plot. The treated plots received three applications of BAS 560 02 F at a nominal rate of 0.16 kg ai/ha (0.32 L/ha) at spray volumes of 150, 600 or 800 L/ha, dependent on trial and application timing.

Samples of grapes were collected immediately before application (untreated only), immediately after final application (treated only) and at 21, 28 and 35 days after the last application (DALA).

All samples were analysed for metrafenone using method 535/2. Overall procedural recoveries for wine grapes were 84.9% (RSD =12, n=36) at fortification levels of 0.01, 0.1, 0.5 and 1.0 mg/kg and 99.4% (RSD=12, n=8) for table grapes at fortification levels of 0.01 and 0.1 mg/kg.

Residues of metrafenone in wine grapes ranged from 0.17-0.83 mg/kg, 0.05-0.65 mg/kg, 0.05-0.34 mg/kg and 0.04-0.36 mg/kg at 0, 20-21, 27-28 and 34-36 DALA, respectively.

Residues of metrafenone in table grapes were not significantly different to those in wine grapes and ranged from 0.30-0.53 mg/kg, 0.09-0.32 mg/kg, 0.08-0.24 mg/kg and 0.05-0.16 mg/kg at 0, 21, 28 and 35 DALA, respectively.

Residues in grapes treated according to GAP were 0.21, 0.23, 0.31 and 0.36 mg/kg for grapes grown in the NEU and 0.05, 0.08, 0.10 and 0.24 mg/kg for grapes grown in the SEU.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: suspension concentrate formulation
Lot/Batch #: 0008024566, Metrafenone: 500.0 g/L
Purity: 500 g/L
CAS#: 220899-03-6
Development code: BAS 560 02 F
Spiking levels: 0.01, 0.1, 0.5 and 1 mg/kg

3. **Test Commodity:** Fruit
Crop: Grapes
Type: Wine and Table
Variety: Johanniter, Weissburgunder, Cabernet Franc, Cabernet Sauvignon, Merlot, Red Glob, Muscat
Botanical name: *Vitis vinifera*
Crop parts(s) or processed commodity: Fruit
Sample size: 1-2 kg, 12 bunches

B. STUDY DESIGN AND METHODS

1. Test procedure

Eight field trials were conducted, two in Germany, two in Northern France, one in Southern France, one in Italy, one in Greece and one on Spain. BAS 560 02 F was applied on three occasions at approximately 125 or 160 g ai/ha in water volumes of 150-800 L/ha.

Details of the test sites are summarised below along with information on the application details for each trial.

Table 6.3.1-1: Test site information

Trial No.	Crop	Variety	Country	State/Province	Geographical Area
L130616	Wine grape	Johanniter	Germany	Rheinhessen	N
L130617	Wine grape	Weissburgunder	Germany	Palatinate	N
L130618	Wine grape	Cabernet Franc	Northern France	Indre et Loire	N
L130619	Wine grape	Cabernet Sauvignon	Northern France	Maine et Loire	N
L130620	Wine grape	Cabernet Sauvignon	Southern France	Gironde	S
L130621	Wine grape	Merlot	Spain	Andalucía/Sevilla	S
L130622	Table grape	Red Glob	Italy	Puglia/Foggia	S
L130623	Table grape	Muscat	Greece	Central Macedonia/Pieria	S

Table 6.3.1-2: Application information

Trial No.	Application No.	Date	Growth stage	Water volume (L/ha)	Rate BAS 560 02 F (L/ha)*	Rate metrafenone (g ai/ha)**
L130616	A1	13.08.2013	81	632	0.340	170
	A2	21.08.2013	83	774	0.310	155
	A3	29.08.2013	85	854	0.340	170
L130617	A1	20.08.2013	78	603	0.322	161
	A2	28.08.2013	79	797	0.319	160
	A3	06.09.2013	81	827	0.331	166
L130618	A1	26.08.2013	79	163	0.347	173
	A2	03.09.2013	83	155	0.331	165
	A3	11.09.2013	85	148	0.315	157
L130619	A1	02.09.2013	83	157	0.334	167
	A2	10.09.2013	83	141	0.302	151
	A3	19.09.2013	85	136	0.291	145
L130620	A1	14.08.2013	79	148	0.248	124
	A2	22.08.2013	83	153	0.256	128
	A3	30.08.2013	85	152	0.253	126
L130621	A1	18.06.2013	77	621	0.259	129
	A2	26.06.2013	77	845	0.264	132
	A3	04.07.2013	79	821	0.257	128
L130622	A1	20.08.2013	81	619	0.258	129
	A2	28.08.2013	81-83	817	0.255	128
	A3	06.09.2013	85	747	0.233	117
L130623	A1	17.07.2013	73	603	0.251	126
	A2	25.07.2013	75	801	0.251	126
	A3	02.08.2013	82	810	0.253	127

2. Description of analytical procedures

Grape samples were analysed using method 535/2. Samples were extracted using a mixture of methanol, water and hydrochloric acid. Aliquots of the initial extracts were centrifuged and partitioned against cyclohexane under alkaline conditions. Final determination of residues was performed by LC-MS/MS using a TSQuantum mass spectrometer with positive ionization polarity. Mass transitions used were 411 to 209 m/z for confirmation and 411 to 229 m/z for quantification. The limit of quantification for metrafenone in grapes was 0.01 mg/kg.

The samples were analysed in series along with fortification experiments. No residues greater than or equal to 0.01 mg/kg were found in control samples, indicating no interferences from the sample material. Fortified control samples were spiked at concentrations of 0.01, 0.1, 0.5 and 1.0 mg/kg and analysed alongside treated samples to determine method efficiency. The results of these procedural recoveries are shown below.

Table 6.3.1-3: Procedural recoveries for wine and table grapes

Matrix	Fortification levels (mg/kg)	metrafenone			
		Mean %	SD	RSD	n
Wine grapes	0.01	83.9	13	15	16
	0.1	87.8	5	6	16
	0.5	72.0	-	-	2
	1.0	82.5	-	-	2
Overall		**84.9**	**10**	**12**	**36**
Table grapes	0.01	109	9	8	4
	0.1	89.5	3	4	4
Overall		**99.4**	**12**	**12**	**8**

Linearity of the method was confirmed using calibration standards at six concentrations from 0.05 to 5.0 ng/mL. The R^2 value was 0.9995. Matrix effects were studied using matrix matched standards against non-matrix matched standards at equivalent concentrations. Suppression, ranged from 6.2 to 29.9%. As some samples did show suppression above 20%, matrix matched standards were used throughout the analytical phase.

II. RESULTS AND DISCUSSION

The maximum storage interval from harvest until analysis was up to 313 days. Samples were previously shown to be stable during this period of frozen storage (Class, T., AC375839 (CL 375839): Storage Stability of AC 375839 Residues at <18ºC in Grapes and Wine, DocID 2000/7000144).

The results of the sample analyses are summarised in the following table.

Table 6.3.1-4: Summary of metrafenone residues in wine and table grapes following treatment with BAS 560 F

Commodity	Sampling occasion	Portion analysed	DALA	Growth stage	n	Range of residues BAS 560 F
Wine grape	1	Fruit	0	BBCH 79-85	6	0.17-0.83
	2	Fruit	20-21	BBCH 85-89	6	0.05-0.65
	3	Fruit	27-28	BBCH 85-89	6	0.05-0.34
	4	Fruit	34-36	BBCH 89	6	0.04-0.36
Table grape	1	Fruit	0	BBCH 82-85	2	0.30-0.53
	2	Fruit	21	BBCH 83-87	2	0.09-0.32
	3	Fruit	28	BBCH 89	2	0.08-0.24
	4	Fruit	35	BBCH 89	2	0.05-0.16

The data from individual trials are detailed in the residue data summaries below.

Table 6.3.1-5: Residue data summary tables

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity / variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**kg as/ha**						
GAP (NEU)						**0.16**	**3**				**28**	
2014/1161029 L130616 Ockheimer, Germany	Wine grape/Johaniter	1 2008 2 20-28.06.13 3 02.10.13	Motor knapsack sprayer		632 774 854	0.17 0.155 0.17	3 29.08.13	85	Grapes Grapes Grapes Grapes	0.48 0.34 0.34 <u>0.36</u>	0 21 27 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**Kg as/ha**						
2014/1161029 L130617 Eschbach, Germany	Wine grape/Weissburgunder	1 2005 2 18-27.06.13 3 07.10.13	Motor knapsack sprayer		603 797 827	0.161 0.160 0.166	3 06.09.13	81	Grapes Grapes Grapes Grapes	0.83 0.65 0.24 0.31	0 20 28 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**Kg as/ha**						
2014/1161029 L130618 La Chappelle Sur Loire, France (north)	Wine grape/Cabernet Franc	1 1980 2 20.06-03.07.13 3 07.10.13	Mist blower		163 155 148	0.173 0.165 0.157	3 11.09.13	85	Grapes Grapes Grapes Grapes	0.22 0.17 0.15 <u>0.23</u>	0 21 28 36	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161029 L130619 Les Ulmes, France (north)	Wine grape/Cabernet Sauvignon	1 1972 2 25.06-04.07.13 3 19.10.13	Mist blower		157 141 136	0.167 0.151 0.145	3 19.09.13	85	Grapes Grapes Grapes Grapes	0.17 0.23 0.15 <u>0.21</u>	0 20 28 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
GAP (SEU)						**0.125**	**3**				**28**	
2014/1161029 L130620 Saint-Loubes, France (south)	Wine grape/Cabernet Sauvignon	1 1988 2 15-30.06.13 3 27.09.13	Atomizer		148 153 152	0.124 0.128 0.126	3 30.08.13	85	Grapes Grapes Grapes Grapes	0.34 0.12 <u>0.10</u> 0.10	0 21 28 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161029 L130621 Utrera, Spain	Wine grape/Merlot	1 2004 2 19-29.04.13 3 13.08.13	Motor knapsack sprayer		621 845 821	0.129 0.132 0.128	3 04.07.13	79	Grapes Grapes Grapes Grapes	0.23 0.05 <u>0.05</u> 0.04	0 20 28 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161029 L130622 Borgo Tressanti Cerignola, Italy	Table grape/Red Glob	1 1997 2 20.05-02.06.13 3 04.10.13	Motor knapsack sprayer		619 817 747	0.129 0.128 0.117	3 06.09.13	85	Grapes Grapes Grapes Grapes	0.53 0.32 <u>0.24</u> 0.16	0 21 28 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161029 L130623 Kato Milia, Greece	Table grape/Muscat	1 1993 2 05-25.05.13 3 20.08-10.09.13	Gas spray with lance		603 801 810	0.126 0.126 0.127	3 02.08.13	82	Grapes Grapes Grapes Grapes	0.30 0.09 <u>0.08</u> 0.05	0 21 28 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

III. CONCLUSION

Following application of BAS 560 02 F to grapes, residues in wine grapes ranged from 0.17 to 0.83 mg/kg at 0 DALA (BBCH 79-85). For wine grapes collected at 20-21 DALA (BBCH 85-89) and 27-28 DALA (BBCH 85-89), residues ranged from 0.05 to 0.65 mg/kg and 0.05 to 0.34 mg/kg, respectively. At the final sampling occasion, 34-36 DALA (BBCH 89), residues in wine grapes ranged from 0.04 to 0.36 mg/kg.

In table grapes, residues immediately after the last application (0 DALA, BBCH 82-85) ranged from 0.3 to 0.53 mg/kg. Samples at 21 DALA (BBCH 83-87) were 0.09 to 0.32 mg/kg and 0.08 to 0.24 mg/kg at 28 DALA (BBCH 89). At the final sampling occasion (35 DALA, BBCH 89), residues ranged from 0.05 to 0.16 mg/kg.

No residues of metrafenone above the limit of quantification (0.01 mg/kg) were found in any of the control samples from wine or table grapes.

Residues in grapes treated according to GAP were 0.21, 0.23, 0.31 and 0.36 mg/kg for grapes grown in the NEU and 0.05, 0.08, 0.10 and 0.24 mg/kg for grapes grown in the SEU.

Report:	CA 6.3.1/2 Oxspring S., 2014a Study on the residue behaviour of BAS 560 F (Metrafenone) in grapes (wine and table) after treatment with BAS 560 02 F under field conditions in Germany, France (North and South), Italy and Spain during 2014 2014/1161529
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5, OECD 509 Crop Field Trial (2009), EEC 7524/VI/95 rev. 2, SANCO/3029/99 rev. 4
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

Eight field trials were conducted in representative grape growing regions of Germany, France (Northern and Southern), Italy and Spain in 2014. Trials were conducted in order to determine the magnitude of residues of metrafenone (BAS 560 F) in or on raw agricultural commodities (RAC) of grapes, both wine and table varieties, under field conditions following four applications of BAS 560 02 F.

Plots were treated with BAS 560 02 F, an SC formulation of metrafenone containing 500 g/L, at a rate of 125g ai/ha (0.25 L of formulated product/ha) on the first two occasions (55-60 and 43-49 days before harvest) and at a higher rate of 160 g ai/ha (0.32 L of formulated product/ha) at 33-40 and 26-29 days before harvest. Spray volumes were 150, 600 or 800 L/ha dependent on trial or application timing.

Samples of whole or part grape bunches were taken immediately before application (untreated plot only) and immediately after the first application (treated plots only). Further samples were taken at 20-22, 26-28 and 34-38 days after the last application (DALA) from both untreated and treated plots.

Samples were transported deep-frozen for analysis for residues of metrafenone (BAS 560 F) using method 535/2 (“Method for the determination of BAS 421 F, BAS 480 F, BAS 500 F, BF 500-3, BAS 505 F, BAS 510 F, BAS 550 F, BAS 555 F and BAS 560 F in plant matrices”).

Using this method, the mean procedural recovery was 99% (RSD=14.7, n=10) at fortification levels of 0.01 and 10 mg/kg.

Residues in the treated samples ranged from 0.22 to 0.80 mg/kg at 0 DALA, 0.08 to 0.49 mg/kg at 20-22 DALA, 0.09 to 0.36 mg/kg at 26-29 DALA and 0.06 to 0.30 mg/kg at 34-38 DALA.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: suspension concentrate formulation
Lot/Batch #: 0008024566
Purity: 500 g/L
CAS#: 220899-03-6
Development code: BAS 560 02 F

2. **Test Commodity:** Fruit
Crop: Grapes
Type: Wine and Table
Variety: Wine grape – Müller-Thurgau, Kerner, Pinot Noir, Gamay, Glemache Noil, Trebbiano
Table grape – Vittoria, Black Magic
Botanical name: *Vitis vinifera*
Crop parts(s) or processed commodity: Fruit
Sample size: 1 kg (0 DBLA/0 DALA), 2 kg (21, 28 and 35 DALA)

B. STUDY DESIGN AND METHODS

1. Test procedure

Eight field trials were conducted, two in Germany, two in Northern France, one in Southern France, two in Italy and one in Spain. BAS 560 02 F was applied on four occasions at rates ranging from 113 to 170 g as/ha in water volumes of 136 to 811 L/ha.

Details of the test sites and information on the application details for each trial are summarised below.

Table 6.3.1-6: Test site information

Trial No.	Crop	Variety	Country	State/Province	Geographical Area
S14-00042-01 (L140080)	Wine grape	Müller-Thurgau	Germany	Baden-wúrttemberg	N
S14-00042-02 (L140081)	Wine grape	Kerner	Germany	Talheimer Hof, Baden-wúrttemberg	N
S14-00042-03 (L140082)	Wine grape	Pinot Noir	Northern France	Loiret	N
S14-00042-04 (L140083)	Wine grape	Gamay	Northern France	Loiret	N
S14-00042-05 (L140084)	Wine grape	Glemache Noil	Southern France	Tarn et Garonne	S
S14-00042-06 (L140085)	Table grape	Vittoria	Italy	Sicily	S
S14-00042-07 (L140086)	Wine grape	Trebbiano	Italy	Emilia Romagna	S
S14-00042-08 (L140087)	Table grape	Black Magic	Spain	Seville	S

Table 6.3.1-7: Application information

Trial No.	Application No.	Date	Growth stage	Water volume (L/ha)	Rate BAS 560 02 F (L/ha)*	Rate metrafenone (g ai/ha)**
S14-00042-01 (L140080)	A1	15.07.2014	75	542	0.23	113
	A2	24.07.2014	77	622	0.26	130
	A3	04.08.2014	78	801	0.32	160
	A4	14.08.2014	79	792	0.32	158
S14-00042-02 (L140081)	A1	18.07.2014	76	600	0.25	125
	A2	31.07.2014	77	594	0.25	124
	A3	07.08.2014	77	797	0.32	159
	A4	19.08.2014	79	795	0.32	159
S14-00042-03 (L140082)	A1	28.07.2014	77	155	0.26	129
	A2	07.08.2014	79	155	0.26	129
	A3	19.08.2014	79	153	0.33	163
	A4	27.08.2014	83	152	0.32	162
S14-00042-04 (L140083)	A1	22.07.2014	75	162	0.27	135
	A2	01.08.2014	77	149	0.25	124
	A3	11.08.2014	77	152	0.32	162
	A4	21.08.2014	81	159	0.34	170
S14-00042-05 (L140084)	A1	16.07.2014	79	143	0.24	119
	A2	28.07.2014	79	145	0.24	121
	A3	07.08.2014	81	136	0.23	113
	A4	14.08.2014	81	143	0.31	153
S14-00042-06 (L140085)	A1	30.05.2014	73	596	0.25	124
	A2	09.06.2014	75	650	0.27	135
	A3	20.06.2014	77	792	0.32	158
	A4	30.06.2014	79	799	0.32	160
S14-00042-07 (L140086)	A1	15.07.2014	79	540	0.23	113
	A2	24.07.2014	81	544	0.23	113
	A3	01.08.2014	81	843	0.34	169
	A4	12.08.2014	83	799	0.32	160
S14-00042-08 (L140087)	A1	19.05.2014	79	587	0.24	122
	A2	28.05.2014	79	606	0.25	126
	A3	06.06.2014	81	803	0.32	161
	A4	17.06.2014	81	811	0.32	162

*Derived rate based on actual rate
** Derived rate based on nominal concentration

2. Description of analytical procedures

Grape samples were removed from their stems and homogenised in the presence of dry ice. Samples were then extracted using a mixture of methanol, water and hydrochloric acid by maceration. Aliquots were removed and centrifuged prior to partitioning in alkaline conditions against cyclohexane. Quantification of residues was by LC-MS/MS using method 535/2.

The limit of quantification for the method 535/2 in grapes was 0.01 mg/kg. The maximum period of frozen storage of study samples from sampling to extraction was 223 days and all samples were analysed within two days of extraction.

Procedural recoveries were performed at fortification levels of 0.01 and 10 mg/kg. Overall mean recovery was 99%, with an RSD of 14.7% (n=10).

II. RESULTS AND DISCUSSION

Residue levels of metrafenone (BAS 560 F) in the grape samples analysed are summarised below.

Table 6.3.1-8: Summary of residues in grape samples treated with metrafenone (BAS 560 F)

Sampling occasion (DALA)[1]	n[2]	Residues of metrafenone (BAS 560 F)
0	16	0.22-0.80
20-22	16	0.08-0.49
26-29	16	0.09-0.36
34-38	16	0.06-0.30

[1]DALA: days after last application
[2]n = number of samples

The data from individual trials are detailed in the residue data summaries below.
In trials, L140087, Vivando was applied to the entire field rather than just the treated plot which resulted in positive residues in control samples. However, treated plots were still treated appropriately and the residues detected here are still valid values.

Table 6.3.1-9: Residue data summary tables (metrafenone in grapes)

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity / variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
GAP (NEU)						**160**	**3**				**28**	
2014/1161529 S14-00042-01 (L140080) Dacsuckel-winzerhof 1 69126 Heidelberg	Wine grape/Müller-Thurgau FB 0269	1 1991 2 Not stated 3 11.09.2014	Foliar	20.8 20.9 15.5 15.7	542 622 801 792	113 130 160 158	4 14.08.2014	79	Grapes Grapes Grapes Grapes	0.36 0.08 <u>0.18</u> 0.13	0 20 28 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-02 (140081) Talheimer Hof, 74388 Talheim Germany	Wine grape/Kerner FB 0269	1 1980 2 01.06-10.06.2014 3 16.09.2014	Foliar	20.8 20.9 15.7 15.6	600 594 797 795	125 124 159 159	4 19.08.2014	79	Grapes Grapes Grapes Grapes	0.34 0.21 <u>0.17</u> 0.15	0 22 28 38	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Northern France	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-03 (L140082) 252 Rue de la Grange 45160 Hilaire St Mesmin Loiret	Wine grape/Pinot Noir FB 0269	1 15.05.1998 2 10.06-20.06.2014 3 24.09.2014	Foliar	8.32 8.32 1.07 1.07	155 155 153 152	129 129 163 162	4 27.08.2014	83	Grapes Grapes Grapes Grapes	0.22 0.09 <u>0.09</u> 0.06	0 21 29 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Northern France	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-04 (L140083) Les Septiers 45630 Beaulieu sur Loire, Loiret	Wine grape/Gamay FB 0269	1 2001 2 25.05-10.06.2014 3 18.09.2014	Foliar	83.3 83.2 107 107	162 149 152 159	135 124 162 170	4 21.08.2014	81	Grapes Grapes Grapes Grapes	0.50 0.33 0.16 0.23	0 21 28 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Northern France	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
GAP (SEU)						**125**	**3**				**28**	
2014/1161529 S14-00042-05 (L140084) 1163 rte de la Françoise 822900 Meauzac Tarn et Garrone	Wine grape/Glemache Noil FB 0269	1 Nov 2000 2 10.06-25.06.2014 3 11.09.2014	Foliar	83.2 83.4 83.1 107	143 145 136 143	119 121 113 153	4 14.08.2014	81	Grapes Grapes Grapes Grapes	0.33 (c0.12) 0.11 0.11 0.08	0 20 26 34	BASF method 535/2 Residues were found in untreated specimen L14008400 01 of 0.12 mg/kg. No residues above the LOQ detected in any of the other untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Italy	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-06 (L140085) Contrada Piano del Fiori 95041 Caltagirone Sicily	Table grape/Vittoria FB 0269	1 2003 2 May 2014 3 28.07.2014	Foliar	20.8 20.8 26.5 26.7	596 650 792 799	124 135 158 160	4 30.06.2014	79	Grapes Grapes Grapes Grapes	0.80 0.49 <u>0.36</u> 0.30	0 21 28 35	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Italy	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-07 (L140086) Via Marana 40050 Granarola Emilia Romagna	Wine grape/Trebbiano FB 0269	1 2002 2 May 2014 3 09.09.2014	Foliar	20.9 20.8 20 20	540 544 843 799	113 113 169 160	4 12.08.2014	83	Grapes Grapes Grapes Grapes	0.45 0.10 0.11 0.06	0 22 27 34	BASF method 535/2 No residues above the LOQ detected in any of the untreated specimens

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wine Grape/Fruit	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Spain	**Other ai in the formulation:**	None
Content of active substance:	500	**(common name and content)**	
Formulation (e.g. WP):	BAS 560 02 F SC	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	**Commodity/ variety/ CODEX code**	**Date of 1 Planting 2 Flowering 3 Harvest**	**Method of treatment**	**Nominal application rate per treatment**			**No. of treatments**	**Growth stage at last treatment**	**Portion analysed**	**Residues**	**PHI (days)**	**Remarks**
				g as/hL	**Water L/ha**	**g as/ha**						
2014/1161529 S14-00042-08 (L140087) C/Dinamarca No 6 41720 Los Palacios Y Villafranca Seville	Table grape/Black Magic FB 0269	1 03.03.2004 2 Not stated 3 15.07.2014	Foliar	20.8 20.8 20.0 20.0	587 606 803 811	122 126 161 162	4 17.06.2014	81	Grapes Grapes Grapes Grapes	0.52 (c0.04) 0.25 (c0.02) 0.23 (c0.02) 0.24 (c0.03)	0 21 28 35	BASF method 535/2 Residues were found in untreated samples ranging from 0.02 to 0.04 mg/kg (in parentheses)

c = residues in control

III. CONCLUSION

Eight field trials were conducted during the growing season of 2014 to determine the level of residues of metrafenone (BAS 560 F) in or on raw agricultural commodities (RAC) of grapes, both wine and table varieties, following four applications of BAS 560 02 F.

Treated samples of grapes were collected immediately after the last application and at 20-22, 26-29 and 34-38 days after the last application. Following extraction, samples were analysed using method 535/2. Residues in grape samples ranged from 0.22 to a maximum of 0.80 mg/kg immediately after the last application declining to 0.30 mg/kg at the final sampling occasion.

Residues in grapes treated according to GAP were 0.09, 0.17, 0.18 and 0.23 mg/kg for grapes grown in the NEU and 0.11(2), 0.24 and 0.36 mg/kg for grapes grown in the SEU.

CA 6.3.2 Crop 2 (Cereals)

Report: CA 6.3.2/1
Trewhitt J.A., 2001a
AC 375839 300 g a.s./L SC (SF10358 (BAS 560 00 F) and SF09957): At harvest residues study on AC 375839 (BAS 560 F) in winter wheat - The Netherlands, 2000
2001/7000487

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
Netherlands	BAS 560 F, SC 300 G/L	2	0.150	0.050	Grain	34	0.04	<0.01	<0.01	<0.01	
2000	(SF 09957)				Straw	34	0.98	<0.1	<0.1	<0.1	2001/ 7000487
00-770-01 (AH)	BAS 560 00 F	2	0.150	0.050	Grain	34	0.04	<0.01	<0.01	<0.01	
	(SF 10358)				Straw	34	0.61	<0.1	<0.1	<0.1	

Report: CA 6.3.2/2
Smalley R., 2001a
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter wheat - North France 2000
2001/7001657

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F-France (North) 2000 00-831-346	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	33	0.03	<0.01	<0.01	<0.01	2001/ 70016574757
					Straw	33	2.32	0.12	<0.1	<0.1	
(AH)	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	33	0.01	<0.01	<0.01	<0.01	
					Straw	33	1.80	0.11	<0.1	<0.1	

Report: CA 6.3.2/3
Smalley R., 2001b
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter wheat - United Kingdom 2000
2001/7001658

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
UK-United Kingdom 2000 00-832-01 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.075	Grain	41	0.03	<0.01	<0.01	<0.01	2001/ 70016584758
					Straw	41	2.04	0.16	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.075	Grain	41	0.04	<0.01	<0.01	<0.01	
					Straw	41	1.72	0.18	<0.1	<0.1	

Report: CA 6.3.2/4
Greenstreet C.A.,Deutsch E., 2002a
BAS 560 F (AC 375839) 300 g a.s./L SC (SF10358) and BAS 560 F 300 g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter wheat, Germany, 2000
2002/7004672

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	BAS 560 F	CL 3000402	CL 434223	CL 376991	Report no.
D-Germany 2000 00-922-01 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	not available	Grain	35	<0.01	<0.01	<0.01	<0.01	2002/ 70046724
					Straw	35	0.40	<0.10	<0.10	<0.10	
	BAS 560 00 F (SF 10358)	2	0.150	not available	Grain	35	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	
					Straw	35	<u>0.67</u>	<u><0.10</u>	<u><0.10</u>	<u><0.10</u>	

Report: CA 6.3.2/5
Smalley R., 2001c
AC 375839 300g a.s./L SC (SF 09957): Decline curve residue study on AC 375839 in winter wheat - Germany 1999
2001/7001675

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
Northern Europe – Decline Curve Trials (DC)											
D-Germany 1999 99-106-02 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0^-	<0.1	<0.1	<0.1	<0.1	2001/ 70016754748
					Whole Plant	0^+	5.63	<0.1	<0.1	<0.1	
					Whole Plant	14	0.74	<0.1	<0.1	<0.1	
					Plant - ears	28	1.38	0.13	<0.1	<0.1	
					Ears	28	0.16	<0.1	<0.1	<0.1	
					Grain	35	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	
					Straw	35	0.89	<u><0.1</u>	<u><0.1</u>	<u><0.1</u>	
					Grain	41	<0.01	<0.01	<0.01	<0.01	
					Straw	41	<u>0.93</u>	<0.1	<0.1	<0.1	

Report: CA 6.3.2/6
Smalley R., 2002b
AC 375839 300g a.s./L SC (SF 09957): Decline curve residue study on AC 375839 in winter wheat - United Kingdom 1999
2002/7004680

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
UK –United Kingdom	BAS 560 F, SC 300 G/L	2	0.200	not available	Whole Plant	0^-	0.17	<0.1	<0.1	<0.1	
1999	(SF 09957)				Whole Plant	0^+	2.62	<0.1	<0.1	<0.1	2002/ 7004680
99-107-01					Whole Plant	14	0.76	<0.1	<0.1	<0.1	
(DC)					Plant - ears	28	1.10	<0.1	<0.1	<0.1	
					Ears	28	<0.1	<0.1	<0.1	<0.1	
					Ears	35	1.43	<0.1	<0.1	<0.1	
					Plant – ears	35	0.04	<0.01	<0.01	<0.01	
					Grain	41	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	
					Straw	41	<u>0.59</u>	<u><0.1</u>	<u><0.1</u>	<u><0.1</u>	
					Grain	49	<0.01	<0.01	<0.01	<0.01	
					Straw	49	0.58	<0.1	<0.1	<0.1	

Report: CA 6.3.2/7
Smalley R., 2002c
AC 375839 300g a.s./L SC (SF 09957): Decline curve residue study on AC 375839 in winter wheat - Netherlands 1999
2002/7004745

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
Northern Europe - Decline Curve Trials (DC) contd.											
NL-Netherlands 1999 99-108-01 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	not available	Whole Plant	0^-	0.11	<0.1	<0.1	<0.1	4746 IIA 6.3.1.2/7
					Whole Plant	0^+	3.25	<0.1	<0.1	<0.1	
					Whole Plant	13	0.58	<0.1	<0.1	<0.1	
					Plant - ears	26	0.94	<0.1	<0.1	<0.1	
					Ears	26	0.17	<0.1	<0.1	<0.1	
					Ears	35	0.01	<0.1	<0.1	<0.1	
					Plant – ears	35	1.10	<0.1	<0.1	<0.1	
					Grain	41	<0.01	<0.01	<0.01	<0.01	
					Straw	41	1.85	<0.1	<0.1	<0.1	

Report: CA 6.3.2/8
Smalley R., 2001d
BAS 560 F (AC 375839) 300g a.s./L SC (SF 10358): Decline curve residue study on BAS 560 F in winter wheat - North France 2000
2001/7001660

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F- France (North)	BAS 560 00 F	2	0.150	0.040	Whole Plant	0^-	<0.1	<0.1	<0.1	<0.1	
2000	(SF 10358)				Whole Plant	0^+	2.59	<0.1	<0.1	<0.1	2001/ 7001660
00-834-347					Whole Plant	14	1.62	<0.1	<0.1	<0.1	
(DC)					Plant - ears	28	1.54	<0.1	<0.1	<0.1	
					Ears	28	0.25	<0.1	<0.1	<0.1	
					Grain	35	<0.01	<0.01	<0.01	<0.01	
					Straw	35	1.43	<0.1	<0.1	<0.1	

Report: CA 6.3.2/9
Smalley R., 2001e
BAS 560 F (AC 375839) 300 g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300 g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter wheat - South France 2000
2001/7001656

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	BAS 560 F	CL 3000402	CL 434223	CL 376991	Report no.
F-France (South) 2000 00-833-290 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	29	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	2001/7001656
					Straw	29	<u>1.25</u>	<u>≤0.1</u>	<u>≤0.1</u>	<u>≤0.1</u>	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	29	<0.01	<0.01	<0.01	<0.01	
					Straw	29	1.07	<0.1	<0.1	<0.1	
F-France (South) 2000 00-833-291 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	23	<0.01	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	2001/7001656
					Straw	23	1.64	<u>≤0.1</u>	<u>≤0.1</u>	<u>≤0.1</u>	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	23	0.01	<0.01	<0.01	<0.01	
					Straw	23	1.09	<0.1	<0.1	<0.1	
F-France (South) 2000 00-833-643 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	34	<u>0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	2001/7001656
					Straw	34	<u>1.10</u>	<u>≤0.1</u>	<u>≤0.1</u>	<u>≤0.1</u>	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	34	<0.01	<0.01	<0.01	<0.01	
					Straw	34	1.07	<0.1	<0.1	<0.1	

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F-France (South) 2000 00-833-644	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	33	<0.01	<0.01	<0.01	<0.01	2001/7001656
					Straw	33	2.12	<0.1	<0.1	<0.1	
(AH)	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	33	0.01	0.01	≤0.01	≤0.01	
					Straw	33	1.69	≤0.1	≤0.1	≤0.1	

Data for the metabolites have been included from the trial using a 23 day PHI 00-833-291 (AH) since the residues were below the limit of quantitation at a more critical GAP.

Report: CA 6.3.2/10
Jones S., 2002a
Study on the residue behaviour of BAS 560 F in cereals after application of BAS 560 00 F under field conditions in France (S), 2001
2002/7004890

Guidelines: UK Guidance on Crop Residue Data Requirements PSD October 1992, EEC 91/414 Annex III 8, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 2, 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

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	**Application**				**Residues (mg/kg)**						**Ref.**
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F-France (South) 2001 FTL/31/01 (AH)	BAS 560 00 F (SF 10358)	2	0.150	0.050	Barley grain	35	0.05	<0.01	<0.01	<0.01	2002/7004890
					Barley straw	35	2.13	0.13	0.04	0.07	
F-France (South) 2001 FTL/32/01 (AH)	BAS 560 00F (SF 10358)	2	0.150	0.050	Wheat grain	35	0.03	<0.01	<0.01	<0.01	2002/7004890
					Wheat straw	35	1.61	0.17	0.11	0.07	

Report: CA 6.3.2/11
Smalley R., 2002d
AC 375839 300g a.s./L SC (SF 09957): Decline curve residue study on AC 375839 in winter wheat - France South 1999
2002/7004740

Guidelines: EEC 7029/VI/95 rev. 5, EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F- France (South) 1999 99-109-295 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0-	0.11	<0.1	<0.1	<0.1	2002/7004740
					Whole Plant	0+	4.07	<0.1	<0.1	<0.1	
					Whole Plant	13	1.21	<0.1	<0.1	<0.1	
					Plant - ears	28	0.83	<0.1	<0.1	<0.1	
					Ears	28	0.23	<0.1	<0.1	<0.1	
					Grain	35	0.01	<0.01	<0.01	<0.01	
					Straw	35	0.89	<0.1	<0.1	<0.1	
					Grain	42	<0.01	<0.01	<0.01	<0.01	
					Straw	42	0.88	<0.1	<0.1	<0.1	
F- France (South) 1999 99-109-296 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0-	0.13	<0.1	<0.1	<0.1	2002/7004740
					Whole Plant	0+	2.88	<0.1	<0.1	<0.1	
					Whole Plant	13	1.13	<0.1	<0.1	<0.1	
					Plant - ears	28	1.06	<0.1	<0.1	<0.1	
					Ears	28	0.13	<0.1	<0.1	<0.1	
					Grain	35	0.01	<0.01	<0.01	<0.01	
					Straw	35	1.08	<0.1	<0.1	<0.1	
					Grain	42	<0.01	<0.01	<0.01	<0.01	
					Straw	42	1.01	<0.1	<0.1	<0.1	

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	BAS 560 F	CL 3000402	CL 434223	CL 376991	Report no.
F- France (South) 1999 99-109-631 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant Whole Plant Whole Plant Plant - ears Ears Grain Straw Grain Straw	0- 0+ 14 28 28 36 36 42 42	<0.1 3.70 0.96 1.11 0.19 <0.01 0.80 <0.01 1.11	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	2002/7004740
F- France (South) 1999 99-109-632 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant Whole Plant Whole Plant Plant - ears Ears Grain Straw Grain Straw	0- 0+ 14 28 28 35 35 42 42	0.10 3.44 1.25 1.90 0.18 <0.01 1.70 <0.01 1.60	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	<0.1 <0.1 <0.1 <0.1 <0.1 <0.01 <0.1 <0.01 <0.1	2002/7004740

Report: CA 6.3.2/12
Smalley R., 2001f
BAS 560 F (AC 375839) 300g a.s./L SC (SF 10358): Decline curve residue study on BAS 560 F in winter wheat - South France 2000
2001/7001676
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
Southern Europe Decline Curve Trials (DC) contd.											
F-France (South) 2000 00-923-292 (DC)	BAS 560 00 F (SF 10358)	2	0.0150	not available	Whole Plant	0^-	<0.1	<0.1	<0.1	<0.1	2001/ 7001676
					Whole Plant	0^+	3.3	<0.1	<0.1	<0.1	
					Whole Plant	14	0.56	<0.1	<0.1	<0.1	
					Plant - ears	28	0.70	<0.1	<0.1	<0.1	
					Ears	28	0.18	<0.1	<0.1	<0.1	
					Grain	35	<0.01	<0.01	<0.01	<0.01	
					Straw	35	0.67	<0.1	<0.1	<0.1	

Report: CA 6.3.2/13
Smalley R., 2002e
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter barley - North France 2000
2002/7004445
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 7029/VI/95 rev. 5
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F-France (North) 2000 00-835-355 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	36	0.15	0.01	<0.01	<0.01	2002/ 7004445
					Straw	36	1.12	<0.1	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	36	0.15	0.01	<0.01	<0.01	
					Straw	36	1.15	<0.1	<0.1	<0.1	

Report: CA 6.3.2/14
Smalley R., 2002f
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter barley - United Kingdom 2000
2002/7004529

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	BAS 560 F	CL 3000402	CL 434223	CL 376991	Report no.
UK-United Kingdom 2000 00-836-01 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.075	Grain	35	0.14	0.02	<0.01	<0.01	2002/ 7004529
					Straw	35	1.10	0.11	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.075	Grain	35	0.16	0.01	<0.01	<0.01	
					Straw	35	1.28	0.13	<0.1	<0.1	
UK-United Kingdom 2000 00-836-02 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.075	Grain	35	0.07	<0.01	<0.01	<0.01	2002/ 7004529
					Straw	35	1.11	<0.1	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.075	Grain	35	0.06	<0.01	<0.01	<0.01	
					Straw	35	0.83	<0.1	<0.1	<0.1	

Report: CA 6.3.2/15
Smalley R., 2002g
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on AC 375839 in winter barley - Germany 2000
2002/7004463

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
D-Germany 2000 00-837-01 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.050	Grain	42	0.01	0.02	<0.01	<0.01	2002/7004463
					Straw	42	1.09	<0.1	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.050	Grain	42	0.11	0.01	<0.01	<0.01	
					Straw	42	0.78	<0.1	<0.1	<0.1	

Report: CA 6.3.2/16
Trewhitt J.A., 2002a
AC 375839 300 g a.s./L SC (SF09957): Decline curve residue study on AC 375839 (BAS 560 F) in winter barley - UK, 1999
2002/7004681

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
UK-United Kingdom 1999 99-110-01 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.070	Whole Plant	0-	0.12	<0.1	<0.1	<0.1	2002/ 7004681
					Whole Plant	0+	4.02	<0.1	<0.1	<0.1	
					Whole Plant	15	0.37	<0.1	<0.1	<0.1	
					Plant - ears	27	0.80	<0.1	<0.1	<0.1	
					Ears	27	0.26	<0.1	<0.1	<0.1	
					Plant – ears	35	0.10	<0.01	<0.01	<0.01	
					Ears	35	0.49	<0.1	<0.1	<0.1	
					Grain	43	0.02	<0.01	<0.01	<0.01	
					Straw	43	0.41	<0.1	<0.1	<0.1	
					Grain	50	0.02	<0.01	<0.01	<0.01	
					Straw	50	0.54	<0.1	<0.1	<0.1	

- = application just prior to final application
+ = application on day 0

Report: CA 6.3.2/17
Smalley R., 2001g
AC 375839 300g a.s./L SC (SF 09957): Decline curve residue study on AC 375839 in winter barley - Germany 1999
2001/7001659

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
D – Germany 1999 99-111-02 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0-	<0.1	<0.1	<0.1	<0.1	2001/ 7001659
					Whole Plant	0+	4.61	<0.1	<0.1	<0.1	
					Whole Plant	14	0.83	<0.1	<0.1	<0.1	
					Plant - ears	28	0.49	<0.1	<0.1	<0.1	
					Ears	28	0.29	<0.1	<0.1	<0.1	
					Grain	35	0.02	<0.01	<0.01	<0.01	
					Straw	35	0.39	<0.1	<0.1	<0.1	
					Grain	44	0.03	<0.01	<0.01	<0.01	
					Straw	44	0.64	<0.1	<0.1	<0.1	
D – Germany 1999 99-111-03 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.070	Whole Plant	0-	0.62	<0.1	<0.1	<0.1	2001/ 7001659
					Whole Plant	0+	6.75	<0.1	<0.1	<0.1	
					Whole Plant	14	1.99	<0.1	<0.1	<0.1	
					Plant - ears	28	1.93	<0.1	<0.1	<0.1	
					Ears	28	1.36	<0.1	<0.1	<0.1	
					Grain	35	0.09	<0.01	<0.01	<0.01	
					Straw	35	1.70	0.10	<0.1	<0.1	
					Grain	44	0.07	<0.01	<0.01	<0.01	
					Straw	44	0.92	<0.1	<0.1	<0.1	

Report: CA 6.3.2/18
Smalley R., 2002h
BAS 560 F (AC 375839) 300g a.s./L SC (SF 10358): Decline curve residue study on BAS 560 F in winter barley - North France 2000
2002/7004922

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F- France (North) 2000 00-840-356 (DC)	BAS 560 00 F (SF 10358)	2	0.150	0.040	Whole Plant	0^-	0.14	<0.1	<0.1	<0.1	2002/ 7004922
					Whole Plant	0^+	2.27	<0.1	<0.1	<0.1	
					Whole Plant	14	1.54	<0.1	<0.1	<0.1	
					Plant - ears	28	3.08	0.25	<0.1	<0.1	
					Ears	28	0.87	<0.1	<0.1	<0.1	
					Grain	36	0.40	0.02	<0.01	<0.01	
					Straw	36	2.01	0.14	<0.1	<0.1	

Report: CA 6.3.2/19
Smalley R., 2002i
BAS 560 F (AC 375839) 300g a.s./L SC (SF10358) and BAS 560 F (AC 375839) 300g a.s./L SC (SF09957): At harvest residue study on BAS 560 F in winter barley - South France 2000
2002/7004525

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	BAS 560 F	CL 3000402	CL 434223	CL 376991	Report no.
F-France (South) 2000 00-839-294 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.065	Grain	34	0.13	0.01	<0.01	<0.01	2002/ 7004525
					Straw	34	1.65	0.12	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.065	Grain	34	0.10	0.01	<0.01	<0.01	
					Straw	34	1.56	0.13	<0.1	<0.1	
F-France (South) 2000 00-839-645 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	34	0.04	<0.01	<0.01	<0.01	2002/ 7004525
					Straw	34	1.02	<0.1	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	34	0.04	<0.01	<0.01	<0.01	
					Straw	34	0.96	<0.1	<0.1	<0.1	
F-France (South) 2000 00-839-645 (AH)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.150	0.040	Grain	36	0.06	0.01	<0.01	<0.01	2002/ 7004525
					Straw	36	1.94	<0.1	<0.1	<0.1	
	BAS 560 00 F (SF 10358)	2	0.150	0.040	Grain	36	0.05	0.01	<0.01	<0.01	
					Straw	36	1.34	<0.1	<0.1	<0.1	

Report: CA 6.3.2/20
Trewhitt J.A., 2001b
AC 375839 300 g a.s./L SC (SF09957): Decline curve residue study on AC 375839 (BAS 560 F) in winter barley, South France, 1999
2001/7000488

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
France - (South) 1999 99-112-297 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0^-	0.10	<0.1	<0.1	<0.1	2001/ 7000488
					Whole Plant	0^+	4.13	<0.1	<0.1	<0.1	
					Whole Plant	13	0.86	<0.1	<0.1	<0.1	
					Plant - ears	26	<0.1	<0.1	<0.1	<0.1	
					Ears	26	0.22	<0.1	<0.1	<0.1	
					Grain	34	0.08	<u>≤0.01</u>	<0.01	<0.01	
					Straw	34	2.81	<u>0.12</u>	<0.1	<0.1	
					Grain	40	<u>0.12</u>	<0.01	<0.01	<0.01	
					Straw	40	<u>4.03</u>	<0.1	<u>0.11</u>	<u>0.10</u>	
France - (South) 1999 99-112-298 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0^-	<0.1	<0.1	<0.1	<0.1	2001/ 7000488
					Whole Plant	0^+	4.56	<0.1	<0.1	<0.1	
					Whole Plant	13	0.53	<0.1	<0.1	<0.1	
					Plant - ears	26	0.63	<0.1	<0.1	<0.1	
					Ears	26	0.13	<0.1	<0.1	<0.1	
					Grain	34	0.05	<0.01	<0.01	<0.01	
					Straw	34	0.12	<0.1	<0.1	<0.1	
					Grain	40	<u>0.68</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	
					Straw	40	<u>0.90</u>	<u>0.10</u>	<u>≤0.1</u>	<u>≤0.1</u>	

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F-France (South) 1999 99-112-633 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0-	0.29	<0.1	<0.1	<0.1	2001/ 7000488
					Whole Plant	0+	2.71	<0.1	<0.1	<0.1	
					Whole Plant	14	0.90	<0.1	<0.1	<0.1	
					Plant - ears	28	3.67	0.16	<0.1	<0.1	
					Ears	28	0.37	<0.1	<0.1	<0.1	
					Grain	35	0.08	<0.01	<0.01	<0.01	
					Straw	35	2.47	0.12	<0.1	<0.1	
					Grain	42	0.06	<0.01	<0.01	<0.01	
					Straw	42	4.25	0.30	<0.1	<0.1	
F-France (South) 1999 99-112-634 (DC)	BAS 560 F, SC 300 G/L (SF 09957)	2	0.200	0.050	Whole Plant	0-	<0.1	<0.1	<0.1	<0.1	2001/ 7000488
					Whole Plant	0+	1.60	<0.1	<0.1	<0.1	
					Whole Plant	14	0.88	<0.1	<0.1	<0.1	
					Plant - ears	28	1.60	0.13	<0.1	<0.1	
					Ears	28	0.35	<0.1	<0.1	<0.1	
					Grain	35	0.04	<0.01	<0.01	<0.01	
					Straw	35	1.22	0.13	<0.1	<0.1	
					Grain	42	0.04	<0.01	<0.01	<0.01	
					Straw	42	0.97	0.12	<0.1	<0.1	

Report: CA 6.3.2/21
Smalley R., 2002j
BAS 560 F (AC 375839) 300g a.s./L SC (SF 10358): Decline curve residue study on BAS 560 F in winter barley - South France 2000
2002/7004744

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005).

CROP	Application				Residues (mg/kg)						Ref.
Country, year (trial no.)	**Formulation**	**No**	**kg as/ha**	**kg as/hl**	**Matrix**	**Day**	**BAS 560 F**	**CL 3000402**	**CL 434223**	**CL 376991**	**Report no.**
F- France (South) 2000 00-841-647 (DC)	BAS 560 00 F (SF10358)	2	0.150	0.040	Whole Plant	0^-	<0.1	<0.1	<0.1	<0.1	2002/ 7004744
					Whole Plant	0^+	2.51	<0.1	<0.1	<0.1	
					Whole Plant	14	0.82	<0.1	<0.1	<0.1	
					Plant - ears	28	0.61	<0.1	<0.1	<0.1	
					Ears	28	0.41	<0.1	<0.1	<0.1	
					Grain	35	0.23	0.02	<0.01	<0.01	
					Straw	35	0.41	<0.1	<0.1	<0.1	

In addition to the twenty-one studies previously evaluated in the EU and briefly summarised above, three further studies supporting the GAP for cereals are available. These additional studies are summarised in full below.

Report:	CA 6.3.2/22 Raunft E. et al., 2004a Study on the residue behaviour of BAS 560 F in cereals after application of BAS 560 00 F under field conditions in Germany, Denmark, France (N) and United Kingdom, 2002 2003/1001354
Guidelines:	EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, 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
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

During the 2002 growing season, a total of four trials were conducted in cereals (wheat and barley). Each trial consisted of three plots, one untreated (control) and two treated plots, plots 2 and 3. Plot 2 was treated with BAS 560 00 F (300 g/L metrafenone, SC) foliar applied two times at a rate of 150 g a.s./ha, at growth stage 37-39 and at growth stage 49-59. This resulted in a maximum seasonal target rate of 300 g a.s./ha. Plot 3 was treated with BAS 560 00 F (300 g/L metrafenone, SC) by a single foliar application at a rate of 150 g a.s./ha at growth stage 49-59. Cereal whole plant samples were collected on the day of the last application from each plot at all locations. Samples of ears and culms (remaining plant) were collected at 34-36 DALA and grain and straw 39-59 DALA. Specimens were analysed for metrafenone using BASF method No. 993/0 (corresponding to RLA12619.03V) which has a limit of quantification of 0.01 mg/kg in all sample materials. The mean procedural recovery was 88% for metrafenone at fortification levels of 0.01 mg/kg and 1.0 mg/kg. Directly after the last application, the residues of metrafenone in the whole plant (without root) samples ranged from between 1.78 and 3.35 mg/kg in the samples treated with two applications and between 1.55 and 2.67 mg/kg after just one application. In cereal grain collected at 39 to 59 DALA, residues were <0.01 mg/kg and 0.019 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Formulation SF10358
Description: Formulated product - metrafenone: 300 g/L SC
Lot/Batch #: R2066-074
Purity: 300 g/L nominal
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels: 0.01, 0.1, 1.0 mg/kg

3. **Test Commodity:**
Crop: Cereals

Type: Winter wheat
Spring wheat
Winter barley
Spring barley

Variety: Winter wheat: Transit
Spring wheat: Vinjett
Winter barley: Siberia
Spring barley: Astoria

Botanical name: Winter wheat: *Triticum hybernum*
Spring wheat: *Triticum aestivum*
Winter barley: *Hordeum vulgare*
Spring barley: *Hordeum vulgare*

Crop parts(s) or processed commodity: Whole plant (without roots), ears, culms, grain and straw
Sample size: 0.5 kg straw, 1 kg plants without roots, ears, culms, grains

B. STUDY DESIGN AND METHODS

1. Test procedure

The objective of the study was to determine the magnitude of residues of metrafenone in cereals. Each trial consisted of a control (untreated) and two treated plots. All applications were made as foliar sprays. The actual application rates achieved in the study were within 10% of the nominal values. Control samples were taken at every time point and were collected prior to collection of the treated samples to avoid possible contamination.

2. Description of analytical procedures

Samples were analysed using method using BASF method 993/0 (corresponds to RLA12619.03V, Determination of BAS 560 F, CL3000402, CL434223 and CL376991 Residues in Cereals using LC-MS Determination). Determination of residues of metrafenone was by HPLC-MS/MS. The limit of quantification was 0.01 mg/kg for all matrices.

Procedural recoveries at fortification levels of 0.01, 0.1 and 1.0 mg/kg were between 75 and 81% for wheat whole plant, 92 to 104% for barley culms, 71 to 94% for wheat straw, 88 to 93% for wheat ears, 77 to 78% for wheat grains and 99 to 105% for barley grains. The overall mean procedural recovery for all matrices was 88% in terms of metrafenone.

The maximum storage interval from harvest until analysis was 18 months.

II. RESULTS AND DISCUSSION

Directly after the last application, the residues of metrafenone in whole plants ranged from 1.78 to 3.35 mg/kg in samples that received two applications and between 1.55 to 2.67 mg/kg following a single application.

In cereal grain collected at 39 to 59 days after the last application residues of metrafenone were between < 0.01 and 0.02 mg/kg.

A summary of the residue results for whole plant, ears, culms, grain and straw for winter and spring wheat and barley are presented in the following table:

Table 6.3.2-1: Summary of residues in winter wheat and barley

Trial Country, year (Variety)	Application					PHI days	Commodity	Metrafenone (mg/kg)	Reference
	Formulation (g as/L)	g as/ha	g as/hL	Water (L/ha)	No				
Wheat and barley									
ALB/01/02 Denmark, 2002 (Spring Wheat - Vinjett)	300 g/L SC	150 150	50 50	300 300	2	0	Whole Plant	1.83	Method 993/0 (RLA 12619.03V), LOQ 0.01 mg/kg
						36	Ears	<0.01	
						36	Culms	0.20	
						59	Grain	<0.01	
						59	Straw	0.11	
		150	50	300	1	0	Whole Plant	2.67	
						36	Ears	<0.01	
						36	Culms	0.09	
						59	Grain	<0.01	
						59	Straw	0.10	
DU2/03/02 Germany, 2002 (Winter Wheat - Transit)	300 g/L SC	150 150	50 50	300 300	2	0	Whole Plant	2.02	Method 993/0 (RLA 12619.03V), LOQ 0.01 mg/kg
						35	Ears	<0.01	
						35	Culms	0.62	
						58	Grain	<0.01	
						58	Straw	0.23	
		150	50	300	1	0	Whole Plant	1.55	
						35	Ears	<0.01	
						35	Culms	0.46	
						58	Grain	<0.01	
						58	Straw	0.14	
FAN/02/02 N. France, 2002 (Spring Barley - Astoria)	300 g/L SC	150 150	50 50	300 300	2	0	Whole Plant	3.35	Method 993/0 (RLA 12619.03V), LOQ 0.01 mg/kg
						34	Ears	<0.01	
						34	Culms	<0.01	
						39	Grain	<u>≤0.01</u>	
						39	Straw	<u>≤0.01</u>	
		150	50	300	1	0	Whole Plant	2.31	
						34	Ears	<0.01	
						34	Culms	<0.01	
						39	Grain	<0.01	
						39	Straw	<0.01	
OAT/05/02 UK, 2002 (Winter Barley - Sibera)	300 g/L SC	150 150	50 50	300 300	2	0	Whole Plant	1.78	Method 993/0 (RLA 12619.03V), LOQ 0.01 mg/kg
						35	Ears	0.03	
						35	Culms	0.24	
						56	Grain	0.02	
						56	Straw	0.24	
		150	50	300	1	0	Whole Plant	2.46	
						35	Ears	0.05	
						35	Culms	0.26	
						56	Grain	0.02	
						56	Straw	0.26	

III. CONCLUSION

During the 2002 growing season, a total of four trials were conducted in cereals (wheat and barley). Each trial consisted of three plots, one untreated (control) and two treated plots, plots 2 and 3. Plot 2 was treated with BAS 560 00 F (300 g/L of metrafenone, SC) was foliar applied two times at a rate of 150 g a.s./ha, at growth stage 37-39 and at growth stage 49-59. This resulted in a maximum seasonal target rate of 300 g a.s./ha.

Directly after the last application, the residues of metrafenone in the whole plant (without root) samples ranged from between 1.78 and 3.35 mg/kg in the samples treated with two applications and between 1.55 and 2.67 mg/kg after just one application.

In cereal grain collected at 39 to 59 DALA, residues were between < 0.01 mg/kg and 0.02 mg/kg.

The maximum storage interval from harvest until analysis was 18 months. Adequate storage stability data for metrafenone are available to support the storage conditions and intervals for the wheat and barley residue samples in this study.

Report:	CA 6.3.2/23 Raunft E. et al., 2004b Study on the residue behaviour of Fenpropimorph and BAS 560 F in cereals after application of BAS 421 12 F, BAS 560 00 F and BAS 564 AF F under field conditions in France, Germany, Denmark, United Kingdom, Italy and Spain, 2003 2004/1010542
Guidelines:	EEC 91/414 (1607/VI/97), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

During the 2003 growing season a total of eight trials were conducted in cereals. Three formulations were compared, a combination product BAS 565 AF F (267 g/L fenpropimorph, 100 g/L metrafenone, EC) and two solo products BAS 421 12 F (750 g/L fenpropimorph, EC) and BAS 560 00 F (300 g/L metrafenone, SC). In each case two applications were made, the first at BBCH growth stage 37 to 45 and the second about 35 days before expected harvest. The application rates were 150 g metrafenone and 400 g fenpropimorph in the combination product, 150 g metrafenone in the BAS 560 00 F solo formulation and 750 g fenpropimorph in the BAS 421 12 F solo formulation. The spray volume used was 300 L/ha. Cereal whole plant specimens were collected directly after the last application (0 DALA) from each plot at all locations. Three more sampling events took place after 4, 5 and 6 weeks where ears and culms (rest of the plant) or grains and straw were taken depending on the maturity of the crop. The specimens were analysed for metrafenone using method 535/0. The limit of quantification for metrafenone was 0.01 mg/kg in all matrices. The mean procedural recovery was 90% for metrafenone at fortification levels of 0.01 and 10 mg/kg. Directly after the last application, residues of metrafenone in whole plant ranged between 1.98 and 5.94 mg/kg following treatment with the combination product and between 1.17 and 4.25 mg/kg in the solo BAS 560 F formulation. In cereal grain collected at 34-42 days after the last application, residues of metrafenone were between <0.01 and 0.08 mg/kg. In cereal straw residues of metrafenone were between 0.07 and 3.91 mg/kg. No significant differences in residue levels were seen between the formulations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 564 AF F (contains 100 g metrafenone/L and 267 g fenpropimorph/L)
BAS 560 00 F (contains 300 g metrafenone/L)
BAS 421 12 F (contains 750 g fenpropimorph/L)

Description: BAS 564 AF F : EC formulation
BAS 560 00 F : SC formulation
BAS 421 12 F : EC formulation

Lot/Batch #: BAS 564 AF F: 2323
BAS 560 00 F : 3000
BAS 421 12 F : 2002-03

Purity: BAS 564 AF F (nominal contents: 100 g metrafenone/L and 267 g fenpropimorph/L)
BAS 560 00 F (nominal contents 300 g metrafenone/L)
BAS 421 12 F (nominal contents 750 g fenpropimorph/L)

CAS#: Metrafenone: 220899-03-6
Fenpropimorph: 67564-91-4
Development code: BAS 560 F (metrafenone)
Spiking levels: 0.01, 0.1 and 1.0 mg/kg

2. Test Commodity:
Crop: Cereals

Type: Winter wheat
Spring wheat
Winter barley
Spring barley

Variety: Winter wheat: Vitromax, Nefer, Malacca
Spring wheat: Triso
Winter barley: Candesse, Orelie
Spring barley: Astoria, Prosa

Botanical name: Winter wheat: *Triticum hybernum*
Barley: *Hordeum vulgare*

Crop parts(s) or processed commodity: Whole plant (without roots), ears, culms, grain and straw
Sample size: 0.5 kg straw, 1 kg plants without roots, ears, culms, grains

B. STUDY DESIGN AND METHODS

1. Test procedure

The objective of the study was to determine the magnitude of residues of metrafenone in cereals comparing two formulations.

Each trial consisted of a control (untreated) and two treated plots. All applications were made as foliar sprays. The actual application rates achieved in the study were within 10% of the nominal values. Control samples were taken at every time point and were collected prior to collection of the treated samples to avoid possible contamination.

2. Description of analytical procedures

Samples were analysed using method using BASF method 993/0 (corresponds to RLA12619.03V, Determination of BAS 560 F, CL3000402, CL434223 and CL376991 Residues in Cereals using LC-MS Determination). Determination of residues of metrafenone was by HPLC-MS/MS. The limit of quantification was 0.01 mg/kg for all matrices.

Procedural recoveries at fortification levels of 0.01, 0.1 and 1.0 mg/kg were between 75 and 81% for wheat whole plant, 92 to 104% for barley culms, 71 to 94% for wheat straw, 88 to 93% for wheat ears, 77 to 78% for wheat grains and 99 to 105% for barley grains. The overall mean procedural recovery for metrafenone from all matrices was 88%.

The maximum storage interval from harvest until analysis was 18 months. The storage stability of metrafenone in wheat was proven over a 29 month period for grain and a 24 month period for straw in study PTRL P 385G “BAS 560 F (AC375839):Storage Stability of BAS 560 F Residues at ≤ -18°C in Cereal Grain and Straw”.

II. RESULTS AND DISCUSSION

Directly after the last application, the residues of metrafenone in whole plants ranged from 1.17 to 4.25 mg/kg in samples that were treated with BAS 560 00 F and between 1.98 to 5.94 mg/kg in samples that were treated with BAS 564 AF F.

In cereal grain collected at 34-42 days after the last application, residues of metrafenone were between < 0.01 and 0.08 mg/kg. In cereal straw residues of metrafenone were between 0.07 and 3.91 mg/kg. No significant differences in residue levels were seen between the formulations.

A summary of the residue results for whole plant, ears, culms, grain and straw for winter and spring wheat and barley are presented in the following table:

Table 6.3.2-2: Summary of residues in winter wheat and barley

Trial Country, year (Variety)	Application					PHI days	Commodity	Metrafenone (mg/kg)	Reference
	Formulation (g as/L)	g as/ha	g as/hL	Water (L/ha)	No				
Wheat and barley									
ACK/04/03 Germany, 2003 (Winter Barley - Cadesse)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	3.11	ASF Method 535/0
						28 28	Ears Culms	0.03 0.08	
						35 35	Grain Straw	0.01 0.11	
						42 42	Grain Straw	0.02 0.07	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	2.00	
						28 28	Ears Culms	0.11 0.84	
						35 35	Grain Straw	0.06 0.95	
						42 42	Grain Straw	0.05 0.83	
ALB/01/03 Denmark, 2003 (Spring Wheat - Triso)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	2.69	ASF Method 535/0
						28 28	Ears Culms	0.09 0.90	
						35 35	Grain Straw	<0.01 0.49	
						41 41	Grain Straw	<0.01 0.47	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	3.67	
						28 28	Ears Culms	0.24 4.89	
						35 35	Grain Straw	<0.01 3.55	
						41 41	Grain Straw	0.01 2.39	
ALO/03/03 Spain, 2003 (Spring Wheat - Vitromax)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	3.78	BASF Method 535/0
						29 29	Ears Culms	0.45 2.01	
						35 35	Grain Straw	<0.01 1.70	
						42 42	Grain Straw	<0.01 1.31	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	3.64	
						29 29	Ears Culms	0.19 3.53	
						35 35	Grain Straw	<0.01 0.96	
						42 42	Grain Straw	<0.01 0.99	
FAN/05/03 N. France, 2003 (Spring Barley - Astoria)	100 g/L EC Formulation BAS 564 AF F	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	Whole plant	2.75	BASF Method 535/0
						28 28	Ears Culms	0.36 1.53	
						36 36	Grain Straw	0.02 1.13	
						42 42	Grain Straw	0.05 1.17	
	300 g/L SC Formulation BAS 560 00 F	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	Whole plant	3.79	
						28 28	Ears Culms	0.35 3.34	
						36 36	Grain Straw	0.02 3.91	
						42 42	Grain Straw	0.05 3.07	

Trial Country, year (Variety)	Application					PHI days	Commodity	Metrafenone (mg/kg)	Reference
	Formulation (g as/L)	g as/ha	g as/hL	Water (L/ha)	No				
Wheat and barley									
FBD/02/03 S. France, 2003 (Winter Barley - Orelie)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	2.71	BASF Method 535/0
						29 29	Ears Culms	0.09 0.78	
						35 35	Grain Straw	0.03 0.44	
						42 42	Grain Straw	0.02 0.46	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	3.73	
						29 29	Ears Culms	0.37 3.43	
						35 35	Grain Straw	0.08 1.29	
						42 42	Grain Straw	0.07 1.48	
FTL/03/03 S. France, 2003 (Winter Wheat - Nefer)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	2.28	BASF Method 535/0
						29 29	Ears Culms	0.06 1.79	
						35 35	Grain Straw	<0.01 0.95	
						42 42	Grain Straw	<0.01 1.10	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	4.25	
						29 29	Ears Culms	0.28 5.58	
						35 35	Grain Straw	<0.01 2.19	
						42 42	Grain Straw	<0.01 3.14	
ITA/03/03 Italy, 2003 (Spring Barley - Prosa)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	5.94	BASF Method 535/0
						28 28	Ears Culms	0.05 0.94	
						35 35	Grain Straw	<0.01 0.60	
						42 42	Grain Straw	<0.01 0.75	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	3.55	
						28 28	Ears Culms	0.25 2.73	
						35 35	Grain Straw	0.02 1.86	
						42 42	Grain Straw	<0.01 1.55	
OAT/02/03 United Kingdom, 2003 (Winter Wheat Malacca)	100 g/L EC Formulation BAS 564 AF F	150 150	50 50	300 300	2	0	Whole plant	1.98	BASF Method 535/0
						27 27	Ears Culms	0.16 2.53	
						34 34	Grain Straw	0.01 2.57	
						41 41	Grain Straw	<0.01 3.35	
	300 g/L SC Formulation BAS 560 00 F	150 150	50 50	300 300	2	0	Whole plant	1.17	
						27 27	Ears Culms	0.17 2.81	
						34 34	Grain Straw	0.01 3.55	
						41 41	Grain Straw	<0.01 2.88	

III. CONCLUSION

During the 2003 growing season a total of eight trials were conducted in cereals. Three formulations were compared, a combination product BAS 565 AF F (267 g/L fenpropimorph, 100 g/L BAS 560 F, EC) and two solo products BAS 421 12 F (750 g/L fenpropimorph, EC) and BAS 560 00 F (300 g/L metrafenone, SC). In each case two applications were made, the first at BBCH growth stage 37 to 45 and the second about 35 days before expected harvest. The application rates were 150 g metrafenone and 400 g fenpropimorph in the combination product, 150 g metrafenone in the BAS 560 00 F solo formulation and 750 g fenpropimorph in the BAS 421 12 F solo formulation. The spray volume used was 300 L/ha.

In cereal grain collected at 34-42 days after the last application, residues of metrafenone were between <0.01 and 0.08 mg/kg. In cereal straw residues of metrafenone were between 0.07 and 3.91 mg/kg. No significant differences in residue levels were seen between the formulations.

Report:	CA 6.3.2/24 White M.T.,Stewart J., 2006a Residue of Metrafenone (BAS 560 F) Fenpropimorph, (BAS 421 F) Epoxiconazol (BAS 480 F) in formulation bridging on wheat, barley after BAS 565 00 F BAS 562 00 F BAS 560 00 F BAS 421 12 F BAS 480 31 F field, in DE, DK, N-FR, S-FR, SE, UK 2005 2005/7004267
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

During the 2005 growing season, eight trials were conducted in cereals. The combination products BAS 565 00 F (75 g/L metrafenone; 200 g/L fenpropimorph; 62.5 g/L epoxiconazole, SE) and BAS 562 00 F (100 g/L metrafenone and 83 g/L epoxiconazole, SC) were compared to three solo formulations: BAS 560 00 F (300 g/L metrafenone SC), BAS 421 12 F (750 g/L fenpropimorph EC), BAS 480 31 F (125 g/L epoxiconazole SC). In each case, two foliar applications were made at crop growth stages BBCH 37-39 and 69-73, targeting a spray volume of 200 L/ha. The targeted application rates for metrafenone, fenpropimorph, and epoxiconazole in the combination products were 150, 400, 125 g as/ha/application, respectively. For the solo formulations, the targeted application rates for metrafenone, fenpropimorph, and epoxiconazole were 150, 750, and 125 g as/ha/application, respectively. Samples of whole plants (without roots) and of panicles (ears) were collected directly after the last application (0 DALA) from each plot at all locations. Three more sampling events took place after about 4, 5 and 6 weeks, where ears and plant without roots (culms) or grain and straw, depending on the maturity of the crop, were taken. The specimens were analysed for metrafenone, fenpropimorph, and epoxiconazole with BASF method No. 535/0 that has a limit of quantification of 0.01 mg/kg for each analyte. Mean procedural recoveries were 101% for metrafenone, 81% for fenpropimorph, and 101% for epoxiconazole at fortification levels between 0.01 mg/kg and 20 mg/kg. At a PHI of 34-43 days, residues of metrafenone in grain ranged from <0.01-0.09 mg/kg and in straw ranged from 0.21-6.71 mg/kg. Formulation type was found to have no impact on the residue levels observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 565 00 F, BAS 562 00 F, BAS 560 00 F, BAS 421 12 F, BAS 480 31 F

Description: BAS 565 00 F: 75 g/L metrafenone; 200 g/L fenpropimorph; 62.5 g/L epoxiconazole, SE
BAS 562 00 F: 100 g/L metrafenone and 83 g/L epoxiconazole, SC
BAS 560 00 F: 300 g/L metrafenone SC
BAS 421 12 F: 750 g/L fenpropimorph EC
BAS 480 31 F: 125 g/L epoxiconazole SC

Lot/Batch #: BAS 565 00 F: batch 4197
BAS 562 00 F: batch 4033
BAS 560 00 F: batch 3000
BAS 421 12 F: batch 9157
BAS 480 31 F: batch 2002-1

Purity: BAS 565 00 F: batch 4197, Metrafenone: 75 g/L nominal; Fenpropimorph: 200 g/L nominal; Epoxiconazole: 62.5 g/L nominal
BAS 562 00 F: batch 4033, Metrafenone: 100 g/L nominal; Epoxiconazole: 83 g/L nominal
BAS 560 00 F: Metrafenone: 100 g/L nominal;
BAS 421 12 F: Fenpropimorph: 750 g/L nominal
BAS 480 31 F: Epoxiconazole: 125 g/L nominal

CAS#: BAS 560 F: 220899-03-6
BAS 421 F: 67564-91-4
BAS 480 F: 133855-98-8

Development code: BAS 560 F: 4037710
BAS 421 F: 108406
BAS 480 F: 205259

Spiking levels: metrafenone: 0.01, 0.05, 0.10, 2 and 10 mg/kg

2. Test Commodity: Cereals
Crop: Wheat, barley
Type: Winter wheat, barley
Variety: Winter wheat: Kris, Isengrain, Caphorn, Royssac
Barley: Scarlett, Orelie, Prestige, Pearl

Botanical name: Winter wheat: *Triticum aestivum*
Barley: *Hordeum vulgare*

Crop parts(s) or processed commodity: Whole plant without root, ear panicle, grain and straw

Sample size: Whole plant without roots: min 1.0 kg
Ear panicles; min 1.0 kg
Grain: min 1.0 kg
Straw: min 0.5 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2005 growing season, eight trials were conducted in cereals to determine the magnitude of residues. Each trial consisted of a control (untreated) plot and one treated plot. All applications were made as foliar sprays.

Combination products and solo formulations were applied to their specified plots at crop growth stage 37-39 and 69-73, at a targeted spray volume of 200 L/ha. Application rates for metrafenone, fenpropimorph and epoxiconazole in the combination products were 150, 400 and 125 g as/ha respectively. Solo formulations were applied at rates of 150, 750 and 125 g as/ha for metrafenone, fenpropimorph and epoxiconazole.

Table 6.3.2-3: Target Application Rates and Timings for BAS 565 00 F, BAS 562 00 F, BAS 560 00 F, BAS 421 12 F, and BAS 480 31 F on Cereals

Plot No.	App. No.	Test Item	App. Rate Product (L/ha)	Active Ingredient	App. Rate A.S. (g/ha)	Water volume (L/ha)	Timing
2	1	BAS 565 00 F	2	Metrafenone	150	200	BBCH 37-39
				Fenpropimorph	400		
				Epoxiconazole	125		
	2	BAS 565 00 F	2	Metrafenone	150	200	BBCH 69
				Fenpropimorph	400		
				Epoxiconazole	125		
3	1	BAS 562 00 F	1.5	Metrafenone	150	200	BBCH 37-39
				Epoxiconazole	125		
	2	BAS 562 00 F	1.5	Metrafenone	150	200	BBCH 69
				Epoxiconazole	125		
4	1	BAS 560 00 F	0.5	Metrafenone	150	200	BBCH 37-39
	2		0.5		150	200	BBCH 69
5	1	BAS 421 12 F	1.0	Fenpropimorph	750	200	BBCH 37-39
	2		1.0		750	200	BBCH 69
6	1	BAS 480 31 F	1.0	Epoxiconazole	125	200	BBCH 37-39
	2		1.0		125	200	BBCH 69

Samples of each commodity were taken at 0 DALA and at 28 DALA, and grain and straw only at 35 and 42 DALA.

All specimens were frozen within 24 h of being taken, and remained frozen at or below -18°C, including during transport, until analysis. The maximum storage interval from harvest until analysis was 9 months.

2. Description of analytical procedures

All samples were maintained frozen from harvest to extraction for analysis. The specimens were homogenised with dry ice prior to analysis. For the analysis of metrafenone, fenpropimorph, and epoxiconazole, BASF method No. 535/0 was used which determines the un-derivatised analytes by means of HPLC-MS/MS

Metrafenone, fenpropimorph, and epoxiconazole are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes is performed by HPLC-MS/MS. The limit of quantification (LOQ) of the method for each analyte is 0.01 mg/kg in all matrices.

II. RESULTS AND DISCUSSION

Residues of metrafenone were 1.82 to 5.75 mg/kg in whole plant and 0.73 to 4.78 mg/kg in ear samples harvested at 0 DALA from plots 2, 3 and 4 (receiving metrafenone at 300 g as/ha/season). During the study, residues generally declined in all matrices. In mature grain samples harvested 5 to 6 weeks after the last application, residues ranged from below the LOQ to 0.09 mg/kg (34-36 DALA) or less than LOQ to 0.06 mg/kg (41-43 DALA). Residues in straw samples at the same timepoints ranged from 0.26-6.71 mg/kg and 0.21-3.81 mg/kg, respectively. No differences were noted between the formulations.

A summary of the residues in matrices following treatment with all the formulations are presented below:

Table 6.3.2-4: Summary of Residues of Metrafenone in Wheat

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Wheat	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	Fenpropimorph+epoxiconazole/epoxiconazole/none
Content of active substance:	75 g/L/100 g/L//300 g/L	**(common name and content)**	200 g/L+62.5 g/L/83 g/L
Formulation (e.g. WP):	BAS 565 00 F SE/BAS 562 00F/BAS 560 00F	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
DK-5500 Midderlfart Fuenen Denmark ALB/11/05	Winter wheat Kris	1 27.09.04 2 28.06-08.07.05 3 10-21.08.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 06.07.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.59 1.12 1.63 0.04 <0.01 1.00 <0.01 1.20	0 0 28 28 35 35 42 42	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.21 1.39 0.86 0.07 <0.01 1.55 <0.01 0.86	0 0 28 28 35 35 42 42	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.18 1.37 1.99 0.15 <u><0.01</u> <u>3.05</u> <0.01 2.75	0 0 28 28 35 35 42 42	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
74193 Baden-Wuttemberg Germany DU2/11/05	Winter wheat Isengrain	1 03.11.04 2 02-12.06.05 3 12-13.08.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 30.06.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	1.82 1.33 <0.01 0.88 0.01 0.68 <u>0.02</u> 0.70	0 0 27 27 34 34 41 41	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.06 1.36 <0.01 0.76 <0.01 0.71 0.01 0.76	0 0 27 27 34 34 41 41	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.56 2.05 0.01 2.28 <0.01 <u>1.76</u> 0.01 1.44	0 0 27 27 34 34 41 41	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
26750 Rhone-Alpes S. France FBD/33/05	Winter wheat Caphorn	1 08.11.04 2 12-30.05.05 3 06-07.07.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 30.05.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	3.63 1.66 3.23 <0.01 <0.01 2.05 <0.01 1.57	0 0 28 28 35 35 42 42	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	4.76 1.98 4.58 <0.01 <0.01 2.64 <0.01 1.50	0 0 28 28 35 35 42 42	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	3.92 1.95 4.79 <0.01 <0.01 6.71 <0.01 3.81	0 0 28 28 35 35 42 42	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
49140 Payes de la Loire N. France FBM/11/05	Winter wheat Royssac	1 24.10.04 2 24-31.05.05 3 08-12.07.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 31.05.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.14 1.59 0.93 0.11 <0.01 0.90 <0.01 1.28	0 0 28 28 35 35 42 42	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.75 1.66 0.33 0.09 <0.01 0.81 <0.01 1.50	0 0 28 28 35 35 42 42	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.47 3.57 1.82 0.09 <0.01 2.96 <0.01 3.54	0 0 28 28 35 35 42 42	

Table 6.3.2-5: Summary of Residues of Metrafenone in Barley

Residue Data Summary from Supervised Trials (Summary)			
Active substance (common name):	Metrafenone (BAS 560 F)	**Commercial Product name:**	BAS 560 02 F
Crop/crop group:	Barley	**Producer of commercial product:**	BASF SE, 67056 Ludwigshafen
Responsible body for reporting (name, address):	BASF SE, 67056 Ludwigshafen	**Indoor/Glasshouse/Outdoor:**	Outdoor
Country	Germany	**Other ai in the formulation:**	Fenpropimorph+epoxiconazole/epoxiconazole/none
Content of active substance:	75 g/L/100 g/L//300 g/L	**(common name and content)**	200 g/L+62.5 g/L/83 g/L
Formulation (e.g. WP):	BAS 565 00 F SE/BAS 562 00F/BAS 560 00F	**Residues calculated as:**	BAS 560 F (metrafenone)

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
67229 Rheinland-Pfalz Germany DU4/11/05	Barley Scarlett	1 15.03.05 2 03-10.06.05 3 27-28.07.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 09.06.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	4.37 NA 0.44 <0.01 <0.01 0.77 <0.01 0.67	0 0 29 29 36 36 42 42	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	4.34 NA 0.73 <0.01 0.02 0.86 <0.01 0.54	0 0 29 29 36 36 42 42	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	5.02 NA 3.32 0.01 0.02 3.55 <0.01 2.87	0 0 29 29 36 36 42 42	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
26750 Rhone-Alpes S. France FBD/34/05	Barley Orelie	1 29.09.05 2 10-29.05.05 3 28-29.06.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 18.05.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	4.16 4.41 0.39 0.02 0.02 0.26 0.03 0.21	0 0 29 29 35 35 41 41	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	4.56 4.78 0.40 0.02 0.02 0.51 0.01 0.32	0 0 29 29 35 35 41 41	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	3.02 3.76 1.37 0.03 0.03 1.09 0.03 0.31	0 0 29 29 35 35 41 41	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
23791 Malmoe Sweden HUS/07/05	Barley Prestige	1 11.04.05 2 01-14.07.05 3 17-27.08.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 14.07.05	69	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	3.80 2.52 0.79 0.22 <u>0.09</u> 1.19 0.06 1.39	0 0 29 29 36 36 43 43	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	5.75 2.70 0.94 0.30 0.05 <u>1.84</u> 0.04 1.16	0 0 29 29 36 36 43 43	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	3.51 0.73 0.76 0.12 0.04 0.71 0.02 0.85	0 0 29 29 36 36 43 43	

BAS Doc ID no. (trial no.)	Commodity/ variety	Date of 1 Planting 2 Flowering 3 Harvest	Method of treatment (Formulation)	Nominal application rate per treatment			No. of treatments	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg as/hL	Water L/ha	g as/ha						
OX27 Oxfordshire UK OAT/16/05	Barley Pearl	1 02.11.04 2 01-15.06.05 3 25-26.07.05	Foliar (BAS 565 00 F)	0.075 0.075	200 200	150 150	2 20.06.05	73	Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.29 1.98 0.38* 0.05 0.05 1.10 0.02 1.21	0 0 28 28 35 35 42 42	BASF method 535/0 No residues above the LOQ detected in any of the untreated specimens
			Foliar (BAS 562 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	2.54 2.04 0.69 0.06 0.04 1.02 0.02 1.05	0 0 28 28 35 35 42 42	
			Foliar (BAS 560 00 F)						Whole plant Ear, panicle Whole plant Ear, panicle Grain Straw Grain Straw	1.98 1.33 1.23 0.09 0.05 1.33 0.04 1.23	0 0 28 28 35 35 42 42	

* - Mean of three determinations (0.47, 0.44, 0.22 mg/ kg)

III. CONCLUSION

Cereal crops treated with combination products including metrafenone, fenpropimorph, and epoxiconazole and solo formulations containing the same single active ingredients showed no significant differences in residues between formulations. Where small differences were noted, these could be attributed to differences in the rate of application.

Highest residues were observed in straw samples, with lower levels found in whole plant samples and ear panicles. Residues in grain samples were low, ranging from below the limit of quantification (0.01 mg/kg) to a maximum of 0.09 mg/kg.

Summary of residues trials in plants

Previous residues trials on grapes reviewed under Directive 91/414 were not included in this review due to the significant change in GAP (number of applications reduced from 8 to 3). A total of 16 residues trials supporting the revised GAP were carried out on wine and table grapes in northern and southern Europe group 1-4). Two slightly different GAPs representing the cGAP (3x0.16 kg as/ha (NEU) or 3x0.125 kg as/ha (SEU)) were used. Statistical analysis using the Kruskal-Wallis test (see below) showed there was no statistically-significant difference between trials conducted in northern or southern Europe or using either of the two GAPs or wine or table grapes i.e. the data points are all from the same population so the data sets were combined to calculate MRLs and other end points.

Group id	Obs	Rank Sum
1	4	49.00
2	4	19.50
3	4	28.50
4	4	39.00

chi-squared = 5.410 with 3 d.f.
probability = 0.1441

chi-squared with ties = 5.442 with 3 d.f.
probability = 0.1422

Residues in ranked order were: 0.05, 0.08, 0.09, 0.10, 0.11(2), 0.17, 0.18, 0.21, 0.23(2), 0.24(2), 0.31 and 0.36(2) mg/kg. The median residue was 0.195 mg/kg and the HR was 0.36 mg/kg.

Twenty five and twenty six trials were conducted in wheat and barley, respectively, in northern and southern Europe. Many of these had been previously assessed for the first EU review of metrafenone under Directive 91/414/EEC. Some trials also included analysis for three metabolites (CL 3000402, CL 434223 and CL 376991). Many of the trials were carried out to assess the impact of different formulation types on residues levels. It can be clearly seen that formulation has no impact and therefore, in these comparison trials, the highest residues from any one formulation have been selected for further assessment, including derivation of MRLs.

The following residues were detected in cereals:

Crop	Region	Number of trials	Residues (mg/kg)	STMR (mg/kg)
Wheat grain	NEU	13	<0.01(8), 0.01, 0.02, 0.03, 0.04(2)	<0.01
Wheat grain	SEU	12	<0.01(7), 0.01(4), 0.03	<0.01
Wheat straw	NEU	13	0.59, 0.67, 0.93, 0.98, 1.43, 1.76, 1.85, 2.04, 2.32, 3.05, 3.54, 3.55(2)	1.85
Wheat straw	SEU	12	0.67, 0.89, 1.08, 1.10, 1.11, 1.25, 1.61, 1.70(2), 2.12, 3.14, 6.71	1.43
Barley grain	NEU	14	<0.01, 0.02(2), 0.03, 0.05(2), 0.06, 0.07, 0.09(2), 0.11, 0.15, 0.16, 0.40	0.065
Barley grain	SEU	12	0.02, 0.03, 0.04(2), 0.05, 0.06, 0.08(2), 0.12, 0.13, 0.23, 0.68	0.07
Barley straw	NEU	14	<0.01, 0.54, 0.64, 0.95, 1.09, 1.11, 1.15, 1.23, 1.28, 1.70, 1.84, 2.01, 3.55, 3.91	1.19
Barley straw	SEU	12	0.41, 0.90, 1.02, 1.09, 1.22, 1.48, 1.65, 1.86, 1.94, 2.13, 4.03, 4.25	1.57

There are no significant differences between residues from northern or southern Europe and therefore these data sets will be combined for further assessment and derivation of MRLs.

Crop	Region	Number of trials	Residues (mg/kg)		
			CL 3000402	CL 434223	CL 376991
Wheat grain	NEU	8	<0.01(8)	<0.01(8)	<0.01(8)
Wheat grain	SEU	10	<0.01(9), 0.01	<0.01(10)	<0.01(10)
Wheat straw	NEU	8	<0.1(6), 0.12, 0.18	<0.1(8)	<0.1(8)
Wheat straw	SEU	10	<0.1(9), 0.17	<0.1(9), 0.11	<0.1(9), 0.07
Barley grain	NEU	8	<0.01(4), 0.01, 0.02(3)	<0.01(8)	<0.01(8)
Barley grain	SEU	9	<0.01(6), 0.01(2), 0.02	<0.01(9)	<0.01(9)
Barley straw	NEU	8	<0.1(5), 0.10, 0.13, 0.14	<0.1(8)	<0.1(8)
Barley straw	SEU	9	<0.1(3), 0.1, 0.12, 0.13(3), 0.30	<0.1(7), 0.04, 0.11	<0.1(7), 0.07, 0.10

Residues of the metrafenone metabolites CL 300402, CL 434223 and CL 376991 were virtually non-detectable in cereal grain samples and considerably lower than residues of the parent compound. Therefore no consideration of human exposure to these metabolites is required. Residues of CL 434223 and CL 376991 were at or around the limit of quantification in straw. However, since straw does not form part of the human diet and these metabolites contribute insignificantly to the residues in ruminant diets, no further consideration of these metabolites is required. Residues up to 0.30 mg/kg of the lactone metabolite CL 3000402 were detected in straw and livestock exposure to this metabolite will be assessed.

CA 6.4 Feeding studies

An assessment of the transfer of residues into products of animal origin has been previously reported in the DAR (UK, 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: yes	Poultry: yes	Pig: yes
Muscle Liver Kidney Fat Milk Eggs	No ruminant feeding study conducted. Metabolism results indicated that residues will not be of significance at N rate.	No hen feeding study conducted. Metabolism results indicated that residues will not be of significance.	No pig feeding study conducted. Metabolism in rat and ruminant similar, ruminant metabolism indicated that residues will not be of significance

This conclusion was subsequently confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498).

> "According to the above mentioned metabolism studies in lactating goats, highest residues according to the residue definition were found in liver (0.27 mg/kg at a 38N dosing rate and 0.11 mg/kg at a 8N dosing rate). It is therefore concluded that significant residues in edible matrices of ruminants are not expected based on the calculated dietary burden (see section 3.2.1). Nevertheless, as there are no validated analytical methods to enforce the residue in food of animal origin, EFSA is not in position to derive MRL proposals at LOQ. For pigs and poultry products however, MRLs are not required because these groups of livestock are not expected to be exposed to significant levels of metrafenone residues."

According to the Pesticides Steering Committee notes from their meeting on 19-20 June 2014,

> "New feed items such as wheat forage, hay… have been introduced in the feedstuff tables. After discussion the meeting agreed that, by default, intended uses on cereals should be understood as "on cereal for grain production" and therefore, only residues in grains and straw considered for the animal burden calculation. Residue data at forage, silage growth stages have therefore not to be requested for uses intended on cereal grain. It was agreed that, when grown for forage, silage, the GAPs are different, and therefore, the GAPs proposed for cereal grains would not be relevant to derive residues in forage or silage."

Therefore, livestock dietary burdens have been calculated taking account of residues in cereal grain and straw only.

Livestock dietary burdens for metrafenone were calculated using the following residues values (processing factors used can be found in section 6.5):

Commodity	**STMR (mg/kg)**	**HR (mg/kg)**
Wheat, rye and triticale grain	0.01	-
Barley and oat grain	0.07	-
Wheat, rye and triticale straw	1.7	6.71
Barley and oat straw	1.255	4.25
Barley brewers grain (pf 0.3)	0.021	-
Barley bran (pf 2.5 pearl barley abrasions)	0.175	-
Wheat milled by-products (pf fine bran 4.2)	0.042	-
Wheat bran and germ (pf fine bran 4.2)	0.042	-
Wheat flour (pf wholemeal flour 1.41)	0.014	-
Wheat middlings and gluten (pf flour type 550 0.19)	0.0019	-

In addition, intakes for the cereal metabolite CL 3000402 were calculated using the following residues values and processing factors for metrafenone:

Commodity	**STMR (mg/kg)**	**HR (mg/kg)**
Wheat, rye and triticale grain	<0.01	-
Barley and oat grain	<0.01	-
Wheat, rye and triticale straw	<0.1	0.18
Barley and oat straw	<0.1	0.30
Barley brewers grain (pf 0.3)	0.003	-
Barley bran (pf 0.2.5 pearl barley abrasions)	0.025	-
Wheat gluten meal and milled by-products (pf flour type 550 0.19)	0.0019	-

CA 6.4.1 Poultry

No livestock feeding study for poultry has been generated for metrafenone.

Livestock dietary burdens of metrafenone for poultry were calculated using the OECD diets.

Poultry broiler – maximum (metrafenone)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Barley grain	GC	0.07	STMR	88	0.08	70	0.056
Wheat milled bypdts	CM/CF	0.042	STMR	88	0.05	20	0.01
Total						90	0.066

Poultry layer – maximum (metrafenone)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Wheat straw	AF/AS	6.71	HR	88	7.63	10	0.763
Barley grain	GC	0.07	STMR	88	0.08	90	0.072
Wheat milled bypdts	CM/CF	0.042	STMR	88	0.05		
Total						100	0.834

In the metabolism study in poultry, hens were fed for 12 days at *ca.* 14 ppm diet (refer to section KCA 6.2.2). mg/kg metrafenone or TRR in eggs and tissues was as shown below:

Tissue	Maximum mg/kg metrafenone (*ca.* 14 ppm)	mg/kg TRR based on an intake of 0.834 ppm diet (DM)
Eggs	0.002	0.0001
Muscle	0.013 (TRR)	0.0008
Skin with fat	0.001	0.00006
Liver	<0.001	<0.00006

Characterised material in the methanol or aqueous extracts would also not be detected at levels above the limit of quantification and therefore no metabolites would be expected to occur at levels above the limit of quantification.

Although the dietary burden of metrafenone in poultry is >0.1 ppm diet (DM), it can be clearly seen from the study on metabolism in hens that a non-detectable residue situation is predicted in products of poultry origin and therefore a feeding study in poultry is not required. MRLs for metrafenone in products of poultry origin should be set at the limit of quantification i.e. 0.01 mg/kg.

Since the cereal metabolite CL 3000402 was detected in cereal grain and straw, livestock dietary burdens for poultry were calculated using the OECD diets.

Poultry broiler– maximum (CL 3000402)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Barley grain	GC	0.01	STMR	88	0.01	70	0.008
Total						70	0.008 (0.0006 mg/kg bw/day)

Poultry layer – maximum (CL 3000402)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Barley straw	AF/AS	0.3	HR	89	0.34	5	0.017
Barley grain	GC	0.01	STMR	88	0.01	90	0.01
Total						100	0.027 (0.0018 mg/kg bw/day)

Since the intakes are well below 0.1 ppm diet DM and also 0.004 mg/kg bw/day, no further consideration of CL 3000402 in the diets of poultry is required since residues would not be expected above the limit of quantification in poultry commodities.

CA 6.4.2 Ruminants

Livestock dietary burdens for ruminants were calculated using the OECD diets.

Beef cattle – maximum

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Rye straw	AF/AS	6.71	HR	88	7.63	20	1.525
Barley grain	GC	0.07	STMR	88	0.08	70	0.056
Wheat milled bypdts	CM/CF	0.042	STMR	88	0.05		
Total						90	1.581

Dairy cattle – maximum

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Rye straw	AF/AS	6.71	HR	88	7.63	20	1.525
Barley grain	GC	0.07	STMR	88	0.08	40	0.032
Wheat milled bypdts	CM/CF	0.042	STMR	88	0.05	30	0.014
Total						90	1.571

In the metabolism study in ruminants, goats were fed for 5 days at *ca.* 8 or 60 and 13 or 87 ppm diet (trimethoxyphenyl or bromophenyl labels, respectively; refer to section KCA 6.2.3). The TRR in muscle was low (<0.01 mg/kg in all dose groups) and therefore it was not characterised. Levels of metrafenone in milk and relevant tissues were as shown below:

Tissue	Maximum mg/kg metrafenone		Dose (ppm diet, label)		mg/kg metrafenone based on an intake of *ca.* 2 ppm diet (DM)
	Trimethyl oxyphenyl	bromophenyl	Trimethyl oxyphenyl	bromophenyl	
Milk	-	<0.005	60	87	0.0001
Adipose tissue	0.019	0.009	60	87	0.0006-0.0002
Liver	0.025	0.035	60	87	0.0008
Kidney	0.005	0.014	60	87	0.0002-0.0003

The EFSA Reasoned Opinion for Article 12 review of MRLs noted that CL 1500698 and CL 1023363 constituted the main residues in ruminant liver and kidney. These were measured together and also included a further unknown which could not be separated from the other two metabolites.

Tissue	Maximum mg/kg ROI-13A1 (CL 1500698) + ROI-13A2 (CL 1023363) + Unknown		Dose (ppm diet, label)		mg/kg metabolite based on an intake of *ca.* 2 ppm diet (DM)
	Trimethyl oxyphenyl	bromophenyl	Trimethyl oxyphenyl	bromophenyl	
Liver	0.106	0.269	60	87	0.004-0.006
Kidney	0.044	0.087	60	87	0.001-0.002

Based on the above calculations, it is not likely that either CL 1500698 or CL 1023363 would be detected in products of animal origin even in a misuse situation. It should be noted that the residues of the metabolites are calculated as total TRR for the two compounds plus an additional unknown compound.

MRLs for metrafenone in products of ruminant origin should be set at the limit of quantification i.e. 0.01 mg/kg.

Since the cereal metabolite CL 3000402 was detected in cereal grain and straw, livestock dietary burdens for cattle were calculated using the OECD diets.

Beef cattle – maximum (CL 3000402)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Barley straw	AF/AS	0.3	HR	89	0.34	30	0.101
Barley grain	GC	0.01	STMR	88	0.01	70	0.008
Total						100	0.109 (0.0026 mg/kg bw/day)

Dairy cattle – maximum (CL 3000402)

Commodity	CC	Residue (mg/kg)	Basis	DM (%)	Residue dw (mg/kg)	Diet content (%)	Residue Contribution (ppm diet DM)
Barley straw	AF/AS	0.3	HR	89	0.34	30	0.101
Barley grain	GC	0.01	STMR	88	0.01	40	0.005
Wheat gluten meal	CM/CF	0.0019	STMR	40	0.0048	20	0.00095
Total						100	0.107 (0.004 mg/kg bw/day)

Since the intakes are at 0.1 ppm diet DM but $\leq$ 0.004 mg/kg bw/day, no further consideration of CL 3000402 in the diets of cattle is required since residues would not be expected above the limit of quantification.

CA 6.4.3 Pigs

Since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly and intakes by pigs are not expected to be significantly higher than other livestock, MRLs for products of porcine origin should be set at the limit of quantification i.e. 0.01 mg/kg.

CA 6.4.4 Fish

Dietary burdens for fish have been calculated using the model developed by the Fraunhofer Institute. Although the log Po/w for metrafenone is >3, the intake by common carp or rainbow trout is <0.1 ppm diet (DM) and therefore no studies to assess the metabolism or residues in fish products are required. The MRL for fish should be set at 0.01 mg/kg.

Dietary Burden Calculation concerning metrafenone
Fraunhofer IME Schmallenberg, Germany, developed 2014 by Judith Klein(Version: 13 November 2014)

INPUT
=====

Target content for Common carp:
Crude fat 10.00%
Crude protein 35.00%

Maximum principal content of components in the diet:

Barley (bran fractions)	100.00%
Brewer's grain (dried)	100.00%
Wheat (Extruded grain)	100.00%
Wheat (bran)	100.00%
Wheat (flour)	100.00%
Wheat (germ)	100.00%
Wheat (middlings)	100.00%
Wheat (gluten)	100.00%

Percent dry matter of components:

Barley (bran fractions)	88.0%
Brewer's grain (dried)	92.0%
Wheat (Extruded grain)	87.7%
Wheat (bran)	88.7%
Wheat (flour)	88.0%
Wheat (germ)	88.7%
Wheat (middlings)	89.4%
Wheat (gluten)	91.4%

metrafenone residues in the components:

Barley (bran fractions)	0.175 mg/kg (STMR-p)
Brewer's grain (dried)	0.021 mg/kg (STMR-p)
Wheat (Extruded grain)	0.010 mg/kg (STMR-p)
Wheat (bran)	0.042 mg/kg (STMR-p)
Wheat (flour)	0.014 mg/kg (STMR-p)
Wheat (germ)	0.042 mg/kg (STMR-p)
Wheat (middlings)	0.0019 mg/kg (STMR-p)
Wheat (gluten)	0.0019 mg/kg (STMR-p)

metrafenone residues in the components (dry matter):

Barley (bran fractions)	0.199 mg/kg (STMR-p /dry matter)
Brewer's grain (dried)	0.023 mg/kg (STMR-P/dry matter)
Wheat (Extruded grain)	0.011 mg/kg (STMR-P/dry matter)
Wheat (bran)	0.047 mg/kg (STMR-P/dry matter)
Wheat (flour)	0.016 mg/kg (STMR-P/dry matter)
Wheat (germ)	0.047 mg/kg (STMR-P/dry matter)
Wheat (middlings)	0.0021 mg/kg (STMR-P/dry matter)
Wheat (gluten)	0.0021 mg/kg (STMR-P/dry matter)

RESULTS
=======

Maximum content dietary burden based on metrafenone is 0.049 mg/kg (dry matter).

The respective composition of the feed is:

Barley (bran fractions)	10.21%
Brewer's grain (dried)	0.00%
Wheat (Extruded grain)	0.00%
Wheat (bran)	59.84%
Wheat (flour)	0.00%
Wheat (germ)	0.00%
Wheat (middlings)	0.00%
Wheat (gluten)	29.95%

The dietary load of metrafenone caused by the individual components is:

Barley (bran fractions)	41.22%
Brewer's grain (dried)	0.00%
Wheat (Extruded grain)	0.00%
Wheat (bran)	57.52%
Wheat (flour)	0.00%
Wheat (germ)	0.00%
Wheat (middlings)	0.00%
Wheat (gluten)	1.26%

Dietary Burden Calculation concerning metrafenone
Fraunhofer IME Schmallenberg, Germany, developed 2014 by Judith Klein(Version: 13 November 2014)

INPUT
=====

Target content for Rainbow trout:

Crude fat	15.00%
Crude protein	42.00%

Maximum principal content of components in the diet:

Barley (bran fractions)	100.00%
Brewer's grain (dried)	100.00%
Wheat (Extruded grain)	100.00%
Wheat (bran)	100.00%
Wheat (flour)	100.00%
Wheat (germ)	100.00%
Wheat (middlings)	100.00%
Wheat (gluten)	100.00%

Percent dry matter of components:

Barley (bran fractions)	88.0%
Brewer's grain (dried)	92.0%
Wheat (Extruded grain)	87.7%
Wheat (bran)	88.7%
Wheat (flour)	88.0%
Wheat (germ)	88.7%
Wheat (middlings)	89.4%
Wheat (gluten)	91.4%

metrafenone residues in the components:

Barley (bran fractions)	0.175 mg/kg (STMR-p)
Brewer's grain (dried)	0.021 mg/kg (STMR-p)
Wheat (Extruded grain)	0.010 mg/kg (STMR-p)
Wheat (bran)	0.042 mg/kg (STMR-p)
Wheat (flour)	0.014 mg/kg (STMR-p)
Wheat (germ)	0.042 mg/kg (STMR-p)
Wheat (middlings)	0.0019 mg/kg (STMR-p)
Wheat (gluten)	0.0019 mg/kg (STMR-p)

metrafenone residues in the components (dry matter):

Barley (bran fractions)	0.199 mg/kg (STMR-p/dry matter)
Brewer's grain (dried)	0.023 mg/kg (STMR-p/dry matter)
Wheat (Extruded grain)	0.011 mg/kg (STMR-p/dry matter)
Wheat (bran)	0.047 mg/kg (STMR-p/dry matter)
Wheat (flour)	0.016 mg/kg (STMR-p/dry matter)
Wheat (germ)	0.047 mg/kg (STMR-p/dry matter)
Wheat (middlings)	0.0021 mg/kg (STMR-p/dry matter)
Wheat (gluten)	0.0021 mg/kg (STMR-p/dry matter)

RESULTS
=======

Maximum content dietary burden based on metrafenone is 0.058 mg/kg (dry matter).

The respective composition of the feed is:

Barley (bran fractions)	18.93%
Brewer's grain (dried)	0.00%
Wheat (Extruded grain)	0.00%
Wheat (bran)	40.38%
Wheat (flour)	0.00%
Wheat (germ)	0.00%
Wheat (middlings)	0.00%
Wheat (gluten)	40.70%

The dietary load of metrafenone caused by the individual components is:

Barley (bran fractions)	65.34%
Brewer's grain (dried)	0.00%
Wheat (Extruded grain)	0.00%
Wheat (bran)	33.19%
Wheat (flour)	0.00%
Wheat (germ)	0.00%
Wheat (middlings)	0.00%
Wheat (gluten)	1.47%

Summary of livestock feeding studies

No livestock feeding studies are available. On the basis of metabolism studies in ruminants and poultry and predicted livestock dietary burdens, residues of metrafenone and its metabolites can be clearly shown to be below the limit of quantitation. Therefore no livestock feeding studies are required. Fish intakes were also shown to be below <0.1 ppm diet and therefore fish feeding studies are not required.

CA 6.5 Effects of Processing

The effects of processing on residues of metrafenone have been previously reported in the DAR (UK, July 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/processed crop	Number of studies	Transfer factor	% Transference *
Wheat	Not required due to low residues in grain (< 0.1 mg/kg)	Not applicable	Not applicable
Barley/ Pearl barley	1	0.14	Not allocated
Barley/ Bran	1	2.9	Not allocated
Barley/ Beer	1	0.14	Not allocated
Grape/ wine (young)	8	0.32	No data n/a
Grape/ wine (stored)	8	0.31	No data n/a
Grape/ raisin	2	0.67	No data n/a

This conclusion was subsequently confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498).

Overview of the available processing studies

Processed commodity	**Number of studies**	**Median PF (a)**	**Median CF (b)**	**Comments**
Enforcement residue definition: metrafenone				
Processing factors recommended (sufficiently supported by data)				
Wine grapes, red wine (unheated)	7	0.32	1.00	EFSA, 2006
Strawberries, jam	4	0.23	1.00	EFSA, 2013
Strawberries, sauce	4	0.17	1.00	The reported study referred to syrup (EFSA, 2013).
Strawberries, canned	4	0.91	1.00	EFSA, 2013
Tomatoes, peeled and canned	4	0.02	1.00	EFSA, 2013
Tomatoes, sauce	4	0.81	1.00	EFSA, 2013
Tomatoes, paste	4	0.37	1.00	EFSA, 2013
Tomatoes, ketchup	4	0.42	1.00	EFSA, 2013
Tomatoes, juice	4	0.33	1.00	EFSA, 2013
Gherkins, canned	4	0.80	1.00	Results were obtained on cucumber (EFSA, 2013).

Processed commodity	Number of studies	Median PF (a)	Median CF (b)	Comments
Enforcement residue definition: metrafenone				
Indicative processing factors (limited dataset)				
Table grapes, dried (raisins)	2	0.67	1.00	EFSA, 2006
Wine grapes, white wine	1	0.28	1.00	EFSA, 2006
Barley, beer	1	0.14	1.00	EFSA, 2006
Barley, pot/pearl	1	0.14	1.00	EFSA, 2006
Barley, bran	1	2.90	1.00	EFSA, 2006

"Studies investigating the magnitude of residues in processed commodities of grapes and barley were reported in the framework of the peer review (EFSA, 2006). Additional data submitted in the framework of a recent MRL application on various crops (EFSA, 2013) were also taken into account. An overview of all available processing studies is available in Table 3-3. Robust processing factors for enforcement and risk assessment were derived for processed commodities of grapes (wine), strawberries (jam & sauce), tomatoes (peeled and canned, sauce, paste, ketchup and juice) and gherkins (canned). For the remaining processed items, no robust processing factors for enforcement and risk assessment could be derived as they were not sufficiently supported by studies (a minimum of 3 processing studies is normally required). Their corresponding processing factors should therefore be considered as indicative only.

Nevertheless, further processing studies are not required in this case as they are not expected to affect the outcome of the risk assessment. If more robust processing factors were to be required by risk managers, in particular for enforcement purposes, additional processing studies would be needed."

CA 6.5.1 Nature of the residue

Report:	CA 6.5.1/1 An D., 2000a AC 375839: Effects of processing on the nature of the residues due to hydrolysis 2000/7000137
Guidelines:	EEC 91/414 Annex II 6.5.1, EEC 91/414 Annex III 8.4, EEC 96/68
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). In a standard nature of the residues study (OECD 507), metrafenone was not degraded during the simulation of pasteurisation (pH 4, 90°C), baking, boiling, brewing (pH 5, 100°C) or sterilisation (pH 6, 120°C).

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Not relevant to cereals and grapes.

CA 6.5.3 Magnitude of residues in processed commodities

Report: CA 6.5.3/1
Trewhitt J.A., 2001c
AC 375839 500 g a.s./L SC (SF09955): Decline curve residue study on AC 375839 in vines (Germany, 1999)
2001/7000344

Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68, EEC 7029/VI/95 rev. 5, BBA IV 3-3.4

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005) under the reference of Ipach, R. 1999 (BN-GE-99-101; BN-713-001). Trials were conducted in representative wine growing regions in Germany utilising red grapes (variety Portugieser, site 01) and white grapes (variety Müller-Thurgau, site 02) which were treated with metrafenone (500 g ai/L SC (SF09955). The test substance was applied to vines eight times at intervals of 12 to 16 days and at dose rates of between 62 and 164 g as/ha. The application volumes ranged between 411 and 858 L/ha. Specimens for wine processing were taken at 28 days after harvest. They were then processed into red or white wine and samples of must, young wine and wine for storage were analysed.

Transfer factors were calculated for processed commodities:

Table 6.5.3-1: Residues of metrafenone in grape process fractions (wine)

Matrix	PHI (days)	Total residue Site 01 (mg/kg)	Concentration factor
Whole Grapes	28	0.15	-
Must	28	<0.05	<0.33
Red wine (young)	28	<0.05	<0.33
Red wine (stored)	28	<0.05	<0.33
Matrix	**PHI (days)**	**Total residue Site 02 (mg/kg)**	**Concentration factor**
Whole Grapes	28	0.18	-
Must	28	0.06	0.33
White wine (young)	28	<0.05	<0.28
White wine (stored)	28	<0.05	<0.28

Report: CA 6.5.3/2
Smalley R., 2002k
BAS 560 01 F (AC 375839) 500g as/L SC (SF 09955): At harvest residue and processing study on BAS 560 F in vines - South France, 2000
2002/7004460
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). One field trial was conducted in southern France in 2000 in which vines were treated eight times with BAS 560 01 F 500 g a.s./L SC at a dose rate of 100 g a.s./ha. Samples from this trial were subjected to two separate processing experiments.

Transfer factors were calculated for processed commodities:

Table 6.5.3-2: Metrafenone Residues (mg/kg) in/on Grape Processed Fractions

Trial	Matrix	Residues (mg/kg)	Transfer factor
00-844-648 VIN 0006 BAS 005	Grapes	0.26	1
	Must	0.21	0.81
	Young wine (red)	<0.05	<0.19
	Wine (red)	<0.05	<0.19
00-844-648 VIN 0006 BAS 007	Grapes	0.27	1
	Must	0.21	0.78
	Young wine (red)	0.08	0.30
	Wine (red)	<0.05	<0.19

Report: CA 6.5.3/3
Smalley R., 2002l
BAS 560 01 F (AC 375839) 500g as/L SC (SF 09955): At harvest residue and processing study on BAS 560 F in vines - Italy, 2000
2002/7004451
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). Two trials were conducted in Italy in 2000 in which vines were treated eight times with BAS 560 01 F 500 g a.s./L SC at a dose rate of 100 g a.s./ha. Samples from the two trials were subjected to two separate processing experiments, making four processing experiments in total.

Transfer factors were calculated for processed commodities:

Table 6.5.3-3: Metrafenone Residues (mg/kg) in/on Grape Processed Fractions

Trial	Matrix	Residues (mg/kg)	Transfer factor
00-845-01 VIN 0007 BAS 005	Grapes	0.30	1
	Must	0.17	0.57
	Young wine	<0.05	<0.17
	Wine (red)	<0.05	<0.17
00-845-01 VIN 0007 BAS 007	Grapes	0.24	1
	Must	0.28	1.17
	Young wine	<0.05	<0.21
	Wine (red)	<0.05	<0.21
00-845-02 VIN 0007 BAS 037	Grapes	0.13	1
	Must	0.10	0.77
	Young wine	<0.05	<0.38
	Wine (red)	<0.05	<0.38
00-845-02 VIN 0007 BAS 039	Grapes	0.07	1
	Must	0.09	1.29
	Young wine	<0.05	<0.71
	Wine (red)	<0.05	<0.71

Report: CA 6.5.3/4
Smalley R., 2002m
BAS 560 01 F (AC 375839) 500g as/L SC (SF 09955): At harvest residue and processing study on BAS 560 F in vines - Spain, 2000
2002/7004459
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). One trial was conducted in Spain in 2000 in which vines were treated eight times with BAS 560 01 F 500 g a.s./L SC at a dose rate of 100 g a.s./ha. Samples of grapes from this field trial were dried in the sun to make raisins in two experiments.

Transfer factors were calculated for processed commodities:

Table 6.5.3-4: Metrafenone Residues (mg/kg) in/on Grape Processed Fractions

Trial	Matrix	Residues (mg/kg)	Transfer factor
00-846-11	Grapes	0.07	1
00-846-11-04A	Raisins	<0.05	<0.71
00-846-11	Grapes	0.08	1
00-846-11-04B	Raisins	<0.05	<0.63

Report: CA 6.5.3/5
Smalley R., 2002n
BAS 560 01 F (AC 375839) 500g as/L SC (SF 09955): At harvest residue and processing study on BAS 560 F in vines - North France, 2000
2002/7004455
Guidelines: EEC 91/414 Annex II 6, EEC 91/414 Annex III 8, EEC 96/68
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). One trial was conducted in northern France in 2000 in which vines were treated eight times with metrafenone 500 g a.s./L SC at a dose rate of 100 g a.s./ha. The crop growth stage at the final application was BBCH 85. Grapes were samples 27 days after the last treatment.

Transfer factors were calculated for processed commodities:

Table 6.5.3-5: Metrafenone Residues (mg/kg) in/on Grape Processed Fractions

Trial	Matrix	DALA (1)	Residues (mg/kg)	Transfer factor
00-843-441 Specimen A	Grapes	27	0.28 (2)	1
	Must	27	<0.05	<0.18
	Still wine (white)	27	<0.05	<0.18
	Sparkling wine	27	<0.05	<0.18
000-843-441 Specimen B	Grapes	27	0.19 (2)	1
	Must	27	0.05	0.26
	Still wine (white)	27	<0.05	<0.26
	Sparkling wine	27	<0.05	<0.26

(1) DALA – Days after last application
(2) The value used for grapes was the specimen obtained from the grape processing specimen

Report: CA 6.5.3/6
Jordan J.M.,Kasiri A., 2006a
Magnitude of BAS 560 F residues in grapes and grape processed fractions following applications of BAS 560 00 F (amended final report)
2006/7007012
Guidelines: EPA 860.1500, EPA 860.1520, EPA 860.1380
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

Twelve trials were carried out in the USA to assess residues of metrafenone in grapes and processed grape commodities and vinification products. Plots received six broadcast foliar applications of the 300g/L SC metrafenone formulation at 0.29-0.31 lb as/A/application (0.32-0.35 kg as/ha/application), with 12- to 15-day retreatment intervals, for a total rate of 1.78-1.84 lb as/A (2.00-2.07 kg as/ha/season). Grape RAC samples were collected from all test sites at 0, 14, and 28 days after the last application, and at one test site, grape RAC samples were also collected at 7 and 21 DALA to assess residue decline. In addition, bulk grape RAC (fresh fruit) and sun-dried raisin samples were collected for processing from one test site. Fresh grapes were harvested at 0 DALA, and RAC samples were sent for processing. The sun-dried raisin samples were collected after the 0 DALA grapes field dried for 28 days. The residues of metrafenone in/on grape RAC and processed commodity samples were determined by HPLC/MS/MS. A comparison of the residues in the RAC with those in each processed fraction indicates that residues of metrafenone concentrate in wet pomace, unwashed raisins, and washed raisin by mean factors ranging from 3.21-3.79X. Metrafenone residues do not concentrate in juice, must, young wine, wine, or yeast (mean processing factors range from 0.07-1.17X).

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: Active substance
Lot/Batch #: 3013
Purity: 298.6 g/L
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels: not relevant

2. **Test Commodity:** Berries and small fruit
Crop: grape
Type: wine
Variety: Catawba, Vidal Blanc, Ruby Seedless, Thompson, Centurian, Zinfandel, Merlot, White Riesling, Pinot Noir
Botanical name: *Vitis vinifera*
Crop parts(s) or processed commodity: Grapes, unwashed and washed raisins, juice, must, young wine, wet pomace, yeast, and wine
Sample size:

B. STUDY DESIGN AND METHODS

1. Test procedure

A total of 12 trials were established on grapes in NAFTA Region 1 (NY, two trials), 10 (CA, eight trials), 11 (WA, one trial), and 12 (OR, one trial) during the 2005 growing season. Samples for processing were taken from one of the trials.

One untreated control plot (Treatment 1) and two treated plots (Treatments 2 and 3) were established at each test site. Each of the treated plots received six broadcast foliar applications of the 300 g/L SC metrafenone formulation at 0.29-0.31 lb as/A/application (0.32-0.35 kg as/ha/application, with 12- to 15-day retreatment intervals, for a total rate of 1.78-1.84 lb as/A (2.00-2.07 kg as/ha/season). Applications were made by ground equipment using either concentrate (52-77 gal/A of water, Treatment 2) or dilute (112-158 gal/A of water, Treatment 3) spray volumes. An adjuvant was not used as BAS 560 00F has an adjuvant included in the formulation. Applications were made using commercial or simulated commercial ground application equipment, and all sprayers were calibrated prior to each application by the volume/time method. The first applications were made at the end of flowering, the inflorescences swelling stage, or when bunches began to hang.

Grape RAC samples (fresh fruit) were collected from all test sites at 0, 14, and 28 days after the last application. At one test site, (RCN R05012, Tulare, CA), grape RAC samples were also collected at 7 and 21 DALA to assess residue decline. A single grape RAC sample was collected from the treated plots at each location and sampling interval.

Control samples were harvested only at 0 DALA. With only one exception, each grape RAC sample weighed ≥2.2 lbs treated samples and ≥5 lbs for the controls. The exception occurred at trial RCN R05019 at the targeted 28 DALA sampling interval, where, because of bird damage, the RAC sample collected from the Treatment 2 plot weighed only 1 lb and no RAC sample was collected from the Treatment 3 plot.

Additional samples were collected for processing from one test site (RCN R05013, Tulare, CA). At this site, bulk RAC samples were collected from the Treatment 3 (dilute spray) plot and from the control plot at 0 DALA. From these samples, two treated and one control were shipped overnight for processing. The remaining samples (two treated, one control) were field dried for 28 days and collected as sun-dried raisins. These raisin samples were then shipped overnight to the food processor for washing.

Processed grape fractions collected at the food processor were the unwashed RAC, unwashed and washed raisins, juice, must, young wine, wet pomace, yeast, and wine. Both white and red wine was made, using processes appropriate to each type.

2. Description of analytical procedures

Residues of metrafenone in grape RAC and processed commodity samples were determined by HPLC/MS/MS Method No. 535/0, the data collection method for plant commodities. The performance of the analytical method was evaluated during each sample set by fortifying control grape RAC and/or processed commodity samples with metrafenone. Concurrent recoveries from control grape RAC and processed commodity samples fortified with metrafenone at 0.01-20.0 mg/kg were 80-127% (n=41).

II. RESULTS AND DISCUSSION

Residues in controls were <0.01 mg/kg (<LOQ) in/on all RAC and processed commodity samples (n=28), with the exception of one wet pomace control which bore apparent residues of metrafenone at 0.02 mg/kg. Recoveries were corrected when apparent residues were detected in the associated control. The maximum frozen (≤-10ºC) storage interval from collection to analysis was 194 days for grape RAC samples and 117 days for grape processed commodities.

Residues of metrafenone were 0.04-4.04 mg/kg (concentrate spray) and 0.11-5.64 mg/kg (dilute spray) in/on 24 treated grape samples harvested 0 days following the last of six broadcast foliar applications of metrafenone (300 g/L SC) at *ca.* 0.30 lb as/A/application, with 12- to 15-day retreatment intervals, totaling 1.78-1.84 lb as/A/season. At the same treatment rate and retreatment intervals, metrafenone residues were 0.02-3.20 mg/kg (concentrate spray) and 0.04-2.96 mg/kg (dilute spray) in/on 24 treated grape samples collected at a 14-day PHI, and were 0.02-4.42 and 0.02-2.26 mg/kg (concentrate and dilute sprays, respectively) in/on 23 treated grape samples collected at a 27 or 28-day PHI.

Residues of metrafenone were <0.01 mg/kg (<LOQ) in/on all control grape RAC samples (n=12). No significant differences in metrafenone residues were observed between the concentrate and dilute spray applications.

Table 6.5.3-6: Metrafenone Residues (mg/kg) in/on Grape Processed Fractions

RAC	Processed commodity	Residues (mg/kg)	Processing factor
Grapes	Unwashed grapes	0.27	-
R0513-0013	Juice	0.01	0.04
	Must	0.04	0.15
	Young wine (white)	0.02	0.07
	Wet pomace	0.75	2.78
	Yeast	0.50	1.85
	White wine	0.02	0.07
Grapes	Unwashed grapes	0.31	-
R0513-0014	Juice	0.02	0.06
	Must	0.01	0.03
	Young wine (red)	0.01	0.03
	Wet pomace	1.11	3.58
	Yeast	0.18	0.58
	Red wine	<0.01	0.03
Grapes	Unwashed grapes	0.27	-
R05013-0016	Unwashed raisins	1.11	4.11
	Washed raisins	0.98	3.63
Grapes	Unwashed grapes	0.31	-
R0513-0017	Unwashed raisins	0.86	2.77
	Washed raisins	1.22	3.94

III. CONCLUSION

The results from this processing study in grapes show that metrafenone residues in commodities processed from grapes bearing detectable residues concentrated in wet pomace, unwashed raisins, and washed raisin by factors ranging from 2.78-4.11X, but did not concentrate in juice, must, young wine or wine (processing factors range from 0.03-0.15X). Results for yeast were variable (processing factors of 0.58 and 1.85X).

Report: CA 6.5.3/7
Pollmann B., 2002a
Determination of residues of BAS 560 F in field samples and in processed goods after application of BAS 560 00 F in summer barley at 4 sites in Germany in 2001
2002/1004080

Guidelines: IVA-Leitlinie Rueckstandsversuche Teil VI (1992), BBA IV 3-3, BBA IV 3-4, EEC 7035/VI/95 rev. 5

GLP: yes
(certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). The objective of the study was to determine the magnitude of metrafenone residues in field samples and processed barley commodities following two applications of BAS 560 03 F.

Transfer factors were calculated for processed commodities:

Table 6.5.3-7: Metrafenone Residues (mg/kg) in/on Cereal Processed Fractions

Sample material	Process	Range of residues (mg/kg)	Transfer factor (mean)
Stored barley grain	Start material for processing	0.03-0.10	-
Malt	Preparation of beer	0.04	0.40
Malt sprouts		0.08	0.80
Brewers grain		0.03	0.30
Spent hops		<0.01	<0.10
Brewers yeast		<0.01	<0.10
Beer		<0.01 (4)	<0.10, <0.13, <0.17, <0.33 (<0.18)
Pearl barley	Preparation of pearl barley	<0.01(2), 0.01, 0.02	<0.13, 0.13, <0.20, 0.22 (<0.17)
Pearl barley abrasions		0.20	2.50

Report:	CA 6.5.3/8 Pollmann B., 2002b Determination of residues of BAS 560 F in field samples and in processed goods after application of BAS 560 00 F in summer wheat at 4 sites in Germany in 2001 2002/1006302
Guidelines:	IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, BBA IV 3-3, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 91/414 (1607/IV/97 Rev. 1)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

A study was conducted to assess the transfer of metrafenone residues from wheat grain to flour, bran and bread. Following processing, residues of metrafenone were concentrated in wholemeal flour, bran flour and fine bran but were reduced in flour type 550, course bran and whole grain bread.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Metrafenone
Description: 300 g/L SC
Lot/Batch #: R2066-074
Purity: 299 g/L
CAS#: 220899-03-6
Development code: BAS 560 F
Spiking levels: 0.01 and 0.1 mg/kg

2. **Test Commodity:**
Crop: wheat
Type: summer
Variety: Perdix, Tasos, Thasus
Botanical name: *Triticum aestivum*
Crop parts(s) or processed commodity: grain, flour, bran, bread
Sample size: 7.08-10.46 kg (grain for processing)

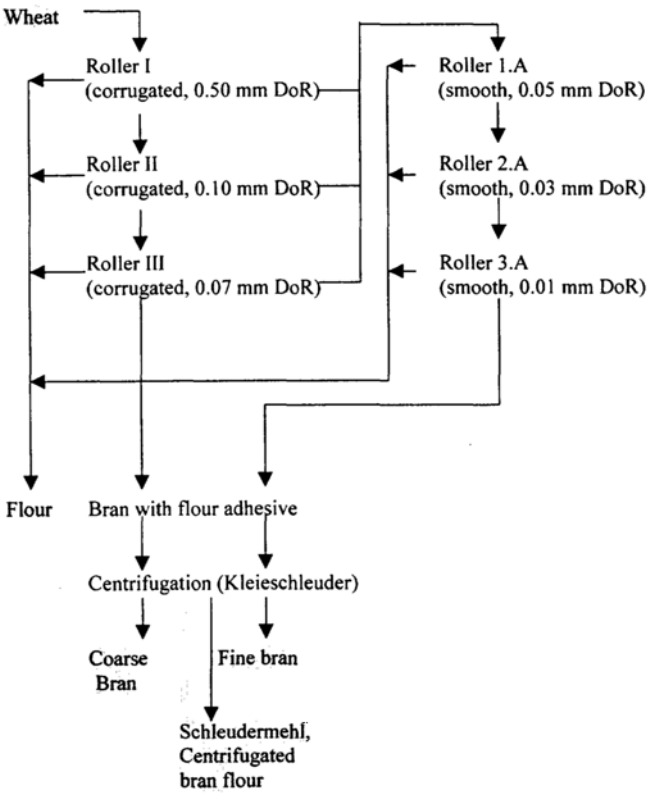
B. STUDY DESIGN AND METHODS

1. Test procedure

The objective of the study was to determine the magnitude and quantitative distribution of metrafenone residues in the various intermediate and end products following simulated commercial processing of summer wheat. BAS 560 00 F was applied with an exaggerated target rate of 10 X the recommended critical good agricultural practice according to the EU label at four sites representing typical German wheat growing areas. Samples for processing were taken 35 $\pm$ 1 day after the final application.

The processing performed in the study was comparable to the processes used for commercial production of the intended intermediates and end products.

Figure 6.5.3-1: Processing of wheat grain to flour and bran fractions



2. Description of analytical procedures

The RAC and processed specimens (grain, whole meal flour, flour type 550, bran flour, coarse bran, fine bran and whole grain bread) were analysed by liquid chromatography/APCI mass spectrometry (LC/MS). Analysis was performed using method RLA 12619.03V (also referred to as method no. 993/0 (Determination of BAS 560 F, CL 3000402, CL 434223 and CL 376991 Residues in Cereals using LC/MS Determination, Edwards J, 2002)).

II. RESULTS AND DISCUSSION

Residue ranges and transfer factors for metrafenone in processed wheat commodities are presented below.

Table 6.5.3-8: Metrafenone Residues (mg/kg) in/on Cereal Processed Fractions

Sample material	Process	Range of residues (mg/kg)	Transfer factor (mean)
Grain	RAC	0.07 – 0.28	-
Wholemeal flour	Milling	0.12 – 0.31	1.11, 1.93, 0.94, 1.71 (1.42)
Flour type 550		0.01 – 0.06	0.21, 0.29. 0.17, 0.14 (0.20)
Bran flour		0.18 – 0.45	1.61, 2.64, 1.33, 2.57 (2.04)
Coarse bran		0.04 – 0.08	0.29, 0.43, 0.33, 0.57 (0.41)
Fine bran		0.37 – 0.97	3.46, 4.93, 2.61, 5.29 (4.07)
Whole grain bread	Baking	0.05 – 0.18	0.64, 1.00, 0.61, 0.71 (0.74)

III. CONCLUSION

Following processing, residues of metrafenone in wheat grain were concentrated in wholemeal flour, bran flour and fine bran but were reduced in flour type 550, course bran and whole grain bread.

Summary of the effects of processing

Processing studies were conducted to assess the transfer of metrafenone residues into processed products produced from grapes, barley and wheat. For some processed commodities such as wine and beer, virtually no processed samples were found to contain detectable residues. There was slightly less transfer of residues into white wine compared to red wine but a large number of wine samples did not contain detectable residues which may have impacted this finding. The processing factor for raisins from the EU study showed a decrease in residues compared to the US study which showed an increase in residues. Since processing into raisins is carried out purely by dehydration, concentration of residues would be expected in this processed fraction. The processing factors and mean processing factors from studies presented in this summary and not previously evaluated in the EU are presented below.

Table 6.5.3-9: Summary of the effects of processing on metrafenone residues

Raw agricultural commodity (RAC)	Processed commodity	Processing factors	Median processing factor
Grapes	Red wine (young)	<0.33, <0.19, 0.30, <0.17, <0.21, <0.38, <0.71, 0.03	<0.26
	Red wine	<0.33, <0.19, <0.19, <0.17, <0.21, <0.38, <0.71, 0.03	<0.20
	White wine (young)	<0.28, 0.07	<0.18
	White wine	<0.28, <0.18, <0.26, 0.07	<0.22
	Sparkling wine	<0.18, <0.26	<0.22
	Juice	0.04, 0.06	0.05
	Raisins	<0.71, <0.63	<0.67
	Unwashed raisins	4.11, 2.77	3.44
	Washed raisins	3.63, 3.94	3.79
Barley	Malt	0.40	0.40
	Brewers grain	0.30	0.30
	Beer	<0.10, <0.13, <0.17, <0.33	<0.15
	Pearl barley	<0.13, <0.13, <0.20, 0.22	<0.17
	Pearl barley abrasions	2.5	2.5
Wheat	Wholemeal flour	1.11, 1.93, 0.94, 1.71	1.41
	Flour type 550	0.21, 0.29. 0.17, 0.14	0.19
	Bran flour	1.61, 2.64, 1.33, 2.57	2.09
	Coarse bran	0.29, 0.43, 0.33, 0.57	0.38
	Fine bran	3.46, 4.93, 2.61, 5.29	4.20
	Whole grain bread	0.64, 1.00, 0.61, 0.71	0.68

CA 6.6 Residues in Rotational Crops

The metabolism of metrafenone in rotational crops has been previously reported in the DAR (UK, July 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)	Not required. Based on rotational crops metabolism data residues in succeeding crops are unlikely if metrafenone is used according to GAP.

This conclusion was subsequently confirmed in EFSA's Reasoned Opinion on metrafenone MRLs following its review under Article 12 (EFSA Journal 2013;11(12):3498).

This requirement is not relevant to grapes as they are a permanent crop.

CA 6.6.1 Metabolism in rotational crops

Report:	CA 6.6.1/1 Zulalian J., 2002b BAS 560 F (AC 375839): Confined rotational crop study with carbon-14 labeled AC 375839 2002/7005187
Guidelines:	EPA 40 CFR 158.290, EPA 860.1850, EEC 91/414 Annex II 6.6, EEC 96/68
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

This study was previously reported in the DAR (UK, July 2005). An outdoor confined rotational crop study with ^{14}C labeled metrafenone was conducted from 1998 to 1999. The study was designed to determine the nature and amount of metrafenone-related residue uptake in rotational crops. The leafy vegetable crop (lettuce), root crop (radish) and oil crop (oilseed rape) were planted back at various time intervals following a single application of bromophenyl or trimethoxyphenyl 14C-metrafenone to the surface of a sandy loam soil at a rate of 625 g a.s./ha. Seeding with the relevant crop at 30, 60, 90 and 365 days following application.

Following application, detectable levels (> 0.004 mg/kg) of radioactivity were present in soil throughout the experiment period. Translocation of ^{14}C-metrafenone residues from soil into the plants was very low for all three crops, ranging from <0.004 - 0.048 mg/kg.

In general, the highest total radioactive residues (TRR) were found in plants sampled from the 30 DAT plant back interval, while the lowest residues (< 0.01 mg/kg) were detected in plants from the 365 DAT plant back interval.

The oilseed rape straw/pod contained the highest residues, while very low residues were detected in oilseed rape seed at all sampling intervals. The TRR in oilseed rape straw/pod ranged from 0.037 - 0.048 mg/kg at 30 DAT and <0.004 mg/kg at 365DAT. The TRR in oilseed rape seed ranged from 0.007 - 0.008 mg/kg at 30DAT and 0.005 - 0.008 mg/kg at 365DAT. The TRR in radish top ranged from 0.015 - 0.025 mg/kg at 30 DAT and 0.005 - 0.007 mg/kg at 365DAT. The TRR in radish root ranged from 0.012 - 0.023 mg/kg at 30 DAT and 0.004 - 0.005 mg/kg at 365 DAT. The TRR in lettuce ranged from 0.005 - 0.006 mg/kg at 30 DAT and was <0.004 mg/kg at 365DAT.

The nature of the residue in the rotational crop samples containing a TRR >0.01 mg/kg was characterized by solvent extraction and reversed phase radio-HPLC analysis of the extracts. With the exception of oilseed rape seed, methanol:water and methanolic HCl were used to extract radiocarbon residues from the crop matrices accounting for 62% to 85.2% and 5.9% to19.9% of the TRR, respectively, with total extractable radioactive residues (ERR) accounting for 64.0% to 87.9% TRR. Hexane was the primary solvent used to extract radioactivity from oilseed rape seed accounting for 41.9% to 86.2% TRR, with additional radioactivity released using methanol:water. The ERR in oilseed rape seed accounted for 41.9% to 86.2% TRR. The solvent nonextractable residues (post extraction solids, PES) ranged from 13.8% to 52.1% TRR in all crop matrices, with the exception of 60 DAT oilseed plants, in which the PES accounted for 23.9% and 100 % TRR for the bromophenyl and trimethoxyphenyl labels, respectively.

The HPLC results showed that the residue was comprised of unchanged parent metrafenone and a prominent polar region designated ROI 1 (Region of Interest 1). Metrafenone accounted for <0.005 mg/kg of the TRR in lettuce and radish roots. ROI 1, which accounted for the major portion of the extractable residues in most of the crops at <0.02 mg/kg, was shown to contain multiple components. Due to the low concentrations of individual peaks, no further investigation for identification was carried out. All other components of the extractable residues were <0.01 mg/kg. The non-extractable residues were less than 0.01 mg/kg for all crops.

The results from the rotational crop study showed that there is no expected accumulation of metrafenone or its degradation products in the parts of plants used for human food or animal feed consumption. The levels of individual metabolites were below 0.01 mg/kg.

CA 6.6.2 Magnitude of residues in rotational crops

Data on metabolism in rotational crops shows that no detectable residues would be expected and therefore field data are not required.

Summary of residues in succeeding crops

The metabolism of metrafenone in rotational crops has been previously reported in the DAR (UK, July 2005). A study using bromophenyl or trimethoxyphenyl 14C-metrafenone with lettuce, radish and oilseed rape as rotational crops indicated that that there is no expected accumulation of metrafenone or its degradation products in the parts of plants used for human food or animal feed consumption. The levels of individual metabolites were below 0.01 mg/kg. Since the metabolism in rotational crops study shows that no detectable residues would be expected, field data are not required.

This data requirement is not relevant to grapes as they are a permanent crop.

CA 6.7 Proposed residue definitions and maximum residue levels

MRLs and residues definitions for metrafenone in grapes, cereals and products of animal origin have been previously reported in the DAR (UK, 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant residue definition for monitoring	Metrafenone
Plant residue definition for risk assessment	Metrafenone
Conversion factor (monitoring to risk assessment)	Not applicable

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	Goat, hen
Animal residue definition for monitoring	Not proposed by RMS
Animal residue definition for risk assessment	Not proposed by RMS[19]
Conversion factor (monitoring to risk assessment)	Not applicable
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Not applicable

[19]should be defined for ruminant liver and kidney (refer to chapter 3.2)

MRLs for metrafenone have also been reviewed under article 12 of Regulation 396/2005 and EFSA have published a Reasoned Opinion (EFSA Journal 2013;11(12):3498) leading to MRLs being updated by SANCO/11973/2014. Following review under Article 12 and by the JMPR in 2014 (CCPR and CAC in 2015), the residues definition for metrafenone in plants and products of animal origin (monitoring and risk assessment) is metrafenone only. JMPR defined the residue as fat soluble.

CA 6.7.1 Proposed residue definitions

Following review under Directive 91/414/EEC, Regulation (EC) 396/2005 and also by the JMPR/CCPR, the residues definitions for both monitoring and risk assessment in plants and products of animal origin were defined as metrafenone only.

Based on plant metabolism studies (refer to section CA 6.2), little or no metabolism was seen in cucumbers with the predominant residue being metrafenone (42.4% TRR, 0.02 mg/kg) with the remaining 37.9% TRR (0.02 mg/kg metrafenone equivalents) being characterized as minor polar or medium polarity metabolites (<0.01 mg/kg). A high level of radioactivity (≥89% TRR was extracted with polar solvents such as methanol and water. Therefore no consideration of any metabolites is required. In grapes, metrafenone was also the dominant residue in leaves at >87% TRR. CL 3000402, CL 1500836 and CL 379395 were also tentatively identified but were not accurately quantified. In juice, radioactivity was found to comprise of multiple minor polar metabolites none of which exceeded 4% TRR (0.003 mg/kg) or 9% TRR (0.006 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. CL 197675 was tentatively identified at a maximum of 9% TRR (0.006 mg/kg) in the bromophenyl labelled samples; no further consideration of this metabolite is necessary due to its low level of occurrence. Parent was the major radioactive residue detected in extracts of the marc from mature grapes accounting for 24.7% TRR (0.109 mg/kg) and 22.8% TRR (0.063 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively. Polar metabolites comprising multiple components were detected at 12% TRR (≤0.05 mg/kg) 17% TRR (≤0.046 mg/kg) from the bromophenyl and trimethoxyphenyl labels, respectively.

CL 3000402 is a lactone metabolite and is also seen in the rat metabolism study. An assessment of its toxicity using a QSAR approach (refer to section CA 5.8.1) shows a trigger for skin sensitisation (not relevant to consumer exposure) but also that read across can be made from metrafenone toxicity data based on their comparable, predicted toxicity. Therefore this metabolite should not be considered as toxicologically significant.

For fruiting vegetables, a residues definition for monitoring and risk assessment of metrafenone only is appropriate. Since grape leaves are considered to be a leafy crop, this residues definition could also be extended to the leafy crop group.

In cereals, metrafenone was the major metabolic product and based on the metabolism study and residues trials determining residues of the metabolites CL 3000402, CL 434223 and CL 376991, metabolites would not be expected to be detectable in wheat grain and CL 3000402 would be expected to reach a maximum of 0.02 mg/kg in barley grain (*cf.* HR of metrafenone of 0.68 mg/kg). Assuming no losses on processing, chronic intakes of CL 3000402 from barley and oat grain would be 0.03 µg/kg bw/day or 0.01% of the ADI for metrafenone (Irish adult) and therefore no further consideration of this metabolite in cereal grains is required. For cereal grains, a residues definition for monitoring and risk assessment of parent only is appropriate.

Some metabolism was seen in straw and residues trials data are also available determining residues of the metabolites CL 3000402, CL 434223 and CL 376991. Residues above 0.1 mg/kg contribute to maximum livestock intakes of 0.034 ppm diet or 0.001 mg/kg bw/day in cattle or poultry and therefore no further consideration of metabolites below this level is necessary as they will not lead to detectable residues in products of animal origin and therefore will not lead to significant consumer exposure. This applies to the following metabolites in straw: CL 1500837, CL 1500836, CL 1500838, CL 1500839, CL 1500832, CL 1500833, CL 1500834, CL 1500835, CL 377160, CL 434223, CL 376991 and CL 1500831. Therefore it is not necessary to further consider their toxicity or inclusion in the residues definition for monitoring or risk assessment in products of animal origin.

CL 3000402 was detected in grain and straw at maximum levels of 0.02 mg/kg in barley grain (non-detectable in wheat grain), 0.18 mg/kg in wheat straw and 0.30 mg/kg in barley straw. However, calculation of intakes in ruminants and poultry showed maximum intakes of $\leq$0.1 ppm diet DM or $\leq$0.004 mg/kg bw/day. Based on these intakes, no detectable levels of CL 3000402 would be predicted in products of animal origin and therefore no further consideration of this metabolite as part of the residues definition or consumer exposure is required. For cereal straw, a residues definition for monitoring and risk assessment of metrafenone only is appropriate.

For products of animal origin, metrafenone was shown to be the dominant residue in goat and hen metabolism studies. Poultry intakes of metrafenone exceeded the 0.1 ppm diet DM trigger. However, based on the metabolism study in hens, residues of metrafenone nor any of its metabolites (which occurred at levels well below those of metrafenone) would be predicted to occur above 0.01 mg/kg in poultry commodities. Therefore no further consideration of the toxicity of these metabolites is required. A similar situation was seen in ruminants. Cattle intakes of metrafenone exceeded the 0.1 ppm diet DM trigger, but based on the metabolism study in the goat, residues of metrafenone would not be predicted to occur above 0.01 mg/kg in ruminant commodities. CL 1500698 and CL 1023363 were seen at levels above those of metrafenone in goat liver and kidney and therefore their likely occurrence in these tissues was considered further. However, residues of these compounds (plus a further unknown which could not be separated from the other two compounds) were predicted to occur at a maximum of 0.006 mg/kg in liver and 0.002 mg/kg in kidney and therefore these do not need to be included in the residues definition or assessed for consumer exposure.

Since metabolism was similar in rats and goats and intakes in pigs are not expected to be significantly different to other livestock, a metabolism study in pigs is not required.

Intakes by fish are predicted to be below 0.1 ppm diet DM and therefore a metabolism study in fish is not required.

Therefore the residue definition for monitoring and risk assessment in products of animal origin should be metrafenone. Metrafenone is fat soluble.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

Grapes: Sixteen trials were carried out in northern and southern Europe. Two slightly different GAPs representing the cGAP (3x0.16 kg as/ha) were used. Statistical analysis using the Kruskal-Wallis test showed there was no statistically-significant difference between trials conducted in northern or southern Europe or using either of the two GAPs, so the data sets were combined to calculate MRLs and other end points.

Residues in ranked order were: 0.05, 0.08, 0.09, 0.10, 0.11(2), 0.17, 0.18, 0.21, 0.23(2), 0.24(2), 0.31 and 0.36(2) mg/kg. The median residue was 0.195 mg/kg and the HR was 0.36 mg/kg. From the OECD calculator, the unrounded MRL is 0.584 mg/kg and the rounded MRL is 0.6 mg/kg. Since the EU MRL of 7 mg/kg is based on an import tolerance (US grapes), it can be clearly demonstrated that the use according to EU GAP is well below this level and therefore no further action is required.

Cereals: Twenty-five trials were conducted in wheat and 26 trials conducted in barley in the EU.

Crop	Region	Number of trials	Residues (mg/kg)	STMR (mg/kg)
Wheat grain	NEU	13	<0.01(8), 0.01, 0.02, 0.03, 0.04(2)	<0.01
Wheat grain	SEU	12	<0.01(7), 0.01(4), 0.03	<0.01
Wheat straw	NEU	13	0.59, 0.67, 0.93, 0.98, 1.43, 1.76, 1.85, 2.04, 2.32, 3.05, 3.54, 3.55(2)	1.85
Wheat straw	SEU	12	0.67, 0.89, 1.08, 1.10, 1.11, 1.25, 1.61, 1.70(2), 2.12, 3.14, 6.71	1.43
Barley grain	NEU	14	<0.01, 0.02(2), 0.03, 0.05(2), 0.06, 0.07, 0.09(2), 0.11, 0.15, 0.16, 0.40	0.065
Barley grain	SEU	12	0.02, 0.03, 0.04(2), 0.05, 0.06, 0.08(2), 0.12, 0.13, 0.23, 0.68	0.07
Barley straw	NEU	14	<0.01, 0.54, 0.64, 0.95, 1.09, 1.11, 1.15, 1.23, 1.28, 1.70, 1.84, 2.01, 3.55, 3.91	1.19
Barley straw	SEU	12	0.41, 0.90, 1.02, 1.09, 1.22, 1.48, 1.65, 1.86, 1.94, 2.13, 4.03, 4.25	1.57

Crop	Region	Number of trials	Residues (mg/kg)	STMR (mg/kg)
Wheat grain	NEU+SEU	25	<0.01(15), 0.01(5), 0.02, 0.03(2), 0.04(2)	<0.01
Wheat straw	NEU+SEU	25	0.59, 0.67(2), 0.89, 0.93, 0.98, 1.08, 1.10, 1.11, 1.25, 1.43, 1.61, 1.70(2), 1.76, 1.85, 2.04, 2.12, 2.32, 3.05, 3.14, 3.54, 3.55(2) 6.71	1.7
Barley grain	NEU+SEU	26	<0.01, 0.02(3), 0.03(2), 0.04(2), 0.05(3), 0.06(2), 0.07, 0.08(2), 0.09(2), 0.11, 0.12, 0.13, 0.15, 0.16, 0.23, 0.40, 0.68	0.065
Barley straw	NEU+SEU	26	<0.01, 0.41, 0.54, 0.64, 0.90, 0.95, 1.02, 1.09(2), 1.11, 1.15, 1.22, 1.23, 1.28, 1.48, 1.65, 1.70, 1.84, 1.86, 1.94, 2.01, 2.13, 3.55, 3.91, 4.03, 4.25	1.255

There were no significant differences between residues from northern and southern trials for wheat grain so these data were combined giving an STMR of <0.01 mg/kg, HR of 0.04 mg/kg, an unrounded MRL of 0.051 mg/kg and a rounded MRL of 0.05 mg/kg. Since this is below the current MRL of 0.07 mg/kg no further action is required. This MRL can also be extrapolated to rye and triticale.

There were no significant differences between residues from northern and southern trials for barley grain so these data were combined giving an STMR of 0.065 mg/kg, HR of 0.68 mg/kg, an unrounded MRL of 0.680 mg/kg and a rounded MRL of 0.7 mg/kg. Since this is slightly above the current MRL of 0.6 mg/kg, an application to increase this MRL will be made. This MRL can also be extrapolated to oats.

Currently, no MRLs are in place for cereal straw. Should these be set in the future, the following would be appropriate:

There were no significant differences between residues from northern and southern trials for wheat straw so these data were combined giving an STMR of 1.7 mg/kg, HR of 6.71 mg/kg, an unrounded MRL of 7.424 mg/kg and a rounded MRL of 8 mg/kg. These values can be extrapolated to rye and triticale straw.

There were no significant differences between residues from northern and southern trials for barley straw so these data were combined giving an STMR of 1.255 mg/kg, HR of 4.25 mg/kg, an unrounded MRL of 6.129 mg/kg and a rounded MRL of 7 mg/kg. These values can be extrapolated to oat straw.

For products of animal origin including fish, MRLs were predicted to be <0.01 mg/kg (refer to CA 6.4). Metrafenone should be defined as fat-soluble based on its log Po/w >3.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The proposed MRLs based on GAP in grapes and cereals are summarized below. Current EU MRLs and those currently under discussion in Europe and at CCPR/CAC are also included for completeness. No change is requested to any current EU MRL except for barley and oat grain.

		Proposed EU MRL	Codex MRL	SANCO/ 11973/2014/ SANTE/ 10377/2015	Reg. (EU) No 500/2013
0150000	**Berries and small fruits**				
0151000	a) grapes	7	5	7	5
0151010	Table grapes	7		7	5
0151020	Wine grapes	7		7	5
0500000	**Cereals**				
0500010	. Barley	**0.7**	0.5	0.6	0.5
0500050	. Oat	**0.7**	0.5	0.6	0.5
0500070	. Rye	0.07	0.06	0.07	0.1
0500090	. Wheat	0.07	0.06	0.07	0.5
	Barley and oat straw	7	6 (dw)		
	Wheat and rye straw	8	10 (dw)		
1000000	**Products of animal origin – terrestrial animals**				0.05*
1010000	. Tissues from			0.01*	0.05*
1011000	. (a) swine			0.01*	0.05*
1011010	. Muscle	0.01*	0.01*	0.01*	0.05*
1011020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1011030	. Liver	0.01*	0.01*	0.01*	0.05*
1011040	. Kidney	0.01*	0.01*	0.01*	0.05*
1011050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1011990	. Others	0.01*	0.01*	0.01*	0.05*
1012000	. (b) bovine			0.01*	0.05*
1012010	. Muscle	0.01*	0.01*	0.01*	0.05*
1012020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1012030	. Liver	0.01*	0.01*	0.01*	0.05*
1012040	. Kidney	0.01*	0.01*	0.01*	0.05*
1012050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1012990	. Others	0.01*	0.01*	0.01*	0.05*
1013000	. (c) sheep			0.01*	0.05*
1013010	. Muscle	0.01*	0.01*	0.01*	0.05*
1013020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1013030	. Liver	0.01*	0.01*	0.01*	0.05*
1013040	. Kidney	0.01*	0.01*	0.01*	0.05*
1013050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1013990	. Others	0.01*	0.01*	0.01*	0.05*

		Proposed EU MRL	Codex MRL	SANCO/ 11973/2014/ SANTE/ 10377/2015	Reg. (EU) No 500/2013
1014000	. d) goat			0.01*	0.05*
1014010	. Muscle	0.01*	0.01*	0.01*	0.05*
1014020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1014030	. Liver	0.01*	0.01*	0.01*	0.05*
1014040	. Kidney	0.01*	0.01*	0.01*	0.05*
1014050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1014990	. Others			0.01*	0.05*
1015000	. (e) equine	0.01*	0.01*	0.01*	0.05*
1015010	. Muscle	0.01*	0.01*	0.01*	0.05*
1015020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1015030	. Liver	0.01*	0.01*	0.01*	0.05*
1015040	. Kidney	0.01*	0.01*	0.01*	0.05*
1015050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1015990	. Others			0.01*	0.05*
1016000	. (f) poultry	0.01*	0.01*	0.01*	0.05*
1016010	. Muscle	0.01*	0.01*	0.01*	0.05*
1016020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1016030	. Liver	0.01*	0.01*	0.01*	0.05*
1016040	. Kidney	0.01*	0.01*	0.01*	0.05*
1016050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1016990	. Others			0.01*	0.05*
1017000	. (g) other farmed terrestrial animals	0.01*	0.01*	0.01*	0.05*
1017010	. Muscle	0.01*	0.01*	0.01*	0.05*
1017020	. Fat tissue	0.01*	0.01*	0.01*	0.05*
1017030	. Liver	0.01*	0.01*	0.01*	0.05*
1017040	. Kidney	0.01*	0.01*	0.01*	0.05*
1017050	. Edible offals (other than liver and kidney)	0.01*	0.01*	0.01*	0.05*
1017990	. Others	0.01*	0.01*	0.01*	0.05*
1020000	. Milk			0.01*	0.05*
1020010	. Cattle	0.01*	0.01*	0.01*	0.05*
1020020	. Sheep	0.01*	0.01*	0.01*	0.05*
1020030	. Goat	0.01*	0.01*	0.01*	0.05*
1020040	. Horse	0.01*	0.01*	0.01*	0.05*
1020990	. Others	0.01*	0.01*	0.01*	0.05*
1030000	. Birds eggs			0.01*	0.05*
1030010	. Chicken	0.01*	0.01*	0.01*	0.05*
1030020	. Duck	0.01*	0.01*	0.01*	0.05*
1030030	. Geese	0.01*	0.01*	0.01*	0.05*
1030040	. Quail	0.01*	0.01*	0.01*	0.05*
1030990	. Others	0.01*	0.01*	0.01*	0.05*
	Fish	0.01*			

CA 6.8 Proposed safety intervals

Pre-harvest interval (in days) for each relevant crop

Cereals: 35 days
Grapes: 28 days

Re-entry period (in days) for livestock, to areas to be grazed

Not applicable. Cereal forage and hay is not recommended for livestock grazing. Grapes are not a grazed crop.

Re-entry period (in hours or days) for man to crops, buildings or spaces treated

Under practical conditions of use, there is no reason for workers to enter the crop shortly after treatment. The general approach of avoiding re-entry until the spray solution has dried is recommended.

Withholding period (in days) for animal feed items

Cereal forage and hay is not recommended for livestock grazing. Residues of metrafenone and its metabolites in cereal feed items (grain, bran and straw) have been considered in the calculated livestock dietary burdens (as detailed under CA 6.4). Intakes are expected to be low and not resulting in detectable residues in products of animal origin. Therefore no specific withholding period for animal feed items is required.

Waiting period (in days) between last application and sowing or planting the crops to be protected

Not applicable. The cereal crops or grapes to be protected have already been planted before application of metrafenone.

Waiting period (in days) between application and handling treated product

No specific waiting period between application and handling treated product is required. The general approach of avoiding handling until the spray solution has dried is recommended.

Waiting period (in days) between last application and sowing or planting succeeding crops

No specific plant-back restriction is required when metrafenone is applied according to GAP (as detailed under CA 6.6).

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Consumer exposure to metrafenone residues has been previously reported in the DAR (UK, 2005).

The agreed end points from the first EU assessment of metrafenone were set out in the EFSA Conclusion on the peer review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72) and are reproduced below:

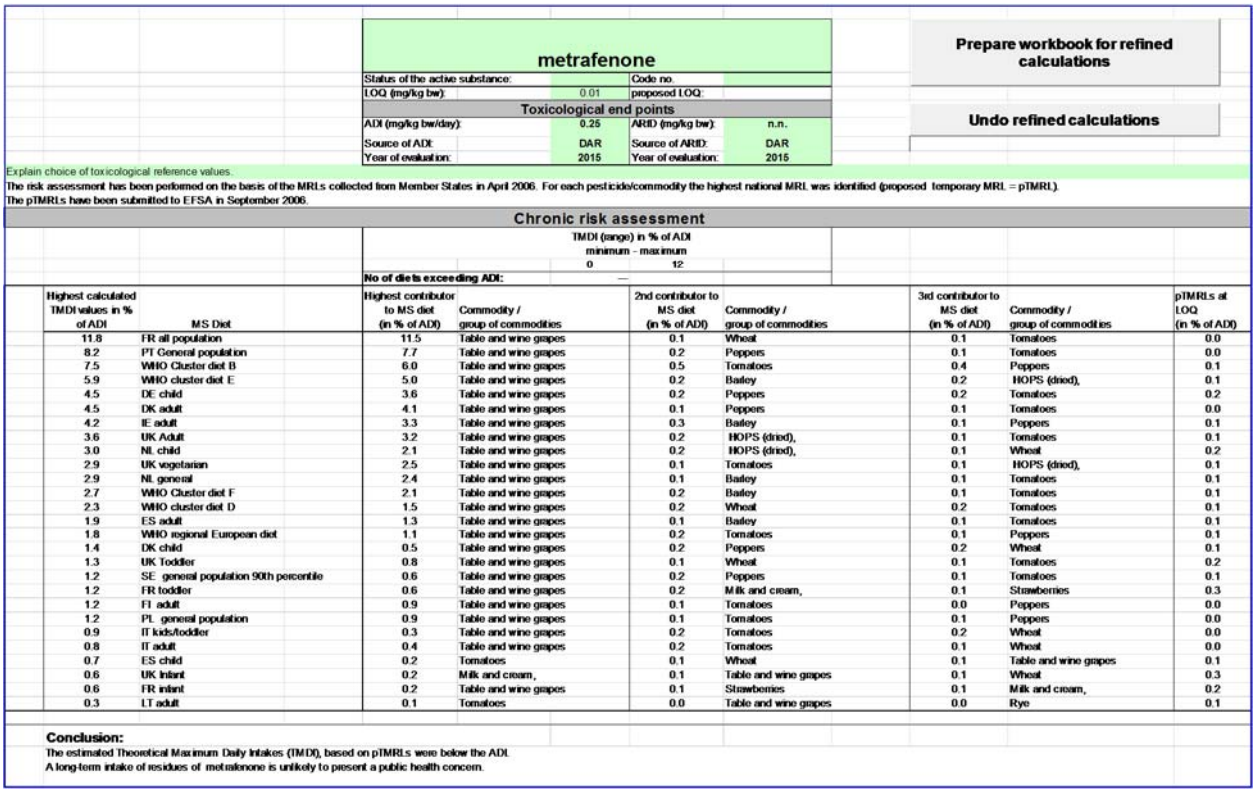
Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)	
ADI	0.25 mg/kg bw/day
TMDI (European Diet) (% ADI)	<1% (UK) for all consumer groups. <1% based on GEMS/Food European diet
NEDI (% ADI)	Not necessary due to low TMDI
Factors included in NEDI	Not necessary
ARfD	Not necessary
Acute exposure (% ARfD)	Not necessary

In the review of MRLs for metrafenone under Article 12 of regulation 396/2005, consumer exposure was also assessed (EFSA Journal 2013;11(12):3498). "Chronic consumer exposure resulting from the authorised uses reported in the framework of this review was calculated using revision 2 of the EFSA PRIMo. For ruminant products, where data were insufficient to derive an MRL at LOQ, EFSA considered the existing EU MRL (also at LOQ) for an indicative calculation (although the existing residue definition is different from the one proposed by EFSA). The highest chronic exposure represented 1.8% of the ADI (French all population). Acute exposure calculations were not carried out because an ARfD was not deemed necessary for this active substance."

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

A theoretical maximum daily intake (TMDI) was calculated for metrafenone using the current MRLs under consideration (detailed in SANCO/10377/2015) plus the proposed MRL for barley and oats. Intakes were calculated using the EFSA PRiMo version 2 model and an acceptable daily intake (ADI) of 0.25 mg/kg bw/day. Where existing MRLs are set at a limit of quantitation (0.01*, 0.02* or 0.05* mg/kg), it has been assumed that a residue occurs at the level of the limit of quantitation.

Table 6.9-1: Chronic consumer exposure for metrafenone (TMDI)



metrafenone

Prepare workbook for refined calculations

Undo refined calculations

Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.25	ARfD (mg/kg bw):	n.n.
Source of ADI:	DAR	Source of ARfD:	DAR
Year of evaluation:	2015	Year of evaluation:	2015

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: 0 – 12

No of diets exceeding ADI: –

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
11.8	FR all population	11.5	Table and wine grapes	0.1	Wheat	0.1	Tomatoes	0.0
8.2	PT General population	7.7	Table and wine grapes	0.2	Peppers	0.1	Tomatoes	0.0
7.5	WHO Cluster diet B	6.0	Table and wine grapes	0.5	Tomatoes	0.4	Peppers	0.1
5.9	WHO cluster diet E	5.0	Table and wine grapes	0.2	Barley	0.2	HOPS (dried),	0.1
4.5	DE child	3.6	Table and wine grapes	0.2	Peppers	0.2	Tomatoes	0.2
4.5	DK adult	4.1	Table and wine grapes	0.1	Peppers	0.1	Tomatoes	0.0
4.2	IE adult	3.3	Table and wine grapes	0.3	Barley	0.1	Peppers	0.1
3.6	UK Adult	3.2	Table and wine grapes	0.2	HOPS (dried),	0.1	Tomatoes	0.1
3.0	NL child	2.1	Table and wine grapes	0.2	HOPS (dried),	0.1	Wheat	0.2
2.9	UK vegetarian	2.5	Table and wine grapes	0.1	Tomatoes	0.1	HOPS (dried),	0.1
2.9	NL general	2.4	Table and wine grapes	0.1	Barley	0.1	Tomatoes	0.1
2.7	WHO Cluster diet F	2.1	Table and wine grapes	0.2	Barley	0.1	Tomatoes	0.1
2.3	WHO cluster diet D	1.5	Table and wine grapes	0.2	Wheat	0.2	Tomatoes	0.1
1.9	ES adult	1.3	Table and wine grapes	0.1	Barley	0.1	Tomatoes	0.1
1.8	WHO regional European diet	1.1	Table and wine grapes	0.2	Tomatoes	0.1	Peppers	0.1
1.4	DK child	0.5	Table and wine grapes	0.2	Peppers	0.2	Wheat	0.1
1.3	UK Toddler	0.8	Table and wine grapes	0.1	Wheat	0.1	Tomatoes	0.2
1.2	SE general population 90th percentile	0.6	Table and wine grapes	0.2	Peppers	0.1	Tomatoes	0.1
1.2	FR toddler	0.6	Table and wine grapes	0.2	Milk and cream,	0.1	Strawberries	0.3
1.2	FI adult	0.9	Table and wine grapes	0.1	Tomatoes	0.0	Peppers	0.0
1.2	PL general population	0.9	Table and wine grapes	0.1	Tomatoes	0.1	Peppers	0.0
0.9	IT kids/toddler	0.3	Table and wine grapes	0.2	Tomatoes	0.2	Wheat	0.0
0.8	IT adult	0.4	Table and wine grapes	0.2	Tomatoes	0.1	Wheat	0.0
0.7	ES child	0.2	Tomatoes	0.1	Wheat	0.1	Table and wine grapes	0.1
0.6	UK Infant	0.2	Milk and cream,	0.1	Table and wine grapes	0.1	Wheat	0.3
0.6	FR infant	0.2	Table and wine grapes	0.1	Strawberries	0.1	Milk and cream,	0.2
0.3	LT adult	0.1	Tomatoes	0.0	Table and wine grapes	0.0	Rye	0.1

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of metrafenone is unlikely to present a public health concern.

Since the TMDI exceeds 10% of the ADI, a refined assessment (national estimate of dietary intake; NEDI) has been calculated using the following STMRs:

Table 6.9-2: STMRs for metrafenone used in NEDI calculation

commodity	STMR (mg/kg)	comment
Table grapes	1.08	STMR (EFSA, 2013)
Wine grapes	0.24	STMR*PF*YF(a) (EFSA, 2013)
Strawberries	0.13	STMR (EFSA, 2013)
Tomatoes	0.1	STMR (EFSA, 2013)
Peppers	0.12	STMR (EFSA, 2013)
Aubergines	0.1	STMR (EFSA, 2013)
Cucurbits – edible peel	0.03	STMR (EFSA, 2013)
Cucurbits – inedible peel	0.04	STMR (EFSA, 2013)
Cultivated fungi	0.11	STMR (EFSA, 2013)
Barley & oats grain	0.065	DAR
Wheat & rye grain	0.01	STMR (EFSA, 2013)
Hops (dried)	22.05	STMR (EFSA 2015)

(a): Consumption figure in the PRIMo model is expressed for the raw commodity (e.g. grape, olive…). A yield factor (YF) is therefore considered to estimate the consumption figure for the processed commodity (e.g. wine, oil…).

EFSA (European Food Safety Authority), 2013. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for metrafenone according to article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013; 11(12):3498

EFSA (European Food Safety Authority), 2015. Reasoned opinion on the setting of a new maximum residue level (MRL) for metrafenone in hop cones. EFSA Journal 2015; 13(4):4078

Table 6.9-3: Chronic consumer exposure for metrafenone (NEDI)

metrafenone			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.25	ARfD (mg/kg bw):	n.n.
Source of ADI:	DAR	Source of ARfD:	DAR
Year of evaluation:	2015	Year of evaluation:	2015

Prepare workbook for refined calculations

Undo refined calculations

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	
0	1
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)
0.8	DE child	0.5	Table grapes	0.1	Milk and cream,	0.1	Pome fruit	0.2
0.7	WHO Cluster diet B	0.2	Wine grapes	0.2	Table grapes	0.1	Tomatoes	0.2
0.7	NL child	0.3	Table grapes	0.1	Milk and cream,	0.1	HOPS (dried),	0.3
0.5	FR all population	0.4	Wine grapes	0.0	Table grapes	0.0	Tomatoes	0.1
0.5	PT General population	0.2	Wine grapes	0.1	Table grapes	0.0	Tomatoes	0.1
0.5	IE adult	0.1	Wine grapes	0.1	Table grapes	0.0	Barley	0.1
0.4	WHO cluster diet E	0.2	Wine grapes	0.1	Table grapes	0.0	HOPS (dried),	0.1
0.4	FR toddler	0.2	Milk and cream,	0.1	Table grapes	0.0	Strawberries	0.3
0.4	UK Toddler	0.1	Table grapes	0.1	SUGAR PLANTS	0.1	Milk and cream,	0.3
0.3	UK Infant	0.2	Milk and cream,	0.0	SUGAR PLANTS	0.0	Root and tuber vegetables	0.3
0.3	WHO cluster diet D	0.1	Table grapes	0.0	Tomatoes	0.0	Wine grapes	0.1
0.3	DK child	0.1	Table grapes	0.1	Milk and cream,	0.0	Wheat	0.1
0.3	NL general	0.1	Table grapes	0.1	Wine grapes	0.0	Milk and cream,	0.1
0.3	WHO regional European diet	0.1	Table grapes	0.0	Tomatoes	0.0	Wine grapes	0.1
0.3	WHO Cluster diet F	0.1	Wine grapes	0.0	Table grapes	0.0	Tomatoes	0.1
0.3	UK Adult	0.1	Wine grapes	0.1	HOPS (dried),	0.0	Table grapes	0.1
0.3	DK adult	0.1	Wine grapes	0.0	Table grapes	0.0	Milk and cream,	0.1
0.3	UK vegetarian	0.1	Wine grapes	0.0	Table grapes	0.0	HOPS (dried),	0.1
0.2	FR infant	0.1	Milk and cream,	0.0	Table grapes	0.0	Root and tuber vegetables	0.2
0.2	PL general population	0.1	Table grapes	0.0	Tomatoes	0.0	Root and tuber vegetables	0.0
0.2	ES child	0.1	Milk and cream,	0.0	Tomatoes	0.0	Wheat	0.1
0.2	SE general population 90th percentile	0.0	Milk and cream,	0.0	Tomatoes	0.0	Root and tuber vegetables	0.1
0.2	ES adult	0.0	Wine grapes	0.0	Tomatoes	0.0	Milk and cream,	0.1
0.2	IT kids/toddler	0.1	Tomatoes	0.0	Table grapes	0.0	Wheat	0.1
0.2	IT adult	0.1	Table grapes	0.0	Tomatoes	0.0	Wheat	0.0
0.1	FI adult	0.0	Wine grapes	0.0	Milk and cream,	0.0	Tomatoes	0.1
0.1	LT adult	0.0	Tomatoes	0.0	Milk and cream,	0.0	Root and tuber vegetables	0.1

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of metrafenone is unlikely to present a public health concern.

Since the predicted chronic intakes are <1% of the ADI, the uses of metrafenone can be considered to result in an acceptable level of risk for consumers.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

An acute exposure assessment is not required since it is not necessary to derive an ARfD for metrafenone.

Summary of consumer exposure

An assessment of consumer exposure has been carried out for metrafenone using an ADI of 0.25 mg/kg bw/day. Assuming all residues occur at the level of the MRL in all crops, the TMDI gave an intake of 11.8% of the ADI for metrafenone. Whilst this shows an acceptable risk for consumers, a refined NEDI calculation was carried out using STMRs for a range of crops. Since the predicted chronic intakes are <1% of the ADI, the uses of metrafenone can be considered to result in an acceptable level of risk for consumers.

An acute exposure assessment is not required since it is not necessary to derive an ARfD for metrafenone.

CA 6.10 Other studies

CA 6.10.1 Effect on the residue level in pollen and bee products

Not required at present since no guideline is available.

BASF
We create chemistry

Metrafenone

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Introduction

Metrafenone is a benzophenone fungicide used for the control of powdery mildew fungi. The biochemical mode of action is not yet known. However, observations indicate that it inhibits growth of mycelium on the leaf surface, leaf penetration, and formation of haustoria and sporulation. In addition, metrafenone has been shown to inhibit sporulation in the fungi and may interfere with a dimorphic switch that allows the fungus to reproduce asexually versus sexually.

Metrafenone was first approved by Commission Directive 2007/6/EC (entry into force: 01 February 2007). This document is submitted to support the renewal of approval of metrafenone and complies with the Table of Contents described in the Annex to Regulation (EU) No 283/2013. It reviews the ecotoxicology studies performed with the active substance.

- For studies performed since the first inclusion of metrafenone in Annex I to Directive 91/414/EEC, the study reports are submitted (See document K-CA) and full study summaries are provided.
- For studies submitted and assessed for the first EU review of metrafenone and are still considered relevant to support renewal of approval, only the critical endpoints and conclusion summary are included in this dossier. For a more detailed assessment, reference can be made either to the summary dossier (Document M-II, Section 6, BASF SE, 2002) submitted for the first EU evaluation or to the Draft Assessment Report (DAR) for metrafenone (UK, 2005). The study reports are not submitted in document K-CA but can be found in the dossier submitted for the first EU Review.

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

CA 7.1.1.1 Aerobic degradation

Adequate data to assess the aerobic route of degradation of metrafenone in soil were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.1.1.1/1 Steinfuehrer T., 2000a AC 375839: Metabolism in soil under aerobic conditions 2000/7000152
Guidelines:	EEC 91/414 Annex II 7.1.1.1.1, EEC 95/36
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report:	CA 7.1.1.1/2 Steinfuehrer T., 2000b 14C-AC 375839 (CL 375839): Rate of degradation in three different soils under aerobic conditions 2000/7000151
Guidelines:	EEC 91/414 Annex II 7.1.1, EEC 95/36 7.1.1.2, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report:	CA 7.1.1.1/3 Steinfuehrer T.,Weis D., 2000a 14C-AC 375839 (CL 375839): Rate of degradation in soil under aerobic conditions at 10°C 2000/7000150
Guidelines:	EEC 91/414 Annex II 7.1.1, EEC 95/36 7.1.1.2, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report:	CA 7.1.1.1/4 Afzal J., 2002a CL 377160 (Metabolite of BAS 560 F): Rate of degradation in three different soils under aerobic conditions 2002/7005909
Guidelines:	EEC 91/414 Annex II 7.1.1.2.1, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

CA 7.1.1.2 Anaerobic degradation

Adequate data to assess the anaerobic route of degradation of metrafenone in soil were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.1.1.2/1 van Dijk A.,Kunz Ch., 2001a 14C-BAS 560 F: Route and rate of degradation in soil under anaerobic conditions 2001/7000460
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC Section 1.2
GLP:	yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report:	CA 7.1.1.2/2 Martin C.A., 2002a 14C-BAS 560 F: Route and rate of degradation in soil under anaerobic conditions (Report amendment #2) 2002/7004411
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC (1995), Section 1.2
GLP:	yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report:	CA 7.1.1.2/3 Huang R.A., 2002a BAS 560 F: Anaerobic soil metabolism 2002/7004949
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC Section 1.2
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 7.1.1.2/4 Ta C., 2012a Response to US EPA data evaluation record of the study entitled: - BAS 560 F: Anaerobic soil metabolism 2012/7005417
Guidelines:	EPA 835.4200
GLP:	no (certified by <none>)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

CA 7.1.1.3 Soil photolysis

The soil photolysis of metrafenone was evaluated during the first EU review following application to soil of the active substance ^{14}C- labelled in the trimethoxyphenyl position in the study of Ta (2001). Contrary to what is reported in the EFSA Conclusion for metrafenone (*EFSA Scientific Report* (2006) 58, 1- 72), the study did not investigate the degradation of metrafenone labelled in the bromophenyl position, and therefore the new study of Adam (2015) has now been performed. A full study summary of Adam (2015) is presented below. The study of Ta (2001) is summarized in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

The soil photolytic metabolite CL 377160 was observed at a maximum concentration of 18.9 % AR in the soil photolysis study of Ta (2001) but was not observed above 0.7 % AR in the dark control. However CL 377160 was not observed at concentrations >5 % AR in the soil photolysis study of Adam (2015), and instead two metabolites, not observed in the study of Ta (2001), were observed at concentrations > 5 % AR. CL 3000402 occurred at mean maximum concentrations of 6.9 – 8.7 % AR, 10 – 17 days after treatment in the irradiated studies performed with both labels, and M2 was observed at mean maximum concentrations of 5.2 – 5.3 % AR at study termination in the irradiated tests performed with both radio-labels. M2 was tentatively identified as 3-(3-bromo-6-methoxy-2-methyl-phenyl)-4,5,6-trimethoxy-3H-isobenzofuran-1-one (CL 1500831) by LC/MS/MS analysis, however a reference standard was not available to confirm this characterization. The reasons for the differences in the route of degradation in the two studies are unclear. However, it is noted that although silt loams were used in both studies, the two soils used were not the same, and in addition that moist soil conditions were not maintained throughout the study in the study of Ta (2001), whereas a soil moisture at pF2 was maintained throughout the study of Adam (2015).

A kinetic re-evaluation of the photolytic degradation rates of metrafenone and its photolytic metabolite CL 377160 from the study of Ta (2001) was performed in accordance with FOCUS Kinetics guidance in the study of Hilton and Callow (2014a), summarized in Section CA 7.1.2.1.1 below. An irradiated DT_{50} value of 12.6 days and dark DT_{50} value of 157.3 days were calculated for metrafenone using SFO kinetics. An irradiated only DT_{50} value of 13.7 days, removing the influence of other processes, was therefore calculated. Because of the low formation of CL 377160 in dark control samples a kinetic evaluation of the degradation of CL 377160 was only possible in the irradiated samples. A DT_{50} value of 5.5 days was calculated for the photolytic

degradation of CL 377160 under the conditions of the test. In the study of Adam (2015) metrafenone degraded with a SFO DT_{50} value of 16.6 days in irradiated soils under the conditions of the test; corresponding to 31.6 days of natural summer sunlight at 30 to 50°N. Metabolite and dark control degradation kinetics were not performed.

Report:	CA 7.1.1.3/1 Ta C., 2001a BAS 560 F (AC 375839): Soil photolysis 2001/7000275
Guidelines:	EPA 161-3, EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36
GLP:	yes (certified by United States Environmental Protection Agency)

Please see Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005) for full summary details. A summary of the route of degradation in soil is presented at the end of this section (Section 7.1.1).

Report: CA 7.1.1.3/2
Adam D., 2015a
[14C] Metrafenone (BAS 560 F): Soil photolytic degradation
2014/1083340

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EPA 835.2410, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides - Part 2 soil photolysis (March 1995), Draft OECD Guideline: Phototransformation of Chemicals in Soil Surfaces (Jan. 2002)

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

Executive Summary

The photolytic degradation of metrafenone in soil was investigated with the [trimethoxyphenyl-U-^{14}C]- and the [bromophenyl-U-^{14}C]-labeled test compounds applied to a 2mm moist soil layer. Individual soil samples on glass plates were placed in a sealed tank and irradiated with a xenon lamp equipped with filters to remove light with wavelengths < 290nm. Test systems were maintained at 20 ± 2 °C, and incubation tanks were aerated with a stream of moistened air and connected to traps for collection of $^{14}CO_2$ and volatile organics. Non-irradiated control samples were incubated in an equivalent system, but kept in the dark. Duplicate samples were collected for both labels immediately after application, at study termination (17 days after treatment), and at four intermediate time-points. Soils were extracted and analyzed by LSC and HPLC. Additional characterization was performed by LC/MS and LC/MS/MS.

Total recoveries were in the range of 91.1 - 106.9 % AR for all samples. Multiple metabolites were observed, however only two were observed at concentrations > 5 % AR. CL 3000402 was observed with mean maximum concentrations of 6.9 % AR – 8.7 % AR, 10 – 17 days after treatment in the irradiated studies performed with both labels. M2, observed at mean maximum concentrations of 5.2 – 5.3 % AR at study termination in the irradiated tests performed with both radio-labels, was tentatively identified as 3-(3-bromo-6-methoxy-2-methyl-phenyl)-4,5,6,-trimethoxy-3H-isobenzofuran-1-one (CL 1500831) by LC/MS/MS analysis. However a reference standard was not available to confirm this characterization. Mineralization remained low throughout the study reaching mean maxima of 0.7 – 3.8 % AR at study termination in irradiated samples. Degradation in dark control samples was very slow or negligible.

The rate of degradation of metrafenone determined according to FOCUS Kinetics guidance gave a DT_{50} value of 16.6 days under the conditions of the test; corresponding to 31.6 days of natural summer sunlight at 30 to 50°N.

I. MATERIAL AND METHODS

A. MATERIALS

Metrafenone (BAS 560 F) labeled in the [trimethoxyphenyl-U-^{14}C]- (Batch No. 911-1301; specific radioactivity: 5.65 MBq/ mg; radiochemical purity 97.2 %) and the [bromophenyl-U-^{14}C]- (Batch No. 1124-1101; specific radioactivity: 1.53 MBq/ mg; radiochemical purity 100.0 %) positions was applied to a 2 mm layer of moist freshly sampled silt loam soil, and incubated at 20 °C ± 2 °C under artificial sunlight. Prior to incubation, the freshly sampled soil was stored at 4 °C under aerobic conditions in the dark.

The Am Fischteich soil used in the study was passed through a 2 mm sieve and characterized. Characteristics of the soil are presented in Table 7.1.1.3-1.

Table 7.1.1.3-1: Soil parameters

Name	Am Fischteich
Sampling Location	Grimmitschau/OT Mannichswalde, Germany
Texture (USDA)	Silt Loam
Clay (<2 µm) (%)	20.70
Silt (50-2 µm) (%)	68.45
Sand (2000 – 50 µm) (%)	10.85
pH (0.01M $CaCl_2$)	5.70
Organic Carbon (%)	1.54
Cation Exchange Capacity (meq/100 g soil)	14.66
Moisture at pF2 (w/w %)	36

B. STUDY DESIGN

A slurry of the soil was added to the surface of a glass plate to form a 2 mm layer with a surface area of 12.5 cm^2. The slurry was then allowed to dry and the soil moisture content was adjusted to pF2.

Soil thin-layers were treated with either [trimethoxyphenyl-U-^{14}C]- or [bromophenyl-U-^{14}C]-labelled metrafenone in acetonitrile, which was evenly distributed on the soil surface. Total amounts of applied test substance were 71.4 µg and 67.4 µg for the trimethoxyphenyl- and bromophenyl- labelled test items respectively, which correspond to respective field rates of 0.571 kg/ha and 0.539 kg/ha.

Treated soil plates were placed into a stainless steel cooling tank, which was sealed with a quartz lid, and placed beneath a xenon lamp with filters to remove light with wavelengths < 290nm. The light intensity of the light source for wavelengths of 290 nm – 400 nm was determined to be equivalent to 1.91 days of natural summer sunlight at 30-50 °N, and the 17 day duration of the test equivalent to 32.5 days of natural summer sunlight at 30-50 °N. Temperature was monitored in soil during the experiment, and maintained at 20 ± 2 °C. Moistened air was drawn through the tank. To trap volatiles, the outgoing air was passed through two glass bottles containing 2N NaOH and ethylene glycol (about 50 mL each). Non-irradiated control samples were incubated in an equivalent system, but kept in the dark.

Duplicate samples were collected for both labels immediately after application, at study termination (17 days after treatment), and at four intermediate time-points. Dark control samples were taken at the same sampling intervals. At all sampling intervals, NaOH volatile trapping solutions were removed for analysis. Duplicate ethylene glycol trapping solution samples were analyzed only once at the end of the incubation period for each label. Samples were generally worked up on the day of sampling. If stored, storage was for a maximum of 1 week in a refrigerator or 4 weeks in a freezer.

On the day of sampling, soil thin-layers were removed from the glass plate and extracted three times by shaking in acetonitrile/ water. Extracts were pooled and the radioactivity measured by LSC. If more than 10% of applied radioactivity remained unextracted after room temperature extractions, reflux extraction using acetonitrile/water was performed. Aliquots of the extracts were submitted for HPLC analysis, equipped with a ^{14}C radio detector and UV detection at 230 nm and 240 nm, without any further sample preparation. The LOD and LOQ for LSC analysis of soil extracts were 0.22 % AR and 0.32 % AR respectively, and for HPLC analysis were 0.26 % AR and 0.52 % AR respectively.

Soil residual radioactivity was determined by combustion and LSC. Radioactivity in trapping solutions was analyzed by LSC.

In order to provide additional/ confirmatory metabolite characterization, pooled sample extracts were subjected to additional sample clean-up and analysis by LC/MS and LC/MS/MS.

The rate of degradation of the test item was determined according to FOCUS Kinetics guidance (2006). The individual duplicate sample measurements from both labels were considered as four individual replicates in a single kinetic evaluation.

II. RESULTS AND DISCUSSION

The recovery and distribution of the radioactivity, and characterization of extracted radioactivity, at the different sampling times is shown in Table 7.1.1.3-2 to Table 7.1.1.3-5.

Table 7.1.1.3-2: Balance of radioactivity (Mean % AR) after the application of [trimethoxyphenyl-U-^{14}C]-metrafenone to irradiated soil samples

	Incubation Time (days)						
	Actual	**0**	**1**	**3**	**7**	**10**	**17**
	Corrected*	**0**	**1.9**	**5.7**	**13.0**	**19.1**	**32.5**
Extractable	A	100.2	92.1	88.8	79.4	72.9	74.7
	B	99.1	91.9	87.5	73.4	72.0	62.4
	Mean	99.6	92.0	88.2	76.4	72.5	68.5
Soxhlet	A	np	np	1.9	3.7	4.4	3.4
	B	np	np	2.1	4.1	3.8	5.9
	Mean	np	np	2.0	3.9	4.1	4.6
Total extractable	**A**	**100.2**	**92.1**	**90.7**	**83.2**	**77.3**	**78.1**
	B	**99.1**	**91.9**	**89.6**	**77.5**	**75.9**	**68.2**
	Mean	**99.6**	**92.0**	**90.2**	**80.3**	**76.6**	**73.2**
Metrafenone	A	95.5	85.4	80.1	67.8	56.3	63.8
	B	95.3	85.7	75.5	56.1	55.1	41.9
	Mean	95.4	85.6	77.8	62.0	55.7	52.8
M2	A	nd	1.7	2.2	3.9	4.8	3.5
	B	nd	1.0	0.7	5.4	4.7	7.1
	Mean	nd	1.3	1.4	4.7	4.8	5.3
M6 (CL 3000402)	A	nd	3.8	3.5	4.8	6.5	4.6
	B	nd	2.9	4.0	7.9	7.4	8.4
	Mean	nd	3.4	3.8	6.4	6.9	6.5
Non-Extractable	**A**	**0.4**	**6.7**	**4.4**	**8.3**	**10.9**	**9.8**
	B	**0.5**	**7.3**	**4.8**	**12.6**	**11.7**	**18.2**
	Mean	**0.5**	**7.0**	**4.6**	**10.5**	**11.3**	**14.0**
$^{14}CO_2$	**A**	**np**	**0.9**	**2.0**	**3.2**	**3.4**	**3.8**
	B	**np**	**0.9**	**2.0**	**3.2**	**3.4**	**3.8**
	Mean	**np**	**0.9**	**2.0**	**3.2**	**3.4**	**3.8**
Other volatiles	**A**	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	B	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	Mean	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
Total Radioactivity	**A**	**100.6**	**99.7**	**97.2**	**94.7**	**91.6**	**91.8**
	B	**99.6**	**100.1**	**96.5**	**93.2**	**91.0**	**90.3**
	Mean	**100.1**	**99.9**	**96.9**	**94.0**	**91.3**	**91.1**

* Corrected to 30 – 50 ºN summer sun
np = not performed
nd = not detected (<LOD of 0.26 % AR)
13 additional metabolite fractions observed, all with maximum mean concentrations ≤2.9 % AR

Table 7.1.1.3-3: Balance of radioactivity (% AR) after the application of [trimethoxyphenyl-U-^{14}C]-metrafenone to dark soil samples

		Incubation Time (days)					
	Actual	**0**	**1**	**3**	**7**	**10**	**17**
Extractable	A	100.2	92.3	94.1	89.9	91.8	91.9
	B	99.1	98.4	93.4	86.6	91.8	92.7
	Mean	99.6	95.3	93.8	88.2	91.8	92.3
Soxhlet	A	np	np	np	2.0	2.1	np
	B	np	np	np	2.0	2.1	np
	Mean	np	np	np	2.0	2.1	np
Total extractable	**A**	**100.2**	**92.3**	**94.1**	**91.9**	**93.9**	**91.9**
	B	**99.1**	**98.4**	**93.4**	**88.6**	**93.9**	**92.7**
	Mean	**99.6**	**95.3**	**93.8**	**90.2**	**93.9**	**92.3**
Metrafenone	A	95.5	91.4	91.9	89.9	91.8	91.9
	B	95.3	98.4	91.1	86.6	91.8	92.7
	Mean	95.4	94.9	91.5	88.2	91.8	92.3
Non-Extractable	**A**	**0.4**	**4.1**	**4.1**	**2.3**	**2.6**	**5.6**
	B	**0.5**	**4.3**	**4.1**	**2.3**	**2.5**	**5.6**
	Mean	**0.5**	**4.2**	**4.1**	**2.3**	**2.6**	**5.6**
$^{14}CO_2$	**A**	**np**	**0.9**	**1.7**	**2.2**	**2.5**	**2.9**
	B	**np**	**0.9**	**1.7**	**2.2**	**2.5**	**2.9**
	Mean	np	**0.9**	**1.7**	**2.2**	**2.5**	**2.9**
Other volatiles	**A**	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	B	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	Mean	np	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
Total Radioactivity	**A**	**100.6**	**97.3**	**99.9**	**96.4**	**99.0**	**100.4**
	B	**99.6**	**103.6**	**99.2**	**93.1**	**98.8**	**101.1**
	Mean	**100.1**	**100.5**	**99.6**	**94.8**	**98.9**	**100.8**

np = not performed
nd = not detected (<LOD of 0.26 % AR)
12 additional metabolite fractions observed, all with maximum mean concentrations ≤1.1 % AR

Table 7.1.1.3-4: Balance of radioactivity (Mean % AR) after the application of [bromophenyl-U-^{14}C]-metrafenone to irradiated soil samples

	Incubation Time (days)						
	Actual	**0**	**1**	**3**	**7**	**10**	**17**
	Corrected*	**0**	**1.9**	**5.7**	**13.0**	**19.1**	**32.5**
Extractable	A	103.0	97.2	95.5	88.8	82.6	69.0
	B	101.3	97.9	92.6	85.3	76.0	71.0
	Mean	102.1	97.5	94.1	87.0	79.3	70.0
Soxhlet	A	np	np	np	3.6	4.3	6.3
	B	np	np	np	4.5	5.0	6.7
	Mean	np	np	np	4.0	4.6	6.5
Total extractable	**A**	**103.0**	**97.2**	**95.5**	**92.4**	**86.8**	**75.3**
	B	**101.3**	**97.9**	**92.6**	**89.7**	**81.0**	**77.7**
	Mean	**102.1**	**97.5**	**94.1**	**91.1**	**83.9**	**76.5**
Metrafenone	A	103.0	91.6	89.3	77.2	72.5	43.3
	B	101.3	92.6	82.7	70.2	59.4	50.2
	Mean	102.1	92.1	86.0	73.7	65.9	46.7
M2	A	nd	2.0	3.4	3.1	4.0	5.4
	B	nd	2.5	3.9	4.4	5.9	5.0
	Mean	nd	2.3	3.6	3.7	4.9	5.2
M6 (CL 3000402)	A	nd	3.6	2.9	4.3	6.6	9.4
	B	nd	2.8	4.0	6.7	9.3	8.1
	Mean	nd	3.2	3.5	5.5	8.0	8.7
Non-Extractable	**A**	**0.4**	**4.8**	**7.2**	**6.6**	**7.9**	**19.3**
	B	**0.4**	**5.3**	**8.7**	**8.8**	**13.0**	**18.4**
	Mean	**0.4**	**5.1**	**7.9**	**7.7**	**10.4**	**18.8**
$^{14}CO_2$	**A**	**np**	**<0.1**	**0.1**	**0.3**	**0.4**	**0.7**
	B	**np**	**<0.1**	**0.1**	**0.3**	**0.4**	**0.7**
	Mean	np	**<0.1**	**0.1**	**0.3**	**0.4**	**0.7**
Other volatiles	**A**	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	B	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	Mean	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
Total Radioactivity	**A**	**103.4**	**102.1**	**102.8**	**99.2**	**95.1**	**95.3**
	B	**101.7**	**103.3**	**101.5**	**98.8**	**94.3**	**96.8**
	Mean	**102.6**	**102.7**	**102.2**	**99.0**	**94.7**	**96.1**

* Corrected to 30 – 50 ºN summer sun
np = not performed
8 additional metabolite fractions observed. All maximum mean concentrations ≤4.0 % AR

Table 7.1.1.3-5: Balance of radioactivity (Mean % AR) after the application of [bromophenyl-U-^{14}C]-metrafenone to dark samples

	Incubation Time (days)						
	Actual	**0**	**1**	**3**	**7**	**10**	**17**
Extractable	**A**	**103.0**	**100.5**	**101.5**	**107.8**	**98.6**	**104.2**
	B	**101.3**	**101.1**	**102.3**	**96.4**	**98.3**	**99.5**
	Mean	**102.1**	**100.8**	**101.9**	**102.1**	**98.5**	**101.9**
Metrafenone	A	103.0	100.5	101.5	106.2	98.6	104.2
	B	101.3	101.1	102.3	95.3	98.3	99.5
	Mean	102.1	100.8	101.9	100.8	98.5	101.9
Non-Extractable	**A**	**0.4**	**1.7**	**2.4**	**2.9**	**3.3**	**4.5**
	B	**0.4**	**1.8**	**2.4**	**2.9**	**3.4**	**4.5**
	Mean	**0.4**	**1.8**	**2.4**	**2.9**	**3.3**	**4.5**
$^{14}CO_2$	**A**	**np**	**<0.1**	**0.1**	**0.2**	**0.3**	**0.5**
	B	**np**	**<0.1**	**0.1**	**0.2**	**0.3**	**0.5**
	Mean	**np**	**<0.1**	**0.1**	**0.2**	**0.3**	**0.5**
Other volatiles	**A**	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	B	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
	Mean	**np**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
Total Radioactivity	**A**	**103.4**	**102.3**	**104.1**	**111.0**	**102.2**	**109.2**
	B	**101.7**	**103.0**	**104.9**	**99.6**	**102.0**	**104.5**
	Mean	**102.6**	**102.7**	**104.5**	**105.3**	**102.1**	**106.9**

np = not performed
1 additional metabolite fraction observed, with a maximum mean concentration of 1.3 % AR.

Mean mass balances were in the range of 91.1 - 106.9 % of the applied radioactivity for all samples. Small or negligible decreases in extracted radioactivity were observed in dark control samples. The total extracted radioactivity decreased in illuminated samples from total initial mean concentrations of 99.6 % AR and 102.1 % AR for the trimethoxyphenyl and bromophenyl labels respectively, to 73.2 % AR and 76.5 % AR respectively at study termination. A corresponding increase in unextracted radioactivity was also observed. Mineralization remained low throughout the study reaching mean maxima of 0.7 – 3.8 % AR at study termination in irradiated samples. The concentration of organic volatiles remained <0.1 % AR throughout the study.

Extracted radioactivity was characterized and quantified by HPLC with retention time comparison to reference standards. Additional metabolite confirmatory analysis was performed by LC/MS and LC/MS/MS. Degradation of metrafenone was observed in irradiated samples with mean concentrations decreasing from 95.4 – 102.1 % AR immediately after application to 46.7 - 52.8 % AR at study termination. Up to 15 metabolite fractions were observed. However, apart from M2 and M6, all mean maximum concentrations of individual metabolites remained ≤ 4.0 % AR. The metabolite M6 was co-chromatographically characterized by HPLC and by LC-MS as CL 3000402. M6 (CL 3000402) was observed with mean maximum concentrations of 6.9 % AR – 8.7 % AR, 10 – 17 days after treatment, in the irradiated studies performed with both labels. A second metabolite, M2, was observed at mean maximum concentrations of 5.2 – 5.3 % AR at study termination in the irradiated tests performed with both radio-labels. When analyzed by LC/MS, M2 did not correspond, by virtue of its m/z and retention time, to any of the available reference standards. However, from the accurate mass and isotopic pattern, M2, is shown to have the same molecular formula as CL 3000402. Based on the MS/MS fragmentation, the component was tentatively identified as 3-(3-bromo-6-methoxy-2-methyl-phenyl)-4,5,6-trimethoxy-3H-isobenzofuran-1-one (CL 1500831). However, a reference standard was not available to confirm this characterization.

In dark control samples metrafenone was observed to degrade only very slowly (trimethoxyphenyl label), or to be essentially stable (bromophenyl label). Up to 13 metabolite fractions were observed, however all individual fractions were present with mean maximum concentrations of ≤1.1 % AR.

The degradation rate of metrafenone in irradiated studies was kinetically evaluated in accordance with FOCUS Kinetics guidance. Replicate values from tests performed with both labels were included in a single kinetic evaluation. Because degradation in dark control samples was very slow or negligible, a kinetic evaluation of such samples was not performed. SFO kinetics provided an excellent statistical and visual fit to the experimental data for the degradation of metrafenone in irradiated samples, and therefore additional kinetic models were not investigated. Calculated DT_{50} and DT_{90} values and fitting statistics are presented in Table 7.1.1.3-6 below.

Table 7.1.1.3-6: Summary of the results of the kinetic determinations for metrafenone in irradiated samples

Parameter	Irradiated samples
Kinetic Model	SFO
χ^2 error (%)	2.9
P	2.7E-10
k	0.0419
DT_{50}	16.6
DT_{90}	55.0
*Corrected DT_{50}	31.6
*Corrected DT_{90}	104.9

* Corrected to natural summer sunlight at 30-50 °N

III. CONCLUSION

Metrafenone degraded in soil photolysis studies with a SFO DT_{50} value of 16.6 days under the conditions of the test, corresponding to 31.6 days of natural summer sunlight at 30 to 50°N. Multiple degradates were detected, of which only two were observed at concentrations > 5 % AR. M6, observed with mean maximum concentrations of 6.9 % AR – 8.7 % AR, 10 – 17 days after treatment in the irradiated studies performed with both labels, was characterized as CL 3000402 by comparison to a reference standard in HPLC and LC/MS analysis. M2, observed at mean maximum concentrations of 5.2 – 5.3 % AR at study termination in the irradiated tests performed with both radio-labels, was tentatively identified as 3-(3-bromo-6-methoxy-2-methyl-phenyl)-4,5,6-trimethoxy-3H-isobenzofuran-1-one (CL 1500831) by LC/MS/MS analysis. However, a reference standard was not available to confirm this characterization.

Summary of Route of Degradation in Soil

In the original review the aerobic route of degradation of metrafenone in soil under dark conditions at 20°C was investigated in two separate studies (Steinfuehrer 2000a & b) in a total of four soils, and in a third study at 10 ºC (Steinfuehrer and Weis 2000) in 1 soil. The route of degradation for both [trimethoxyphenyl-U-^{14}C]- and [bromophenyl-U-^{14}C]- labelled metrafenone was only investigated in a single soil, at 20 ºC. The route of degradation in the remaining soils was investigated using only the bromophenyl radiolabelling position. This is in compliance with guidelines under Regulation 1107/2009.

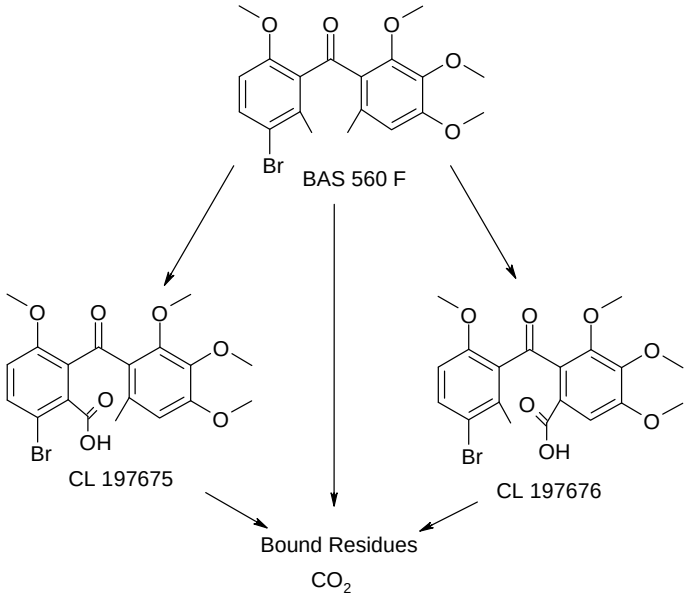
Metrafenone was observed to degrade slowly at 20°C in all four soils in the aerobic degradation studies, such that 56.1 – 69.0% AR was still present as the parent material at 120 DAT, for both labels. Mineralization was relatively low with 1.5 - 5.3% AR present as CO_2 at 120 DAT, with unextracted radioactivity reaching up to 17.4 - 24.8% AR at 120 DAT. Mass balances were acceptable throughout both studies. Very low levels of identified metabolites were found in 1 soil in the study of Steinfuehrer 2000b, with combined levels of CL 197675 and CL 197676 reaching a maximum of 0.15% AR at 210 DAT for both labels. Neither metabolite was detected at 120 DAT. Unidentified radioactivity reached a maximum of 6.6 - 8.7% AR at 60 DAT. These values were calculated as the differences between the total extractable residues and those characterized as parent and metabolites. They comprised small amounts radioactivity in various non-analyzed extracts, as well as radioactivity remaining at the TLC origin and radioactivity lost during sample work-up. It is likely to be composed of a number of minor compounds. No information on metabolite formation was available from the remaining aerobic rate of degradation studies. There appeared to be little indication of differences in metabolite, CO_2 or unextracted residue formation with either radiolabel.

At 10 ºC degradation proceeded at a slower rate than at 20°C, with 82.0% AR remaining as parent at 120 DAT. Whilst radioactivity as ‘others’ reached a peak of 11.5% AR, no defined peak or fraction could be identified on analysis, and it is likely that this category comprised numerous small components. CO_2 and unextracted residues accounted for 1.4% AR and 8.2% AR respectively at 120 DAT.

Since the original review, the guidelines for evaluating the relevance of metabolites has changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study and are present at concentrations >5 % AR (Sanco/221/2000). However, no metabolite peaks were observed at concentrations > 0.15 % AR, and therefore there are no metabolites which trigger additional risk assessments as a result of these changes.

The proposed aerobic route of degradation is given in Figure 7.1.1.3-1.

Figure 7.1.1.3-1: Proposed route of degradation of metrafenone (BAS 560 F) under dark aerobic soil conditions



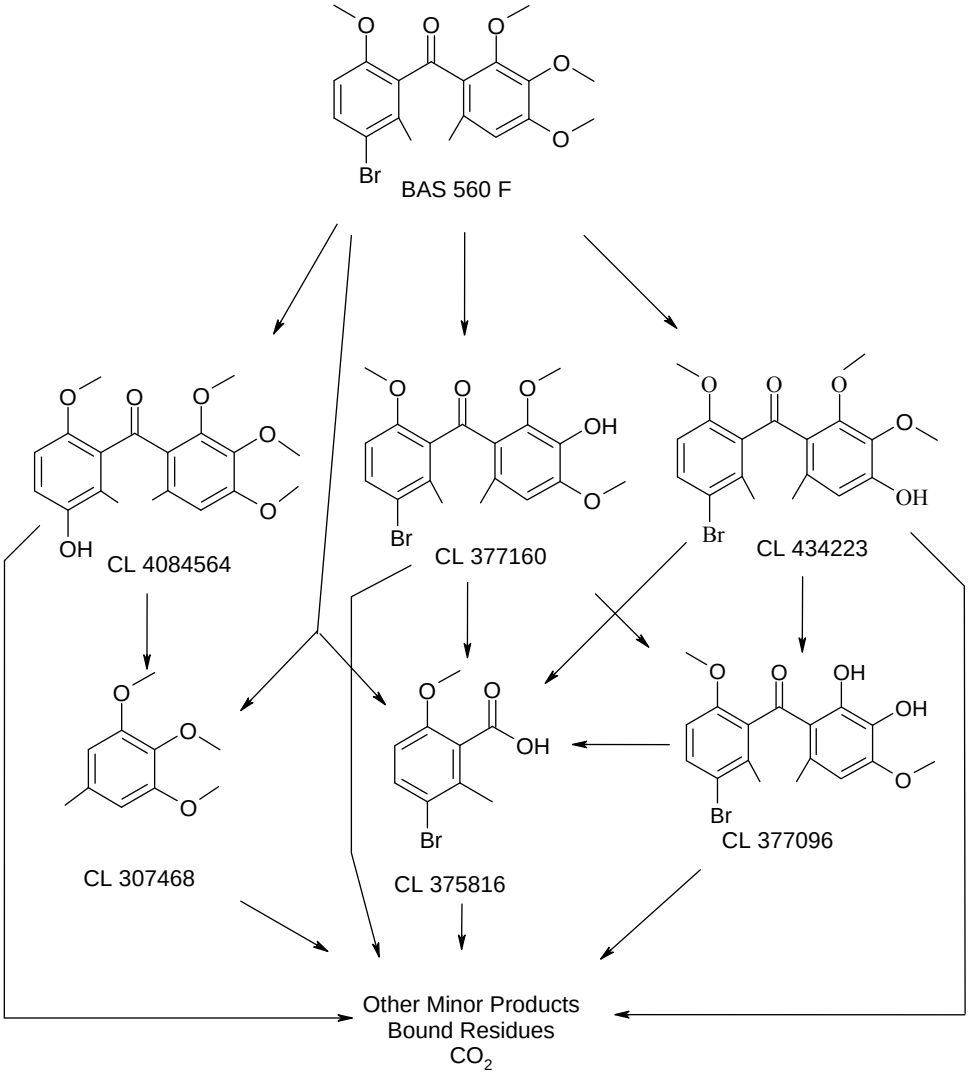
For the first EU review of metrafenone, two anaerobic soil degradation studies were conducted at 20°C using both [trimethoxyphenyl-U-^{14}C]- and [bromophenyl-U-^{14}C]- labelled metrafenone. In the first study, metrafenone was observed to degrade relatively rapidly compared to aerobic conditions, and a number of metabolite and metabolite 'fractions' were found to occur. As a sequence of increasingly harsh extractions were performed, results of extractions using acid or alkaline conditions are not considered relevant as the components within such extracts are unlikely to be biologically available. However, it is noted that some metabolite fractions, M5.2, M6 and 'others', exceeded 10% AR at certain sample times, M6 reaching 45.1% AR at 15 DAT, when harsh extractions were considered in addition to the milder extraction techniques.

When only the 'bioavailable' extracts were considered, three metabolites were identified, CL 377160 found at a maximum of 5.2% AR at 15 DAT, CL 434223 at a maximum of 8.2% AR (may contain CL 377160) at 8 DAT, and CL 375816 at 0.1% AR at 28 DAT. Four metabolite fractions were also detected, which contained multiple minor components. Of these fractions, M6 occurred at the highest level, at 5.7% AR at 15 DAT. M6 comprised all radioactivity at the origin of TLC analysis (Rf = 0.0) and was likely to comprise multiple polar components. The other metabolite fractions did not exceed 2.4% AR. At 120 DAT, total volatile compounds represented 0.4 – 1.3% AR, and unextracted residues 29.5 – 30.0% AR. Mass balance was > 90 %AR at all time-points in the system treated with bromophenyl labelled material. The mass balance was marginally lower than 90% AR between 28 – 120 DAT with the trimethoxyphenyl labelled material (87.6 – 89.3% AR), but was not considered to compromise the results of the study.

Degradation of metrafenone in a second anaerobic study was also relatively rapid when compared to aerobic degradation. Six metabolites were identified (CL 377160, CL 434223, CL 377096, CL 307468, CL 375816 and CL4084564). CL 4084564 occurred at the highest level (7.3% AR at 28 days) and CL 377160 occurred at 5.3% AR at 7 DAT. However, neither of these metabolites exceeded 5 % AR at consecutive time-points, and neither were increasing at the study termination. None of the other identified metabolites exceeded 5% AR. Four main metabolite fractions were also found, Met 1, Met 2, Met 3 and 'Others'. Met 2, Met 3 and 'others' reached levels exceeding 10% AR and up to 45.0% AR in the case of 'others'. Further HPLC analysis was conducted on the fractions which separated them into multiple individual components, none of which exceeded 8.3% AR for Met 3 (although chromatograms indicate that it may be composed of more than a single component) and 5.6 % AR for Met 2. The 'Others' fraction consisted of multiple polar components, none of which exceeded 7.8 % AR. At study end (120 DAT), unextracted residues reached 34.5 – 38.3% AR and mineralization 0.2 – 0.5% AR. The mass balance was acceptable.

The proposed anaerobic route of degradation is shown in Figure 7.1.1.3-2.

Figure 7.1.1.3-2: Proposed route of degradation of metrafenone (BAS 560 F) under dark anaerobic soil conditions



Given the apparent persistence of metrafenone under aerobic conditions, it is possible that on certain soil types, soil residues of metrafenone will experience anaerobic conditions. This is most likely to occur in cereals rather than vines, the latter crop tending to be grown on better drained soils. Under anaerobic conditions, it would be expected that metrafenone would degrade more rapidly and lead to the formation of a large number of minor metabolites. As discussed above, since the original review, the guidelines for evaluating the relevance of metabolites has changed. However, in the bioavailable fractions no metabolite peaks or metabolite fractions were observed at concentrations > 5.0 % AR at two consecutive time-points, and neither were they increasing at study termination. Therefore there are no anaerobic metabolites which trigger additional risk assessments as a result of these changes. Anaerobic conditions would be expected to occur mainly in the winter; typical application timing of metrafenone, i.e. spring/summer, is likely to avoid significant occurrence of anaerobic conditions at application.

For the first EU review, the assessment of the photolytic degradation in soil was based upon the single study of Ta (2001), in which the degradation of metrafenone ^{14}C-labelled in the trimethoxyphenyl position was investigated. The photolytic degradation of metrafenone labelled in the bromophenyl ring was not investigated, and since in the study of Ta (2001) the maximum extracted and unidentified radioactivity was >31 % AR (all present as unidentified minor metabolites), the presence of metabolites specific to the bromophenyl label position could not be ruled out. The study of Adam (2015) was therefore performed; in which the photolytic degradation in soil of metrafenone labelled in both the trimethoxyphenyl and bromophenyl positions was investigated.

In the study of Ta (2001) the metabolite CL 377160 was produced at up to 18.9% AR under illuminated conditions compared to 0.7% AR under dark conditions. Six unknown metabolites were observed at concentrations up to a maximum of 6.6% AR (Unknown 1). A second unidentified metabolite ('Unknown 5') was observed at 5.1 % AR 14 days after treatment. The sum of 'other' degradates, composing numerous separate components, each less than 3.7 % AR, accounted for up to 15.2% AR. Mineralization and unextracted residues at the study end (30 days) were 2.9% AR and 24.7% AR respectively; in the dark control, quantification of CO_2 was not performed, but unextracted residues were only 7.3% AR at the study end. The mass balance was acceptable.

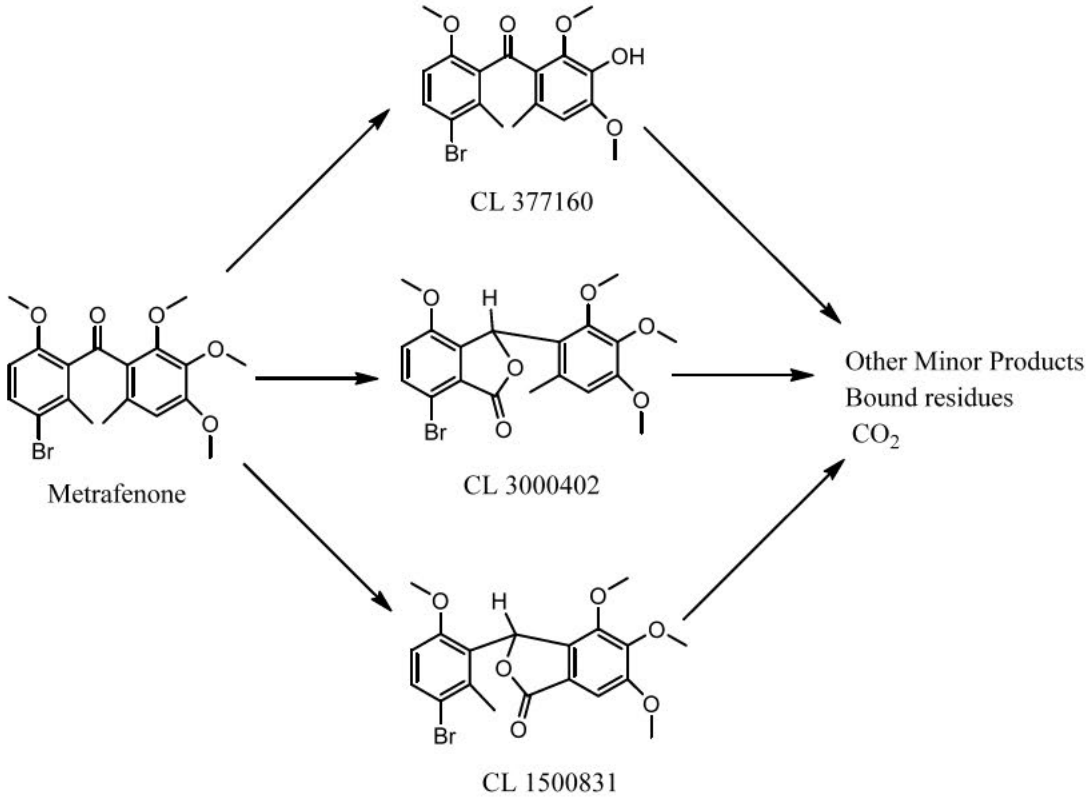
Under the new guidelines only the metabolite 'Unknown 1' would trigger assessment (in addition to CL 377160) since it is increasing at study termination (though present at a maximum concentration of > 5 % AR, 'Unknown 5' does not exceed 5 % AR in consecutive samples and is not increasing at study termination). However this metabolite is observed at equivalent concentrations in the dark control, and therefore it must be considered to be an aerobic metabolite. More extensive and relevant studies were performed to study the aerobic degradation in soil of metrafenone, and as discussed above, no relevant metabolites were observed. Based on its elution time, the metabolite is also considered to be highly polar. Therefore it is not necessary to consider Unknown 1 further.

In the study of Adam (2015) mineralization in the illuminated samples performed with both labels was 0.7 – 3.8 % AR at study termination, and maximum unextracted residues were 14.0 – 18.8 % AR at study termination (17 DAT). CL 377160 was not observed at concentrations >5 % AR; instead two metabolites, not identified in the study of Ta (2001) were observed at concentrations > 5 % AR. CL 3000402 was observed with mean maximum concentrations of 6.9 – 8.7 % AR, 10 – 17 days after treatment in the irradiated studies performed with both labels. M2 was observed at mean maximum concentrations of 5.2 – 5.3 % AR at study termination in the irradiated tests performed with both radio-labels. M2 was tentatively identified as 3-(3-bromo-6-methoxy-2-methyl-phenyl)-4,5,6-trimethoxy-3H-isobenzofuran-1-one (corresponding to CL 1500831) by LC/MS/MS analysis, however a reference standard was not available to confirm this characterization, and identification is ongoing. The proposed soil photolytic metabolic pathway of metrafenone (including the structures of CL 300402 and that proposed for M2) is presented in Figure 7.1.1.3-3.

The reasons for the differences in the route of degradation in the two studies are unclear. However, it is noted that though silt loams were used in both studies, the two soils used were not the same. In addition moist soil conditions were not maintained throughout the study in the study of Ta (2001), whereas soil moisture was maintained at pF2 throughout the study of Adam (2015).

Metrafenone degraded at a faster rate under illuminated conditions than in a dark control in both studies, and at a faster rate than in aerobic soil degradation studies (see Section CA 7.1.2 summary). Therefore the soil photolysis metabolites CL 377160, CL 3000402 and M2 (tentatively identified as CL 1500831) are considered in risk assessments.

Figure 7.1.1.3-3: Proposed soil photolytic pathway for metrafenone (BAS 560 F)



A separate soil degradation study was conducted on CL 377160, the major metabolite in the soil photolysis study, to investigate its degradation under dark, aerobic conditions. This study demonstrated that the metabolite dissipates rapidly, with >90% dissipation occurring within the first 7 days of the study; the main route of dissipation appeared to be to unextracted residues. As the first two sample times were at 0 and 7 DAT, the degradation rate on all three soils tested could only be expressed as DT_{90} <7 days.

CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

Adequate data to assess the rate of the aerobic degradation of metrafenone in the laboratory were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). However since the original assessment in the Draft Assessment Report for the Annex I listing assessment of metrafenone was finalized in October 2003, the FOCUS Degradation Kinetics Report (FOCUS 2006), which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from laboratory and field studies, has been finalized. Therefore the degradation rates of metrafenone are required to be re-calculated in accordance with current FOCUS Kinetics guidance. This has been done in the report of Hilton and Callow (2014a) which is summarized below.

Report:	CA 7.1.2.1.1/1 Steinfuehrer T., 2000a AC 375839: Metabolism in soil under aerobic conditions 2000/7000152
Guidelines:	EEC 91/414 Annex II 7.1.1.1.1, EEC 95/36
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.1.1/2 Steinfuehrer T., 2000b 14C-AC 375839 (CL 375839): Rate of degradation in three different soils under aerobic conditions 2000/7000151
Guidelines:	EEC 91/414 Annex II 7.1.1, EEC 95/36 7.1.1.2, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.1.1/3 Steinfuehrer T.,Weis D., 2000a 14C-AC 375839 (CL 375839): Rate of degradation in soil under aerobic conditions at 10°C 2000/7000150
Guidelines:	EEC 91/414 Annex II 7.1.1, EEC 95/36 7.1.1.2, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report: CA 7.1.2.1.1/4
Hilton M.,Callow B., 2014a
Determination of rates of decline for Metrafenone and its metabolite CL377160 in soil aerobic, anaerobic and photolysis degradation studies according to the FOCUS Kinetics Guidance Document
2014/1083467

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

The degradation of metrafenone in four laboratory aerobic soils and two laboratory anaerobic soils was kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document. In addition the degradation of metrafenone and its soil photolytic metabolite CL 377160 was kinetically evaluated for the single soil studied in the soil photolysis study of Ta (2001).

The SFO model provided a very good description of the degradation of metrafenone in both aerobic and anaerobic soils, and for the photolytic degradation of metrafenone in soil. In all cases the chi^2 % error was significantly < 15%, and visual fits and plots of the residuals confirmed the very good fits. P values for the SFO rate constant were < 0.05 in all cases. SFO non-normalized laboratory soil aerobic DT_{50} values at 20 °C were in the range 154.7 – 275.3 days; corresponding DT_{50} values normalized to standard moisture (pF2) and temperature (20 °C) conditions were in the range 154.7 – 252.1 days, with a calculated geometric mean of 200.9 days. SFO laboratory anaerobic DT_{50} values at 20 °C were 7.3 – 15.6 days. The photolytic soil DT_{50} for metrafenone under the continuous irradiation conditions of the test was 12.6 days, which when corrected for aerobic degradation in the dark control of the same study was 13.7 days.

The SFO model provided an acceptable description of the decline of the soil photolysis metabolite CL 377160. The calculated DT_{50} under the conditions of the test was 5.5 days with a formation fraction of 0.52. The metabolite was not observed at concentrations which allowed a kinetic evaluation in the dark control, and therefore no correction for aerobic degradation in the dark control was performed.

I. MATERIAL AND METHODS

A. MATERIALS

The degradation of metrafenone in four laboratory aerobic soils from the studies of Steinfuehrer (2000a&b) and Steinfuehrer and Weis (2000), and two laboratory anaerobic soils from the studies of van Dijk and Kunz (2001) and Huang (2002) was kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document. In addition the degradation of metrafenone and its soil photolytic metabolite CL 377160 was kinetically evaluated for the single soil studied in the soil photolysis study of Ta (2001). Therefore for this test alone parent metrafenone was assumed to degrade directly to CL 377160, as well as to a sink compartment. CL 377160 was also assumed to degrade to a sink compartment.

B. STUDY DESIGN

Data from the experimental studies were treated in accordance with the recommendations of FOCUS Kinetics Guidance. Therefore radioactivity extracted but remaining unidentified and unextracted soil radioactivity were added to extracted radioactivity identified as metrafenone at t=0. In addition where the same soils were treated with different radio-labelled forms of metrafenone in the same study, measured concentrations of metrafenone in % AR were treated as replicates.

The kinetic modelling was performed using KINGUII vers. 2. The approach used followed that given in Chapters 7 & 8 of the FOCUS Kinetics Guidance Document for the determination of both persistence and modelling end-points. The suitability of the fit of the models was evaluated both visually, based on a graphical plot of the degradation and in a plot of the residuals, and statistically by calculating the minimum % error required to pass the χ^2 test at a probability of 0.05. For SFO kinetics a t-test was also performed to evaluate whether the determined parameters were significantly different to 0. T-test statistics are not appropriate to describe the FOMC fitting parameters alpha and beta, and therefore confidence intervals were reported. Statistical parameters were compared to the acceptability criteria as indicated in FOCUS Kinetics guidance. However, it is also stated within that guidance that acceptability criteria should not be considered as absolute cut-off criteria.

For the determination of the persistence end-point FOCUS Kinetics guidance recommends that both SFO and FOMC models are initially applied to the data. However, in this case for the aerobic soils visual and statistical parameters for SFO fits indicated an excellent fit, which it was considered could not be improved upon using biphasic kinetics. Therefore only SFO kinetics were applied to aerobic soils data.

Only the SFO model was applied to the metabolite observed in the soil photolysis study. The kinetics for the metabolism scheme were determined in a sequential manner. Initially those for metrafenone were fitted. These were then fixed, and those for the metabolite CL 377160 were then determined using the data from all time-points. The initial values were first set to those obtained and then the parameters for all the substances were fitted to the data.

DT_{50} values were normalized to standard temperature and moisture conditions of 20 °C and field capacity (pF2) for use in FOCUS modelling. Temperature correction was performed using the Arrhenius equation, with an activation energy of 65.4 $kj.mol^{-1}$, which corresponds to a Q10 of 2.58. Correction to standard soil moisture was performed with the Walker equation using a Walker exponent of 0.7 and the default values presented in FOCUS groundwater guidance (FOCUS 2012).

II. RESULTS AND DISCUSSION

The results of the determinations were evaluated using visual and statistical methods. Summaries of the results and kinetic fitting parameters are summarized in Table 7.1.2.1.1-1 for aerobic incubations, Table 7.1.2.1.1-2 for anaerobic incubations, and Table 7.1.2.1.1-3 for the photolytic study incubations.

For all aerobic soil incubations, optimization using SFO kinetics provided an excellent statistical and visual fit to the data. For the two anaerobic soils kinetic fitting with both SFO and FOMC kinetics was performed. The fits using SFO kinetics were concluded to be better based upon the chi-squared values, and because FOMC alpha and beta parameters appeared more uncertain than the rate constant from the SFO kinetic fit.

For the kinetic fitting of the irradiated test in the soil photolysis study of Ta 2001, fits with SFO and FOMC kinetics were performed. SFO kinetics were concluded to provide the better fit by virtue of the chi-squared statistics, the visual fits, and the level of uncertainty as indicated by the t-test results for SFO fits and the confidence intervals for both alpha and beta parameters in FOMC fits. The chi-squared statistic for the photolytic degradation of CL 377160 was high. However examination of visual fits demonstrated that this is likely to be due to the large scatter of the data, which demonstrate a random scatter of data points rather than any systematic deviation. The t-test statistic was also < 0.1, and is therefore acceptable according to FOCUS Kinetics guidance (FOCUS 2006). Overall the metabolite fit was considered acceptable.

It should be noted that for the aerobic soils all DT_{50} and DT_{90} values, and for the photolytic degradation the DT_{90} values, were extrapolated beyond the study durations and therefore exact values should be treated with a degree of caution. Temperature and moisture normalized DT_{50} values for the calculation of modelling endpoints are summarized in Table 7.1.2.1.1-4 for the aerobic soils.

Table 7.1.2.1.1-1: Summary of the results of the kinetic determinations for metrafenone in the aerobic soils incubated in Steinfuehrer 2000a, Steinfuehrer 2000b, and Steinfuehrer and Weis 2000

Model	Parameter	Engelstadt/ Benz silty loam 20°C & 50% MWHC	Sporkenheim loamy sand 20°C & 50% MWHC	Binger Pfad sandy loam 20°C & 50% MWHC	Gensingen clay loam 20°C & 50% MWHC	Sporkenheim loamy sand 10°C & 40 - 50% MWHC
SFO	χ^2 error (%)	2.9	5.9	5.1	5.0	2.7
	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
	k	0.00294	0.00448	0.00252	0.00285	0.00130
	DT_{50}	236.0	154.7	275.3	243.1	532.1
	DT_{90}	784.0	513.9	914.4	807.4	1767.5

Table 7.1.2.1.1-2: Summary of the results of the kinetic determinations for metrafenone in the anaerobic soils incubated in van Dijk & Kunz, 2001 (with amendments in Martin, 2002a) and Huang 2002

Model	Parameter	Engelstadt/ Benz silty loam 20°C	Stetten silty clay loam 20 °C
SFO	χ^2 error (%)	11.2	8.0
	P	< 0.05	< 0.05
	k	0.0950	0.0445
	DT_{50}	7.3	15.6
	DT_{90}	24.2	51.8
FOMC	χ^2 error (%)	11.4	8.2
	α	469.2 ± 2980	317.8 ± 3219
	β	4934 ± 31374	7139 ± 72424
	DT_{50}	7.3	15.6
	DT_{90}	24.3	51.9

Table 7.1.2.1.1-3: Summary of the results of the kinetic determinations for metrafenone and CL 377160 in the soil photolysis study of Ta 2001

Model	Parameter	Engelstadt/ Benz silty loam 20°C		
		Irradiated		Dark
		Metrafenone	CL 377160	Metrafenone
SFO	χ^2 error (%)	9.3	40.0	2.3
	P	< 0.05	< 0.05	< 0.05
	k	0.0551	0.1259	0.00441
	DT_{50}	12.6	5.5	157.3
	DT_{90}	41.8	18.3	522.6
	FF	-	0.52	-
FOMC	χ^2 error (%)	9.3	-	-
	α	782.8 ± NA	-	-
	β	14288 ± NA	-	-
	DT_{50}	12.7	-	-
	DT_{90}	42.1	-	-

NA = values not available – too high to be calculated.

Table 7.1.2.1.1-4: Summary of the calculated modelling DT_{50} values from the kinetic determinations normalized to 20 °C and field capacity (pF2)

Soil	Incubation Temperature (°C)	Incubation soil moisture (%)	Soil Field Capacity (%)	Study DT_{50} (d)	Normalized DT_{50} (20 °C/ pF2)
Engelstadt/ Benz silty loam	20 °C	50 % MWHC = 23.7	27	236.0	215.4
Sporkenheim loamy sand	20 °C	50 % MWHC = 16.4	14	154.7	154.7
Binger Pfad sandy loam	20 °C	50 % MWHC = 16.8	19	275.3	252.1
Gensingen clay loam	20 °C	50 % MWHC = 20.3	28	243.1	194.1
Sporkenheim loamy sand	10 °C	40 - 50 % MWHC* = 17.1	14	532.6	206.1
Geometric mean =					200.9**

* As a worst case assumption soil moisture content in the study was assumed as 50 % MWHC for the entire study duration.
** The moisture and temperature normalized geometric mean DT_{50} value at 20°C was calculated with the exclusion of the Sporkenheim soil test performed at 10 °C.

III. CONCLUSION

The SFO model provided a very good description of the degradation of metrafenone in both aerobic and anaerobic soils, and for the photolytic degradation of metrafenone in soil. In all cases the chi^2 % error was significantly < 15%, and visual fits and plots of the residuals, and t-test statistics confirmed the very good fits. SFO non-normalized laboratory soil aerobic DT_{50} values at 20 °C were in the range 154.7 – 275.3 days; corresponding DT_{50} values normalized to standard moisture (pF2) and temperature (20 °C) conditions were in the range 154.7 – 252.1 days, with a calculated geometric mean of 200.9 days. SFO laboratory anaerobic DT_{50} values at 20 °C were 7.3 – 15.6 days. The photolytic soil DT_{50} for metrafenone under the continuous irradiation conditions of the test was 12.6 days, which when corrected for aerobic degradation in the dark control of the same study was 13.7 days.

The SFO model provided an acceptable description of the decline of the soil photolysis metabolite CL 377160. The calculated DT_{50} under the conditions of the test was 5.5 days with a formation fraction of 0.52.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Adequate data to assess the rate of the aerobic degradation of the metabolites of metrafenone in the laboratory were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). Though the original Draft Assessment Report for the Annex I listing assessment of metrafenone was finalized in October 2003, prior to the finalization of the FOCUS Degradation Kinetics Report (FOCUS 2006) which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from laboratory and field studies, the degradation rates and DT_{50} values for the aerobic degradation of the sole soil metabolite of metrafenone CL 377160 (Afzal 2002) are unaffected by this change. This is because worst case DT_{90} values of <7 days were assumed in all soils, and reported in the list of end-points in the EFSA Conclusion for metrafenone (EFSA 2006), due to the very rapid aerobic degradation of CL 377160. Therefore the degradation rates of CL 377160 are not required to be re-calculated here. The worst case DT_{90} value is appropriate to derive DT_{50} values for use in modelling.

Report:	CA 7.1.2.1.2/1 Afzal J., 2002a CL 377160 (Metabolite of BAS 560 F): Rate of degradation in three different soils under aerobic conditions 2002/7005909
Guidelines:	EEC 91/414 Annex II 7.1.1.2.1, SETAC Europe Part 1 Section 1.1 (March 1995)
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.1.2/2 Hilton M.,Callow B., 2014a Determination of rates of decline for Metrafenone and its metabolite CL377160 in soil aerobic, anaerobic and photolysis degradation studies according to the FOCUS Kinetics Guidance Document 2014/1083467
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

Please see the full summary details presented at Section CA 7.1.2.1.1 above. A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

CA 7.1.2.1.3 Anaerobic degradation of the active substance

Adequate data to assess the rate of the anaerobic degradation of metrafenone in the laboratory were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). However since the original assessment in the Draft Assessment Report for the Annex I listing assessment of metrafenone was finalized in October 2003, the FOCUS Degradation Kinetics Report (FOCUS 2006), which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from laboratory and field studies, has been finalized. Therefore the degradation rates of metrafenone are required to be re-calculated in accordance with current FOCUS Kinetics guidance. This has been done in the report of Hilton and Callow (2014a) which is summarized in CA 7.1.2.1.1 above. SFO laboratory anaerobic DT_{50} values for metrafenone at 20 °C were 7.3 – 15.6 days.

Report:	CA 7.1.2.1.3/1 van Dijk A.,Kunz Ch., 2001a 14C-BAS 560 F: Route and rate of degradation in soil under anaerobic conditions 2001/7000460
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC Section 1.2
GLP:	yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.1.3/2 Martin C.A., 2002a 14C-BAS 560 F: Route and rate of degradation in soil under anaerobic conditions (Report amendment #2) 2002/7004411
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC (1995), Section 1.2
GLP:	yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.1.3/3 Huang R.A., 2002a BAS 560 F: Anaerobic soil metabolism 2002/7004949
Guidelines:	EEC 91/414 Annex II 7.1.1.1.2, EEC 95/36, SETAC Section 1.2
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 7.1.2.1.3/4 Ta C., 2012a Response to US EPA data evaluation record of the study entitled: - BAS 560 F: Anaerobic soil metabolism 2012/7005417
Guidelines:	EPA 835.4200
GLP:	no (certified by <none>)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil is presented at the end of this section (Section 7.1.2).

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Adequate data to assess the rate of the anaerobic degradation of the metabolites of metrafenone in the laboratory were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. Metrafenone was degraded much faster under anaerobic conditions than under aerobic conditions, with three metabolites (CL 377160, CL 4084564, CL 434223) being formed with maximum concentrations > 5 % AR. However no metabolites were considered to require risk assessment in the previous EU evaluation, and since none exceeded 5 % AR in consecutive samples it is still considered that no anaerobic soil metabolites will require an exposure assessment in either soil or groundwater. Consequently a kinetic evaluation of these metabolites is not required. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

Adequate data to assess the dissipation in soil of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). However since the original assessment in the Draft Assessment Report for the Annex I listing assessment of metrafenone was finalized in October 2003, the FOCUS Degradation Kinetics Report (FOCUS 2006), which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from laboratory and field studies, has been finalized. Therefore for the Approval Renewal assessment of metrafenone the degradation rates of metrafenone are required to be re-calculated in accordance with current FOCUS Kinetics guidance. This has been done in the report of Hilton and Callow (2014b) which is summarized below.

Report:	CA 7.1.2.2.1/1 Jones S.D., 2002a BAS 560 F (AC 375839) 200 g a.s./L SC (SF09956): Rate of dissipation study of AC 375839 in soil (Germany 1999) 2002/7004671
Guidelines:	EEC 91/414 Annex II 7.1.1.2.2, EEC 91/414 Annex III 9.1.1.2, EEC 95/36
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes field dissipation, is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.2.1/2 Smalley R., 2002a BAS 560 F (AC 375839) 200 g a.s./L SC (SF09956): Rate of dissipation study of BAS 560 F in soil (United Kingdom 1999) 2002/7004650
Guidelines:	EEC 91/414 Annex II 7, EEC 91/414 Annex III 9, EEC 95/36, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes field dissipation, is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.2.1/3 Bamber A., 2002a AC 375839 200 g a.i./L SC (SF 09956): Rate of dissipation study of AC 375839 in soil (Denmark, 1999) 2002/7004652
Guidelines:	EEC 91/414 Annex II 7.1.1.2.2, EEC 91/414 Annex III 9.1.1.2, EEC 95/36, BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes field dissipation, is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.2.1/4 Bamber A., 2002b AC 375839 200 g a.i./L SC (SF 09956): Rate of dissipation study of AC 375839 in soil (France-North, 1999) 2002/7004651
Guidelines:	EEC 91/414 Annex II 7.1.1.2.2, EEC 91/414 Annex III 9.1.1.2, EEC 95/36, BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 7.1.2.2.1/5 Bamber A., 2002c Report Amendment No. 1 - AC 375839 200 g a.i./L SC (SF 09956): Rate of dissipation study of AC 375839 in soil (France-North, 1999) 2002/7008500
Guidelines:	EEC 91/414 Annex II 7.1.1.2.2, EEC 91/414 Annex III 9.1.1.2, EEC 95/36, BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes field dissipation, is presented at the end of this section (Section 7.1.2).

Report: CA 7.1.2.2.1/6
Hilton M.,Callow B., 2014c
Determination of rates of decline for Metrafenone in field dissipation studies according to the FOCUS Kinetics Guidance Document
2014/1083469

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
(certified by none)

Executive Summary

A kinetic evaluation was performed in accordance with FOCUS kinetics guidance for field dissipation studies conducted following applications of metrafenone at trial sites in Germany, the UK, Northern France and Denmark. Kinetic evaluations were performed solely for the purpose of deriving persistence end-points for comparison against regulatory trigger values, and therefore normalization to standard soil temperature and moisture conditions was not performed. No metabolites were observed above the LOQ in any of the field dissipation studies, and hence, kinetic fitting for metrafenone alone was performed.

The SFO kinetic model provided a good description of the degradation of metrafenone at both the German and UK field trial sites. While the chi-squared error value was higher than 15%, values were only marginally improved when using FOMC kinetics. Visual fits and plots of the residuals did not display any systematic deviation, instead indicating that the chi-squared values were related to the large random scatter of measured concentrations. P values for the SFO rate constant were < 0.05 in both cases.

For the trials conducted in Northern France and Denmark biphasic FOMC kinetics displayed better statistical and visual fits than SFO kinetics. Consequently DFOP kinetics were also evaluated, and based upon statistical data and visual fits, displayed the best fit for the trial conducted in Denmark. However FOMC kinetics provided the best fit for the trial conducted in Northern France.

Reported DT_{50} values for metrafenone were in the range 22.2 - 145 days (n=4). Reported DT_{90} values were in the range 473 – 1221 days (n=3). KINGUII could not calculate the DT_{90} value for metrafenone associated with the Denmark trial site. The value of 1221 days from the Northern France site is extrapolated beyond the study duration and therefore should be treated with a degree of caution.

I. MATERIAL AND METHODS

A. MATERIALS

The degradation of metrafenone in four field dissipation studies conducted at sites in Germany, the UK, Northern France and Denmark and reported in the studies of Jones (2002a), Smalley (2002j) and Bamber (2002a&b) was kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document. No residues of the metabolite CL 377160 above the LOQ (0.02 mg/kg) were observed in any soil specimen from any trial. As a result kinetic evaluation was only possible for parent metrafenone. No residues of metrafenone above the LOQ (0.02 mg/kg) were observed below the 0-10 cm horizon in any soil specimen from any trial. The rate of degradation of metrafenone was therefore determined based upon concentrations in the 0-10 cm soil layer alone.

B. STUDY DESIGN

Treatment of raw data from the field dissipation studies in accordance with FOCUS Kinetics guidance was not required for the trials conducted in Germany, the UK and Denmark. For the trial conducted in Northern France two samples were reported as < LOQ, and therefore, in accordance with FOCUS Kinetics guidance, values were set to ½ x LOQ for the purposes of kinetic evaluation.

The kinetic modelling was performed solely for the determination of persistence end-points for comparison against regulatory trigger values, using KINGUII vers 2. The approach used followed that given in Chapters 7 & 9 of the FOCUS Kinetics Guidance Document. The suitability of the fit of the models was evaluated both visually, based on a graphical plot of the degradation and in a plot of the residuals, and statistically by calculating the minimum % error required to pass the χ^2 test at a probability of 0.05. For SFO and DFOP kinetics a t-test was also performed to evaluate whether the determined parameters were significantly different to 0. T-test statistics are not appropriate to describe the FOMC fitting parameters alpha and beta, and therefore confidence intervals were reported. Statistical parameters were compared to the acceptability criteria as reported in FOCUS Kinetics guidance; however it is also stated within that guidance that acceptability criteria should not be considered as absolute cut-off criteria.

For the determination of the persistence end-point FOCUS Kinetics guidance recommends that both SFO and FOMC models are initially applied to the data. If the SFO model gave an acceptable visual and statistical fit then this was accepted. If the FOMC model gave a more appropriate fit DFOP kinetics were also applied to the data. The results of the FOMC and DFOP models were then compared to determine which gave the best fit. The model which was considered to best represent the data was then selected.

II. RESULTS AND DISCUSSION

The results of the determinations were evaluated using visual and statistical methods. Summaries of the results and kinetic fitting parameters are summarized in Table 7.1.2.2.1-1.

For the trials conducted in Germany and the UK, SFO chi-squared % error values were only marginally higher than those from the respective FOMC fits. While the chi-squared error value for both the SFO and FOMC fits for the UK trial are high, examination of the visual fits and plots of residuals for the SFO kinetics demonstrated no systematic deviation for either the UK or German trials. The high chi-squared error values for the UK trial are therefore considered to demonstrate the large random scatter of the measured concentrations. In addition comparison of t-test statistics for SFO fits and error values for alpha and beta parameters in FOMC demonstrated that SFO fits were more certain. Overall it was concluded that SFO kinetics provided the best fits for both the German and UK trials.

For the trials conducted in Northern France and Denmark comparison of chi-squared error values, visual fits and residual plots, for the SFO and FOMC fits indicated that FOMC kinetics provided the better fits. Therefore additional kinetic fits were performed for both trials with biphasic DFOP kinetics.

For the trial conducted in Northern France, DFOP kinetics did not improve upon the FOMC kinetic fit. FOMC kinetics were therefore concluded to provide the best fit at the Northern France trial. For the Danish trial, DFOP kinetics provided a better fit to the data based on comparison of both the chi-squared error values and the visual fits. Consequently for the Danish trial DFOP kinetics were concluded to provide the best fit.

It should be noted that for the Northern France site FOMC kinetic fit the DT_{90} value of 1221 days is extrapolated significantly beyond the study termination. Hence, the reported DT_{90} value for the Northern France site should be treated with caution. The DT_{90} value for the DFOP fit for the Denmark site could not be calculated by KINGUII, indicating a large degree of uncertainty. This uncertainty is reflected in the error value associated with t-test statistic for k_{slow} for the DFOP fit, which is 0.457. In reality, it is likely that the DT_{90} value is significantly shorter than indicated by the k_{slow} value of the Danish DFOP fit, since the g statistic of 0.597 means that effectively the slow phase is only represented by the latter data points which display a large variation when compared to the slow decline calculated.

Table 7.1.2.2.1-1: Summary of the results of the kinetic determinations for metrafenone in the field dissipation studies conducted in Germany, the UK, Northern France and Denmark

Model	Parameter	Germany	UK	Northern France	Denmark
SFO	χ^2 error (%)	**12.6**	**31.5**	28.2	21.6
	P	**<0.05**	**<0.05**	<0.05	<0.05
	k	**0.00486 ± 0.00175**	**0.00477 ± 0.00505**	0.01001 ± 0.00783	0.00317 ± 0.00273
	DT_{50}	**143**	**145**	69.2	219
	DT_{90}	**473**	**483**	230	727*
FOMC	χ^2 error (%)	11.4	30.9	**22.6**	11.0
	α	0.91219 ± 1.37132	0.69157 ± 1.74679	**0.42419 ± 0.31989**	0.23845 ± 0.11791
	β	95.997 ± 242.69	69.211 ± 301.12	**5.38648 ± 12.5453**	3.58358 ± 6.86267
	DT_{50}	109	119	**22.2**	62.0
	DT_{90}	1102*	1863*	**1221***	55986*
DFOP	χ^2 error (%)	-	-	23.2	**9.6**
	k_{fast}	-	-	0.18333 ± 0.25740	**0.03486 ± 0.02227**
	k_{slow}	-	-	0.0047637 ± 0.00545	**0.00007 ± 0.00118**
	g	-	-	0.478	**0.597**
	P-k_{fast}	-	-	0.100	**<0.05**
	P-k_{slow}	-	-	0.063	**0.457**
	DT_{50}	-	-	17.4	**51.7**
	DT_{90}	-	-	347	**NC**

Fits presented in bold are those considered to be the best fits.
NC = not calculated by KINGUII
* = value extrapolated beyond study duration

III. CONCLUSION

A kinetic evaluation was performed in accordance with FOCUS kinetics guidance for four field dissipation studies conducted with metrafenone. Values were derived for persistence end-points of metrafenone for comparison against regulatory trigger values.

Based upon fitting statistics and visual fits the SFO kinetic model provided the best description of the degradation of metrafenone at two field trial sites. For the trial conducted in Northern France biphasic FOMC kinetics displayed the best fit, while DFOP kinetics displayed the best fit for the trial conducted in Denmark.

Reported DT_{50} values for metrafenone were in the range 22.2 - 145 days (n=4). Reported DT_{90} values were in the range 473 – 1221 days (n=3). KINGUII could not calculate the DT_{90} value associated with the Denmark trial site. The DT_{90} value of 1221 days is extrapolated beyond the study duration and therefore this value should be treated with a degree of caution.

CA 7.1.2.2.2 Soil accumulation studies

Data to assess the field accumulation in soil of metrafenone were evaluated during the first EU review. However the four studies (Smalley, 2002m; Young, 2002a & b; Jones, 2002b) summarized in the Draft Assessment Report for metrafenone were not finalized at that time, nor were they finalized at the time the EFSA Conclusion (EFSA 2006) was produced. The studies are now finalized as Johnston 2006a-d, and therefore a full summary for the four studies is presented below. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005), the final addendum to the DAR for metrafenone (UK, 2005), and the EFSA Conclusion for metrafenone (EFSA 2006).

In addition to the field soil accumulation studies the modelling study of Beigel (2002b), with additional information in Beigel and Ta (2003), was conducted to assess the field accumulation of metrafenone, and is summarized in the original DAR. Modelling was performed using FOCUS PEARL version 1.1.1 and the relevant standard FOCUS groundwater scenarios for applications to spring cereals, winter cereals and vines. Applications were made at rates of 2 x 150 g as/ ha/ year to winter and spring cereals, and 8 x 100 g as/ ha/ year for vines. Appropriate crop interception values were considered.

While the FOCUS scenarios are designed to be a realistic worst case for leaching, predicted accumulation in the soil layer can also be derived from the pesticide mass balance tables. In this case the worst case values of a K_{om} of 3223 L/kg (corresponding to the worst case K_{oc} of 5556 L/kg) and a 1/n of 0.85, with a DT_{50} value of 345.4 d (the worst case laboratory DT_{50} from the original review corrected to 20 °C and pF2), were input in to the model. The amount of metrafenone leaching below the 1 m layer was less than the limit of accuracy of the model (< 0.001 µg/L). Therefore despite the model's realistic worst case nature with respect to leaching, leaching did not contribute significantly to losses from the top 1m soil layer in the modelling performed, and therefore does not unduly affect the soil accumulation in this case.

The accumulation plateau was reached after approximately 10 years of annual applications. The authors then assumed that all metrafenone in the top 1m of soil was present in the top 5 cm of the soil layer as a further worst case assumption for the calculation of accumulated PEC_{soil} values. A soil bulk density of 1.4 g/ cm^3 was assumed in the report, but correction was made by the RMS to a standard soil bulk density of 1.5 g/ cm^3. Peak accumulated PEC_{soil} values were then presented in the DAR by the RMS for all FOCUS scenarios relevant to the applications to vines and winter and spring cereals. Peak accumulated PEC_{soil} values were 0.426 – 0.832 mg/ kg, 0.453 – 0.843 mg/kg, and 0.656 – 1.336 mg/kg for the modelled applications to winter cereals, spring cereals and vines respectively. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005), and the EFSA Conclusion for metrafenone (EFSA 2006).

By comparison to the peak concentrations observed in the field accumulation studies (Johnston 2006a-d), it can be seen that the modelled accumulated PEC_{soil} values are significantly higher than the maximum soil concentrations observed in field accumulation studies, the maximum concentration from which was 0.65 mg/ kg in the upper 10 cm soil layer, or 0.69 mg/kg when expressed for total residue, following application to bare soil in Italy at a rate of 8 x 100 g as/ ha/ year. It is therefore concluded that the peak accumulated PEC_{soil} values derived from the modelling study provide worst case accumulated PEC_{soil} values.

Report:	CA 7.1.2.2.2/1 Johnston R.L., 2006a BAS 560 F (AC 375839) 500 g ai/L SC (SF 09955): Accumulation of BAS 560 F residues in soil (Italy, 1999-2005) 2006/7011060
Guidelines:	EEC 91/414 Annex II 7, EEC 91/414 Annex III 9, EEC 95/36, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by United States Environmental Protection Agency)

See combined summary below.

Report:	CA 7.1.2.2.2/2 Johnston R.L., 2006b BAS 560 F (AC 375839) 500 g ai/L SC (SF 10358): Accumulation of BAS 560 F residues in soil (Germany, 1999 - 2005) 2006/7011058
Guidelines:	EEC 91/414 Annex II 7, EEC 91/414 Annex III 9, EEC 95/36, SETAC
GLP:	yes (certified by United States Environmental Protection Agency)

See combined summary below.

Report:	CA 7.1.2.2.2/3 Johnston R.L., 2006c BAS 560 F (AC 375839) 300 g ai/L SC (SF 10358): Accumulation of BAS 560 F residues in soil (Germany, 1999-2005) 2006/7011059
Guidelines:	EEC 91/414 Annex II 7, EEC 91/414 Annex III 9, EEC 95/36, BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by United States Environmental Protection Agency)

See combined summary below.

Report:	CA 7.1.2.2.2/4 Johnston R.L., 2006d BAS 560 F (AC 375839) 500 g ai/L SC (SF 09955): Accumulation of BAS 560 F residues in soil (Spain, 1999-2005) 2006/7011061
Guidelines:	EEC 91/414 Annex II 7, EEC 91/414 Annex III 9, EEC 95/36, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

Four soil accumulation studies were conducted; two at a site in Schwabenheim Germany, on plots cropped with vines and cereals, and two bare soil plots in Italy and Spain. Spray applications of varying SC formulations of metrafenone were applied at all trial sites. At the first Schwabenheim trial, eight applications were made to vines every year, for 6 consecutive years (1999 – 2004 inclusive), with individual application rates of between 60 – 160 g as/ ha. For the second Schwabenheim trial two applications of 200 g as/ ha were made to a field cropped with cereals for seven years between 1999 and 2005 inclusive, with the exception of 2001, when no applications were made. For the Italian and Spanish trials eight applications of 100 g as/ ha were applied to bare soil every year for six years between 1999 and 2004 inclusive.

Where crops were present cultivation methods during the trials were according to good agricultural practice, with the exception that at harvest crops were incorporated back into the soil/ worked in. No cultivation was performed for the Italian and Spanish bare soil trials.

Samples were collected annually from both treated and untreated plots in the form of 20 x 30 cm soil cores. A final sample was collected approximately 1 year after the 1st application in the season of final application. All samples from all four trials were frozen at ≤ -18 °C within 24 hours of collection prior to shipment to the analytical facility. Upon receipt at the analytical facility samples were stored frozen at ≤ -18 °C until analysis.

The majority of samples were separated into 0-5 cm, 5-10 cm, 10 – 20 cm, and 20 – 30 cm soil layers. The upper two layers were analyzed for residues of metrafenone and its photolytic soil metabolite CL 377160. If positive residues at or above the limit of quantitation (LOQ) were detected in the uppermost layers, the 10 to 20 cm layer was analyzed. If there were no detectable residues above the LOQ in the 0-10 or 10-20 cm layers, the following soil layer was not analyzed. For the Schwabenheim trials soil cores collected in 1999 and 2000, for the Italian bare soil trial soil cores collected in 1999 – 2001, and for the Spanish trial soil cores collected in 1999 – 2002, were divided into 10 cm segments, such that the top layer was 0-10 cm.

Soil samples were extracted by vortexing, firstly with acetonitrile, and then with triethylamine/water/acetonitrile. After centrifugation aliquots of extracts were combined, and further centrifuged and filtered prior to analysis by LC/MS/MS. Samples collected prior to 2002 (Italy) or 2003 (Spain) were extracted by heating in a microwave oven with a mixture of triethylamine/water/acetonitrile. After filtration extracts were cleaned-up using C18 solid phase extraction cartridges. Final determination of metrafenone and CL 377160 was performed by HPLC/MS. The LOQ was 0.005 mg/kg for the Schwabenheim trials, and 0.02 mg/ kg for the Italian and Spanish trials for both methods of analysis.

The sum of total residue concentrations of metrafenone from all soil layers were calculated for the purposes of assessing the total soil accumulation of metrafenone. This was done by summing all quantified residue concentrations in individual soil layers. Where residues were < LOQ or a soil layer was not analyzed, a concentration of 0 mg/kg was assumed for that soil layer. Where residue concentrations were reported for the 0 – 5 cm and 5 – 10 cm layers, concentrations for these two layers were corrected by multiplying by 0.5, in order to make the concentrations reported for these 5 cm soil layers equivalent to those reported for the 10 cm soil layers. Total residue concentrations for metrafenone are summarized in Table 7.1.2.2.2-1 and Table 7.1.2.2.2-2. The total soil residue concentration of metrafenone in three of the four trials was observed to have reached a plateau by the study termination. In the case of the Schwabenheim cereal trial, a definitive judgement is complicated by the samples from 2004 which were lost and could not be analyzed. However, with the exception of the single sample taken 0+DAT11, comparison of equivalent sample concentrations from 2003 to 2005 indicated that a plateau had been reached, or only very small increases in total metrafenone concentrations had occurred over the two year period. The maximum accumulated metrafenone soil concentration of 0.65 mg/kg was observed in the upper 0 – 10 cm soil layer at the Italian trial immediately after the final application in the third season.

No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample for three of the four studies, and in the Schwabenheim were only rarely observed in the 0 – 5 cm soil layer, with a maximum concentration in that soil layer of 0.007 mg/kg.

No residues of either metrafenone or its metabolite CL 377160 were observed above the LOQ in untreated plots in any trial, with the single exception of the 184+ DAT25 in the Italy trial, where the untreated plot contained apparent residues in the 10 – 20 cm soil layer of 0.03 mg/kg.

Table 7.1.2.2.2-1: Total Residue Concentrations of metrafenone at the Schwabenheim vine and cereal soil accumulation trials

Year	Schwabenheim - Vine		Schwabenheim – Cereal	
	Sample	Total metrafenone (mg/kg)	Sample	Total metrafenone (mg/kg)
1999	0-DAT1	<0.005	0-DAT1	<0.005
	0+DAT1	<0.005	0+DAT1	0.009
	0-DAT4	0.039	0-DAT2	0.018
	0+DAT4	0.046	0+DAT2	0.024
	0-DAT8	0.162	-	-
	0+DAT8	0.155	-	-
2000	0-DAT9	0.114	0-DAT3	0.030
	0+DAT9	0.149	0+DAT3	0.049
	0-DAT12	0.197	0-DAT4	0.053
	0+DAT12	0.164	0+DAT4	0.064
	0-DAT16	0.172	154+DAT4	0.038
	0+DAT16	0.198	-	-
2001	0-DAT17	0.188	330+DAT4	0.034
	0+DAT17	0.216	-	-
	0-DAT20	0.180	-	-
	0+DAT20	0.166	-	-
	0-DAT24	0.240	-	-
	0+DAT24	0.257	-	-
2002	0-DAT25	0.224	0-DAT5	0.022
	0+DAT25	0.247	0+DAT5	0.071
	0-DAT28	0.207	0-DAT6	0.060
	0+DAT28	0.262	0+DAT6	0.088
	0-DAT32	0.305	-	-
	0+DAT32	0.270	-	-
2003	0-DAT33	0.315	0-DAT7	0.059
	0+DAT33	0.253	0+DAT7	0.169
	0-DAT36	0.230	0-DAT8	0.193
	0+DAT36	0.278	0+DAT8	0.192
	0-DAT40	0.256	56+DAT8	0.185
	0+DAT40	0.274	-	-
2004	0-DAT41	N/A	0-DAT9	N/A
	0+DAT41	N/A	0+DAT9	N/A
	0-DAT44	0.360	0-DAT10	N/A
	0+DAT44	0.380	0+DAT10	N/A
	0-DAT48	0.224	71+DAT10	0.136
	0+DAT48	0.256	-	-
	254+DAT48	0.240	-	-
2005	-	-	0-DAT11	0.065
	-	-	0+DAT11	0.245
	-	-	0-DAT12	0.179
	-	-	0+DAT12	0.206
	-	-	65+DAT12	0.140
	-	-	307+DAT12	N/A

N/A – Samples not analyzed

Table 7.1.2.2.2-2: Total Residue Concentrations of metrafenone at the Sasso Morelli and Fuentiduena de Tajo bare soil accumulation trials

Year	Sasso Morelli, Italy – Bare soil		Fuentiduena de Tajo, Spain – Bare soil	
	Sample	Total metrafenone (mg/kg)	Sample	Total metrafenone (mg/kg)
1999	0-DAT1	<0.02	0-DAT1	<0.02
	0+DAT1	0.02	0+DAT1	0.05
	0-DAT8	0.14	0-DAT8	0.05
	0+DAT8	0.13	0+DAT8	0.09
	182+DAT1	0.05	183+DAT1	0.08
2000	0-DAT9	0.07	0-DAT9	0.03
	0+DAT9	0.10	0+DAT9	0.14
	0-DAT16	0.20	0-DAT16	N/A
	0+DAT16	0.29	10+DAT16	0.12
	175+DAT9	0.33	183+DAT9	0.04
2001	0-DAT17	0.11	0-DAT17	0.03
	0+DAT17	0.16	0+DAT17	0.08
	0-DAT24	0.66	0-DAT24	0.12
	0+DAT24	0.69	0+DAT24	0.13
	255+DAT17	0.12	181+DAT17	0.11
2002	0-DAT25	0.12	0-DAT25	0.07
	0+DAT25	0.16	0+DAT25	0.07
	0-DAT32	0.16	0-DAT32	0.13
	0+DAT32	0.31	0+DAT32	0.19
	184+DAT25	0.14	181+DAT25	0.07
2003	1-DAT33	0.18	1-DAT33	0.09
	0+DAT33	0.22	0+DAT33	0.11
	0-DAT40	0.31	0-DAT40	0.13
	0+DAT40	0.35	0+DAT40	0.19
	181+DAT33	0.13	182+DAT33	0.06
2004	0-DAT41	0.21	0-DAT41	0.02
	0+DAT41	0.27	0+DAT41	0.08
	1-DAT48	0.25	0-DAT48	0.07
	0+DAT48	0.31	0+DAT48	0.13
	182+DAT41	0.21	188+DAT41	0.04
	369+DAT41	0.13	375+DAT41	0.03

N/A – Samples not analyzed

I. MATERIAL AND METHODS

A. MATERIALS

Four soil accumulation studies were conducted; two at a site in Schwabenheim, Germany, on plots of approximately 477 - 480 m^2, and two in Southern Europe; at Sasso Morelli, Italy, and at Fuentiduena de Tajo, Spain, both on plots of 200 m^2. Untreated plots of similar dimensions acted as controls for each of the German trials, with the area of control plots in the Italian trial comprising half the area of the treated plot. Soil characteristics for the sites are shown in Table 7.1.2.2.2-3.

Table 7.1.2.2.2-3: Summary of the soil properties for the soil accumulation studies with metrafenone

Study	**Johnston 2006a**	**Johnston 2006b**	**Johnston 2006c**	**Johnston 2006d**
Location	**Schwabenheim, Germany**	**Schwabenheim, Germany**	**Sasso Morelli, Italy**	**Fuentiduena de Tajo, Spain**
Crop	Vines	Cereals	Bare soil	Bare soil
Soil characteristics:				
Soil Type (USDA)	Silt	Silt	Clay	Sandy loam
% Sand	15.4	14.8	4.0	52.0
% Silt	59.9	58.5	36.0	31.0
% Clay	24.7	26.7	60.0	17.0
% OM	4.41	3.73	2.1	0.4
% OC	2.56	2.17	1.2	0.2
pH ($CaCl_2$)	7.5	7.2	7.4*	8.0*
Cation Exchange Capacity (mEq/100g)	173	84.0	13.4	4.4
Maximum Water Holding Capacity (%)	48.9	44.7	35.4	12.9

*pH measured in 0.1N KCl

B. STUDY DESIGN

At the first Schwabenheim trial in Johnson 2006a, spray applications of a 500 g as/L SC formulation of metrafenone were made to vines, eight times every year, for 6 consecutive years (1999 – 2004 inclusive). Dose rates varied between 60 – 160 g as/ ha, with application rates generally increasing throughout the season. Applications were made from early May to late August, and between BBCH crop growth stages 13 – 87, with application intervals which varied, but were approximately 14 days.

For the second Schwabenheim trial reported in Johnson 2006b, spray applications of various SC formulations, containing 200 – 300 g as/ L metrafenone were applied to a field cropped with either wheat, barley or oilseed rape every year for seven years between 1999 and 2005 inclusive, with the exception of 2001, when no applications were made. Two spray applications were made per year at individual application rates of 200 g as/ ha, and between late April and early June, at BBCH growth stages 29 – 73. Application intervals varied but were usually approximately 14 days, with the exception of the applications made in 2002 and 2005, where the application interval was between 7 - 8 weeks.

For the Italian and Spanish trials reported in Johnson 2006c & 2006d, spray applications of different batches of an SC formulation, containing 300 or 500 g as/ L metrafenone, were applied to bare soil every year for six years between 1999 and 2004 inclusive. Eight spray applications were made per year at individual application rates of approximately 100 g as/ ha, and between early/mid-May and mid-/late August in the Italian trial, and mid-April/ mid-June to mid-July/mid-September in the Spanish trial. Application intervals varied but were approximately 14 days at both sites.

The actual formulations differed throughout the duration of the studies; however this does not affect the fate and behavior of the active substance. Actual application dates, rates, and crop growth stages at application are presented in Table 7.1.2.2.2-4 - Table 7.1.2.2.2-7 alongside measured soil residues.

Weather data for the trials at Schwabenheim were collected from the weather station at Schwabenheim 2 - 4 km from the trial sites in 1999, or from Frankfurt, 60 km from the trial site, thereafter. Weather data for the Italian trial were collected from the weather station at Casola Canina approximately 4.5 km from the trial site, and additionally in 2004, at S. Agata sul Santerno and Sasso Morelli 11.5 km and 1 km from the trial site respectively. Data for the Spanish trial were collected from the Madrid Aeropuerto weather station 50 km from the trial site. Weather patterns were considered normal in comparison to historical data.

Cultivation methods during the trials were according to good agricultural practice in those trials where crops were present, with the exception that at harvest crops were incorporated back into the soil/ worked in. No cultivation was performed for the Italian and Spanish bare soil trials.

Samples were collected in the form of 20 x 30 cm soil cores, from treated plots immediately prior to, and immediately after the 1st, 4th and 8th applications in a season for the Schwabenheim vine trial, and immediately pre- and post- both applications in a season to cereals. For the Italian and Spanish bare soil trials, samples were collected immediately before and after the 1st and 8th applications every season, with additional annual samples generally collected 180 days after the 1st treatment every season. A final sample was collected approximately 1 year after the 1st application in 2004.

At the Schwabenheim trials soil cores from the untreated plots were collected immediately prior to the 1st application every year. An additional sample was collected immediately after the last application in the final year at the Schwabenheim vine trial. At the Italian and Spanish bare soil trials untreated plot samples were collected annually before the first and last applications, and generally approximately 180 days after the first application in each year. An additional sample was collected approximately 1 year after the first application in 2004.

Additional soil samples from the Schwabenheim cereal trial were taken from both treated and untreated plots in the 2000/2001 season when no applications were made, and post-harvest in July 2003 and 2004, and August 2005, and in early April 2006. Straw samples were also collected at harvest in treated and untreated plots in 2000.

For all trials samples from untreated plots consisted of a total of 20 soil cores. For both Schwabenheim trials cores were collected from 3 - 5 different sub-plots, and for the vine trial all cores were collected from bare ground beneath the vine plant (i.e. in the 'dripping zone'). All samples from all four trials were frozen at ≤ -18 ºC within 24 hours of collection prior to shipment to the analytical facility. Upon receipt at the analytical facility samples were stored frozen at ≤ -18 ºC until analysis.

For the Schwabenheim trial site soil cores collected in 1999 and 2000 were divided into 10 cm segments and the upper layer analyzed for residues of metrafenone and its photolytic soil metabolite CL 377160. If positive residues at or above the limit of quantitation (LOQ) were detected in the uppermost layer, the 10 to 20 cm layer was analyzed. If there were no detectable residues above the LOQ in the 0-10 or 10-20 cm layers, the following soil layer was not analyzed. Specimens collected in 2001 onwards were separated into 0-5 cm, 5-10 cm, 10 – 20 cm, and 20 – 30 cm soil layers. All soil samples were definitively analyzed by method M3441 (modified). Samples were extracted by vortexing, firstly with acetonitrile, and then with triethylamine/water/acetonitrile. After centrifugation aliquots of extracts were combined, and further centrifuged and filtered prior to analysis by LC/MS/MS, with quantification by comparison to external standards. The LOQ was 0.005 mg/kg.

For the Italian bare soil trial, soil cores collected in 1999 – 2001, and for the Spanish trial soil cores collected in 1999 – 2002, were divided into 10 cm segments, analyzed with the same procedure described for the Schwabenheim trials, and utilizing the method RLA 12618V. In brief; samples were extracted by heating in a microwave oven with a mixture of triethylamine/water/acetonitrile. After filtration extracts were cleaned-up using C18 solid phase extraction cartridges. Final determination of metrafenone and CL 377160 was performed by HPLC/MS, with quantification by comparison to external standards. Samples collected from 2002 (Italy) or 2003 (Spain) onwards were separated into 0 - 5 cm, 5 – 10 cm, 10 – 20 cm, and 20 – 30 cm soil layers and analyzed with the method M3441 (modified) method described above. The stated LOQ for all samples was 0.02 mg/kg.

Straw specimens from the Schwabenheim cereal trial were analyzed for residues of metrafenone and the metabolites CL 3000402, CL 376991 and CL 434223 with method RLA 12560.00. Straw samples were extracted with (80:20) methanol: water, filtered, cleaned-up with an anion exchange cartridge, with final analysis by LC/MS. The LOQ was 0.10 mg/kg.

II. RESULTS AND DISCUSSION

Results for the analysis of soil cores taken from the treated plots in the soil accumulation studies are presented in Table 7.1.2.2.2-4 - Table 7.1.2.2.2-7 together with full details of the applications made. The sum of total residue concentrations of metrafenone from all soil layers have been calculated for the purposes of assessing the total soil accumulation of metrafenone. This was done by summing all quantified residue concentrations in analyzed soil layers. Where residues were < LOQ or a soil layer was not analyzed, a concentration of 0 mg/kg was assumed for that soil layer. Where residue concentrations were reported for the 0 – 5 cm and 5 – 10 cm layers, concentrations for these two layers were corrected by multiplying by 0.5, in order to make the concentrations reported for these 5 cm soil layers equivalent to those reported for the 10 cm soil layers. Total residue concentrations are also presented in Table 7.1.2.2.2-4 - Table 7.1.2.2.2-7 and in graphical form in Figure 7.1.2.2.2-1 - Figure 7.1.2.2.2-4.

In the Schwabenheim vine trial quantifiable residues of metrafenone were found in the upper soil layer prior to the 4th application of metrafenone in the first season, but were not observed in lower soil layers until the second season's application. By study termination, regular but low concentrations of metrafenone were observed in the 20 – 30 cm soil layer. The maximum concentration in this layer was 0.026 mg/kg immediately after the 4th application made in the 5th season. The total residue concentration of metrafenone was observed to have reached a plateau by the study termination by virtue of the comparison of total residues before and after the final application in the seasons. No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample over the 6 year study period.

In the Schwabenheim cereal trial quantifiable residues of metrafenone were found in the upper soil layer immediately after the 1st application of metrafenone in the first season, but were not observed in the 10 – 20 cm soil layer until the fifth season. Quantifiable concentrations of metrafenone were never observed in the 20 – 30 cm soil layer. With the exception of the single sample taken 0+DAT11 comparison of equivalent sample total residue concentrations from 2003 to 2005 indicated that a plateau had been reached, or only very small increases in total metrafenone concentrations were occurring. Comparison could not be made to samples collected in 2004, since those samples were lost. The study could not be extended beyond the 6 year duration because there was not enough room remaining in the plots to collect undisturbed soil samples in an unbiased manner. Only sporadic quantifiable residues of metabolite CL 377160 were observed in 0-5 cm soil layers over the 6 year study period. The maximum concentration was 0.007 mg/ kg in the sample taken 71 DAT10, with quantifiable concentrations of 0.005 mg/ kg also observed 0-DAT12 and 0+DAT12. Quantifiable residues of CL 377160 were never observed above the LOQ below the 0 – 5 cm soil layer. A metrafenone concentration of 1.32 mg/kg straw was reported in the sole straw sample analyzed. No metabolites were observed above the LOQ in straw.

In the Italian bare soil trial quantifiable residues of metrafenone were found in the upper soil layer after the first application of metrafenone in the first season, but were not observed in the 10 – 20 cm soil layer until after the 8th application in the second season. Quantifiable concentrations of metrafenone were only observed once in the 20 – 30 cm soil layer (0.04 mg/kg 175 DAT16). Total metrafenone concentrations were observed to have plateaued by the end of year 3 by virtue of the measured soil concentration immediately after the final application, and by the end of the 4th season's application by virtue of the sample taken 180 days after the first treatment in a season. The maximum concentration in the upper soil layer immediately after the final application was 0.65 mg/kg in the 3rd season. No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample over the 6 year study period.

In the Spanish bare soil trial quantifiable residues of metrafenone were found in the upper soil layer after the first application of metrafenone in the first season, but were never observed above the LOQ in the 10 – 20 cm soil layer. In the final two years of the trial when the 0 - 5 cm and 5 – 10 cm soil layers were analyzed separately, only low residues were observed in the 5 – 10 cm layer (< 0.02 mg/kg – 0.06 mg/kg) confirming that the vast majority of un-metabolized metrafenone remained in the upper soil layer. Total metrafenone concentrations were observed to have plateaued by the end of year 3 by virtue of the measured soil concentration approximately 180 days after the first treatment of the season, and by the end of the 4th season's application by virtue of the samples taken immediately after the last application made in a season. The maximum concentration in the upper soil layer immediately after the final application was 0.33 mg/kg for the 0 – 5 cm layer in the 5th season. No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample over the 6 year study period.

No residues of either metrafenone or its metabolite CL 377160 were observed above the LOQ in untreated plots in any trial, with the single exception of the 184+ DAT25 in the Italy trial, where the untreated plot contained apparent residues in the 10 – 20 cm soil layer of 0.03 mg/kg.

Table 7.1.2.2.2-4: Summary of the field study on metrafenone soil accumulation following application to vines at Schwabenheim

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No cores per sample depth (cm)	Residue, parent				Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
Johnson 2006a BASF Doc ID: 2006/7011058	Schwabenheim, Germany Silt, pH 7.5, 2.56 % OC Silt, pH 7.5, 2.56 % OC	500g as/l SC formulation	spray to vines GS 14-15	1	62.85	19/5/99	0-DAT1	20/ 30	<0.005	<0.005	N/A	<0.005	
			spray to vines GS 55	2	76.77	1/6/99	0+ DAT 1	20 / 30	<0.005	<0.005	N/A	<0.005	Procedural recovery at LOQ (0.005 mg/kg): 93-114% parent, 59-95% CL 377160
			spray to vines GS 63	3	99.96	16/6/99							
			spray to vines GS 71-72	4	121.60	30/6/99	0- DAT 4	20 / 30	0.039	<0.005	N/A	0.039	
			spray to vines GS 74-75	5	122.35	13/7/99	0+ DAT 4	20 / 30	0.046	<0.005	N/A	0.046	No residues of CL 377160 ≥LOQ at any depth
			spray to vines GS 75	6	136.69	28/7/99							
			spray to vines GS 81	7	159.85	11/8/99	0- DAT 8	20 / 30	0.162	<0.005	N/A	0.162	
			spray to vines GS 81-82	8	159.45	25/8/99	0+ DAT 8	20 / 30	0.155	<0.005	N/A	0.155	
			spray to vines GS 53	9	58.28	15/5/00	0- DAT9	20 / 30	0.114	<0.005	N/A	0.114	
			spray to vines GS 55	10	83.44	26/5/00	0+ DAT 9	20 / 30	0.149	<0.005	N/A	0.149	
			spray to vines GS 69	11	100.42	8/6/00							
			spray to vines GS 73	12	123.48	19/6/00	0- DAT 12	20 / 30	0.186	0.011	<0.005	0.197	
			spray to vines GS 75	13	121.83	3/7/00	0+ DAT 12	20 / 30	0.155	0.009	<0.005	0.164	
			spray to vines GS 77	14	144.65	18/7/00							
			spray to vines GS 79	15	170.23	3/8/00	0- DAT 16	20 / 30	0.172	<0.005	N/A	0.172	
			spray to vines GS 83	16	157.32	21/8/00	0+ DAT 16	20 / 30	0.174	0.007	0.017	0.198	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment Method kind	Trt No.	g as/ha	Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent (mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	Remarks:
Johnson 2006a BASF Doc ID: 2006/ 7011058	Schwabenheim, Germany Silt, pH 7.5, 2.56 % OC Silt, pH 7.5, 2.56 % OC	500g as/l SC formulation	spray to vines GS 15	17	60.03	16/05/01	0-DAT17	20/ 30	0.297	0.053	0.013	<0.005	0.188	
			spray to vines GS 53	18	79.59	29/05/01	0+ DAT 17	20 / 30	0.327	0.037	0.018	0.016	0.216	Procedural recovery at LOQ (0.005 mg/kg): 93-114% parent, 59-95% CL 377160
			spray to vines GS 59	19	101.45	12/06/01								
			spray to vines GS 69	20	125.50	25/06/01	0- DAT 20	20 / 30	0.285	0.042	0.008	0.008	0.180	
			spray to vines GS 73-75	21	123.27	09/07/01	0+ DAT 20	20 / 30	0.269	0.036*	0.013	<0.005	0.166	No residues of CL 377160 ≥LOQ at any depth
			spray to vines GS 77	22	144.51	24/07/01								
			spray to vines GS 79	23	161.54	08/08/01	0- DAT 24	20 / 30	0.351	0.060	0.025	0.009	0.240	
			spray to vines GS 81	24	160.13	23/08/01	0+ DAT 24	20 / 30	0.390	0.068	0.019	0.009	0.257	
			spray to vines GS 13-16	25	59.85	15/05/02	0- DAT25	20 / 30	0.387	0.040	0.010	<0.005	0.224	
			spray to vines GS 53	26	80.15	29/05/02	0+ DAT 25	20 / 30	0.408	0.056	0.015	<0.005	0.247	
			spray to vines GS 59	27	100.06	15/06/02								
			spray to vines GS 71-73	28	120.07	02/07/02	0- DAT 28	20 / 30	0.307	0.074	0.009	0.007	0.207	
			spray to vines GS 77-79	29	120.17	19/07/02	0+ DAT 28	20 / 30	0.420	0.064	0.013	0.007	0.262	
			spray to vines GS 79	30	140.70	02/08/02								
			spray to vines GS 79-83	31	159.47	14/08/02	0- DAT 32	20 / 30	0.489	0.064	0.018	0.010	0.305	
			spray to vines GS 83	32	161.01	29/08/02	0+ DAT 32	20 / 30	0.411	0.067	0.024	0.007	0.270	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent					Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
Johnson 2006a BASF Doc ID: 2006/7011058	Schwabenheim, Germany Silt, pH 7.5, 2.56 % OC	500g as/l SC formulation	spray to vines GS 14	33	59.86	06/05/03	0-DAT 33	20/ 30	0.397	0.126	0.040	0.013*	0.315	
			spray to vines GS 16	34	81.30	21/05/03	0+ DAT 33	20 / 30	0.345	0.098	0.018	0.013*	0.253	Procedural recovery at LOQ (0.005 mg/kg): 93-114% parent, 59-95% CL 377160
			spray to vines GS 63-65	35	100.74	05/06/03								
			spray to vines GS 73	36	119.77	19/06/03	0- DAT 36	20 / 30	0.313	0.105	0.021	<0.005	0.230	
			spray to vines GS 81	37	120.42	03/07/03	0+ DAT 36	20 / 30	0.354	0.099	0.025	0.026	0.278	No residues of CL 377160 ≥LOQ at any depth
			spray to vines GS 83-85	38	140.10	21/07/03								
			spray to vines GS 83-85	39	158.99	05/08/03	0- DAT 40	20 / 30	0.366	0.101	0.012	0.010	0.256	
			spray to vines GS 85-87	40	160.06	21/08/03	0+ DAT 40	20 / 30	0.381	0.114	0.019	0.007	0.274	
			spray to vines GS 15	41	59.96	19/05/04	0- DAT 41	20 / 30	N/A	N/A	N/A	N/A	-	
			spray to vines GS 55	42	80.51	04/06/04	0+ DAT 41	20 / 30	N/A	N/A	N/A	N/A	-	
			spray to vines GS 68	43	100.31	21/06/04								
			spray to vines GS 69	44	120.43	01/07/04	0- DAT 44	20 / 30	0.496	0.139	0.024	0.018	0.360	
			spray to vines GS 75	45	120.81	13/07/04	0+ DAT 44	20 / 30	0.491	0.146	0.041	0.020	0.380	
			spray to vines GS 81	46	141.69	27/07/04								
			spray to vines GS 83	47	162.43	11/08/04	0- DAT 48	20 / 30	0.336	0.071	0.015	0.005	0.224	
			spray to vines GS 83-85	48	160.65	25/08/04	0+ DAT 48	20 / 30	0.393	0.061	0.020	0.009	0.256	
							254+ DAT 48	20 / 30	0.363	0.087	0.009	0.006	0.240	

* Average of duplicate analyses
N/A – Samples not analyzed

Table 7.1.2.2.2-5: Summary of the field study on metrafenone soil accumulation following application to cereals at Schwabenheim

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No cores per sample depth (cm)	Residue, parent				Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
Johnson 2006b BASF Doc ID: 2006/7011059	Schwabenheim, Germany Silt, pH 7.2, 2.17 % OC	200g as/l SC formulation	spray to spring barley GS 31	1	200	20/05/99	0-DAT1	20/ 30	<0.005	<0.005	N/A	<0.005	
			spray to spring barley GS 61	2	200	07/06/99	0+ DAT 1	20 / 30	0.009	<0.005	N/A	0.009	Procedural recovery at LOQ (0.005 mg/kg): 84 – 107 % parent, 64 – 90 % CL 377160
							0- DAT 2	20 / 30	0.018	<0.005	N/A	0.018	
							0+ DAT 2	20 / 30	0.024	<0.005	N/A	0.024	
		300g as/l SC formulation	spray to winter wheat GS 45 - 47	3	207.30	15/05/00	0- DAT3	20 / 30	0.030	<0.005	N/A	0.030	No residues of CL 377160 ≥LOQ at any depth
			spray to winter wheat GS 61 - 63	4	199.69	31/05/00	0+ DAT3	20 / 30	0.049	<0.005	N/A	0.049	
							0- DAT 4	20 / 30	0.053	<0.005	N/A	0.053	
							0+ DAT 4	20 / 30	0.064	<0.005	N/A	0.064	
							64+ DAT 4	Straw	1.32*	-	-		

dy Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment Method kind	Trt No.	g as/ha	Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent (mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	Remarks:
Johnson 2006b BASF Doc ID: 2006/ 7011059	Schwabenheim, Germany Silt, pH 7.2, 2.17 % OC	300g as/l SC formulation	spray to winter wheat GS 61 - 63				154+ DAT4	20 / 30	0.047	0.028	<0.005	N/A	0.038	
							330+ DAT4	20 / 30	0.043	0.024	<0.005	N/A	0.034	Procedural recovery at LOQ (0.005 mg/kg): 84 – 107 % parent, 64 – 90 % CL 377160
		300g as/l SC formulation	spray to winter wheat GS 31	5		23/04/02	0-DAT5	20 / 30	0.026	0.018	<0.005	<0.005	0.022	
			spray to winter wheat GS 61 - 65	6		04/06/02	0+ DAT5	20 / 30	0.119	0.022	<0.005	<0.005	0.071	
							0- DAT 6	20 / 30	0.093	0.027	<0.005	<0.005	0.060	
							0+ DAT 6	20 / 30	0.150	0.026	<0.005	N/A	0.088	
		300g as/l SC formulation	spray to winter barley GS 39	7	202.58	05/05/03	0- DAT 7	20 / 30	0.058	0.043	0.008	<0.005	0.059	No residues of CL 377160 ≥LOQ at any depth
			spray to vines GS 47 - 59	8	199.25	14/05/03	0+ DAT 7	20 / 30	0.267	0.050	0.010	<0.005	0.169	
							0- DAT 8	20 / 30	0.330	0.039	0.008	<0.005	0.193	
							0+ DAT 8	20 / 30	0.316	0.052	0.008	<0.005	0.192	
							56+ DAT 8	20 / 30	0.292	0.053	0.012	<0.005	0.185	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment Method kind	Trt No.	g as/ha	Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent (mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	Remarks:
Johnson 2006b BASF Doc ID: 2006/ 7011059	Schwabenheim, Germany Silt, pH 7.2, 2.17 % OC	300g as/l SC formulation	spray to winter oilseed rape GS 65 - 69	9	202.69	29/04/04	0-DAT 9	20/ 30	N/A*	N/A*	N/A*	N/A*	-	
			spray to winter oilseed rape GS 71 - 73	10	200.33	18/05/04	0+ DAT 9	20 / 30	N/A*	N/A*	N/A*	N/A*	-	Procedural recovery at LOQ (0.005 mg/kg): 93-114% parent, 59-95% CL 377160
							0- DAT 10	20 / 30	N/A*	N/A*	N/A*	N/A*	-	
							0+ DAT 10	20 / 30	N/A*	N/A*	N/A*	N/A*	-	No residues of CL 377160 ≥LOQ at any depth apart from in the 0-5 cm soil layer 71 DAT10, 0-DAT12, and 0+DAT12, when residues were 0.007, 0.005, and 0.005 mg/kg respectively layer 71 DAT10, 0-DAT12, and 0+DAT12, when residues were 0.007, 0.005, and 0.005 mg/kg respectively.
							71+ DAT 10	20 / 30	0.192	0.061	0.009	<0.005	0.136	
		300g as/l SC formulation	spray to winter wheat GS 29 - 30	11	197.78	18/04/05	0- DAT 11	20 / 30	0.067	0.047	0.008	<0.005	0.065	
			spray to winter wheat GS 61	12	198.61	08/06/05	0+ DAT 11	20 / 30	0.396	0.067	0.013	<0.005	0.245	
							0- DAT 12	20 / 30	0.270	0.069	0.009	<0.005	0.179	
							0+ DAT 12	20 / 30	0.311	0.077	0.012	<0.005	0.206	
							65+ DAT 12	20 / 30	0.189	0.070	0.010	<0.005	0.140	
							307+ DAT12	20 / 30	N/A*	N/A*	N/A*	N/A*	-	

N/A – Samples not analyzed
* Value for straw in mg as/kg straw. No residues of any metabolite were observed ≥LOQ

Table 7.1.2.2.2-6: Summary of the field study on metrafenone soil accumulation following application to bare soil at Sasso Morelli, Italy

Study Reference	Location/ soil properties/ plot size	Test material	Application rate per treatment			Applic-ation dates	Sample DAT No.	No cores per sample	Residue, parent				Remarks:
		Type	Method kind	Trt No.	g as/ha			depth (cm)	(mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
Johnson 2006c BASF Doc ID: 2006/ 7011060	Sasso Morelli, Italy Clay, pH 7.4, 1.2 % OC	500g as/l SC formulation	spray to bare soil	1	100	12/05/99	0-DAT1	20/ 30	<0.02	<0.02	N/A	<0.02	
				2	100	28/05/99	0+ DAT 1	20 / 30	0.02	<0.02	N/A	0.02	Procedural recovery at LOQ (0.02 mg/kg) for RLA 12618V: 96 – 114 % parent, 72 - 100 % CL 377160
				3	100	09/06/99							
				4	100	24/06/99	0- DAT 8	20 / 30	0.14	<0.02	N/A	0.14	
				5	100	07/07/99	0+ DAT 8	20 / 30	0.13	<0.02	N/A	0.13	
				6	100	23/07/99							No residues of CL 377160 ≥LOQ at any depth
				7	100	05/08/99							
				8	99.48	19/08/99	182+ DAT1	20 / 30	0.05	<0.02	N/A	0.05	
		500g as/l SC formulation	spray to bare soil	9	99.79	11/05/00	0- DAT9	20 / 30	0.07	<0.02	N/A	0.07	
				10	99.69	25/05/00	0+ DAT9	20 / 30	0.10	<0.02	N/A	0.10	
				11	99.79	08/06/00							
				12	99.69	23/06/00	0- DAT 16	20 / 30	0.20	<0.02	N/A	0.20	
				13	99.69	05/07/00	0+ DAT 16	20 / 30	0.22	0.07	<0.02	0.29	
				14	99.69	19/07/00							
				15	99.90	03/08/00							
				16	99.69	18/08/00	175+DAT 9	20 / 30	0.23	0.06	0.04	0.33	

Study Reference	Location/ soil properties/ plot size	Test material	Application rate per treatment			Applic-ation dates	Sample DAT No.	No cores per sample	Residue, parent				Remarks:
		Type	Method kind	Trt No.	g as/ha			depth (cm)	(mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
		500g as/l SC formulation	spray to bare soil	17	96.88	24/05/01	0- DAT17	20 / 30	0.11	<0.02	<0.02	0.11	
				18	95.83	07/06/01	0+ DAT17	20 / 30	0.16	<0.02	N/A	0.16	
				19	96.88	20/06/01							
				20	97.40	04/07/01	0-DAT24	20 / 30	0.63	0.03	<0.02	0.66	
				21	97.40	19/07/01	0+ DAT24	20 / 30	0.65	0.04	<0.02	0.69	
				22	96.88	01/08/01							
				23	95.83	16/08/01							
				24	98.96	30/08/01	255+DAT17	20 / 30	0.12	<0.02	N/A	0.12	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment Method kind	Trt No.	g as/ha	Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent (mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	Remarks:
Johnson 2006c BASF Doc ID: 2006/ 7011060	Sasso Morelli, Italy Clay, pH 7.4, 1.2 % OC	500g as/l SC formulation	spray to bare soil	25	96.09	23/05/02	0- DAT 25	20 / 30	0.09*	0.09*	0.03	<0.02	0.12	Procedural recovery at LOQ (0.02 mg/kg) for M3441 (modified): 76.5 – 106.3 % parent, 73.6 – 99.2 % CL 377160
				26	100	05/06/02	0+ DAT 25	20 / 30	0.27	0.04	<0.02	N/A	0.16	
				27	98.96	19/06/02								
				28	98.70	03/07/02	0- DAT 32	20 / 30	0.21	0.03	0.04	<0.02	0.16	
				29	97.92	18/07/02	0+ DAT 32	20 / 30	0.43	0.06	0.06	<0.02	0.31	
				30	94.79	31/07/02								No residues of CL 377160 ≥LOQ at any depth
				31	96.88	15/08/02								
				32	97.40	28/08/02	184+ DAT 25	20 / 30	0.25	0.03	<0.02	N/A	0.14	
		500g as/l SC formulation	spray to bare soil	33	95.42	14/05/03	1-DAT33	20 / 30	0.31	0.04	<0.02	N/A	0.18	
				34	98.44	27/05/03	0+DAT33	20 / 30	0.39	0.04	<0.02	N/A	0.22	
				35	94.27	11/06/03								
				36	96.35	25/06/03	0-DAT40	20 / 30	0.45	0.08	0.04	<0.02	0.31	
				37	98.96	09/07/03	0+DAT40	20 / 30	0.57	0.07	0.03	<0.02	0.35	
				38	98.96	23/07/03								
				39	96.88	06/08/03								
				40	98.96	20/08/03	181DAT33	20 / 30	0.25	<0.02	<0.02	<0.02	0.13	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent					Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-5 cm depth	(mg/kg) 5-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
		500g as/l SC formulation	spray to bare soil	41	91.7	19/05/04	0-DAT41	20 / 30	0.27	0.07	0.04	<0.02	0.21	
				42	97.9	02/06/04	0+DAT41	20 / 30	0.41	0.05	0.02	0.02	0.27	
				43	97.9	16/06/04								
				44	98.4	29/06/04	1-DAT48	20 / 30	0.40	0.05	0.02	<0.02	0.25	
				45	101.6	14/07/04	0+DAT48	20 / 30	0.51	0.05	0.03	<0.02	0.31	
				46	99.0	28/07/04								
				47	95.3	12/08/04	182+DAT41	20 / 30	0.31	0.05	0.03	<0.02	0.21	
				48	98.1	25/08/04	369+DAT41	20 / 30	0.23	0.03	<0.02	<0.02	0.13	

* 0-10 cm layer analyzed, therefore concentration relevant to both 0-5 cm and 5-10 cm layers.
N/A – Samples not analyzed

Table 7.1.2.2.2-7: Summary of the field study on metrafenone soil accumulation following application to bare soil at Fuentiduena de Tajo, Spain

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment Method kind	Trt No.	g as/ha	Applic-ation dates	Sample DAT No.	No cores per sample depth (cm)	Residue, parent (mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	Remarks:
Johnson 2006d BASF Doc ID: 2006/ 7011061	Fuentiduena de Tajo, Spain Sandy loam, pH 8.0, 0.2 % OC	500g as/l SC formulation	spray to bare soil	1	102.03	17/05/99	0-DAT1	20/ 30	<0.02	<0.02	N/A	<0.02	
				2	107.81	31/05/99	0+ DAT 1	20 / 30	0.05	<0.02	N/A	0.05	Procedural recovery at LOQ (0.02 mg/kg) for RLA 12618V: 87 – 110 % parent, 73 – 103 % CL 377160
				3	107.08	14/06/99							
				4	97.50	28/06/99	0- DAT 8	20 / 30	0.05	<0.02	N/A	0.05	
				5	101.56	12/07/99	0+ DAT 8	20 / 30	0.09	<0.02*	N/A	0.09	
				6	92.18	26/07/99							
				7	102.60	09/08/99							
				8	113.02	23/08/99	183+ DAT1	20 / 30	0.08	<0.02	N/A	0.08	
		500g as/l SC formulation	spray to bare soil	9	109.30	18/04/00	0- DAT9	20 / 30	0.03	<0.02	N/A	0.03	No residues of CL 377160 ≥LOQ (0.02 mg/kg) at any depth
				10	94.65	03/05/00	0+ DAT9	20 / 30	0.14	<0.02	N/A	0.14	
				11	104.71	15/05/00							
				12	105.66	30/05/00	0- DAT 16	20 / 30	N/A‡	N/A‡	N/A‡	-	
				13	103.45	13/06/00	10+ DAT 16	20 / 30	0.12	<0.02	N/A	0.12	
				14	108.03	27/06/00							
				15	106.34	11/07/00							
				16	100.38	24/07/00	183+DAT 9	20 / 30	0.04	<0.02	N/A	0.04	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No cores per sample depth (cm)	Residue, parent				Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-10 cm depth	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
		500g as/l SC formulation	spray to bare soil	17	100.63	14/06/01	0- DAT17	20 / 30	0.03*	<0.02*	N/A	0.03	
				18	100.00	28/06/01	0+ DAT17	20 / 30	0.08*	<0.02*	N/A	0.08	
				19	100.21	12/07/01							
				20	99.79	26/07/01	0-DAT24	20 / 30	0.12*	<0.02*	N/A	0.12	
				21	99.79	09/08/01	0+ DAT24	20 / 30	0.13*	<0.02*	N/A	0.13	
				22	99.79	23/08/01							
				23	99.38	06/09/01							
				24	100.0	20/09/01	181+DAT17	20 / 30	0.11*	<0.02*	N/A	0.11	

Study Reference	Location/ soil properties/ plot size	Test material	Application rate per treatment			Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent					Remarks:
		Type	Method kind	Trt No.	g as/ha				(mg/kg) 0-5 cm depth†	(mg/kg) 5-10 cm depth†	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
Johnson 2006d BASF Doc ID: 2006/ 7011061	Fuentiduena de Tajo, Spain Sandy loam, pH 8.0, 0.2 % OC	500g as/l SC formulation	spray to bare soil	25	100.00	28/05/02	0- DAT 25	20 / 30	0.07*	0.07*	<0.02*	N/A	0.07	Procedural recovery at LOQ (0.02 mg/kg) for M3441 (modified): 91 - 111 % parent, 81 - 103 % CL 377160
				26	99.58	13/06/02	0+ DAT 25	20 / 30	0.07*	0.07*	<0.02*	N/A	0.07	
				27	100.21	27/06/02								
				28	100.63	11/07/02	0- DAT 32	20 / 30	0.13*	0.13*	<0.02*	N/A	0.13	
				29	101.04	23/07/02	0+ DAT 32	20 / 30	0.19*	0.19*	<0.02*	N/A	0.19	
				30	100.21	07/08/02								
				31	99.79	21/08/02								
				32	100.83	04/09/02	181+ DAT 25	20 / 30	0.07	0.07	<0.02	N/A	0.07	
		300g as/l SC formulation	spray to bare soil	33	100.21	28/05/03	1-DAT33	20 / 30	0.13	0.04	<0.02	N/A	0.09	No residues of CL 377160 ≥LOQ (0.02 mg/kg) at any depth
				34	100.00	11/06/03	0+DAT33	20 / 30	0.16	0.06	<0.02	N/A	0.11	
				35	100.21	25/06/03								
				36	100.00	09/07/03	0-DAT40	20 / 30	0.24	0.02	<0.02	N/A	0.13	
				37	99.79	23/07/03	0+DAT40	20 / 30	0.33	0.05	<0.02	N/A	0.19	
				38	100.00	07/08/03								
				39	99.58	21/08/03								
				40	99.79	03/09/03	182+DAT3 3	20 / 30	0.11	<0.02	<0.02	N/A	0.06	

Study Reference	Location/ soil properties/ plot size	Test material Type	Application rate per treatment			Applic-ation dates	Sample DAT No.	No. cores per sample depth (cm)	Residue, parent					Remarks:
			Method kind	Trt No.	g as/ha				(mg/kg) 0-5 cm depth†	(mg/kg) 5-10 cm depth†	(mg/kg) 10-20 cm depth	(mg/kg) 20-30 cm depth	(mg/kg) Total	
		500g as/l SC formulation	spray to bare soil	41	101.25	04/06/04	0-DAT41	20 / 30	0.04	<0.02	<0.02	N/A	0.02	
				42	99.79	16/06/04	0+DAT41	20 / 30	0.14	0.02	<0.02	<0.02	0.08	
				43	99.79	30/06/04								
				44	98.96	14/07/04	0-DAT48	20 / 30	0.13	<0.02	<0.02	<0.02	0.07	
				45	99.58	28/07/04	0+DAT48	20 / 30	0.26	<0.02	<0.02	<0.02	0.13	
				46	100.20	11/08/04								
				47	100.00	25/08/04	188+DAT41	20 / 30	0.07	<0.02	<0.02	<0.02	0.04	
				48	100.40	09/09/04	375+DAT41	20 / 30	0.06	<0.02	<0.02	<0.02	0.03	

* Mean of two or more analyses.
‡ Samples taken but not analyzed due to breakdown of freezer
†For 2002 samples the 0-10 cm layer analyzed, therefore concentration relevant to both 0-5 cm and 5-10 cm layers.
N/A – Samples not analyzed

Figure 7.1.2.2.2-1: Concentrations of metrafenone in soil in the soil accumulation study at Schwabenheim, Germany following application of metrafenone to vines

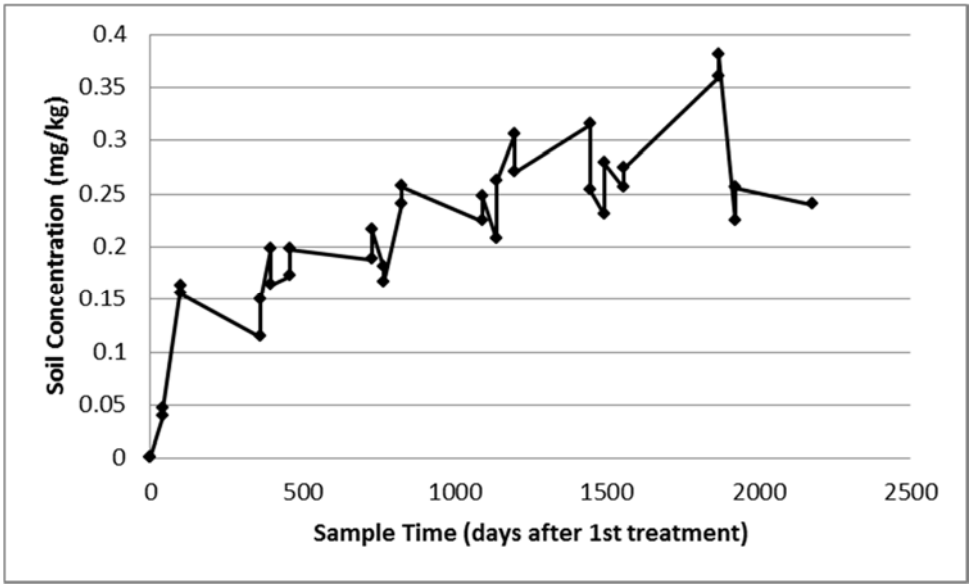


Figure 7.1.2.2.2-2: Concentrations of metrafenone in soil in the soil accumulation study at Schwabenheim, Germany following application of metrafenone to cereals

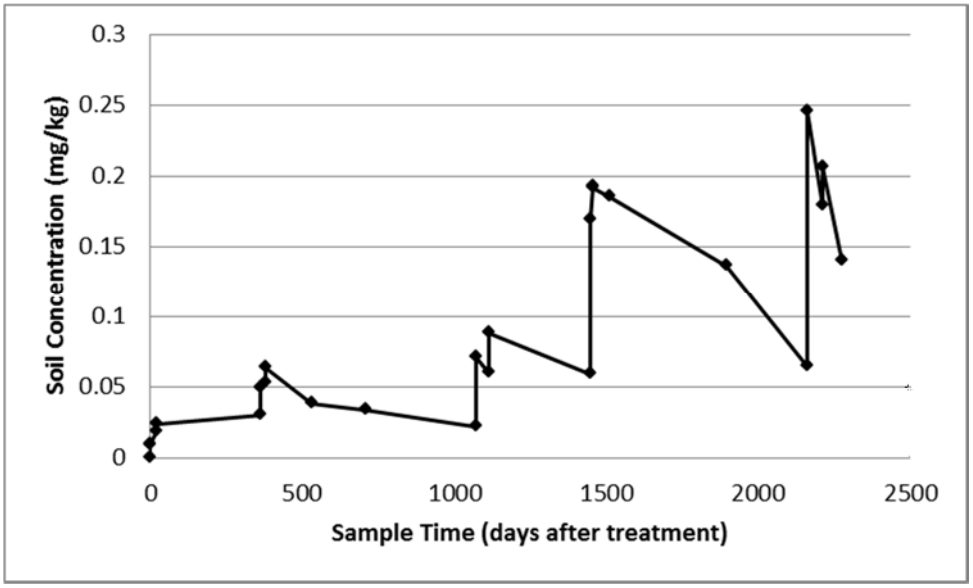


Figure 7.1.2.2.2-3: Concentrations of metrafenone in soil in the bare soil accumulation study at Sasso Morelli, Italy

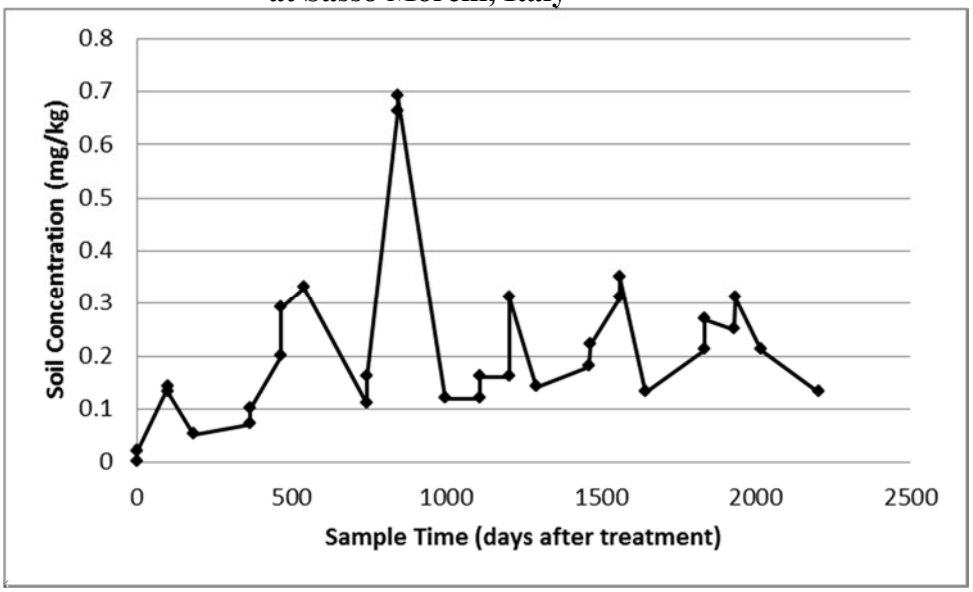
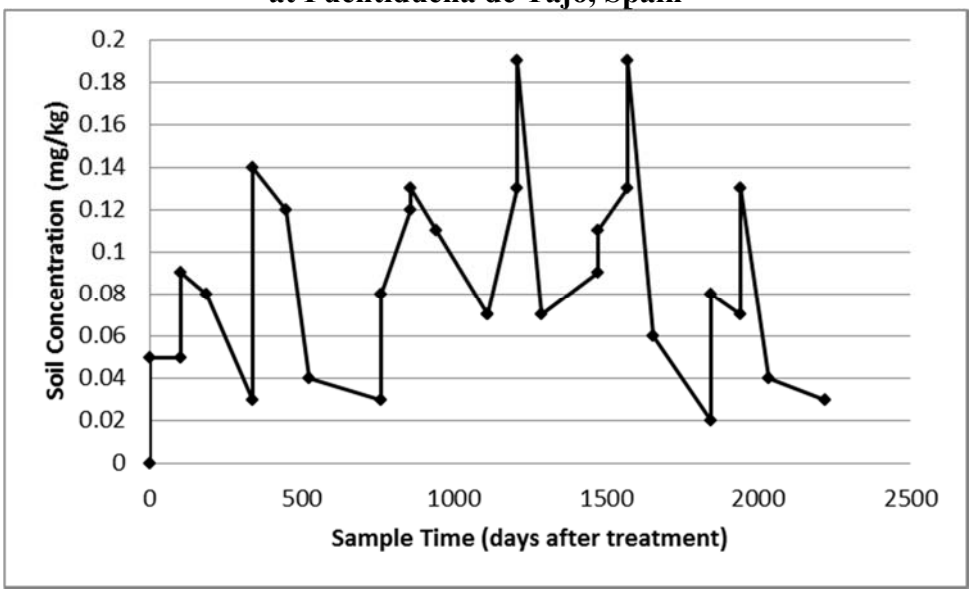


Figure 7.1.2.2.2-4: Concentrations of metrafenone in soil in the bare soil accumulation study at Fuentiduena de Tajo, Spain



III. CONCLUSION

The total soil residue concentration of metrafenone in three of the four trials was observed to have reached a plateau by the study termination. In the case of the Schwabenheim cereal trial with the exception of the single sample taken 0+DAT11, comparison of equivalent sample concentrations from 2003 to 2005 indicated that a plateau had been reached, or only very small increases in total metrafenone concentrations had occurred over the two year period. The maximum accumulated metrafenone soil concentration of 0.65 mg/kg was observed in the upper 0 – 5 cm soil layer at the Italian trial immediately after the final application in the third season.
No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample for three of the four studies, and in the Schwabenheim were only rarely observed in the 0 – 5 cm soil layer, with a maximum concentration in that soil layer of 0.007 mg/kg.

No residues of either metrafenone or its metabolite CL 377160 were observed above the LOQ in untreated plots in any trial, with the single exception of the 184+ DAT25 in the Italy trial, where the untreated plot contained apparent residues in the 10 – 20 cm soil layer of 0.03 mg/kg.

Report:	CA 7.1.2.2.2/5 Beigel C., 2002a Estimation of the potential accumulation of BAS 560 F in EU soils following applications to cereals and vines using the FOCUS groundwater scenarios Shell PEARL 2002/7005185
Guidelines:	none
GLP:	no

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes soil accumulation, is presented at the end of this section (Section 7.1.2).

Report:	CA 7.1.2.2.2/6 Beigel C.,Ta C., 2003b Response to UK Pesticides Safety Directorate request for clarification, concerning application for provisional approval for the UK use of BAS 560 00 F and Annex 1 listing for Metrafenone 2003/1020208
Guidelines:	none
GLP:	no

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the rate of degradation in soil, which includes soil accumulation, is presented at the end of this section (Section 7.1.2).

Summary of Rate of Degradation in Soil

For the first EU review, the rate of degradation of metrafenone in aerobic soil was investigated in the studies of Steinfuehrer, 2000a & b, in a total of four soils incubated at 20° C, and in a third study in 1 soil incubated at 10 °C (Steinfuehrer and Weis 2000). Since the original assessment in the Draft Assessment Report (UK, 2005) metrafenone was finalized, the FOCUS Degradation Kinetics Report (FOCUS 2006), which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from laboratory and field studies, has been adopted. Therefore the degradation rates of metrafenone were re-calculated in accordance with current FOCUS Kinetics guidance in the report of Hilton and Callow (2014).

The degradation rates, re-calculated in accordance with FOCUS Kinetics guidance, and normalized to 20 °C and pF2 are presented in Table 7.1.2.2.2-8. The SFO model provided a good visual and statistical fit in all soils, and the results confirmed the persistent nature of metrafenone in laboratory aerobic soils, with a geometric mean DT_{50}, normalized to 20 °C and pF2, of 200.9 days.

Table 7.1.2.2.2-8: Normalized laboratory DT_{50}s for metrafenone at 20°C and pF 2

Soil	Temp. (°C)	Incubation soil moisture (% MWHC)	DT_{50} (days)	DT_{90} (days)	χ^2 % error	Normalized DT_{50} (days)
Engelstadt/ Benz silty loam	20	50	236.0	784.0	2.9	215.4
Sporkenheim loamy sand	20	50	154.7	513.9	5.9	154.7
Binger Pfad sandy loam	20	50	275.3	914.4	5.1	252.1
Gensingen clay loam	20	50	243.1	807.4	5.0	194.1
Sporkenheim loamy sand	10	40 – 50*	532.6	1767.5	2.7	206.1
Geometric mean =						200.9**

* As a worst case assumption soil moisture content in the study was assumed as 50 % MWHC for the entire study duration.
** The moisture and temperature normalized geometric mean DT_{50} value at 20°C was calculated with the exclusion of the Sporkenheim soil test performed at 10 °C.

For the first EU review, the aerobic soil degradation of the soil photolysis metabolite CL 377160, was investigated in three soils in the study of Afzal, 2002. CL 377160 was rapidly degraded in all three soils under aerobic conditions, with concentration in solvent extracts <10 % AR in all three soils after 7 days incubation. Thus, it was not possible to calculate reliable degradation rates for CL 377160, and a worst case DT_{90} value of 7 days was estimated. As an extreme worst case, a DT_{50} value of 7 days was considered in exposure assessments. Because of the low number of data points, re-calculation of degradation rates for CL 377160 in accordance with FOCUS Kinetics guidance is not possible; however, the worst case aerobic soil DT_{50} value of 7 days remains an appropriate worst case value for use in exposure assessments.

The soil photolysis metabolites CL 3000402 and M2 (considered as CL 1500831 pending confirmatory analysis) were observed in the study of Adam (2015) at concentrations > 5 %AR and trigger further assessment. A soil aerobic degradation study, performed in accordance with OECD 307, is ongoing for CL 3000402 and the results will be reported when they are available. The identity of M2 as CL 1500831 was not confirmed due to the lack of an available standard, and final confirmation of its identity is ongoing. However, as discussed, based upon the LC/MS/MS fragmentation pattern, M2 is considered most likely to be CL 1500831. CL 3000402 (lactone of the bromophenyl ring) and CL 1500831 (lactone of the trimethoxyphenyl ring) are structurally very similar (see degradation pathway in Figure 7.1.1.3-3 for structures). The physical/ chemical properties calculated for the two metabolites using the OECD QSAR Toolbox v3.3 (See Pallizzaro and Da Silva, 2015, summarized in M-CA Section 5.8.1) are the same (See Table 7.1.2.2.2-9). Taking into account additional K_{oc} information calculated using EPIWEB 4.1, it can be predicted that the degradation rates of the two compounds will be very similar. Therefore extrapolation of aerobic soil degradation DT_{50} values from the study conducted with CL 3000402 to CL 1500831 is appropriate for use in exposure modelling when results are available.

Table 7.1.2.2.2-9: Physical/ chemical properties for the soil photolysis metabolites CL 3000402 and CL 1500831 calculated using EPISUITE 4.1

	CL 3000402	CL 1500831
Molecular Mass (g/mol)	423.3	423.3
Vapor Pressure at 25 °C (Pa)	3.29×10^{-8}	3.29×10^{-8}
Water Solubility at 25 °C; K_{ow} method (mg/ L)	2.86	2.86
Water Solubility at 25 °C; estimated from fragments (mg/ L)	0.063	0.063
Koc; K_{ow} method (L/kg)	693.3	693.3
Koc; MCI method (L/kg)	2.81×10^{4}	2.81×10^{4}
Log K_{ow}	3.38	3.38
Experimental LogK_{ow}	3.0	-

The rate of degradation of metrafenone under laboratory anaerobic soil conditions was previously investigated in two studies and a total of two soils incubated at 20 °C. Anaerobic soil degradation rates of metrafenone were re-calculated in accordance with current FOCUS Kinetics guidance in the report of Hilton and Callow (2014). The SFO kinetic model provided the best fit to the data on the basis of a statistical and visual assessment, and the re-calculated degradation rates confirmed the faster degradation of metrafenone under anaerobic soil conditions. Re-calculated anaerobic soil DT_{50} values for metrafenone at 20 °C were 7.3 days and 15.6 days.

Based upon the soil photolysis study of Ta (2001), performed with metrafenone labelled in the trimethoxyphenyl ring, the first EU review concluded that soil photolysis would also contribute to the degradation of metrafenone in soil, on the basis of its much faster degradation under photolytic conditions. The rates of soil photolytic degradation of metrafenone and CL 377160 in the study of Ta (2001) were re-calculated in accordance with current FOCUS Kinetics guidance in the report of Hilton and Callow (2014). The additional study of Adam (2015), in which the soil photolytic rate of degradation of metrafenone, ^{14}C-labelled in both the bromophenyl and trimethoxy rings, was investigated, has now also been performed.

Following a kinetic re-evaluation, SFO DT_{50} values for metrafenone were calculated as 12.6 days and 157.3 days for irradiated and dark conditions, respectively, in the Engelstadt/Benz soil from the study of Ta (2001). An irradiated only DT_{50} value of 13.7 days was therefore calculated. In the study of Adam (2015) metrafenone degraded in Am Fischteich silt loam soil with a SFO DT_{50} value of 16.6 days under the conditions of the test; corresponding to 31.6 days of natural summer sunlight at 30 to 50°N. Dark control degradation rates were not calculated because of the very slow degradation observed. Therefore, metrafenone degraded at a faster rate under illuminated conditions than in a dark control in both studies.

Because of the low formation of CL 377160 in dark control samples, a kinetic evaluation of the degradation of CL 377160 was only possible in the irradiated samples of the study of Ta (2001). A DT_{50} value of 5.5 days was calculated for the soil photolytic degradation of CL 377160 under the conditions of the test. It was not possible to calculate reliable photolytic degradation rates for metabolites observed in the study of Adam (2015) because of the lack of a decline phase being observed.

Adequate data to assess the dissipation in field soil of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, and therefore no further data are considered necessary. The field dissipation trials were conducted at four sites in Europe: located in Germany, United Kingdom, Denmark and Northern France. A single application of an SC formulation of metrafenone was applied to bare soil at a rate of approximately 0.4 kg as/ha. Soil samples, in the form of soil cores with a depth of 54 – 60 cm, were collected up to 482 – 487 days after application. Cores were separated into 10 cm depth segments and analyzed for metrafenone and CL 377160. An LOQ of 0.02 mg/kg was reported for both analytes, which was equivalent to 8.7 – 11.8 % of the maximum metrafenone residues observed in the individual trials. Metrafenone was not observed above the LOQ below the 0-10 cm soil layer in any of the trials. CL 377160 was not observed above the LOQ in any soil layer in any of the trials.

The EFSA Conclusion from the original review reports that because applications were made to bare soil, the shorter DT_{50} values observed in field dissipation studies, compared to laboratory aerobic soil degradation studies, may be due to the contribution of soil photolysis. It was concluded that since the field studies were performed on bare soils and the representative uses are for developed crops where the foliage will shadow the field, laboratory studies should be considered to derive the DT_{50} for use in risk assessments.

The field degradation rates of metrafenone were re-calculated in accordance with FOCUS Kinetics guidance for the derivation of end-points for comparison to persistence triggers, in the study of Hilton and Callow 2014b. Because the previous review considered that field studies were not appropriate for deriving modelling end-points, a kinetic evaluation was not re-performed for their derivation. On the basis of the statistical and visual fit, SFO kinetics returned the best fits for the trials conducted in Germany and the UK, whereas trials conducted in Northern France and Denmark displayed biphasic degradation for metrafenone, with FOMC kinetics providing the best fit for Northern France, and DFOP for Denmark. Field dissipation DT_{50} and DT_{90} values are presented in Table 7.1.2.2.2-10.

Table 7.1.2.2.2-10: Field dissipation rates for metrafenone

Site	Model	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)
Germany	SFO	143	473	12.6
UK	SFO	145	483	31.5
Northern France	FOMC	22.2	1221*	22.6
Denmark	DFOP	51.7	NC	9.6

NC = Not calculated by KINGUII
* = value extrapolated beyond study duration

Data to assess the field accumulation in soil of metrafenone were evaluated during the first EU review. However the four studies (Smalley, 2002m; Young, 2002a & b; Jones, 2002b) summarized in the Draft Assessment Report for metrafenone were not finalized at that time, nor were they finalized at the time the EFSA Conclusion (EFSA 2006) was produced. The studies are now finalized as Johnston 2006a-d, and are therefore included in this submission. Study summaries are presented CA 7.1.2.2.2.

The four soil accumulation studies were conducted on bare soil plots in Italy and Spain, and on two plots at a site in Schwabenheim Germany; the first cropped with vines and the second cropped with cereals. Spray applications of varying SC formulations of metrafenone were applied at all trial sites. At the first Schwabenheim trial, eight applications were made to vines every year, for 6 consecutive years (1999 – 2004 inclusive), with individual application rates of between 60 – 160 g as/ ha. For the second Schwabenheim trial two applications of 200 g as/ ha were made to a field cropped with cereals for seven years between 1999 and 2005 inclusive, with the exception of 2001, when no applications were made. For the Italian and Spanish trials eight applications of 100 g as/ ha were applied to bare soil every year for six years between 1999 and 2004 inclusive. Where crops were present cultivation methods during the trials were according to good agricultural practice, with the exception that at harvest crops were incorporated back into the soil/ worked in. No cultivation was performed for the Italian and Spanish bare soil trials.

Samples were collected annually from both treated and untreated plots in the form of 20 x 30 cm soil cores. A final sample was collected approximately 1 year after the 1st application in the season of final application. The majority of samples were separated into 0-5 cm, 5-10 cm, 10 – 20 cm, and 20 – 30 cm soil layers. The LOQ was 0.005 mg/kg for the Schwabenheim trials, and 0.02 mg/ kg for the Italian and Spanish trials.

The sum of total residue concentrations of metrafenone from all soil layers were calculated for the purposes of assessing the total soil accumulation of metrafenone. The total soil residue concentration of metrafenone in three of the four trials (Schwabenheim vine trial, Spanish and Italian bare soil trials) was observed to have reached a plateau by the study termination. In the case of the Schwabenheim cereal trial, a definitive judgement is complicated by the samples from 2004 which were lost and could not be analyzed. However, with the exception of the single sample taken 0+DAT11, comparison of equivalent sample concentrations from 2003 to 2005 indicated that a plateau had also been reached in this fourth trial. The maximum total accumulated metrafenone soil concentrations observed were 0.19 - 0.69 mg/kg.

No quantifiable residues of metabolite CL 377160 were ever observed in any layer of any sample for three of the four studies, and in the Schwabenheim were only rarely observed in the 0 – 5 cm soil layer, with a maximum concentration in that soil layer of 0.007 mg/kg.

In the absence of complete field soil accumulation studies, the additional modelling study of Beigel (2002b), with additional information in Beigel and Ta (2003), was considered as part of the original review. Modelling was performed using FOCUS PEARL version 1.1.1 and the relevant standard FOCUS groundwater scenarios for applications to spring cereals, winter cereals and vines, to assess the field accumulation of metrafenone. Applications were made at rates of 2 x 150 g as/ ha/ year to winter and spring cereals, and 8 x 100 g as/ ha/ year for vines. Appropriate crop interception values were considered. Worst case values of a K_{om} of 3223 L/kg (corresponding to the worst case K_{oc} of 5556 L/kg) and a 1/n of 0.85, with a DT_{50} value of 345.4 days (the worst case laboratory DT_{50} from the original review corrected to 20 °C and pF2), were input in to the model for metrafenone.

The accumulation plateau was reached after approximately 10 years of annual applications. As a worst case assumption, all metrafenone in the top 1m of soil was considered to be present in the top 5 cm of the soil layer. A soil bulk density of 1.4 g/ cm^3 was assumed in the report, but correction was made by the RMS to a standard soil bulk density of 1.5 g/ cm^3. Peak accumulated PEC_{soil} values for the FOCUS scenarios were 0.426 – 0.832 mg/ kg, 0.453 – 0.843 mg/kg, and 0.656 – 1.336 mg/kg for the modelled applications to winter cereals, spring cereals and vines, respectively.

Comparison of the peak concentrations observed in the field accumulation studies to those from modelling demonstrates the conservative nature of the modelling previously submitted. Nevertheless, both the soil accumulation studies and the PEARL modelling were performed for cereal and vine GAPs in which a larger annual total dose was applied than is being proposed in the critical GAPs of 300 g as/ ha (2 x 150 g as/ ha) to cereals, and 480 g as/ ha (3 x 160 g as/ ha) to vines. Peak accumulated soil concentrations arising from the applied for uses would therefore be anticipated to be lower than those observed in soil accumulation studies. Maximum accumulated PEC_{soil} values for metrafenone for the proposed GAPs are additionally presented in the respective M-CP document for BAS 560 00 F and BAS 560 02 F at point CP 9.1.3.

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Adequate data to assess the adsorption/desorption of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.1.3.1.1/1 Fang C., 2001a BAS 560 F (AC 375839): Adsorption/desorption on soils (report amendment #1) 2001/7000271
Guidelines:	EEC 91/414 Annex II 7.1.2, EEC 95/36, OECD 106
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the adsorption and desorption in soil is presented at the end of this section (Section 7.1.3).

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Adequate data to assess the adsorption/desorption of the metabolites of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.1.3.1.2/1 Voelkel W., 2002a 14C-CL 377160: Adsorption/desorption on soils 2002/7005915
Guidelines:	EEC 91/414 Annex II 7.1.2, EEC 94/37, OECD 106, EPA 163-1
GLP:	yes (certified by Swiss Agency for the environment, forests, and landscape; Berne, Switzerland)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the adsorption and desorption in soil is presented at the end of this section (Section 7.1.3).

Report:	CA 7.1.3.1.2/2 Voelkel W., 2002b 14C-CL 377160: Adsorption/desorption on soils (Report amendment #1) 2002/7005921
Guidelines:	EEC 91/414 Annex II 7.1.2, EEC 94/37, OECD 106, EPA 163-1
GLP:	no

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the adsorption and desorption in soil is presented at the end of this section (Section 7.1.3).

CA 7.1.3.2 Aged sorption

Adequate data to assess the adsorption/desorption of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

Adequate data to assess the column leaching of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Adequate data to assess the column leaching of the metabolites of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.1.4.2 Lysimeter studies

Adequate data to address the data requirement for lysimeter studies were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.1.4.3 Field leaching studies

Adequate data to address the data requirement for field leaching studies were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Summary of adsorption/ desorption and mobility in soil

For the first EU review of metrafenone batch adsorption studies were conducted to investigate the adsorption of metrafenone and its metabolite CL377160.

The batch adsorption study conducted on metrafenone (Fang 2001) was performed on five soils with pH values of 5.8 – 7.6 when measured in 0.01 M $CaCl_2$, and organic carbon contents of 1.09 – 4.65 %. Adsorption K_{foc} values ranged from 1592 – 5556 mL/g with 1/n values of 0.85 – 0.95. In accordance with the EFSA guidance document for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values (EFSA Journal 2014;12(5):3662, implemented 1st May, 2015) a geometric mean adsorption K_{foc} of 2812 mL/g was calculated for metrafenone, with an arithmetic mean 1/n of 0.91. On initial inspection, it appears that adsorption of metrafenone may be pH dependent. However, this is not a strict correlation, and the structure of the molecule and its physical and chemical properties indicate that its adsorption would be unlikely to be affected by changes in pH. Thus it is considered that the apparent trend is not a real pH effect.

A batch adsorption study was also conducted on CL 377160 on five soils of pH 5.42 – 7.53 (0.01 M $CaCl_2$), and organic carbon contents of 1.22 – 2.98 %. Adsorption K_{foc} values ranged from 2199 – 21649 mL/g with 1/n values of 0.982 – 1.130. In accordance with EFSA guidance a geometric mean adsorption K_{foc} of 4061 mL/g and an arithmetic mean 1/n of 1.01 were calculated for CL 377160. There was no pH dependence.

Summaries of the K_{oc} values for metrafenone and its photolytic soil metabolite CL377160 are presented in Table 7.1.4.3-1 and Table 7.1.4.3-2 respectively.

Table 7.1.4.3-1: A summary of the adsorption characteristics of metrafenone in different soil types

Soil Type (USDA)	OC %	Soil pH ($CaCl_2$)	K_d (mL/g)	K_{doc} (mL/g)	K_f (mL/g)	K_{foc} (mL/g)	1/n
Inveresk Sandy loam	4.65	5.8	615	13226	258	5556	0.85
Speyer 2.2 Loamy sand	2.29	5.9	110	4803	86.9	3794	0.95
Engelstadt/Benz Silty loam	2.27	7.4	49.4	2176	36.2	1592	0.92
Ingelhein/Moers Sandy loam	1.33	7.6	38.4	2887	31.5	2367	0.94
Schwabenheim Silty loam	1.09	5.9	35.9	3294	24.1	2214	0.89
Geometric mean						2812	-
Arithmetic mean						-	0.91

Table 7.1.4.3-2: A summary of the adsorption characteristics of CL377160 in different soil types

Soil Type	OC %	Soil pH ($CaCl_2$)	K_d (mL/g)	K_{doc} (mL/g)	K_f (mL/g)	K_{foc} (mL/g)	1/n
Mechtildshausen Loam	1.22	7.27	127.8	10475	264.1	21649	1.130
Mussig Clay Loam	2.98	7.53	78	2617	73.5	2465	0.982
Bretagne 2 Silt loam	1.91	5.52	67.6	3539	66.1	3459	0.982
Huffoltz Silty clay loam	2.67	5.42	74.9	2805	63.1	2199	0.986
LUFA 2.2 Loamy sand	2.19	5.80	66.7	3046	62.1	2722	0.990
Geometric mean						4061	-
Arithmetic mean						-	1.014

The soil photolysis metabolites CL 3000402 and M2 (considered as CL 1500831 pending confirmatory analysis) were observed in the study of Adam (2015) at concentrations > 5 %AR and trigger further assessment. A batch adsorption/ desorption study, performed in accordance with OECD 106, is ongoing for CL 3000402 and the results will be reported when they are available. The identity of M2 as CL 1500831 was not confirmed due to the lack of an available standard, and final confirmation of its identity is ongoing. However, based upon the LC/MS/MS fragmentation pattern, M2 is considered most likely to be CL 1500831.

CL 3000402 (lactone of the bromophenyl ring) and CL 1500831 (lactone of the trimethoxyphenyl ring) are structurally very similar (see degradation pathway in Figure 7.1.1.3-3 for structures). The physical/ chemical properties calculated for the two metabolites using the OECD QSAR Toolbox v3.3 (See Pallizzaro and Da Silva, 2015, summarized in M-CA Section 5.8.1) are the same (See Table 7.1.2.2.2-9). Taking into account additional K_{oc} information calculated using EPIWEB 4.1, it is assumed that the adsorption characteristics of the two compounds will be the same. Therefore extrapolation of batch adsorption K_{oc} values from the study conducted with CL 3000402 to CL 1500831 is appropriate for use in exposure modelling.

No column leaching, lysimeter or field leaching studies were conducted, and none are required in accordance with the environmental fate and behavior data requirements for Regulation 1107/2009 set out under Regulation 283/2013.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

Adequate data to assess the hydrolytic degradation of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.2.1.1/1 An D., 1999a AC 375839: Hydrolysis 1999/7000284
Guidelines:	EEC 91/414 2.9.1, EEC 94/37, EEC 91/414 Annex II 7.2.1.1, EEC 95/36, OECD 111
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

CA 7.2.1.2 Direct photochemical degradation

Adequate data to assess the direct photochemical degradation of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

No aqueous photolysis metabolites exceeded 10 % AR, and therefore for the original review no aqueous photolysis metabolite required further assessment. Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study and are present at concentrations >5 % AR. Multiple metabolites were observed in the study of Fung (2002a), however, only CL4084564 exceeded 5 %AR on consecutive occasions, reaching a maximum of 8.7 % AR, and now requires further assessment.

Report:	CA 7.2.1.2/1 Fung C.H., 2002a BAS 560 F (AC 375839): Aqueous photolysis 2002/7005112
Guidelines:	EPA 161-2, EEC 91/414 Annex II 2.9.2, EEC 91/414 Annex II 2.9.3, EEC 94/37, SETAC Europe Part 1 Section 10
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

CA 7.2.1.3 Indirect photochemical degradation

Adequate data to assess the indirect photochemical degradation of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.2.1.3/1 Huang R., 2002a BAS 560 F: Natural water photolysis 2002/7004458
Guidelines:	EEC 91/414 Annex II 7.2.1.2, EEC 96/46, EPA 161-2, SETAC Europe Part 1 Section 10
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

Adequate data to assess the ready biodegradation of metrafenone were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.2.2.1/1 Bradley B.,Yan Z., 1999a AC 375839 ready biodegradability - modified sturm test 1999/7000306
Guidelines:	EEC 91/414 Annex II 7.2.1.3.1, EEC 92/69 C.4 C, EEC 95/36, OECD 301 B, EPA 835.3110
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

CA 7.2.2.2 Aerobic mineralisation in surface water

Report:	CA 7.2.2.2/1 Adam D., 2014b [14C] Metrafenone (BAS 560 F): Aerobic mineralisation in surface water - Simulation biodegradation test 2014/1083339
Guidelines:	OECD 309 (April 2004), SANCO 11802/2010/ rev.7 amending EC 1107/2009, EEC 79/117, EEC 91/414
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

The aerobic metabolism in surface waters of [trimethoxyphenyl-U-^{14}C]- and [bromophenyl-U-^{14}C]-labeled metrafenone was investigated in a freshly sampled, sieved pond water. Test systems were maintained in the dark at 21 ± 0.2 °C, and were continuously and gently stirred throughout the study duration. Individual test systems were connected to traps for collection of $^{14}CO_2$ and volatile organics, and were aerated with a stream of moistened air. Individual test flasks were set-up to investigate the aerobic mineralization of metrafenone for a high dose, a low dose, and a sterilized high dose. Total recoveries were in the range of 91.7 - 106.7 % of the applied radioactivity (% AR) for the majority of samples treated with ^{14}C-metrafenone. Metrafenone was stable, or degraded only very slowly, under the conditions of the test. No metabolites were observed, with the exception of small amounts of $^{14}CO_2$ (max formation 5.0 % AR) and other volatiles (maximum formation 0.7 % AR).

I. MATERIAL AND METHODS

A. MATERIALS

Metrafenone (BAS 560 F) labeled in the [trimethoxyphenyl-U-^{14}C]- (Batch No.: 911-1301; specific radioactivity: 5.65 MBq/mg; radiochemical purity: 98.1%) and the [bromophenyl-U-^{14}C]- (Batch No.: 1124-1101; specific radioactivity: 1.53 MBq/mg; radiochemical purity: 98.0%) positions was applied to freshly sampled water from a pond located in Rheinfelden, Switzerland. The pond water was filtered through a 0.1 mm sieve, characterized, and stored under aerated conditions in the dark at 4 °C for 2 weeks prior to use. Characteristics of the water are presented in Table 7.2.2.2-1.

Table 7.2.2.2-1: Water parameters

Origin/Source		Fröschweiher, Rheinfelden, Switzerland
Temperature [1]	[°C]	8.0
pH [1]	-	7.73
Redox Potential [1]	[mV]	207
Oxygen content [1]	[mg/L]	14.4
Total organic carbon (TOC) [2]	[mg/L]	3.09
Dissolved organic carbon (DOC) [2]	[mg/L]	2.98

[1] measured at field sampling.
[2] determined by AgroLab GmbH, 6037 Root, Switzerland (non GLP).
Note redox potential was measured with platinum/silver chloride electrode (not corrected for pH). In order to obtain redox potential of the hydrogen electrode, +211 mV had to be added to the measured values.

B. STUDY DESIGN

Pond water (100 mL) was dispensed into individual test flasks, and samples incubated in the dark at 21 ± 0.2 ºC. Aerobic conditions were maintained by aeration with moistened air. Samples were continuously and gently stirred to maintain particulate and micro-organisms in suspension and to facilitate oxygen transfer from the headspace to the aqueous phase. For sterile samples, pond water was additionally sterilized with an autoclave.

Individual test flasks for four test systems were set-up to investigate the aerobic mineralization of both radiolabels. The incubated test systems comprised a high dose, a low dose, a sterilized high dose, and a reference sample incubated to test the microbial activity of the surface water, with a substance known to easily biodegrade; [^{14}C(U)]-benzoic acid was the reference substance applied for this purpose. Sterilized high dose samples were incubated for [trimethoxyphenyl-U-^{14}C]-labelled metrafenone only and incubated and maintained in the same manner as the non-sterilized samples, with the exception that single rather than duplicate samples were sacrificed for all samples taken. Untreated control samples were also incubated under the same conditions.

Applications of the test substances were made drop-wise to the surface water of samples at a concentration of 0.099 mg/ L and 0.010 mg/ L for [trimethoxyphenyl-U-^{14}C]-metrafenone high and low dose test systems; and 0.102 mg/ L and 0.010 mg/ L for the [bromophenyl-U-^{14}C]-metrafenone high and low dose test systems. The benzoic acid reference standard was applied at a rate of 0.0153 mg/ L.

Following application, individual test systems were connected to a trapping system comprising an ethylene glycol trap and a 2 N NaOH trap, to trap organic volatiles and $^{14}CO_2$ in outgoing air respectively. Test systems were then maintained as described above. Duplicate samples (single for sterilized samples) treated with the test items were taken immediately after application, at study termination after 61 days, and at 5 intermediate time-points. Benzoic acid treated test systems were removed and analyzed after 7 and 16 days. At each sampling interval the pH and oxygen concentration of the samples were determined. The radioactivity in solution was determined by LSC, and characterized by HPLC with radioactive detection and UV detection at 218 nm. Additional characterization was performed for selected samples with TLC. Radioactivity in trapping solutions was determined by LSC.

II. RESULTS AND DISCUSSION

The oxygen content, and pH of the test systems were consistent for the full study duration, and consistent with values reported for the untreated control systems. After 7 days incubation, the reference substance [^{14}C (U)]-benzoic acid was present at concentrations < LOD of 1.0 % AR, confirming the microbially active nature of the test systems. The recovery and distribution of the radioactivity at the different sampling times is shown in Table 7.2.2.2-2 to Table 7.2.2.2-6.

Table 7.2.2.2-2: Balance of radioactivity (Mean % AR) after the application of [trimethoxyphenyl-U-^{14}C]-metrafenone (high dose)

	Incubation Time (days)						
	0	**1**	**3**	**7**	**14**	**30**	**61**
Aqueous Phase	**95.4**	**95.4**	**96.7**	**94.9**	**96.3**	**92.2**	**98.0**
Parent	95.4	95.4	96.7	94.9	96.3	92.2	98.0
$^{14}CO_2$	**-**	**0.2**	**0.4**	**1.0**	**1.2**	**1.5**	**5.0**
Other volatiles	**-**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**
Total	**95.4**	**95.7**	**97.1**	**95.9**	**97.5**	**93.6**	**103.0**

Table 7.2.2.2-3: Balance of radioactivity (% AR) after the application of [trimethoxyphenyl-U-^{14}C]-metrafenone (high dose, sterile)

	Incubation Time (days)						
	0	**1**	**3**	**7**	**14**	**30**	**61**
Aqueous Phase	**101.8**	**101.1**	**99.2**	**91.9**	**100.1**	**104.5**	**99.3**
Parent	101.8	101.1	99.2	91.9	100.1	104.5	99.3
$^{14}CO_2$	**-**	**<0.2**	**<0.2**	**0.3**	**0.5**	**<0.2**	**2.8**
Other volatiles	**-**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**
Total	**101.8**	**101.2**	**99.2**	**92.3**	**100.6**	**104.6**	**102.2**

Table 7.2.2.2-4: Balance of radioactivity (Mean % AR) after the application of [trimethoxyphenyl-U-^{14}C]-metrafenone (low dose)

	Incubation Time (days)						
	0	**1**	**3**	**7**	**14**	**30**	**61**
Aqueous Phase	**95.6**	**97.2**	**102.1**	**98.8**	**93.2**	**89.9**	**103.0**
Parent	95.6	97.2	102.1	98.8	93.2	89.9	103.0
$^{14}CO_2$	**-**	**0.2**	**<0.2**	**1.1**	**<0.2**	**1.7**	**3.5**
Other volatiles	**-**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**
Total	**95.6**	**97.5**	**102.2**	**99.9**	**93.2**	**91.7**	**106.7**

Table 7.2.2.2-5: Balance of radioactivity (Mean % AR) after the application of [bromophenyl-U-^{14}C]-metrafenone (high dose)

	Incubation Time (days)						
	0	**1**	**3**	**7**	**14**	**30**	**61**
Aqueous Phase	**96.1**	**103.1**	**98.7**	**96.6**	**97.1**	**94.2**	**96.0**
Parent	96.1	103.1	98.7	96.6	97.1	94.2	96.0
$^{14}CO_2$	**-**	**<0.2**	**<0.2**	**0.2**	**0.2**	**0.3**	**1.0**
Other volatiles	**-**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**	**<0.2**
Total	**96.1**	**103.1**	**98.7**	**96.8**	**97.4**	**94.4**	**97.1**

Table 7.2.2.2-6: Balance of radioactivity (Mean % AR) after the application of [bromophenyl-U-^{14}C]-metrafenone (low dose)

	Incubation Time (days)						
	0	**1**	**3**	**7**	**14**	**30**	**61**
Aqueous Phase	**94.2**	**104.2**	**85.9**	**101.8**	**105.3**	**106.1**	**98.4**
Parent	94.2	104.2	85.9	101.8	105.3	106.1	98.4
$^{14}CO_2$	**-**	**<0.2**	**0.5**	**<0.2**	**0.6**	**0.3**	**3.3**
Other volatiles	**-**	**<0.2**	**<0.2**	**<0.2**	**0.4**	**<0.2**	**0.7**
Total	**94.2**	**104.4**	**86.5**	**101.9**	**106.4**	**106.4**	**102.4**

Total recoveries were in the range of 91.7 - 106.7 % of the applied radioactivity (% AR) for all samples, with the exception of the sample taken 3 days after treatment for the low dose bromophenyl-metrafenone study, which was just under 90 % AR at 86.5 % AR. The slightly low recovery of this single sample does not affect the reliability of the study. All of the radioactivity which remained in the aqueous phase was characterized as parent metrafenone in all test systems (i.e. for both bromophenyl- and trimethoxyphenyl- labelled metrafenone, high and low dose systems, sterile and non-sterile systems) for the entire study duration. No metabolites were observed in the aqueous phase. Small amounts of $^{14}CO_2$ were detected with maximum formations of 1.0 %AR to 5.0 % AR at study termination in all test systems. Other volatiles were not detected above the LOD of 0.2 %AR at any time-point, apart from in the low dose bromophenyl-metrafenone study, where a maximum of 0.7 % AR was observed in the ethylene glycol trap at study termination.

The results of the study demonstrate that that metrafenone is stable, or degrades only very slowly, under the conditions of the test. Reliable degradation rates could not be calculated for metrafenone because of the stable nature of the compound under the conditions of the test.

III. CONCLUSION

Metrafenone is stable, or degrades only very slowly, under the conditions of the test. No metabolites were observed in the aqueous phase. Small amounts of $^{14}CO_2$ (max formation 5.0 % AR) and other volatiles (maximum formation 0.7 % AR) were detected in volatile traps.

CA 7.2.2.3 Water/sediment studies

Adequate data to assess degradation of metrafenone in water/ sediment studies were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions are required to be considered, as well as those which reach their maximum at the final time-point of the study and are present at concentrations >5 % AR. Multiple metabolites were observed in the study of Yan (2001) (updated in Huang (2002b)), however, no metabolite exceeded 5 % AR in the water phase, and though CL 375816 and CL 377160 exceeded 5 % AR in the sediment phase (maximum concentrations of 6.4 % AR and 6.2 % AR respectively) neither did so on consecutive occasions, or were increasing at study termination. Nevertheless, when the whole system is considered, both CL 377160 (maximum concentration 6.5 % AR) and CL 375816 (maximum concentration 8.4 % AR) exceeded 5 % AR in consecutive samples, and therefore both are considered further in exposure assessments.

Since the original assessment in the Draft Assessment Report for the Annex I listing assessment of metrafenone was finalized in October 2003, the FOCUS Degradation Kinetics Report (FOCUS 2006), which presents structured guidance for the calculation of rates of degradation for both parent compounds and metabolites from water/ sediment studies, has been finalized. Therefore the degradation rates of metrafenone and its metabolite CL 377160 are required to be re-calculated in accordance with current FOCUS Kinetics guidance. This has been done in the report of Hilton and Montesano (2014) which is summarized below. Whole system DT_{50} values for metrafenone were 8.4 – 9.9 days (geometric mean = 9.2 days). Whole system DT_{50} values for the metabolite CL 377160 could only be calculated for the two radio-labelled forms in the river system. DT_{50} values of 8.2 days and 13.6 days (geometric mean = 10.6 days) were derived.
It was not possible to calculate reliable whole system degradation rates for CL 375816, because concentrations were increasing at the study termination in the river system test, and the maximum formation in pond system was only 3.1 % AR.

Report:	CA 7.2.2.3/1 Yan Z., 2001a BAS 560 F (AC 375839): Aerobic transformation in water-sediment systems 2001/7000872
Guidelines:	EEC 91/414 Annex II 7.2.1.3.2, EEC 95/36, SETAC Part I 8.2, OECD draft proposal for a new guideline: Aerobic-anaerobic transformation in water-sediment systems (Nov. 1998)
GLP:	yes (certified by United States Environmental Protection Agency)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

Report:	CA 7.2.2.3/2 Huang R.A., 2001a BAS 560 F (AC 375839): Aerobic transformation in water-sediment systems (Report Amendment #1) 2002/7005254
Guidelines:	EEC 91/414, EEC 95/36, OECD, FIFRA 40CFR part 160, SETAC
GLP:	no

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in aquatic systems is presented at the end of this section (Section 7.2.).

Report:	CA 7.2.2.3/3 Hilton M.,Montesano V., 2014a Determination of rates of decline for Metrafenone in sediment-water studies according to the FOCUS Kinetics Guidance Document 2014/1083468
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

The degradation of metrafenone and its metabolite CL 377160 in two laboratory sediment/ water systems each performed with [trimethoxyphenyl-U-^{14}C]- and [bromophenyl-6-^{14}C]- labelled metrafenone was kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document. The decline in the whole system was modelled using the P-I (M-I) step guidance.

The SFO model provided a good description of the degradation of metrafenone in the whole system. In all cases the chi^2 % error value was < 17%, and visual fits and plots of the residuals confirmed the good fits. P values for the SFO rate constant were < 0.05 in all cases. Whole system SFO DT_{50} values at 20 ºC for metrafenone were 8.4 – 9.9 days.

The peak formation of CL 377160 was not well described when it was considered in the same degradation scheme as parent metrafenone, and consequently it was not possible to derive an acceptable fit. When peak-down SFO kinetics were employed acceptable visual and statistical fits (chi^2 % error value < 12%, P value < 0.05) were obtained for the two whole river system incubations. The calculated DT_{50} values under the conditions of the test were 8.2 days and 13.6 days. The formation of CL 377160 in the two pond system incubations was < 5 % AR, and consequently acceptable fits could not be obtained.

I. MATERIAL AND METHODS

A. MATERIALS

The degradation of metrafenone ([trimethoxyphenyl-U-^{14}C]- (Batch No.: AC 11662-17; specific radioactivity: 37.2 µCi/mg; radiochemical purity: 99.7%) and [bromophenyl-6-^{14}C]- (Batch No.: AC 11662-18; specific radioactivity: 40.6 µCi/mg; radiochemical purity: 99.6%)), and its metabolite CL 377160, in the whole system of two laboratory water/ sediment systems from the study of Yan (2001), were kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document.

B. STUDY DESIGN

The kinetic modelling was performed using KINGUII vers. 2. The approach used followed that given in Chapter 10 of the FOCUS Kinetics Guidance Document for the determination of both persistence and modelling end-points. The suitability of the fit of the models was evaluated both visually, based on a graphical plot of the degradation and in a plot of the residuals, and statistically by calculating the minimum % error required to pass the χ^2 test at a probability of 0.05. For SFO kinetics a t-test was also performed to evaluate whether the determined parameters were significantly different to 0. T-test statistics are not appropriate to describe the FOMC fitting parameters alpha and beta, and therefore confidence intervals were reported. Statistical parameters were compared to the acceptability criteria as reported in FOCUS Kinetics guidance indicates, however it is also stated within that guidance that acceptability criteria should not be considered as absolute cut-off criteria.

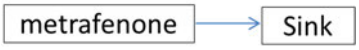
Two levels of kinetics are proposed in FOCUS Kinetics guidance for the determination of rates of degradation from sediment/water studies. P-I (M-I for metabolites) is for single compartment approaches and P-II (M-II for metabolites) is proposed for multi-compartment approaches where degradation in both the water and sediment individually is considered. In this exercise only the single compartment (P-I/ M-I) approach was used for the kinetic evaluation of the degradation of metrafenone and CL 377160 in the whole system.

Prior to running the kinetic evaluation, the appropriate metabolism scheme was investigated. Based on the study of Yan (2001) a degradation scheme in which metrafenone degraded to a sink compartment and the metabolite CL 377160, which in turn also degraded to a sink compartment, was initially investigated. Kinetic fitting was initially performed for parent metrafenone, the derived parameters were fixed and the metabolite CL 377160 was added. However using this approach it was not possible to determine reliable kinetic fits for the metabolite, and consequently a top-down approach was taken. The degradation schemes considered in the kinetic evaluation are presented in Figure 7.2.2.3-1.

For the determination of both persistence and modelling end-points FOCUS Kinetics guidance recommends that both SFO and FOMC models are initially applied to the data. If the SFO model gives an acceptable visual and statistical fit then this is accepted. In this case SFO kinetics provided acceptable fits in all systems, and therefore further investigation with other bi-phasic kinetic models was not required.

Figure 7.2.2.3-1: Schemes used for the kinetic evaluations

Scheme 1: Evaluation of metrafenone without metabolites



Scheme 2: Evaluation of metrafenone and metabolite CL 377160

Scheme 3: Evaluation of metabolite CL 377160 (top down)

II. RESULTS AND DISCUSSION

The results of the determinations were evaluated using visual and statistical methods. Summaries of the results and kinetic fitting parameters are summarized in Figure 7.2.2.3-1.

Good visual and statistical fits were obtained for parent metrafenone in both systems following treatment with both ^{14}C-labelled forms of metrafenone. The initial peak formation of the metabolite CL 377160 was not well described when considered in the degradation scheme, and as a result it was not possible to derive a good fit. However, an acceptable visual and statistical fit was obtained for the two river systems with DT_{50}s of 13.6 and 8.2 days when the top-down approach was considered. Acceptable kinetic evaluations for CL 377160 could not be determined in the pond systems because the maximum observed concentrations of the metabolite were ≤ 4.2%. This is demonstrated by the χ^2 error (%) values which are above 20 %, the poor visual fits, and the t-test statistics, which indicate uncertain fits.

Table 7.2.2.3-1: Summary of the results of the kinetic determinations for the degradation of metrafenone and CL 377160 from the whole system

	Bromophenyl label		Trimethoxyphenyl label	
	River	**pond**	**River**	**pond**
Metrafenone (SFO)				
χ^2 error (%)	**16.8**	**11.0**	**11.6**	**11.7**
k (day^{-1}) *	**0.0829 (0.00229)**	**0.076 (0.000273)**	**0.074 (0.000458)**	**0.070 (0.000473)**
DT_{50} (day)	**8.4**	**9.1**	**9.4**	**9.9**
DT_{90} (day)	**27.8**	**30.2**	**31.2**	**32.9**
Metrafenone (FOMC)				
χ^2 error (%)	17.9	11.7	12.4	12.5
α*	$8.952x10^2$ (±39059.1)	$4.278x10^2$ (±9159.7)	$1.218x10^3$ (±60998.6)	$5.707x10^2$ (±17552)
β*	$1.079x10^4$ (±471140.3)	$5.596x10^3$ (±119932.6)	$1.648x10^4$ (±825657.9)	$8.140x10^3$ (±250564.7)
DT_{50} (day)	8.4	9.1	9.4	9.9
DT_{90} (day)	27.8	30.2	31.2	32.9
CL 377160 top down (SFO)**				
χ^2 error (%)	**9.1**	20.6	**11.8**	31.0
k (day^{-1}) *	**0.0509 (0.0035)**	0.0138 (0.1069)	**0.0847 (0.0236)**	0.0212 (0.253)
DT_{50} (day)	**13.6**	50.1	**8.2**	32.6
DT_{90} (day)	**45.3**	166.5	**27.2**	108.5

*P value from the t-test (SFO) and CI (FOMC) are given in brackets.

** Only the metabolite is considered for the determination of DT_{50}

Selected fit shown in bold font

III. CONCLUSION

The SFO model provided a good description of the degradation of metrafenone in both whole water/ sediment systems. DT_{50} values of 8.4 – 9.9 days were derived. The fits were acceptable for the metabolite CL 377160 in the two river systems when top down SFO kinetics were considered. DT_{50} values of 8.2 and 13.6 days were calculated. The low formations of this metabolite in the pond systems meant that reliable fits were not possible in these two systems.

CA 7.2.2.4 Irradiated water/sediment study

The requirement for irradiated water/ sediment studies is a new, but optional, data requirement under Commission Regulation (EC) No 283/2013, which sets out the data requirements in accordance with regulation (EC) No 1107/2009. Data to assess the degradation of metrafenone in irradiated water/ sediment studies are therefore not required. Adequate data to assess degradation of metrafenone in aquatic systems were evaluated during the first EU review. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

CA 7.2.3 Degradation in the saturated zone

The requirement to address degradation in the saturated zone is a new, but optional, data requirement under Commission Regulation (EC) No 283/2013, which sets out the data requirements in accordance with regulation (EC) No 1107/2009. Data to assess the degradation of metrafenone in the saturated zone are therefore not required. Adequate data to assess degradation of metrafenone in aquatic systems were evaluated during the first EU review. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Summary of fate and behavior in water and sediment

A hydrolysis study conducted at 50°C and pH 4, 7 and 9 in sterile buffer solution (An, 1999) indicated that there was virtually no degradation of metrafenone over a five day period. It is concluded that metrafenone is stable with respect to hydrolysis.

Two aqueous photolysis studies were conducted on metrafenone, one in sterile aqueous buffer solution at pH 7 with both trimethoxyphenyl- and bromophenyl- ^{14}C labelled metrafenone (Fung 2002a) and the second in natural water with bromophenyl ^{14}C-labelled metrafenone only (Huang 2002a). In the sterile study, metrafenone was found to degrade with a half-life of 6.2 days of 12 hour light/dark cycles with light intensity equivalent to early autumn sunshine at 40°N latitude. The quantum yield was 2.29×10^{-4}. Using the EPA programme GCSOLAR, the theoretical photolytic half-life of metrafenone in the top 30cm of aqueous systems was calculated to be 13.5 days in summer at 50°N and 128 days in winter at 40°N.

The relatively rapid degradation under illuminated conditions was accompanied by formation of a large number of metabolites. None of these exceeded 10% AR at any time in the study, the largest individual compound being the metabolite CL 4084564 which occurred at a maximum of 8.7% AR, and was observed in tests performed with both radiolabelled forms of metrafenone. One chromatographic region constituted up to 47.9% AR, but on further analysis was found to comprise more than 14 separate compounds, none occurring at greater than 8.4% AR. Multiple other metabolites were observed, however none exceeded 5 % AR. Since the original review, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions should be considered as well as those which reach their maximum at the final time-point of the study and are present at concentrations >5 % AR. Therefore the metabolite, CL4084564, which exceeded 5 % AR on consecutive occasions, now triggers further assessment.

In the natural water photolysis study, ^{14}C-bromophenyl labelled metrafenone degraded with a half-life of 5.2 days of 12 hour light/dark cycles. As with the sterile water photolysis study, a large number of metabolites were formed, but generally, individual compounds were formed at lower levels than in the sterile water study, and all were observed at maximum concentrations of < 5 % AR.

These studies indicate that degradation of metrafenone in natural surface water systems is likely to be influenced by sunlight. However, incident solar radiation varies between different locations and at different times of year. In addition, it is known that natural surface waters are often turbid, with aqueous photolysis reactions likely to be limited to the top few millimeters of the water body. Thus, it is difficult to accurately predict the influence of photolysis in such systems on an EU-wide basis. Nevertheless, as CL 408564 was observed at concentrations > 5 % AR at consecutive sample times, PEC_{sw} values will be calculated for CL 4084564 with FOCUS SW and using worst case assumptions.

A ready biodegradability study (Bradley and Yan, 1999) indicated that there was no significant mineralization during the 28 day duration of the test. Consequently, metrafenone would be classified as 'not readily biodegradable'.

In accordance with the new data requirements in Commission Regulation (EU) No 283/2013, a new aquatic mineralization study (Adam, 2014) was performed with [trimethoxyphenyl-U-^{14}C]- and [bromophenyl-U-^{14}C]- labeled metrafenone, in accordance with OECD 309. Metrafenone was stable, or degraded only very slowly, under the conditions of the test. No metabolites were observed, with the exception of small amounts of $^{14}CO_2$ (max formation 5.0 % AR) and other volatiles (maximum formation 0.7 % AR).

A dark sediment/water study (Yan (2001); Huang (2002b)) was conducted at 20°C with both trimethoxyphenyl- and bromophenyl- ^{14}C-labelled metrafenone applied to two aquatic systems. Metrafenone was found to dissipate relatively rapidly from water, with the major route of dissipation being by partitioning to sediment of the parent from the water phase, with up to 56.9% AR as parent in sediment at 3 DAT. The highest levels of parent in sediment occurred in the system with the higher level of organic carbon in the sediment. Degradation in sediment occurred, such that from peak levels of 40.0-56.9% AR, parent had declined to 6.2 – 11.6% AR at study termination (100 DAT). The degradation rate of metrafenone was evaluated in accordance with FOCUS Kinetics guidance in the study of Hilton and Montesano (2014). Whole system DT_{50} values for metrafenone were calculated to be 8.4 – 9.9 days, with a geometric mean of 9.2 days.

Metabolism of metrafenone in the study was extensive, with more than 20 degradation products detected. Formation of individual metabolites in the water phase was generally low, with a metabolite fraction, Met-1, occurring at the highest metabolite level of 4.0% AR. However, this fraction was likely to be composed of 2-3 separate components. The highest occurring identified metabolite in water was CL 375816 at 3.7% AR at study end. Radioactivity expressed as 'others' was found at up to 19.3% AR, but was found on further analysis to be comprised of at least 15 separate components, each <5%AR.

The metabolites found in the water phase were also found in the sediment phase, but generally at higher levels. The highest occurring identified metabolites in sediment were CL 375816 at 6.4% AR at 56 DAT and CL 377160 at 6.2% AR at 7 DAT; neither metabolite exceeded 5 % AR on consecutive occasions or were increasing at study termination in sediment. A metabolite fraction, Met-1, was found up to 10.8% AR in sediment at 28 DAT, but was found to comprise 2-3 components on further analysis. 'Others' occurred at 38.0% AR at 56 DAT, but comprised at least 15 separate components, each of which was <5% AR.

Since the first EU review of metrafenone, the guidelines for evaluating the relevance of metabolites have changed. Metabolites occurring at >5% on two or more consecutive occasions are required to be considered, as well as those which reach their maximum at the final time-point of the study and are present at concentrations >5 % AR. Based on the individual water and sediment phases no metabolite triggers further assessment, however when the whole system is considered both CL 377160 (maximum concentration 6.5 % AR) and CL 375816 (maximum concentration 8.4 % AR) trigger further assessment since both exceed 5 % AR in consecutive samples.

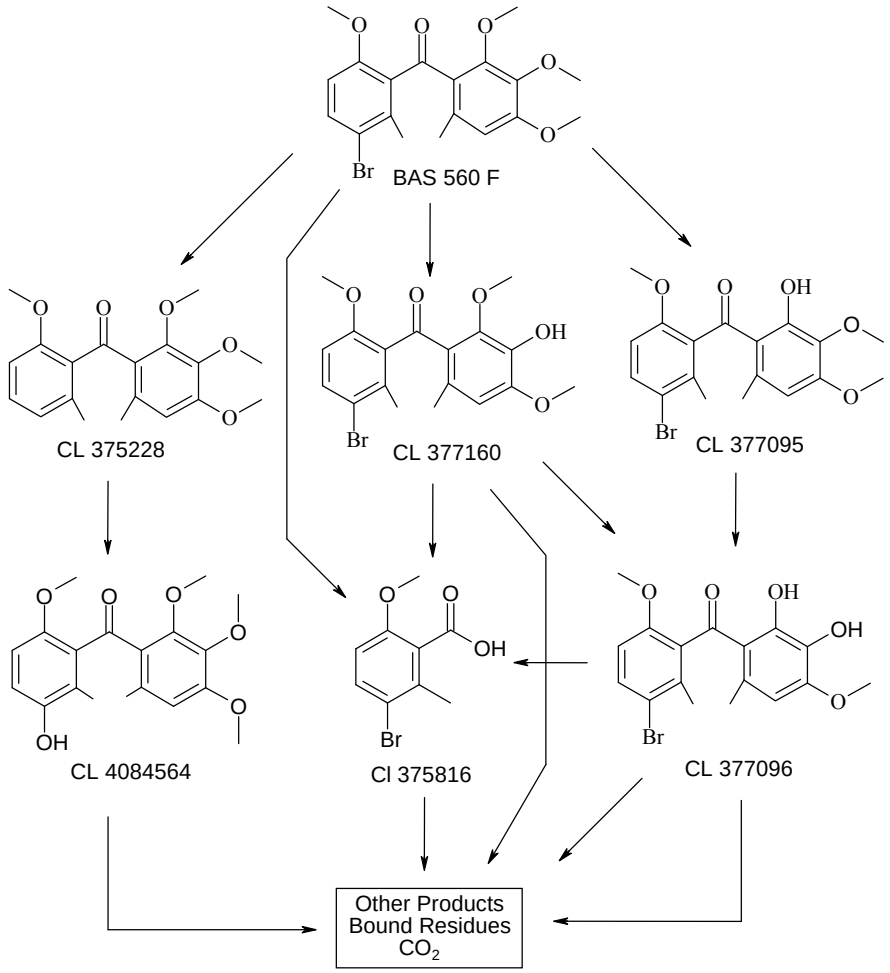
Unextracted residues were formed up to 15.7 – 26.4% AR at study end. Slightly higher levels of unextracted residues were found in the higher organic carbon sediment, and with the trimethoxyphenyl- ^{14}C-labelled material. Mineralization was 2.6 – 12.4% AR at study end, with greater mineralization with the trimethoxyphenyl- ^{14}C-labelled material. Mass balance was generally acceptable.

The whole system degradation rates of the metabolite CL 377160 were calculated in accordance with current FOCUS Kinetics guidance in the study of Hilton and Montesano (2014). Whole system DT_{50} values for CL 377160 in the river system were calculated to be 8.2 days and 13.6 days for the two radio-labelled forms, with a geometric mean of 10.6 days. Acceptable fits could not be obtained in the pond system tests because the formation of CL 377160 was < 5 % AR. It was not possible to obtain reliable kinetic evaluations for the metabolite CL 375816 because concentrations were increasing at the study termination in the river system test, and the maximum formation in pond system was only 3.1 % AR.

The proposed metabolic pathway of metrafenone in sediment/water systems is given in Figure 7.2.3-1.

Overall, the dominant route of degradation in aquatic systems in the field is anticipated to be adequately described by the studied water/ sediment systems, though in some rare situations it is plausible that metrafenone will be photolytically degraded in the surface layer of aquatic systems. As a result of the revision of triggers for metabolite assessment, PEC_{sw} and PEC_{sed} values for CL 375816, which exceeded 5 % AR in sediment on consecutive occasions, will be calculated with FOCUS SW and using worst case assumptions. The metabolite CL 377160 was observed in sediment/ water systems at a maximum concentration of 6.2 % AR in sediment on a single occasion only, and at < 5 % AR in the water phase; however PEC_{sw} and PEC_{sed} values will be calculated because of its formation at concentrations > 10 % AR in soil photolysis studies, and possible subsequent inputs to surface water bodies by run-off and drainflow. In addition, as a precautionary approach, PEC_{sw} values will be calculated for the aqueous photolysis metabolite CL 4084564 with FOCUS SW, and using worst case assumptions.

Figure 7.2.3-1: Proposed metabolic pathway of metrafenone (BAS 560 F) in sediment/water systems.



CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

Adequate data to assess degradation of metrafenone in air were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.3.1/1 Beigel C., 2002b BAS 560 F: Calculation of Henry Law Constant 2002/7004412
Guidelines:	EEC 91/414 Annex II 2.3.2, EEC 94/37
GLP:	no

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in air is presented at the end of this section (Section 7.3.).

CA 7.3.2 Transport via air

Adequate data to assess the transport of metrafenone in air were evaluated during the first EU review. The guidance for the conduct of such studies has not substantively altered since the first review, therefore no further data are considered necessary. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Report:	CA 7.3.2/1 Mangels G., 2001a Estimation of the photochemical oxidative degradation of BAS 560 F in the atmosphere (QSAR estimates) 2001/7000334
Guidelines:	EEC 91/414 Annex II 2.10, EEC 94/37
GLP:	no

Executive Summary

The calculated half-life for photochemical oxidative degradation in the atmosphere by the method of Atkinson was 0.63 hours. Please see the full summary details presented in Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005). A summary of the route and rate of degradation in air is presented at the end of this section (Section 7.3.).

CA 7.3.3 Local and global effects

The requirement to address local and global effects is a new data requirement under Commission Regulation (EC) No 283/2013, which sets out the data requirements in accordance with regulation (EC) No 1107/2009. Metrafenone is not applied in large amounts and is not expected to be subject to long range transport in air, therefore additional data to assess local and global effects are not required. Adequate data to assess fate and behavior of metrafenone in air were evaluated during the first EU review. For further details, please refer to Volume 3, Section B.8 of the DAR for metrafenone (UK, 2005).

Summary of fate and behavior in air

Metrafenone has a vapor pressure of 1.53×10^{-4} Pa at 20 °C and calculated Henry's Law Constant of 0.132 Pa m^3/mol. As such, metrafenone may be prone to some volatilization from soil or water surfaces. However, it was noted in radiolabelled soil and water studies that significant radioactivity as organic volatiles was not found in volatile traps. This may be the result of the relatively strong adsorption of the active substance. In addition, the calculated half-life for photochemical oxidative degradation in the atmosphere by the method of Atkinson was 0.63 hours. This would suggest that, even if metrafenone were to enter the atmosphere, it would be rapidly degraded, and would not be subject to long-range transport.

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

The definition of the residue for risk assessment in the environmental compartments soil, groundwater, surface water, sediment and air are as follows:

Soil:	Metrafenone, CL 377160, CL 3000402 & M2 (tentatively identified as CL 1500831 – identity to be confirmed)
Groundwater:	Metrafenone, CL 377160, CL 3000402 & M2 (tentatively identified as CL 1500831 – identity to be confirmed)
Surface water:	Metrafenone, CL 377160, CL 4084564, CL 375816, CL 3000402 & M2 (tentatively identified as CL 1500831 – identity to be confirmed)
Sediment:	Metrafenone, CL 377160, CL 375816, CL 3000402 & M2 (tentatively identified as CL 1500831 – identity to be confirmed)
Air:	Metrafenone

CA 7.4.2 Definition of the residue for monitoring

The definition of the residue for risk assessment in the environmental compartments soil, groundwater, surface water, sediment and air are as follows:

Soil:	Metrafenone
Groundwater:	Metrafenone
Surface water:	Metrafenone
Sediment:	Metrafenone
Air:	Metrafenone

CA 7.5 Monitoring data

No data were evaluated during the first EU review, and none were required.

In accordance with Commission Regulation (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Regulation (EC) No. 1107/2009, monitoring data are required to be submitted where they are available. The study of Fernandez *et al.* (2014), which reports fungicide concentrations in surface water, was identified following a literature review. This paper was regarded as relevant with a reliability rating of 2 ((reliable with restrictions according to Klimisch et al[i].), since no recognised guideline was followed and mean recoveries for one of the sampling techniques were low (34 %). A summary of this published paper is presented below.

Report: CA 7.5/1
Fernandez D. et al., 2014a
Calibration and field application of passive sampling for episodic exposure to polar organic pesticides in streams
2014/1326852
Guidelines: none
GLP: no

Executive Summary

The study was performed to investigate the suitability of passive sampling to quantify episodic exposures driven by run-off events. Event-driven water samples (EDS) were collected and extracted to assess exposure to 15 fungicides (including metrafenone) and 4 insecticides in 17 streams in a German vineyard area during 4 rainfall events. Simultaneously, Empore™ styrene-divinylbenzene reverse phase sulfonated disks (SDB disks) were deployed as passive water samplers in the same locations and for the same time-periods. Concentrations of all 19 analytes were reported as mean TWA concentrations from the SDB disks, and maximum concentrations from the EDS water samples.

A microcosm experiment was also conducted to determine the SDB-disk sampling rates and provide a free-software solution to derive sampling rates under time-variable exposure.

Sampling rates ranged from 0.26 to 0.77 L/d (0.54 L/d for metrafenone), peak concentrations from EDS samples ranged from <LOD to 2.11 µg/L (1.237 µg/L for metrafenone). For metrafenone the mean time weighted average (TWA) concentration was reported as 0.151 µg/L. The 2 sampling systems were in good agreement and EDS exceeded TWA concentrations on average by a factor of 3. The report concludes that passive sampling is suitable to quantify episodic exposures from polar organic pesticides.

I. MATERIAL AND METHODS

The study was conducted in 17 streams in the South of the federal state of Rhineland-Palatinate (southwest Germany), which is the largest German vine growing region, characterised by 23,000 ha of vineyards. Fungicides are known to be applied every 10-14 days from the end of April to the middle of August. The Palatinate forest, is located upstream of the vineyards and is the source of all streams in the region so that other than vinicultural inputs, pesticide input can largely be excluded. The selected streams covered a presumed gradient of fungicide exposure including 4 reference sites without exposure, located in the Palatinate Forest.

Monitoring was based on precipitation information and both passive samplers and EDS were deployed 1 to 2 days preceding forecasted precipitation events (>10 mm/day). Samplers were retrieved within 2 days after precipitation events (except for the fourth precipitation event, where samplers were retrieved after 5 days due to logistical constraints).

The EDS sampling system consisted of 2 x 1 litre brown glass bottles that were fixed to a steel bar and placed in the stream with the bottle openings approximately 10 and 20 cm above the normal water level. Bottle lids were fixed 1 cm above the opening to prevent rainfall to enter the bottle and dilute the sample. Stream water samples were retrieved after the 4 monitored rain events, stored in a fridge and solid-phase extracted within 24 h after retrieval. When the 2 bottles were filled, the lowest one was discarded as the peak concentration of pesticides occur simultaneously to the increase in water level.

Passive SDB disk samplers were pre-conditioned in methanol and ultra-pure water, before being deployed by fixing, in duplicate, to the stream bed by means of a metal stake. Upon retrieval, disks were stored in acetone at -21°C. Additional microcosm experiments were performed to assess the sampling rates of passive samplers.

Sites were monitored for 15 fungicides and 4 insecticides in 2012 from July to September, and physico-chemical variables at the sampling location were also measured.

Prior to extraction, EDS water samples were centrifuged to remove large particles and then acidified with HCl to pH 2. Oasis® HLB 6 cc 500 mg extraction cartridges (Waters, Milford, USA), pre-conditioned with methanol, and ultra-pure water were used for extraction. Finally, the cartridges were dried for 30 min in a nitrogen stream and stored at 21 °C. Cartridges were washed and dried prior to elution with methanol.

Initial extraction of the passive sample SDB disks was in the acetone in which it was stored, by shaking, followed by a 2nd extraction in methanol. Extracts were combined, evaporated to dryness and reconstituted in methanol.

Extracts from both sample techniques were analysed by LC-HRMS, and results were adjusted to the cartridge recovery determined for each pesticide (mean recovery: EDS = 34% ± 8 RSD; passive SDB samples = 77% ± 8 RSD). For passive samples concentrations were additionally adjusted for matrix effects. Such effects were not significant for the EDS samples.

The authors postulate that the low recovery from the EDS samples presumably resulted from an erroneous washing step with distilled water prior to elution with methanol and that recoveries should be higher if omitting this step. However, due to acceptable RSDs (<15% for all compounds except azoxystrobin and dimethoate) and the good correlation with TWA concentrations, EDS results were deemed reliable.

Prior to extraction, EDS water samples were centrifuged to remove large particles and then acidified with HCl to pH 2. Oasis® HLB 6 cc 500 mg extraction cartridges (Waters, Milford, USA), pre-conditioned with methanol, and ultra-pure water were used for extraction. Finally, the cartridges were dried for 30 min in a nitrogen stream and stored at 21 ºC. Cartridges were washed and dried prior to elution with methanol.

Initial extraction of the passive sample SDB disks was in the acetone in which it was stored, by shaking, followed by a 2nd extraction in methanol. Extracts were combined, evaporated to dryness and reconstituted in methanol.

Extracts from both sample techniques were analysed by LC-HRMS, and results were adjusted to the cartridge recovery determined for each pesticide (mean recovery: EDS = 34% ± 8 RSD; passive SDB samples = 77% ± 8 RSD). For passive samples concentrations were additionally adjusted for matrix effects. Such effects were not significant for the EDS samples.

The authors postulate that the low recovery from the EDS samples presumably resulted from an erroneous washing step with distilled water prior to elution with methanol and that recoveries should be higher if omitting this step. However, due to acceptable RSDs (<15% for all compounds except azoxystrobin and dimethoate) and the good correlation with TWA concentrations, EDS results were deemed reliable.

II. RESULTS AND DISCUSSION

The pesticide concentrations obtained with the two sampling methods were significantly correlated ($r^2 = 0.87$, $p < 0.01$, $n = 175$), and for metrafenone demonstrated a significant relationship ($r^2 = 0.78$, $p<0.01$). Peak concentrations from the EDS exceeded TWA concentrations on an average by a factor of 2.6.

Duration of the linear uptake phase of the SDB disks was approximately 5 days. Sampling rates for the SDB disks ranged from 0.26 – 0.77 L/day (0.54 L/d for metrafenone).

Metrafenone was detected in 69 % of all samples collected from vineyard sites and 0 % of samples collected in the forest sites. The mean TWA from passive samplers was 0.151 µg/L while the maximum TWA concentration from the passive samples was 1.237 µg/L. Maximum concentrations from EDS samples were not reported.

The pesticide concentrations obtained with the two sampling methods were significantly correlated. Duration of the linear uptake phase of the SDB disks was approximately 5 days. Sampling rates for the SDB disks ranged from 0.26 – 0.77 L/day (0.54 L/d for metrafenone).

Metrafenone was detected in 69 % of all samples collected from vineyard sites and 0 % of samples collected in the forest sites. The mean TWA from passive samplers was 0.151 µg/L while the maximum TWA concentration from the passive samples was 1.237 µg/L. Maximum concentrations from EDS samples were not reported.

The study appears to be well conducted and reported, with the exceptions that it was not performed according to a guideline, and that the mean recovery from EDS samples was low (34 %).

III. CONCLUSION

The report concludes that passive sampling is suitable to quantify episodic exposures from polar organic pesticides.

[i] Klimisch, H-J., Andreae, M. & Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology **25** pp 1-5

We create chemistry

Metrafenone

Document M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

Metrafenone is a benzophenone fungicide used for the control of powdery mildew fungi. The biochemical mode of action is not yet known. However, observations indicate that it inhibits growth of mycelium on the leaf surface, leaf penetration, and formation of haustoria and sporulation. In addition, metrafenone has been shown to inhibit sporulation in the fungi and may interfere with a dimorphic switch that allows the fungus to reproduce asexually versus sexually.

Metrafenone was first approved by Commission Directive 2007/6/EC (entry into force: 01 February 2007). This document is submitted to support the renewal of approval of metrafenone and complies with the Table of Contents described in the Annex to Regulation (EU) No 283/2013. It reviews the ecotoxicology studies performed with the active substance.
:

- For studies performed since the first inclusion of metrafenone in Annex I to Directive 91/414/EEC, the study reports are submitted (See document K-CA) and full study summaries are provided.
- For studies submitted and assessed for the first EU review of metrafenone and are still **considered relevant to support renewal of approval, only the critical endpoints and conclusion summary are included in this dossier. For a more detailed assessment, reference** can be made either to the summary dossier (Document M-II, Section 6, BASF SE, 2002) submitted for the first EU evaluation or to the Draft Assessment Report (DAR) for metrafenone (UK, 2005). The study reports are not submitted in document K-CA but can be found in the dossier submitted for the first EU Review.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effect on birds

Avian toxicity data submitted for the first EU evaluation of metrafenone consisted of acute oral and short-term dietary toxicity studies with bobwhite quail and mallard duck; and a reproductive toxicity study with bobwhite quail. More recently, an acute oral toxicity study with the zebra finch and a reproductive toxicity study with mallard duck have been performed. The available data on avian toxicity are summarized in Table 8.1.1-1.

Table 8.1.1-1: Summary of effects of metrafenone on birds

Test species	Test substance	Test System	Exposure	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Metrafenone	Acute	Single dose	LD_{50} > 2250 mg a.s./kg bw	KCA 8.1.1.1/1 2000/7000117
Mallard duck (*Anas platyrhynchos*)	Metrafenone	Acute	Single dose	LD_{50} > 2250 mg a.s./kg bw	KCA 8.1.1.1/2 2000/7000115
Zebra finch (*Taeniopygia guttata*)	Metrafenone	Acute	Single dose	LD_{50} > 2000 mg a.s./kg bw	KCA 8.1.1.1./3 2011/1263863
Bobwhite quail (*Colinus virginianus*)	Metrafenone	Short-term	8-days	LC_{50} > 5314 ppm (>948.4 mg a.s./kg bw/d)	KCA 8.1.1.2/1 2000/7000126
Mallard duck (*Anas platyrhynchos*)	Metrafenone	Short-term	8-days	LC_{50} > 5314 ppm (>1483 mg a.s./kg bw/d)	KCA 8.1.1.2/2 2000/7000125
Bobwhite quail (*Colinus virginianus*)	Metrafenone	Reproduction	22-weeks	NOEC = 1350 ppm (125.4 mg a.s./kg bw/d)	KCA 8.1.1.3/1 2002/7005090
Mallard duck (*Anas platyrhynchos*)	Metrafenone	Reproduction	22-weeks	NOEC = 900 ppm (114.7 mg a.s./kg bw/d)	KCA 8.1.1.3/2 2006/1018046

CA 8.1.1.1 Acute oral toxicity to birds

Report:	CA 8.1.1.1/1 [redacted], 2000a Avian acute oral toxicity test with AC 375839 technical in northern bobwhite (Colinus virginianus) 2000/7000117
Guidelines:	EPA 71-1, EPA 850.2100, EEC 91/414 Annex II 8.1.1, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The LD_{50} for northern bobwhite (*Colinus virginianus*) exposed to metrafenone was determined to be > 2250 mg a.s./kg body weight. A no-observed-effect level (NOEL) for mortality was determined to be 2250 mg a.s./kg body weight, the highest dose tested, as there were no test substance related effects on mortality, morbidity, signs of intoxication and body weight change observed in any dose group during the 14 day observation period. Please refer to Document M-II, Section 6, Point 8.1.1 of the summary dossier dated 2002 for further details.

Report:	CA 8.1.1.1/2 [redacted] 2000b Avian acute oral toxicity test with AC 375839 technical in mallard duck (Anas platyrhynchos) 2000/7000115
Guidelines:	EPA 71-1, EPA 850.2100, EEC 91/414 Annex II 8.1.1, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The LD_{50} for mallard duck (*Anas platyrhynchos*) exposed to metrafenone was determined to be > 2250 mg a.s./kg body weight. A no-observed-effect level (NOEL) for mortality was established to be 2250 mg a.s./kg body weight, the highest dose tested as there were no test substance related mortality, morbidity, signs of intoxication, body weight change and feed consumption observed in any dose group during the 14 day observation period. Please refer to Document M-II, Section 6, Point 8.1.1 of the summary dossier dated 2002 for further details.

Report: CA 8.1.1.1/3
[redacted] 2011a
BAS 560 F (Metrafenone) - Acute toxicity in the zebra finch (Taeniopygia guttata) after single oral administration (LD50)
2011/1263863
Guidelines: EPA 71-1, EPA 850.2100
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A laboratory study was conducted to evaluate the acute toxicity of BAS 560 F (metrafenone technical) administered to the zebra finch (*Taeniopygia guttata*) as a single oral dose. Ten northern bobwhite, five males and five females, were assigned to each of the treatment groups (500, 1000 or 2000 mg a.s./kg bw) and the control group. Birds were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days after exposure.

Analytical verification of the test substance concentration in the diet demonstrated that measured concentrations were within a range of 92.3% to 97.5% of the nominal concentrations during the test. The biological results are therefore based on the nominal concentrations.

No substance-related mortality, impairment of feed uptake or reduction in body weights occurred throughout the duration of the study in all treatment groups. Clinical signs were only observed in the highest test group, where five birds per sex were observed to be tumbling during the first hour of observation only. All birds were free of substance-related findings during the post-mortem examinations. Although one bird died, in the treatment group exposed to 1000 mg a.s./kg, this was not considered substance related. Hence, the highest dose causing no substance related mortality was 2000 mg a.s./kg bw for males and females.

In an acute oral toxicity test with the zebra finch (*Taeniopygia guttata*), the LD_{50} of metrafenone was found to be > 2000 mg a.s./kg bw. The highest dose without mortality was determined to be 2000 mg a.s./kg bw. A no-observed-effect level (NOEL) based on signs of toxicity (tumbling) was established to be 1000 mg a.s./kg bw.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid, beige

2. Test concentrations: 0 (control), 500, 1000 or 2000 mg a.s./kg body weight (bw)

3. Vehicle: 0.5% aqueous carboxy methyl cellulose

4. Test organism

Species:	Zebra finch (*Taeniopygia guttata*)
Age:	Approximately two months old, before their first breeding season
Source:	Kölle-Zoo, Ludwigshafen, Germany
Acclimation period:	Approximately six weeks
Diet:	Commercial diet for exotic birds in grain form, supplied by 'Versale-Laga nv', Deinze, Belgium. The birds received the food *ad libitum*, except for a fasting period of 19 to 21 hours prior to dosing
Housing:	Cages composed of stainless steel with stainless steel floors and covered with card board, measuring 1.67 m deep x 0.76 m wide x 0.76 m high (total area: 1.3 m^2)

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	19.8-26.1°C
Relative humidity:	42-99%
Photoperiod:	8h light: 16h darkness, with light intensity between 52 and 138 lux

2. Animal assignment and treatment:

Fifteen days prior to dosing, birds were weighed individually and then randomly allocated to the test groups. Per test group, ten birds were included; five males and five females. At test initiation, birds were administered the test substance by gavage into the crop. The birds were observed for at least one hour after dosing for regurgitation and then the next fourteen days. During these fourteen days, the birds were offered feed *ad libitum*.

3. Dose preparation:

For each test item group, 50 g of a preparation of the test substance in carboxy methyl cellulose (CMC) in drinking water was prepared separately. The test substance was suspended in 0.5% CMC with an ultra turrax stirrer. The birds were administered single doses of 500, 1000 or 2000 mg a.s./kg bw. The control group received CMC dispersion only.

4. Measurements/observations:

During the observation period of fourteen days, assessment of mortality and signs of clinical toxicity was carried out four times on the day of dosing and daily thereafter. Assessment of body weight was carried out on the day of dosing and on day seven and fourteen. Mean food consumption was calculated from the weekly food consumption, separately for male and female birds. Gross-pathological post-mortem examinations of all birds were performed at study termination on day fourteen after dosing.

For analysis of test item concentration, a sample was obtained from each test item concentration and the control dispersions. The analysis was performed using HPLC.

5. Statistics:
No statistical calculation of the LD_{50} was performed, since no substance related mortality (above 50%) was observed. Food consumption was not examined statistically, since the food consumption was evaluated only per cage and not per bird. Body weight data were statistically analyzed using the Dunnett test, with the program ToxData of the PDS Pathology Data Systems Ltd..

II. RESULTS AND DISCUSSION

A. MORTALITIES AND BEHAVIOURAL OBSERVATIONS

No substance related mortality occurred throughout the duration of the study in the control and in all test substance groups. The single mortality (female bird on Day 13) in the test group exposed to 1000 mg a.s./kg b.w. was considered not substance related. The highest concentration causing no substance related mortality was 2000 mg a.s./kg bw for males and females. No substance related clinical signs were observed in the control and in all test substance concentration groups, except in the highest test group where five birds per sex were observed to be tumbling during the first hour of observation. These symptoms were no longer noted after the 2-hour post dosing observation period.

B. FOOD CONSUMPTION AND BODY WEIGHT

There was no substance-related impairment of feed uptake in comparison to the control observed in any of the dose groups. There was no statistically significant substance-related reduction of the body weights in any of the dose groups at day seven and day fourteen (sacrifice), and the body weight development was not impaired in comparison to the control group. The results are presented in the table below.

Table 8.1.1.1-1: Mean food consumption per day and body weight change of zebra finch (*Taeniopygia guttata*) exposed to BAS 560 F

Nominal concentration (mg a.s./kg bw)	Mean food consumption (g/bird/day)		Overall mean body weight gain (g) compared between day 0 and day 14	
	Males	Females	Males	Females
Negative control	3.8	3.9	1.0	1.1
500	4.1	3.8	1.5	0.8
1000	3.9	3.6	1.5	0.2
2000	3.6	4.0	1.0	1.1

C. POST-MORTEM EXAMINATIONS

One surviving female of the control group had a high-grade enlarged liver. All other surviving birds were free of findings. The bird that died on day 13 (dose group 1000 mg/kg bw) had a grey white encrustation on the liver, which was not considered to be treatment-related.

D. ANALYSIS

The results of the analytical verification of the test substance concentration in the diet were within a range of 92.3% to 97.5% of the nominal concentrations during the test. The biological results are therefore based on the nominal concentrations. The concentrations of the test substance BAS 560 F in stock solutions observed in the analysis are shown in the table below.

Table 8.1.1.1-2: Measured concentrations of BAS 560 F in stock solutions of test samples

Nominal concentration (g a.s./50 g)	Nominal concentration (g test substance/50 g)*	Measured concentration (mg test substance/L)	Measured as % of nominal
2.500	2.650	2.445	92.3
5.000	5.300	4.999	94.3
10.000	10.600	10.331	97.5

* The concentration of the test substance BAS 560 F, containing 94.2% of the active substance metrafenone

E. DEFICIENCIES

None.

III. CONCLUSION

The LD_{50} for zebra finch (*Taeniopygia guttata*) exposed to BAS 560 F was found to be > 2000 mg a.s./kg body weight. The no-observed-effect level (NOEL) was 1000 mg a.s./kg body weight, based on signs of toxicity (tumbling).

CA 8.1.1.2 Short-term dietary toxicity to birds

Report:	CA 8.1.1.2/1 [redacted] 2000c Avian dietary toxicity test with AC 375839 in northern bobwhite (Colinus virginianus) 2000/7000126
Guidelines:	EPA 71-2, EPA 850.2200, OECD 205, EPA 40 CFR 158.490, EEC 91/414 Annex II 8.1.2, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The LC_{50} for northern bobwhite (*Colinus virginianus*) exposed to metrafenone was > 5314 ppm (> 948.4 mg/kg bw/d). The no-observed-effect concentration (NOEC) was determined to be 5314 ppm (948.4 mg/kg bw/d), the highest concentration tested, based on the lack of effects on survival, weight gain, feed consumption and no test substance-related gross pathological lesions. Please refer to Document M-II, Section 6, Point 8.1.2 of the summary dossier dated 2002 for further details.

Report:	CA 8.1.1.2/2 [redacted], 2000d Avian dietary toxicity test with AC 375839 in mallard duck (Anas platyrhynchos) 2000/7000125
Guidelines:	EPA 71-2, EPA 850.2200, OECD 205, EPA 40 CFR 158.490, EEC 91/414 Annex II 8.1.2, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The LC_{50} for mallard duck (*Anas platyrhynchos*) after exposure to metrafenone was determined to be > 5314 ppm (> 1483 mg/kg bw/d). The no-observed-effect concentration (NOEC) was determined to be 5314 ppm (948.4 mg/kg bw/d), the highest concentration tested, based on the lack of effects on survival, weight gain and feed consumption, and the lack of test substance-related gross pathological lesions. Please refer to Document M-II, Section 6, Point 8.1.2 of the summary dossier dated 2002 for further details.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Report: CA 8.1.1.3/1
[redacted] 2002a
BAS 560 F: Assessment to determine the effects on reproduction in the northern bobwhite (Colinus virginianus)
2002/7005090
Guidelines: EPA 71-4, EPA 850.2300, EEC 91/414 Annex II 8.1.3, EEC 96/12, OECD 206
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Conclusion

The no-observed-effect concentration (NOEC) for BAS 560 F in an avian reproduction study with northern bobwhite quail (*Colinus virginianus*) was determined to be 1350 ppm a.s. in the diet (125.4 mg a.s./kg bw/d), as dietary administration of BAS 560 F at concentrations up to 1350 ppm had no effects on the health and reproductive performance of adult birds, and on the health and growth of their chicks. The lowest-observable effect concentration (LOEC) was > 1350 ppm (> 125.4 mg a.s./kg bw/d). Please refer to Document M-II, Section 6, Point 8.1.2 of the summary dossier dated 2002 for further details.

Report:	CA 8.1.1.3/2 [redacted] 2006a BAS 560 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchus) by administration in the diet 2006/1018046
Guidelines:	OECD 206, EPA 540/9-82-024, EPA 71-4, EPA 540/9-86-139, EPA 850.2300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The reproductive toxicity of BAS 560 F to mallard duck (*Anas platyrhynchos*) was determined in a one-generation reproduction study, for 22 weeks. Groups of sixteen male/female pairs were exposed to dietary concentrations of 450, 900 and 1350 mg a.s./kg (corresponding to 50.9, 114.7, 167.7 mg a.s./kg bw), in parallel with a control. During the study, the effects of adult exposure to the test item were evaluated for adult health, body weight and feed consumption. In addition, the number of eggs laid, eggshell thickness and egg fertility, embryo viability, hatch rates, offspring survival and offspring weight were assessed.

The measured concentration of test substance in the diet ranged from 92.7% to 98.9% of the nominal concentration. Hence, the biological results were based on the nominal concentrations.

No substance-related effects in the parent generation on mortality, birds' health, food consumption and body weight could be detected in any treatment group. Avoidance of feed was not observed. In the offspring, no biologically relevant substance-related effects were observed in the 450 and 900 mg a.s./kg diet groups. In the highest test group, effects noted were a reduction in the number of eggs laid per female per week and the survival during hatching, and an increase of the number of chicks found dead in shell.

The no-observed-effect concentration (NOEC) during this study was 900 mg/kg feed (114.7 mg a.s./kg bw/d) and the lowest-observable-effect concentration (LOEC) was 1350 mg/kg diet (167.7 mg a.s./kg bw/d), based on reproductive performance.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F
Batch number: 220899-03-6
Purity: 99.4% metrafenone
Description: Solid, beige

2. Test concentrations: 0 (control), 450, 900 and 1350 mg a.s./kg diet (corresponding to 0, 50.9, 114.7 and 167.7 mg a.s./kg bodyweight)

3. Vehicle: None, as the test item was immediately mixed through the diet

4. Test organism

Species:	Mallard duck (*Anas platyrhynchus*)
Age:	Approximately five months old and approaching their first breeding season
Source:	Geflügelhof Knerr, Rieschweiler-Mühlback, Germany
Acclimation period:	Two weeks
Diet:	Experimental diet, feed for ducks in meal form, supplied by Provimi kliba SA, Basel, Switzerland. Additionally, adult birds were offered mussel shell grit *ad libitum.* Municipal drinking water was offered *ad libitum* and during the exposure period additionally with a water bath.
Housing:	Adults: Cages composed of galvanized or stainless steel wire, with stainless steel wire floors, measuring 1.3 m deep x 0.65 m width x 1.3 m high (total area: 0.85 m^2). Hatchlings: Pens made of galvanized or stainless steel wire with stainless steel wire mesh

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	Adults: 16.3-22.7ºC; Eggs: 37.8 ± 0.1ºC; Hatchlings: 37.7-38.1ºC
Relative humidity:	Adults: 28-93%; Eggs: 60-70%; Hatchlings: 80-90%
Photoperiod:	Adults: 7h light: 17h darkness (week one to seven); 14h light: 10h darkness (week eight and nine); 17h light: 7h darkness (week ten to twenty-two). Hatchlings: 17h light: 7h darkness

2. Animal assignment and treatment:

Sixteen replicates per concentration group, with one male and one female per replicate, were exposed during a pre-egg period of ten weeks and a subsequent egg-laying period of twelve weeks. During these twenty-two weeks, the birds were offered feed with test substance *ad libitum*. The eggs were incubated using a commercial incubator with automatic egg rotation. After hatching, the chicks were moved to pens and observed for fourteen days. These chicks received the same feed as the adult birds (without test item) in pelleted form, *ad libitum*.

3. Dose preparation:

The concentrations used in the test were 450, 900 and 1350 mg a.s./kg diet, which were mixed separately with diet. The test substance was weighed and then mixed with the diet using a laboratory mixer, for ten minutes. The diet/test substance mixtures were prepared once a week. The control group received feed without test substance.

4. Measurements/observations:
For the adult birds, observations were made daily for mortality and clinical observations (general condition, signs of toxic effects and abnormal behavior). Food consumption was determined weekly, along with routine observations concerning palatability. In addition, body weights were recorded at test initiation, at the end of weeks two, four, six and eight of the pre-egg production period, and at test termination. All birds which died during the test or were sacrificed at the end of the test were necropsied and subjected to gross-pathological assessment.

All eggs produced were collected daily during the 12-week egg-laying period starting at the beginning of week 11 and ending at the end of week 22. These eggs were also weighed per cage and examined visually for their quality. One egg per pair was collected in the weeks 1, 3, 5, 7, 9 and 11 of the egg-laying period and was examined for egg shell thickness. All eggs (not cracked, broken, abnormal or taken for egg shell measurements) were placed in an incubator at weekly intervals and were candled on days 14 and 21 of the incubation period for evaluation of infertilities, as well as early and late embryonic deaths.

For the chicks, the number hatched and any abnormalities were recorded. These chicks were reared until fourteen days old, and mortality and symptoms (toxic signs) were checked daily during this period. In addition, their body weights were recorded when the chicks were removed from the hatcher and fourteen days after hatching.

To verify if the samples were homogeneously mixed, samples were collected at the beginning of the exposure period from the upper, middle and lower layer of the highest and lowest test item concentration diets. Otherwise, samples (from freshly made diets) were obtained from each test item concentration and the control diets, at three different time points (1: test initiation (start of substance feeding), 2: start of egg-laying period (ten weeks after test initiation) and 3: eighteen weeks after test initiation), for analysis of test item concentration. The analysis was performed using HPLC with variable wavelength detection set at 205 nm.

5. Statistics:
For body weight and food consumption of the adult birds, as well as for egg weight, egg shell thickness and chicks' body weight, a comparison between each test item concentration group and the control group was performed using the Dunnett's test. For count data (i.e. number of eggs and hatched chicks) and proportions (number of fertile eggs of eggs initially set) the Wilcoxon test was used. The SAS-System was used for the statistical analyses. For the analyses of body weight and food consumption of the adult birds, the DATATOX F1-System was used.

II. RESULTS AND DISCUSSION

A. MORTALITIES AND BEHAVIOURAL OBSERVATIONS

Only one bird died during the pre-egg-laying period; a female exposed to 900 mg a.s./kg diet. This was not considered to be related to the test substance exposure. No abnormalities in appearance and behavior were observed in any of the groups during the study.

B. FOOD CONSUMPTION AND BODY WEIGHT

The food consumption was in the normal range during both the pre-egg and the egg-laying period. The uptake was only statistically significantly different (increased) from the control group for adult birds exposed to 900 mg a.s./kg diet during weeks four and seven. No rejection of food was observed. Body weight did not differ significantly between male and female birds exposed to BAS 560 F and the birds in the control group. The results are presented in the table below.

Table 8.1.1.3-1: Mean food consumption and body weight change of mallard duck (*Anas platyrhynchus*) exposed to BAS 560 F for 22 days

Nominal concentration (mg a.s./kg diet)	Mean feed consumption (g/bird/day)	Overall mean body weight change (%) compared between day 0 and day 22	
		Males	Females
Negative control	139.0	-5.66	+1.85
450	133.6	-3.91	+4.56
900	150.9	-4.26	+2.63
1350	146.0	-1.50	-0.90

C. REPRODUCTIVE RESULTS

The groups exposed to 450 and 900 mg a.s./kg diet did not show statistically significant differences to the control group for any of the reproduction parameters. For the group exposed to 1350 mg a.s./kg diet, statistically significant differences to the control group were observed for number of eggs laid/female/week, survival during hatch and number of chicks ‘dead-in-shell’. The results are presented in the following tables:

Table 8.1.1.3-2: Reproductive performance of mallard duck (*Anas platyrhynchus*) exposed to BAS 560 F

Reproductive parameter	Experimental group (mg a.s./kg diet)			
	Control	**450**	**900**	**1350**
No. of eggs laid/group	870	819	811	640
No. of cracked and broken eggs/group	14	17	2	5
Mean egg weight (g)	56.5	55.4	57.5	58.2
Mean egg shell thickness (mm)	0.39	0.39	0.39	0.40
No. of eggs incubated/group	785	739	742	583
No. of fertile eggs/group	577	568	538	524
No. of infertile eggs/group	208	171	204	59
No. of early embryonic mortalities/group	26	11	35	19
No. of viable 14-day old embryos/group	551	557	504	505
No. of late embryonic mortalities/group	4	5	14	8
No. of viable 21-day old embryos/group	547	552	490	497
No. of total embryonic mortalities/group	30	16	48	27
No. of 'dead-in-shell'/group	109	120	90	158
No. of chicks hatched/group	438	432	400	339
No of 14-day surviving chicks/group	438	422	396	336
No. of eggs laid/female/week	4.5	4.3	4.2	3.3*
No of chicks hatched/female/week	2.3	2.3	2.1	1.8
No of 14-day surviving chicks/female/week	2.3	2.2	2.1	1.8
Mean body weight of chicks at hatching (g)	33.8	32.8	34.5	33.3
Mean body weight of chicks 14 days after hatching (g)	280.9	283.5	286.8	290.2

* Statistically significantly different to the control group ($p<0.05$)

Table 8.1.1.3-3: Reproductive performance of mallard duck (*Anas platyrhynchus*) exposed to BAS 560 F expressed as percentages

Reproductive parameter	Experimental group (mg a.s./kg diet)			
	Control	**450**	**900**	**1350**
% fertile eggs of incubated eggs	72.4	82.0	69.2	89.7
% viable 14-day old embryos of eggs incubated	68.9	80.2	65.1	85.5
% viable eggs at day 21 of eggs incubated at day 14	99.4	99.2	96.8	98.0
% hatched chicks of eggs incubated at day 21	83.1	70.5	79.0	60.4*
% 14-day survivors of chicks hatched	99.4	97.5	99.2	99.2
% cracked and broken eggs of eggs laid	1.7	1.8	0.2	1.0
% early embryonic mortalities of fertile eggs	6.7	2.2	6.4	4.3
% late embryonic mortalities of fertile eggs	0.5	0.7	2.5	1.9
% 'dead-in-shell' of fertile eggs	15.7	28.6	16.7	36.8*
Hatchability (% chicks hatched of total eggs incubated)	55.5	56.3	51.9	52.6
Hatchability (% chicks hatched of fertile eggs)	77.0	68.5	74.4	57.0*

* Statistically significantly different to the control group ($p<0.05$)

D. ANALYSIS

Analysis of the samples collected from the upper, middle and lower layer of the diets showed measured concentrations of 445.2, 434.6 and 448.4 mg test substance/kg for the lowest test substance concentration and 1307.5, 1349.2 and 1302.7 mg/kg for the highest test substance concentration for the respective layers. These values demonstrate a homogeneous distribution of the test substance in the diet.

The measured concentration of test substance in the diet (with samples taken at three time points) ranged from 92.7% to 98.9% of the nominal concentrations. The biological results are therefore based on the nominal concentrations. The concentrations of the test substance BAS 560 F observed in the analysis are shown in the table below.

Table 8.1.1.3-4: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./kg)	Nominal concentration* (mg test substance/kg)	Time point**	Measured concentration (mg test substance/L)	Measured as % of nominal test substance concentration	Mean measured as % of nominal test substance concentration
0 (negative control)	0 (negative control)	1 2 3	< LOQ	-	-
450	453	1 2 3	442.7 448 433	97.7 98.9 95.6	97.4
900	905	1 2 3	850.8 839 869	94.0 92.7 98.6	94.2
1350	1358	1 2 3	1319.8 1312 1339	97.2 96.6 98.6	97.5

* The concentration of the test substance BAS 560 F, containing 99.4% of the active substance metrafenone
** Samples were taken from freshly made diets at three different time points (1: test initiation (start of substance feeding), 2: start of egg-laying period (ten weeks after test initiation) and 3: eighteen weeks after test initiation)
LOQ = Limit of quantification (1.86 mg/L, corresponding to 18.6 mg/kg feed)

E. DEFICIENCIES

None.

III. CONCLUSION

The no-observed-effect concentration (NOEC) for mallard duck (*Anas platyrhynchos*) exposed to BAS 560 F was 900 mg a.s./kg diet (114.7 mg a.s./kg bw/d) and the lowest-observable-effect concentration (LOEC) was 1350 mg a.s./kg diet (167.7 mg a.s./kg bw/d), based on reproductive performance.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

The table below gives the relevant mammalian toxicity endpoints for metrafenone, as taken from the list of endpoints for this active substance presented in the EFSA Conclusion for metrafenone (*EFSA Scientific Report* (2006) 58, 1- 72). These endpoints are adequate for the mammalian risk assessment (please see the respective M-CP Section 10 documents for BAS 560 00 F and BAS 560 02 F). For further information on toxicity of metrafenone to mammals please see M-CA Section 5 (toxicological evaluation).

Table 8.1.2-1: Summary of effects of metrafenone to mammals

Test species	Test substance	Test System	Exposure	Results	Reference
Rat	Metrafenone	Acute	Single dose	LD_{50} > 5000 mg a.s./kg bw	[redacted] (1999a) MCA 5.2.1/1
Rats	Metrafenone	Reproduction	2-generation	NOAEL = 10000 ppm (811 mg a.s./kg bw/d)	[redacted] (2002a) MCA 5.6.1/2

CA 8.1.2.1 Acute oral toxicity to mammals

Please refer to Document M-CA, Section 5.2.1 for study summary details on the acute oral toxicity data for metrafenone.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Please refer to Document M-CA, Section 5.5 for study summary details on the long-term toxicity data for metrafenone.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

In M-CP Section 10, the risks to birds and mammals from secondary poisoning have been assessed and found acceptable. Therefore, no further data are required to address this point.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No additional data submitted.

CA 8.1.5 Endocrine disrupting properties

A full review of the ecotoxicological and toxicological dataset as well as any other additional information on the toxicity profile has been undertaken to determine whether metrafenone has the potential to cause endocrine disruption in terrestrial organisms. Although the ecotoxicology studies are of limited sensitivity in detecting endocrine-relevant effects, there is no evidence that metrafenone causes endocrine disruption based on the evaluation of the available avian reproduction studies.

When considering the available toxicological studies (M-CA Section 5), in the two-generation rat study an increased proportion of abnormal sperm was seen only in F1 males and only at the highest dose level of 10000 ppm (811 mg/kg/day). However, for these males no effects on reproductive performance were observed, all other sperm parameters were comparable to controls and no effects were observed at histopathological examination of the testes and epididymides. In developmental toxicity studies with rats and rabbits, metrafenone did not show teratogenic potential.

Overall ecotoxicological and toxicological data do not indicate any relevant endocrine mode of action for metrafenone. Metrafenone is not classified as a carcinogen or reproductive toxin and does not therefore meet the interim EU criteria for an endocrine disruptor. Although final criteria for the definition of 'endocrine disruptor' have yet to be agreed, based on the above assessment, it is unlikely that metrafenone would be considered to be an endocrine disruptor in the context of Regulation (EC) No 1107/2009. Therefore, further specific testing for endocrine disruption is not required. However this conclusion should be re-assessed once agreed criteria become available.

CA 8.2 Effects on aquatic organisms

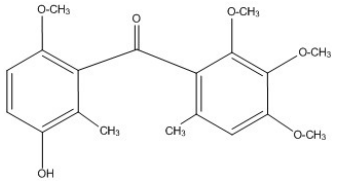
A number of studies with aquatic organisms exposed to the active substance (metrafenone) and potentially relevant surface water metabolites (CL375816 and CL4084564) were submitted and reviewed for the first EU Review of metrafenone. Additional data on fish, aquatic invertebrates, sediment dwellers, algae and higher aquatic plants (*Lemna)* are now available and are included with this submission. No data are available for the soil photo metabolites CL 1500831, CL 3000402 or CL 377160, which are potentially relevant in surface water. Since CL 377160 is structurally similar to CL 4084564 and parent, no additional data were deemed necessary. The two lactone metabolites CL 3000402 and CL 1500831 are structurally very similar to each other and would be expected to have very similar toxicity towards aquatic organisms. Therefore, for the purposes of the aquatic assessment, CL 3000402 and CL 1500831 are considered in combination and data on one would also address the aquatic toxicity of the other. Attempts to perform studies on CL 3000402 with *Daphnia* and algae were not successful (refer to points CA 8.2.4.1 and 8.2.6.1). In the absence of data on CL 3000402 and CL 1500831, data on the parent is used (worst-case toxicity scenario). This assumption is supported by QSAR values (ECOSAR Version 1.00, included in EPI Suite Version 4.1), which indicated the parent to be more toxic than the metabolites (see Table 8.2-1below). The QSAR values were calculated using the SMILES notations of the molecules.

Table 8.2-1: QSAR endpoint for metrafenone and its potentially relevant metabolites CL 300402 and CL 1500831

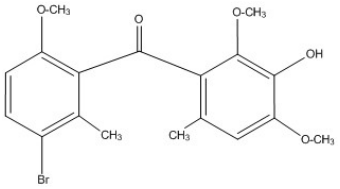
Organisms/endpoint	CL 300402 (mg/L)	CL 1500831 (mg/L)	Metrafenone (QSAR) (mg/L)	Metrafenone (Experimental) (mg/L)
Fish / LC_{50}	9.4 *	9.4 *	1.3	> 0.82
Daphnia / EC_{50}	16.1 *	16.1 *	1.1	> 0.92
Algae / EC_{50}	5.9 *	5.9 *	1.7	> 0.36

* The compound may not be sufficiently soluble to achieve this dose

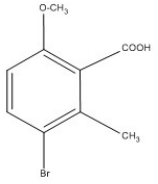
Please refer to the figure below for the molecular structures of parent metrafenone and its metabolites which could occur in surface water.



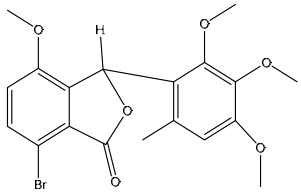
CL 4084564



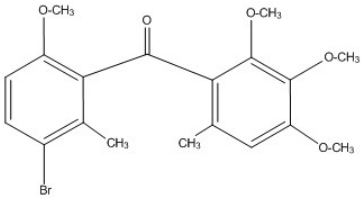
CL 377160



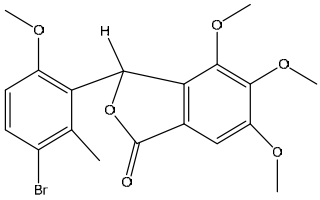
CL 375816



CL 3000402



Parent



CL 1500831

Figure 8.2-1: Comparison of the molecular structures of metabolites CL 377160, CL 084564, CL 1500831 and CL 4084564 with parent

All available data on aquatic toxicity are summarized in Table 8.2-2.

Table 8.2-2: Summary of the toxicity of metrafenone and its metabolites to aquatic organisms

Test material	Species	Time scale, study type	End-point	Result (mg a.s./L)	Reference
Fish					
Metrafenone technical	Rainbow trout (*Oncorhynchus mykiss*)	96 hrs flow-through	LC_{50}	> 0.82 (mm)	KCA 8.2.1/1 1999/7000289
Metrafenone technical	Bluegill sunfish (*Lepomis macrochirus*)	96 hrs flow-through	LC_{50}	> 0.87 (mm)	KCA 8.2.1/2 1999/7000286
Metrafenone technical	Fathead minnows (*Pimephales promelas*)	96 hrs flow-through	LC_{50}	> 0.51 (mm)	KCA 8.2.1/3 2011/1281328
Metrafenone technical	Sheepshead minnow (*Cyprinodon variegatus*)	96 hrs flow-through	LC_{50}	> 0.35[a] (mm)	KCA 8.2.1/4 2005/7003439
CL 375816	Rainbow trout (*Oncorhynchus mykiss*)	96 hrs static	LC_{50}	> 99 (mm)	KCA 8.2.1/5 2002/1004394
CL4084564	Rainbow trout (*Oncorhynchus mykiss*)	96 hrs static	LC_{50}	16.4 (mm)	KCA 8.2.1/6 2002/1005255
Metrafenone technical	Fathead minnows (*Pimephales promelas*)	32 days flow-through	ELS - NOEC	0.228 (mm)	KCA 8.2.2.1/1 2000/7000128
Metrafenone technical	Fathead minnows (*Pimephales promelas*)	33 days flow-through	ELS - NOEC	0.204 (mm)	KCA 8.2.2.1/2 2012/1009601
Metrafenone technical (radio-labelled)	Bluegill sunfish (*Lepomis macrochirus*)	28 days flow-through bioconcentrat-ion	BCF	530 (whole fish)	KCA 8.2.2.3/1 2001/7000274

Test material	Species	Time scale, study type	End-point	Result (mg a.s./L)	Reference
Aquatic invertebrates					
Metrafenone technical	*Daphnia magna*	48 hrs static	EC_{50}	> 0.92 (mm)	KCA 8.2.4.1/1 1999/7000287
CL 375816	*Daphnia magna*	48 hrs static	EC_{50}	> 100 (nom)	KCA 8.2.4.1/2 2002/1004870
CL4084564	*Daphnia magna*	48 hrs static	EC_{50}	46 (mm)	KCA 8.2.4.1/3 2002/1004869
Metrafenone technical	*Crassostrea virginica*	96 hrs flow-through	EC50	0.22 (mm)	KCA 8.2.4.2/1 2005/7003442
Metrafenone technical	*Americamysis bahia*	96 hrs flow-through	EC50	> 0.33 (mm)	KCA 8.2.4.2/2 2011/7000913
Metrafenone technical	*Daphnia magna*	21 days static-renewal	NOEC	0.225 (mm)	KCA 8.2.5.1/1 2000/7000130
Metrafenone technical	*Daphnia magna*	21 days static-renewal	NOEC	0.300 (nom)	KCA 8.2.5.1/2 2011/1260868
Metrafenone technical	*Americamysis bahia*	28 days flow-through	NOEC	0.022 (mm)	KCA 8.2.5.2/1 2007/7009454
Sediment dwellers					
Metrafenone technical	*Chironomus riparius*	28 days static	NOEC	2.0 (nom)	KCA 8.2.5.3/1 2007/1018942
Metrafenone technical	*Chironomus riparius*	40 days static	NOEC	1.0 (nom)	KCA 8.2.5.4/1 2001/7000462
Metrafenone technical	*Chironomus riparius*	28 days static with spiked sediment	NOEC	296 mg a.s./kg sediment dw (mm)	KCA 8.2.5.4/2 2010/1145509
Metrafenone technical (radio-labelled)	*Leptocheirus plumulosus*	10 days static with spiked sediment	EC_{50} NOEC	> 1.7 mg a.s./kg sediment dw (mm) 0.76 mg a.s./kg sediment dw (mm)	KCA 8.2.5.4/3 2011/7000373

Test material	Species	Time scale, study type	End-point	Result (mg a.s./L)	Reference
Algae					
Metrafenone technical	*Selenastrum capricornutum*	72 hrs static	E_rC_{50} E_bC_{50}	> 0.870 (mm) 0.711 (mm)	KCA 8.2.6.1/1 2000/7000122
Metrafenone technical	*Pseodokirchneriella subcapitata*	96 hrs static	E_rC_{50} E_yC_{50}	> 0.339 (mm) > 0.339 (mm)	KCA 8.2.6.1/2 2011/1254828
CL375816	*Pseodokirchneriella subcapitata*	72 hrs static	ERC50 EBC50	> 100 (nom) > 100 (nom)	KCA 8.2.6.1/3 2002/1004873
CL4084564	*Pseodokirchneriella subcapitata*	72 hrs static	ERC50 EBC50	38.7 (mm) 27.8 (mm)	KCA 8.2.6.1/4 2002/1004872
Metrafenone technical	*Anabaena flos-aquae*	96 hrs static	E_rC_{50} E_bC_{50}	> 0.86 (imm) > 0.86 (imm)	KCA 8.2.6.2/1 2005/7003441
Metrafenone technical	*Anabaena flos-aquae*	96 hrs static	E_rC_{50} E_yC_{50}	> 0.450 (mm) > 0.450 (mm)	KCA 8.2.6.2/2 2011/1254829
Metrafenone technical	*Navicula pelliculosa*	96 hrs static	E_rC_{50} E_bC_{50}	> 0.91 (imm) > 0.91 (imm)	KCA 8.2.6.2/3 2005/7003436
Metrafenone technical	*Navicula pelliculosa*	96 hrs static	E_rC_{50} E_yC_{50}	> 0.357 (mm) > 0.357 (mm)	KCA 8.2.6.2/4 2011/1254831
Metrafenone technical	*Skeletonema costatum*	72 hrs [b] static	E_rC_{50} E_bC_{50}	0.67 (imm) 0.50 (imm)	KCA 8.2.6.2/5 2005/7003443
Metrafenone technical	*Skeletonema costatum*	96 hrs static	E_rC_{50} E_yC_{50}	> 0.364 (mm) > 0.364 (mm)	KCA 8.2.6.2/6 2011/1254830
Lemna					
Metrafenone technical	*Lemna gibba*	7 days static-renewal	E_rC_{50} E_bC_{50}	> 0.76 (mm) > 0.76 (mm)	KCA 8.2.7/1 2005/7003440
Metrafenone technical	*Lemna gibba*	7 days static	E_rC_{50} E_yC_{50}	> 0.327 (mm) > 0.327 (mm)	KCA 8.2.7/2 2011/1254832

[a] Based on centrifuged samples. Non-centrifuged samples resulted in a 96-he LC_{50} of > 0.65 mg a.s./L/
[b] Test was conducted up to 96 hrs, but lowest endpoints at 72 hrs
mm = mean measured, nom = nominal, imm = initial mean measured, dw = sediment dry weight

CA 8.2.1 Acute toxicity to fish

Report: CA 8.2.1/1
[redacted] 1999a
Acute toxicity of AC 375839 to rainbow trout, Oncorhynchus mykiss, under flow-through test conditions
1999/7000289
Guidelines: EEC 91/414 Annex II 8.2.1, EEC 96/12, EPA 72-1
GLP: yes
(certified by United States Environmental Protection Agency)

Conclusion

The LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to metrafenone for 96 hours under flow-through conditions was > 0.82 mg a.s./L, based on mean measured concentrations. The no-observed-effect concentration (NOEC) was determined to be 0.25 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.1 of the summary dossier dated 2002 for further details.

Report: CA 8.2.1/2
[redacted] 1999b
Acute toxicity of AC 375839 to bluegill, Lepomis macrochirus, under flow-through test conditions
1999/7000286
Guidelines: EEC 91/414 Annex II 8.2.1, EEC 96/12, EPA 72-1
GLP: yes
(certified by United States Environmental Protection Agency)

Conclusion

The LC_{50} for bluegill sunfish (*Lepomis macrochirus*) exposed to metrafenone for 96 hours under flow-through conditions was determined to be > 0.87 mg a.s./L, based on mean measured concentrations. The no-observed-effect concentration (NOEC) was determined to be 0.45 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.1 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.1/3 [redacted] 2012a BAS 560 F (Metrafenone) - Acute toxicity study in fathead minnow (Pimephales promelas) 2011/1281328
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 (1992), EPA 540/9-82-024, EPA 850.1075, EPA 72-1
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 96-hour acute toxicity laboratory study, fathead minnows (*Pimephales promelas*) were exposed to 100% of a saturated solution of the test substance BAS 560 F (metrafenone) and a water control. The fish were exposed under flow-through conditions, in groups of ten fish, with two replicates for the control and three replicates for the test substance treatment group. Fish were observed for survival and symptoms of toxicity within 1 hour after test initiation and after 6, 24, 48, 72 and 96 hours.

The overall mean measured concentration of the test substance was 0.51 mg/L, equal to 108% of the reported water solubility. The biological results were based on the mean measured concentration.

After 96 hours of exposure, no mortality and no toxic effects were observed in the control and the test item treatment group.

In this flow-through acute toxicity study with fathead minnow exposed for 96 hours, the LC_{50} of BAS 560 F was > 0.51 mg/L (mean measured), based on the limit of solubility in the test system. The no-observed-effect concentration (NOEC) was determined to be 0.51 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2 ± 1%
Description: Solid, beige

2. Test concentrations: 0 (control) and 100 % of a saturated solution (corresponding to a mean measured concentration of 0.51 mg a.s./L)

3. Reference item: None

4. Dilution water: Charcoal-filtered tap water mixed with deionized water
Vehicle: None

5. Test organism:

Species:	Fathead minnow (*Pimephales promelas*)
Age:	Approximately six months old
Weight:	0.65 g (0.54 - 0.87 g)
Length:	4.1 cm (3.8 - 4.5 cm)
Source:	Fish were hatched in the testing facility
Acclimation period:	Fish were acclimatized prior to test initiation
Diet:	Not fed during the test
Test vessels:	9 L stainless steel aquaria (29 x 21 x 22 cm) filled with approximately 9 L of test water. Exposure occurred under flow-through conditions, with a flow rate of 37 to 38 mL/min
Loading:	0.72 g fish/L

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	24 ºC
pH:	7.4-8.0
Dissolved oxygen:	7.2-7.8 mg/L
Hardness:	100 mg $CaCO_3$/L
Photoperiod:	16h light: 8h darkness (96-451 lux)
Aeration:	No aeration

2. Animal assignment and treatment:
Fish were randomly placed in the aquaria, with ten fish per replicate aquarium. Three replicate aquaria were exposed to the test substance at a concentration of 100% of a saturated solution, which corresponds to a mean measured concentration of 0.51 mg a.s./L. A control group consisting of 2 replicate aquaria was also included in the test and the control solution consisted of water only. The exposure period was 96 hours under flow-through conditions.

3. Dose preparation:
As the test substance is poorly soluble in water, the test solution was prepared using a saturation column, in order to achieve a saturated solution of the test substance. The saturation column was prepared by dissolving 10 g of test substance in 200 mL acetone. This solution was then poured over glass wool and the solvent was completely evaporated, leaving the test substance adhered to the glass wool. The treated glass wool was placed in a glass column. A similarly prepared column, without test substance, was used for the control group. The outflow of the column was distributed equally among the test vessels and analyzed to monitor test concentrations. The flow-through system was allowed to saturate for several days and exposure was started once stable analytical results were obtained (approximately after ten days).

4. Measurements and observations:
Mortality and symptoms of toxicity were recorded within one hour after test initiation and 6, 24, 48, 72 and 96 hours after test initiation.

Samples were collected within 30 minutes before insertion of the fish to the test vessels, and 48 and 96 hours after test initiation. Samples were analyzed by HPLC with MS-detection.

5. Statistics:
Descriptive statistics were used, since the data were not appropriate for the use of computerized methods for the statistical calculations.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

After 96 hours of exposure, no mortality and no toxic effects were observed in the control and the test item treatment group.

B. ANALYSIS

Analytical verification of test substance concentration was conducted in each replicate at test initiation, and after 48 and 96 hours. The overall mean measured concentration of BAS 560 F was 0.51 mg/L (range: 0.47-0.53 mg/L), corresponding to 108% of the reported water solubility limit. The biological results are based on the mean measured concentration. The results of the analytical verification of test substance concentrations are presented in the table below.

Table 8.2.1-1: Measured concentrations of BAS 560 F in test samples

Test group	Rep-licate no.	Sampling time (hours)	Measured concentration (mg test substance/L)	Overall mean measured concentration (mg test substance/L)	% of reported water solubility*
Negative control	1	0 48 96	< LOQ	-	-
Negative control	2	0 48 96	< LOQ	-	-
100% saturated test substance solution	1	0 48 96	0.53 0.56 0.49	0.51	108
100% saturated test substance solution	2	0 48 96	0.53 0.53 0.47		
100% saturated test substance solution	3	0 48 96	0.53 0.51 0.47		

LOQ = Limit of quantification (0.001 mg a.s./L)
* % of reported water solubility = 0.474 mg/L

C. DEFICIENCIES

None.

III. CONCLUSION

The LC_{50} for fathead minnow (*Pimephales promelas*) exposed to BAS 560 F (metrafenone) for 96 hours under flow-through conditions was > 0.51 mg/L (mean measured), based on the limit of solubility in the test system. The no-observed-effect concentration (NOEC) was determined to be 0.51 mg/L (mean measured), the highest concentration tested.

Report: CA 8.2.1/4
[redacted] 2005e
BAS 560 F: A 96-hour flow-through acute toxicity test with the sheepshead minnow (Cyprinodon variegatus)
2005/7003439
Guidelines: EPA 850.1075
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The acute toxicity of BAS 560 F (metrafenone) to the sheepshead minnow (*Cyprinodon variegatus*) was determined in a 96 hour flow-through test. BAS 560 F was tested at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s/L, which was to the limit of solubility in the test system. A negative control (filtered saltwater) and a solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The fish were tested in groups of ten fish per replicate, with two replicates in each treatment and control group.

Results of analyses showed that concentrations generally remained stable over the 96-hour period. The mean measured concentrations were 0.072, 0.13, 0.24, 0.32 and 0.65 mg a.s./L. Since precipitate was visible in the two highest test concentrations (0.5 and 1.0 mg a.s./L (nominal)) these samples were also analyzed following centrifugation; the mean measured concentrations were 0.13 and 0.35 mg a.s./L, respectively. The results were based on the mean measured concentrations of both centrifuged and uncentrifuged samples.

The sheepshead minnows in the control groups and in all BAS 560 F treatment groups appeared normal throughout the test, with no mortalities or signs of toxicity noted.

The test was conducted to the limit of solubility in the test system. The no-mortality concentration and the no-observed-effect concentration (NOEC) were both 0.65 mg a.s./L (0.35 mg a.s./L based on centrifuged samples), the highest mean measured concentration tested. LC_{50} values at 24, 48, 72 and 96 hours were estimated to be > 0.65 mg a.s./L (> 0.35 mg a.s./L based on centrifuged samples), the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured: 0, 0.072, 0.13, 0.24, 0.32 (0.13 centrifuged) and 0.65 (0.35 centrifuged) mg a.s./L)

3. Reference item: None

4. Dilution water: Natural seawater collected at Indian River Inlet, Delaware filtered and diluted to a salinity of ~20‰ with well water
Vehicle: Dimethyl formamide (DMF)

5. Test organism:

Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Age:	Juveniles. All fish were from the same source and year class.
Weight:	0.23 g (0.10-0.31 g) average wet weight at the end of the test
Length:	2.2 cm (1.8-2.5 cm) average total length at the end of the test
Source:	Aquatic BioSystems, Inc. Fort Collins, Colorado
Acclimation period:	At least 14 days
Diet:	Commercially-prepared diet and *Artemia nauplii*. Not fed at least two days prior to and during the test
Test vessels:	9 L glass aquaria filled with approximately 7 L of test water
Loading:	0.33 g fish/L

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	21.7-22.4 °C
Salinity:	20 – 21‰
pH:	8.2 – 8.3
Dissolved oxygen:	≥ 4.9 mg/L (≥ 63% of saturation)
Photoperiod:	16 h light : 8 h darkness (255 lux at test initiation)
Aeration:	By Day 2 gentle aeration was added to maintain dissolved oxygen above 60% of saturation throughout the remainder of the test

2. Animal assignment and treatment:
Twenty impartially distributed fish per test group (two replicate chambers containing ten fish each) were exposed to the test substance at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative (filtered saltwater) control and a solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The exposure period was 96 hours under flow-through conditions.

3. Dose preparation:
A primary stock solution was prepared by mixing a calculated amount of test substance into dimethyl formamide (DMF) at a nominal concentration of 10 mg a.s./L. Four additional solutions (at nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./mL) were prepared in DMF by proportional dilution of the primary stock. The five stock solutions were injected into the diluter mixing chambers where they were mixed with saltwater to achieve the desired test concentrations. The solvent control was prepared by injecting DMF into the mixing chamber for the solvent control. The concentration of DMF in the solvent control and all BAS 560 F treatment groups was 0.1 mL/L. The diluter was adjusted so that each test chamber received approximately 13 volume additions of test water every 24 hours.

4. Measurements and observations:
Observations were made 5.5, 24, 48, 72 and 96 hours after test initiation, to determine the number of mortalities in each treatment group. The number of individuals exhibiting signs of toxicity or abnormal behavior was also evaluated.

Samples were collected prior to test initiation from one test chamber of each treatment and control groups to confirm the operation of the diluter. Additional samples were collected from alternating replicate test chambers at 0, 48 and 96 hours to measure concentrations of the test substance. Samples were diluted with saltwater, as needed, and analyzed by HPLC using wavelength detection at 220 nm.

Temperature, dissolved oxygen and pH were measured every 24 hours during the test.

5. Statistics:
As no mortalities were observed during the test, LC_{50} values could not be calculated but were estimated to be greater than the highest concentration tested. The no-mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The sheepshead minnows in both control groups and in each BAS 560 F treatment groups appeared normal throughout the test, with no mortalities or signs of toxicity noted. The results are presented in the table below.

Table 8.2.1-2: Mortality and effects of sheepshead minnows (*Cyprinodon variegatus*) exposed to BAS 560 F

Mean measured concentration (mg a.s./L)	5.5 hours		24 hours		48 hours		72 hours		96 hours	
	No. Dead[a]	Effects[b]	No. Dead[a]	Effects[b]	No. Dead[a]	Effects[b]	No. Dead[a]	Effects[b]	No. Dead[a]	Effects[b]
Negative control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
Solvent control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.072	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.13	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.24	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.32[c]	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.65[c]	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN

[a] Cumulative number of dead fish
[b] Observed effects: AN = appear normal
[c] Non-centrifuged mean measured concentration

B. ANALYSIS

At nominal concentrations ≤ 0.25 mg a.s./L, measured concentrations ranged from approximately 93% to 119% of nominal. At nominal concentrations of 0.5 and 1.0 mg a.s./L, results ranged from approximately 56% to 70% of nominal in samples analyzed without centrifugation. Since a precipitate was visible in the 0.50 and 1.0 mg a.s./L test chambers, samples were also analyzed following centrifugation (at 14000 rpm). The measured concentrations of the centrifuged samples ranged from approximately 20 to 37% of nominal. The lower recoveries and the presence of a precipitate in the 0.50 and 1.0 mg a.s./L test chambers indicate that the test was conducted to the limit of water solubility in the test system (0.3 mg a.s./L). The concentrations of BAS 560 F observed in the analysis are shown in the table below.

Table 8.2.1-3: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	Measured as % of nominal	Mean measured concentration (mg a.s./L)	Mean measured as % of nominal
0 (negative control)	0 48 96	< LOQ	-	-	-
0 (solvent control)	0 48 96	< LOQ	-	-	-
0.063	0 48 96	0.0712 0.0749 0.0707	113 119 112	0.072	114
0.13	0 48 96	0.137 0.133 0.127	105 102 97.7	0.13	100
0.25	0 48 96	0.242 0.233 0.242	96.8 93.0 97.0	0.24	96
0.50	0 48 96	0.320 0.338 0.296	64.1 67.6 59.1	0.32	64
1.0	0 48 96	0.558 0.697 0.694	55.8 69.7 69.4	0.65	65
0.50*	0 48 96	0.147 0.141 0.0998	29.4 28.1 20.0	0.13	26
1.0*	0 48 96	0.313 0.362 0.366	31.3 36.2 36.6	0.35	35

LOQ = Limit of quantification (0.040 mg a.s./L)
* Samples centrifuged at 14000 rpm

C. DEFICIENCIES

None.

III. CONCLUSION

The acute toxicity of BAS 560 F to sheepshead minnow (*Cyprinodon variegatus*) was assessed under flow-through conditions, to the limit of solubility in the test system. The 96-hour LC_{50} value was > 0.65 mg a.s./L (mean measured; corresponding to > 0.35 mg a.s./L for centrifuged samples), the highest concentration tested. The no-mortality concentration and the no-observed-effect concentration (NOEC) were both 0.65 mg a.s./L (0.35 mg a.s./L when based on centrifuged samples).

Report:	CA 8.2.1/5 [redacted] 2002a Reg,No. 40744484 (metabolite of BAS 560 F) - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2002/1004394
Guidelines:	EPA 72-1, EPA-SEP 540/9-85-006, EEC 92/69 A V C 1, OECD 203
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to CL 375816 (metabolite of metrafenone) for 96 hours under static conditions was determined to be > 99 mg a.s./L, the highest mean measured concentration tested. The no-observed-effect concentration (NOEC) was 99 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.1 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.1/6 [redacted] 2002b CL 4084 564 (metabolite of BAS 560 F) - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in static system over 96 hours 2002/1005255
Guidelines:	EPA 72-1, EEC 92/69 A V C 1, OECD 203
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to CL 4084564 (metabolite of metrafenone) for 96 hours under static conditions was calculated to be 16.4 mg a.s./L, based on mean measured concentrations. The no-observed-effect concentration (NOEC) was determined to be 9.2 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.1 of the summary dossier dated 2002 for further details.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Report: CA 8.2.2.1/1
[redacted] 2000a
Toxicity of AC 375839 during the early life-stages of the fathead minnow (Pimephales promelas)
2000/7000128
Guidelines: EPA 72-4(a), EEC 91/414 Annex II 8.2.2.2, EEC 96/12
GLP: yes
(certified by United States Environmental Protection Agency)

Conclusion

The no-observed-effect concentration (NOEC) for fathead minnow (*Pimephales promelas*) exposed to metrafenone for 32 days (28 days post-hatching) under flow-through conditions in an early life-stage (ELS) test was 228 µg a.s./L (mean measured) and the lowest-observable-effect concentration (LOEC) was determined to be 419 µg a.s./L (mean measured), based on mean total length and wet weights. Please refer to Document M-II, Section 6, Point 8.2.2 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.2.1/2 [redacted] 2012b BAS 560 F (Metrafenone) - Early life-stage toxicity test on the fathead minnow (Pimephales promelas) in a flow through system 2012/1009601
Guidelines:	OECD 210, EPA 540/9-86-138, EPA 72-4 (a), EPA 850.1400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 8.2.2.1/3 Obermann M., 2012a Concentration control analysis of BAS 560 F (Metrafenone) in mixing-water, GV/T project-no. 50F0437/01E002 2012/1016030
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The chronic toxicity of metrafenone to fathead minnow (*Pimephales promelas*) was evaluated in a 33-day early life-stage (ELS) test under flow-through conditions. Embryos were exposed to 16, 25, 40, 63 and 100 % of a saturated solution of the active ingredient as nominal concentrations (corresponding to mean measured concentrations of 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L). A dilution water control was tested in parallel. Hatchability, pre- and post-hatch survival rate, time to hatch and swim-up, signs of toxicity and growth parameters of fathead minnow embryos were assessed throughout the study.

The measured concentrations in the analyzed samples were within the range of ±20% of the overall mean measured concentration, except for samples in the two highest test groups with deviations of 77% and 79% of the mean measured concentration on the last day of the exposure. The biological results were based on mean measured concentrations.

No test substance-related effect was observed on the time to start or end of hatching and the time to swim-up. The survival from hatch to the end of swim-up and from the end of swim-up to the end of exposure (day 6 – 33) as well as the overall survival (day 0 - 33) was not statistically significantly reduced in the treatment group in comparison to the control group. There were no observable test substance related signs of toxicity or abnormalities (sublethal effects) in any of the tested concentration groups. In comparison to the control group, the mean wet weights and the total body lengths of the surviving fish at the end of the exposure period were statistically significantly reduced in the two highest test concentrations of 0.364 and 0.535 mg a.s./L.

In a 33-day early life stage study with fathead minnow (*Pimephales promelas*), the overall no-observed-effect concentration (NOEC) for metrafenone was determined to be 0.204 mg a.s./L and the lowest-observable-effect concentration (LOEC) was 0.364 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Metrafenone (BAS 560; Reg. No. 4 037 710)
Batch number: AC12053-29
Purity: 94.2%
Description: Beige solid

2. Test concentrations: 0 (control), 16%, 25%, 40%, 63% and 100% of a saturated solution of the a.s. (nominal), (mean measured: 0, 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L)

3. Reference item: None

4. Dilution water: Non-chlorinated, filtered drinking water (diluted with deionized water)
Vehicle: Dilution water

5. Test organism:
Species: Fathead minnow (*Pimephales promelas*)
Age: Embryos (fertilized, less than four hours old)
Source: Parents were obtained from Osage Catfisheries Inc., USA
Diet: Live brine shrimp nauplii (*Artemia* sp.) and fine milled commercial fish diet ("Tetramin") from day 6 on, twice daily
Test vessels: Cylindrical glass vessels, water volume: 1.7 L (until day 15); stainless steel aquaria (29 x 21 x 22 cm), water volume: 9.0 L (from day 15 until test termination)

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 24.2 °C – 25.6 °C
pH: 7.9 – 8.2
Dissolved oxygen: 5.7 – 8.4 mg/L
Hardness: 1.02 – 1.05 mmol $CaCO_3$/L
Photoperiod: 16 h light: 8 h darkness (80 – 196 lux)
Aeration: Slight aeration via glass tubes

2. Animal assignment and treatment
Fertilized eggs (embryos) and larvae were exposed in cylindrical glass vessels and were transferred into stainless steel aquaria on day fifteen. The test solution flowed continuously from the mixing tank into an "udder", which divided the test solution into four equal parts for the four replicate test aquaria (containing 25 embryos each). Flow rates were 150 mL/minute/treatment group and 2.25 L/hour/test vessel (providing a 6-fold exchange rate of the water volume in each larger test vessel (9 L) every 24 hours). The fish were exposed to test concentrations of 16%, 25%, 40%, 63% and 100% of a saturated nominal solution of the active substance, corresponding to 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L (mean measured). A control group (dilution water only) was tested in parallel. The fish were exposed for 33 days under flow-through conditions.

3. Dose preparation:
As the test substance is poorly soluble in water, a saturated solution of the test substance was prepared using a saturation column. The saturation column was prepared by dissolving 10 g of test substance in 200 mL acetone. This solution was then poured over glass wool and the solvent acetone was completely evaporated, leaving the test substance adhered to the glass wool. The treated glass wool was placed in a glass column. The outflow of the column was collected in a stock solution tank. A metering pump delivered the stock solution to each mixing tank, where it was continuously diluted with aerated dilution water to generate the nominal test concentration for each test material concentration group. From there the solution was distributed equally among the four test vessels per treatment group. A similarly prepared column, without test substance, was used for the control group.

4. Measurements and observations
Throughout the exposure period, hatching, swim-up, mortality, signs of toxicity and abnormal behavior were assessed daily. After 33 days, the fish were sacrificed and the body length and weight of surviving individuals were determined.

Analytical verification of test item concentrations was conducted on samples taken (at least) every week, which were analyzed either directly or after centrifugation, using a reversed-phase HPLC-method with MS-detection.

Temperature was recorded daily. Dissolved oxygen and pH were measured every three days.

5. Statistics
Dunnett`s test (two-sided) was carried out for statistical evaluation of the weight and length data. For the survival data, Fisher's exact test (one-sided) was used to compare the exposed groups to the control group. Wilcoxon-test (one-sided) was performed to examine variability between replicates.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No test substance-related effect was observed on the time to start or end of hatching and the time to swim-up. The survival from hatch to the end of swim-up and from the end of swim-up to the end of exposure (day 6 – 33) as well as the overall survival (day 0 - 33) was not statistically significantly decreased in the treatment group in comparison to the control group. There were no observable test substance related signs of toxicity or abnormalities (sublethal effects) in any of the tested concentration groups. In comparison to the control group the mean wet weights and the total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in the two highest test concentrations of 0.364 and 0.535 mg a.s./L. The results are summarized in the table below.

Table 8.2.2.1-1: Chronic toxicity of metrafenone to fathead minnow (*Pimephales promelas*) in a fish early life stage test (33 d)

Concentration (mean measured) (mg a.s./L)	**Control**	**0.095**	**0.150**	**0.204**	**0.364**	**0.535**
Hatching success (%)	96	96	94	93	95	92
Survival of larvae from hatch until end of swim-up (day 6) (%)	97	98	99	100	96	99
Post-hatch survival of young fish (day 6 to 33) (%)	94	94	97	91	96 [a]	94
Survival from day 0 to test termination (day 33) (%)	87	88	90	85	87	85
Start of hatch (day) End of hatch (day)	3 5	3 5	3 5	3 5	3 5	3 5
Start of swim-up End of swim-up	5 6	5 6	5 6	5 6	5 6	5 6
Symptoms	none	none	none [b]	none	none	none
Mean weight (33 d) (mg)	197	201	211	202	182	168 **
% of control	100	102	107	103	93	85
Mean length (33 d) (cm)	2.8	2.8	2.8	2.7	2.6 **	2.5 **
% of control	100	100	101	99	95	92
	Endpoints (mg a.s./L) (mean measured)					
$NOEC_{overall}$ (33 d)	0.204					

[a] One fish killed by handling was subtracted from the number of swim-up larvae at risk.
[b] One fish with a vertebral deformation was seen; however this cannot be related to the test substance as no abnormalities were observed in higher concentrations.
** Statistically significantly different compared to the control (Dunnett's test; $p \leq 0.01$)

B. ANALYSIS

The individually measured concentrations in uncentrifuged samples were within the range of 80 to 120% of the overall mean measured concentration, as shown in the table below.

Samples were also analyzed after centrifugation (17700 G, 20 minutes), to confirm the absence of undissolved test substance in the test solution. The mean measured concentrations of centrifuged samples were 4.3 to 9.7% lower than the corresponding mean measured concentrations of uncentrifuged samples. The minimal loss after centrifugation and the high degree of consistency of measured concentrations among test vessel replicates over the exposure period support the conclusion that no undissolved material was present in the test solution.

The biological results were based on mean measured concentrations of the uncentrifuged samples.

Table 8.2.2.1-2: Measured concentrations of BAS 560 F in test samples

Nominal concentration (%)	Sampling time (days)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of mean measured stock solution	Measured concentration as % of nominal concentration
0 (negative control)	0 8 15 22 29 33	< LOD	-	-	-
16	0 8 15 22 29 33	0.112 0.092 0.104 0.125 0.154 0.136	0.095	16.4	102.5
25	0 8 15 22 29 33	0.180 0.139 0.166 0.125 0.154 0.136	0.150	26.0	104
40	0 8 15 22 29 33	0.241 0.202 0.232 0.165 0.196 0.185	0.204	35.3	88.25
63	0 8 15 22 29 33	0.423 0.341 0.414 0.336 0.391 0.281	0.364	63.2	100.3

Nominal concentration (%)	Sampling time (days)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of mean measured stock solution	Measured concentration as % of nominal concentration
100	0 8 15 22 29 33	0.598 0.541 0.637 0.500 0.511 0.423	0.535	92.7	92.7
Stock solution	0 8 15 22 29 33	0.627 0.541 0.627 0.512 0.642 0.511	0.577	-	-

LOD = Limit of detection (0.001 mg/L)

C. DEFICIENCIES

None.

III. CONCLUSION

In an early life stage study, the overall no-observed-effect concentration (NOEC) for fathead minnow (*Pimephales promelas*) exposed to metrafenone under flow-through conditions was determined to be 0.204 mg a.s./L, based on mean measured concentrations. The lowest-observable-effect concentration (LOEC) was 0.364 mg a.s./L (mean measured).

CA 8.2.2.2 Fish full life cycle test

No data submitted.

CA 8.2.2.3 Bioconcentration in fish

Report:	CA 8.2.2.3/1 [redacted] 2001a BAS 560 F (AC 375839): Uptake, depuration, bioconcentration and metabolism of carbon-14 labeled AC 375839 in bluegill sunfish (Lepomis macrochirus) under flow-through conditions 2001/7000274
Guidelines:	OECD 305, EPA 850.1730
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

Bluegill sunfish (*Lepomis macrochirus*) were exposed to radio-labelled BAS 560 F under flow-through conditions at test concentrations of 5 and 50 µg a.s./L for 28 days. The bioconcentration factor (BCF) for the total radioactively-labelled BAS 560 F was determined to be 530 in whole fish. There was no bioconcentration of the BAS 560 F metabolites and degradation products. The time to reach 95% clearance (CT_{95}) was 2.3 days. This indicates an intensive metabolic clearance of BAS 560 F. Hence, the potential for accumulation in fish or other aquatic organisms is low, because of the rapid excretion of the parent compound and its metabolites. Please refer to Document M-II, Section 6, Point 8.2.3 of the summary dossier dated 2002 for further details.

CA 8.2.3 Endocrine disrupting properties

A full review of the data as well as any other additional information on the toxicity profile has been undertaken to determine whether metrafenone is a potential endocrine disruptor in aquatic organisms. Although the ecotoxicology studies are of limited sensitivity in detecting endocrine-relevant effects, there is no evidence that metrafenone causes endocrine disruption based on the evaluation of the available aquatic data (fish ELS study and chronic *Daphnia*).

Metrafenone is not classified as a carcinogen or reproductive toxin and therefore does not meet the interim EU criteria for an endocrine disruptor. Although final criteria for the definition of 'endocrine disruptor' have yet to be agreed, based on the above assessment it is unlikely that metrafenone would be considered to be an endocrine disruptor in the context of Regulation (EC) No 1107/2009. Although further specific testing for endocrine disruption is not justified at present; this conclusion may require reassessment once criteria are agreed.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

Report: CA 8.2.4.1/1
Palmer S.J. et al., 1999c
Acute toxicity of AC 375839 to Daphnia magna under static test conditions
1999/7000287
Guidelines: EEC 91/414 Annex II 8.2.4, EEC 96/12, EPA 72-2
GLP: yes
(certified by United States Environmental Protection Agency)

Conclusion

The EC_{50} for *Daphnia magna* exposed to metrafenone for 48 hours under static conditions was > 0.92 mg a.s./L, the highest mean measured concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.92 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.4 of the summary dossier dated 2002 for further details.

Report: CA 8.2.4.1/2
Jatzek H.-J., 2002a
CL 375 816 (metabolite of BAS 560 F, Benzophenone) - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS
2002/1004870
Guidelines: OECD 202 Part I (1984), EPA 850.1010, ISO 6341, ISO/DIS 10706, EEC 92/32 A V C 2
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The EC_{50} for *Daphnia magna* exposed to CL 375816 (metabolite of metrafenone) for 48 hours under static conditions was > 100 mg /L (nominal), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 100 mg/L, based on nominal concentrations. Please refer to Document M-II, Section 6, Point 8.2.4 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.4.1/3 Jatzek H-.J., 2002a CL 4084 564 (metabolite of BAS 560 F) - Determination of the acute effect on the swimming abilitiy of the water flea Daphnia magna STRAUS 2002/1004869
Guidelines:	OECD 202 Part I (1984), EPA 850.1010, ISO/DIS 10706, ISO 6341, EEC 92/32 A V C 2
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The EC_{50} for *Daphnia magna* exposed to CL 4084564 (metabolite of metrafenone) for 48 hours under static conditions was 46 mg/L, based on mean measured concentrations. The no-observed-effect concentration (NOEC) was determined to be 23.2 mg/L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.4 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.4.1/4 Dabrunz A., 2015a S15-02612 (Algae growth inhibition test) and S15-02611 (Daphnia acute toxicity test) with CL3000402 / Reg.No. 4110838: Results of Non-GLP pre-tests regarding solubility and toxicity 2015/1177630
Guidelines:	<none>
GLP:	no (certified by <none>)

Conclusion

A laboratory study on the acute toxicity of CL 3000402 was attempted. It was not possible to obtain a clear solution, free of particles in M4 Medium, as well as in several solvents (acetone, DMF and DMSO). Only by using a filtration approach it was possible to obtain a stock solution which was stable for 48 hours and showed a concentration of the dissolved test item of approximately 16 µg/L. When this filtrate was tested on *Daphnia* for 48 hours in a range-finder, no effects on mobility of the daphnids were observed. However, this study does not accurately demonstrate the true toxicity of the metabolite, hence a definitive study has not been conducted.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Report: CA 8.2.4.2/1
Palmer S.J. et al., 2005a
BAS 560 F: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
2005/7003442
Guidelines: EPA 850.1025
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The acute toxicity of BAS 560 F (metrafenone) to the eastern oyster (*Crassostrea virginica*) was determined under flow-through conditions. The oysters were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L for 96 hours. A negative control (filtered saltwater) and solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The oysters were tested in groups of ten per test chamber, with one test chamber per test group.

Results of analyses showed mean measured concentrations between 80 and 85% of nominal concentrations for the three lowest test substance concentrations and mean measured concentrations of 56 and 57 % for the two highest test substance concentrations. This indicates that the test was conducted to the limit of solubility in the test system. In addition, a precipitate was observed for the highest test substance concentration (1.0 mg a.s./L (nominal)) and therefore these samples were also analyzed following centrifugation. The mean measured concentration after centrifugation was 33% of nominal concentration. The results were based on the mean measured concentrations of both centrifuged and uncentrifuged samples.

All the oysters in the control groups and in the BAS 560 F exposed groups appeared normal throughout the test, without any mortalities or signs of toxicity. Inhibition of shell growth for the oysters exposed to BAS 560 F was calculated relative to the pooled control and showed an increase in inhibition of 5.6% at the lowest test substance concentration of 0.051 mg a.s./L (mean measured) to 100 % inhibition at the highest test substance concentration of 0.57 mg a.s./L (mean measured).

The LC_{50} for *Crassostrea virginica* exposed to BAS 560 F for 96 hours was 0.22 mg a.s./L, based on mean measured concentrations of uncentrifuged and centrifuged samples. The no-observed-effect concentration (NOEC) was determined to be 0.11 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L); (mean measured: 0, 0.051, 0.11, 0.20, 0.28 and 0.57 mg a.s./L)

3. Reference item: None

4. Dilution water: Natural seawater collected at Indian River Inlet, Delaware, filtered and diluted to a salinity of 20‰ with well water

Vehicle: Dimethyl formamide (DMF)

5. Test organism:

Species: Eastern oyster (*Crassostrea virginica*)
Age/life stage: Not described
Length: 38.1 ± 5.1 mm
Source: Taylor Shellfish Farms, Shelton, USA
Acclimation period: 12 days
Diet: Suspension of marine microalgae, provided continuously during testing at a rate of 5.8 x 10^9 cells/oyster/day
Test vessels: 54 L glass aquaria filled with 27 L of test water

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 19.8 – 20.8 ºC
Salinity: 19 - 20‰
pH: 8.1 – 8.3
Dissolved oxygen: 5.2 – 7.2 mg/L (4.8 mg/L represents 60% saturation at 20ºC in saltwater with salinity of 20‰)
Photoperiod: 16 h light: 8 h darkness (192 lux, at test initiation)

2. Animal assignment and treatment:

At test initiation, oysters were randomly distributed to test chambers, until each group contained 20 oysters. The oysters were placed in the test chambers with flat valves facing up and umbos away from the flow of the water. The test included one test chamber per test group. The oysters were exposed for 96 hours under flow-through conditions.

3. Dose preparation:

A primary stock solution was prepared by mixing test substance into dimethyl formamide (DMF) at a nominal concentration of 10 mg a.s./L. Four additional solutions (at nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./mL) were prepared in DMF by proportional dilution of the primary stock. The five stock solutions were injected into the diluter mixing chambers, where they were mixed with saltwater to achieve the desired test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. The solvent control was prepared by injecting DMF into the mixing chamber for the solvent control. The concentration of DMF in the solvent control and all BAS 560 F treatment groups was 0.1 mL/L. A test water only control was also included in the test. The diluter was adjusted so that each test chamber received 19 volume additions of test water every 24 hours.

4. Measurements and observations:
Observations were made at 6, 24, 48, 72 and 96 hours after test initiation to determine the number of mortalities, as well as the number of individuals with sublethal signs of toxicity. At test termination, the longest finger of new shell growth on each oyster was measured to the nearest 0.1 mm, to obtain the shell growth.

Samples were collected from each treatment and control group at 0, 48 and 96 hours to measure concentrations of the test substance. Samples were analyzed by HPLC using wavelength detection set at 220 nm.

5. Statistics:
The concentration of test substance that would reduce shell deposition by 50% relative to the (pooled) control (EC_{50}) was calculated using linear interpolation. Shell growth data in the treatment groups were compared to the (pooled) control data using analysis of variance (ANOVA) and Bonferroni's t-test to identify significant differences. The no-observed-effect concentration (NOEC) was determined from the statistical analysis and an assessment of the concentration-response pattern. The statistical analyses were conducted using TOXSTAT computer program (1996).

II. RESULTS AND DISCUSSION

A. MORTALITY AND APPEARANCE

No oysters died during the test, in the control groups and in all test substance groups. All oysters appeared normal throughout the test, without any signs of toxicity.

B. SHELL DEPOSITION

Shell deposition was not statistically significantly different between the seawater and the vehicle control, hence the two control groups were pooled. Inhibition of shell growth for the oysters exposed to BAS 560 F was calculated relative to the pooled control and the inhibition increased from 5.6% at the lowest test substance concentration of 0.051 mg a.s./L (mean measured) to 100 % inhibition at the highest test substance concentration of 0.57 mg a.s./L (mean measured). The results are presented in the table below. The 96-hour EC_{50} value was calculated to be 0.22 mg a.s./L, with a 95% confidence interval of 0.20 to 0.24 mg a.s./L, based on mean measured concentrations of both uncentrifuged and centrifuged samples. The NOEC was considered to be 0.11 mg a.s./L (mean measured).

Table 8.2.4.2-1: Mean shell deposition and shell growth inhibition of eastern oyster (*Crassostrea virginica*) exposed to BAS 560 F

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Shell deposition Mean ± sd [a] (mm)	Shell growth inhibition (%) [b]
Negative control	Negative control	3.16 ± 1.41	-
Solvent control	Solvent control	2.61 ± 0.97	-
Pooled control	Pooled control	2.88 ± 1.23	-
0.063	0.051	2.72 ± 0.96	5.6
0.13	0.11	2.33 ± 0.99	19
0.25	0.20	1.76 ± 0.76	39*
0.50	0.28	0.50 ± 0.76	83*
1.0	0.57	0.00 ± 0.00	100*

[a] Mean and standard deviation for 20 oysters
[b] Percent inhibition from the pooled control
[*] Statistically significantly different from the pooled control using Bonferroni's t-test ($p \leq 0.05$)

C. ANALYSIS

At nominal concentrations ≤ 0.25 mg a.s./L, mean measured concentrations ranged from 80 to 85% of nominal concentrations. At nominal concentrations of 0.5 and 1.0 mg a.s./L, mean measured concentration were 56 and 57% of nominal concentrations, respectively. Since a precipitate was visible in the highest test substance concentration of 1.0 mg a.s./L, samples for this test substance concentration were also analyzed following centrifugation at 14000 rpm for five minutes. The mean measured concentration of these centrifuged samples was 33% of the nominal concentration. The lower recoveries and the presence of a precipitate in the 0.50 and 1.0 mg a.s./L test chambers indicate that the water solubility in the test system was approximately 0.3 mg a.s./L. The results of the study were based on mean measured concentrations of both uncentrifuged and centrifuged samples. The concentrations of BAS 560 F observed in the analysis are shown in the table below.

Table 8.2.4.2-2: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of nominal
0 (negative control)	0 48 96	< LOQ	-	-
0 (solvent control)	0 48 96	< LOQ	-	-
0.063	0 48 96	0.0506 0.0560 0.0462	0.051	81
0.13	0 48 96	0.115 0.0929 0.117	0.11	85
0.25	0 48 96	0.200 0.202 0.207	0.20	80
0.50	0 48 96	0.256 0.306 0.280	0.28	56
1.0	0 48 96	0.570 0.588 0.546	0.57	57
1.0*	0 48 96	0.313 0.336 0.335	0.33	33

LOQ = Limit of quantification (0.040 mg a.s./L)
* Samples centrifuged at 14000 rpm for five minutes

D. DEFICIENCIES

None.

III. CONCLUSION

The LC_{50} for eastern oyster (*Crassostrea virginica*) exposed to BAS 560 F (metrafenone) for 96 hours under flow-through conditions was 0.22 mg a.s./L (mean measured), based on the limit of solubility in the test system. The no-observed-effect concentration (NOEC) was determined to be 0.11 mg a.s./L, based on mean measured concentrations.

Report:	CA 8.2.4.2/2 Claude M.B. et al., 2011a BAS 560 F - A 96-hour flow-through acute toxicity test with the saltwater mysid (Americamysis bahia) 2011/7000913
Guidelines:	EPA 850.1035
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study, saltwater mysids (*Americamysis bahia*) were exposed to BAS 560 F (metrafenone) under flow-through conditions. The mysids were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A water control and a solvent control (0.1 mL/L dimethylformamide) were tested in parallel. Each test group consisted of two replicates, with ten animals per replicate. Mysids were observed for survival and symptoms of toxicity 4, 24, 48, 72 and 96 hours after start of exposure.

Due to the presence of a precipitate in the 0.50 and 1.0 mg a.s./L mixing chambers, all samples were centrifuged prior to analysis. Mean measured concentrations of centrifuged samples ranged from 33 to 51% of nominal concentrations. The biological results are based on these mean measured concentrations.

After 96 hours of exposure no mortality or other toxic effects were observed in the control, the solvent control and at mean measured concentrations of up to 0.12 mg a.s./L. Mortality rates of 10% and 35% were observed after 96 hours at the two highest test concentrations of 0.21 and 0.33 mg a.s./L, respectively. Signs of toxicity observed among mysids in the 0.33 mg a.s./L groups at test termination included erratic swimming and loss of equilibrium.

The EC_{50} for *Americamysis bahia* exposed to BAS 560 F (metrafenone) for 96 hours was > 0.33 mg a.s./L (mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.12 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	BAS 560 F (metrafenone; Reg. No. 4037710)
	Batch number:	AC12053-29
	Purity:	94.2%
	Description:	Solid
2. Test concentrations:		0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured concentrations: 0, 0.032, 0.066, 0.12, 0.21 and 0.33 mg a.s./L from centrifuged samples)

3. Reference item: None

4. Dilution water: Sand filtered natural seawater mixed with freshwater from a well
Vehicle: Dimethyl formamide (DMF)

5. Test organism:
Species: Saltwater mysid (*Americamysis bahia*)
Age/life stage: Less than 24 hours old juveniles
Source: In-house culture
Diet: Live brine shrimp nauplii (Artemia sp.), provided two times daily
Test vessels: 9.0 L glass aquaria, test volume 5.0 L

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 25.0 – 25.5 °C
Salinity: 20 - 21‰
pH: 7.9 – 8.0
Dissolved oxygen: 6.5 – 7.4 mg/L
Photoperiod: 16 hours light and 8 hours darkness (649 lux)

2. Animal assignment and treatment:
At test initiation, the juvenile mysids were collected from the cultures and randomly assigned to test vessels, until each test vessel contained ten mysids. The mysids were exposed for 96 hours to nominal test material concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, under flow-through conditions. A dilution water control and a solvent control (DMF) were also included in the test. Each test item group and control group contained two replicate test vessels.

3. Dose preparation:
First a stock solution at a nominal test concentration of 10 mg a.s./L was prepared by mixing the test material in dimethylformamide (DMF). This stock solution was proportionally diluted to obtain the four other stock solutions at nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./L. These five stock solutions were injected into the diluter mixing chambers at a rate of 12.50 µL/minute, where they were mixed with dilution water delivered at a rate of 125 mL/minute to achieve the five desired test concentrations. The solvent control was prepared by delivering DMF to the mixing chamber. The concentration of DMF in the solvent control and all BAS 560 F treatment groups was 0.1 mL/L.

4. Measurements and observations:
Mortality and symptoms of toxicity or abnormal behavior were recorded 4, 24, 48, 72 and 96 hours after test initiation.

Samples were taken at test initiation and at 48 and 96 hours for analytical verification of test item concentrations, using a HPLC-method with wavelength detection set at 220 nm.

Dissolved oxygen and pH were measured every 24 hours during the test.

5. Statistics:
There was less than 50% mortality in all BAS 560 F treatment groups, hence an LC_{50} value could not be calculated. The no-observed mortality concentration (NOEC) was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

All mysids in the negative control and the solvent control appeared normal throughout the exposure period. For the treatment groups, mortality was only observed at the two highest test concentrations of 0.21 and 0.33 mg a.s./L and signs of toxicity (erratic swimming and loss of equilibrium) were only observed among mysids in the 0.33 mg a.s./L groups at test termination. The results are shown in the table below.

Table 8.2.4.2-3: Mortality and signs of toxicity of saltwater mysids (*Americamysis bahia*) exposed to BAS 560 F (metrafenone) for 96 hours

Concentration (mg/L) (nominal)	**Control**	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Concentration (mg/L) (mean measured)	**Control**	**0.032**	**0.066**	**0.12**	**0.21**	**0.33**
Mortality (96 h) (%)	0	0	0	0	10	35
Symptoms *	none	none	none	none	none	E, N
	Endpoints (mg a.s./L) (mean measured)					
EC_{50} (96 h)	> 0.33					
NOEC (96 h)	0.12					

* Symptoms: E = erratic swimming; N = loss of equilibrium.

B. ANALYSIS

Due to the presence of a precipitate in the 0.50 and 1.0 mg a.s./L mixing chambers, all samples were centrifuged prior to analysis. Measured concentrations of centrifuged samples ranged from 29.9 to 56.9% of nominal concentrations, as shown in the table below. The biological results are based on mean measured concentrations from the centrifuged samples.

Table 8.2.4.2-4: Measured concentrations of BAS 560 F in centrifuged test samples

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of nominal
0 (negative control)	0 48 96	< LOQ	-	-
0 (solvent control)	0 48 96	< LOQ	-	-
0.063	0 48 96	0.0261 0.0344 0.0346	0.032	51
0.13	0 48 96	0.0543 0.0739 0.0694	0.066	51
0.25	0 48 96	0.0967 0.128 0.138	0.12	48
0.50	0 48 96	0.154 0.224 0.257	0.21	42
1.0	0 48 96	0.299 0.381 0.316	0.33	33

LOQ = Limit of quantification (0.030 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

The EC_{50} for saltwater mysids (*Americamysis bahia*) exposed to metrafenone for 96 hours under flow-through conditions was > 0.33 mg a.s./L (mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.12 mg a.s./L, based on mean measured concentrations.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Report:	CA 8.2.5.1/1 Barker C. et al., 2000b Toxicity of AC 375839 during the life-cycle of the cladoceran (Daphnia magna) 2000/7000130
Guidelines:	EPA 72-4 (b), EEC 91/414 Annex II 8.2.5, EEC 96/12
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The no-observed-effect concentration (NOEC) for *Daphnia magna* exposed to metrafenone for 21 days under static-renewal test conditions was 225 µg a.s./L, based on mean measured concentrations. The lowest-observable-effect concentration (LOEC) was determined to be 462 µg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.5 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.5.1/2 Janson G.-M., 2012a Chronic toxicity of BAS 560 F (Metrafenone) to Daphnia magna STRAUS in a 21 day semi-static test 2011/1260868
Guidelines:	OECD 211, EPA 850.1300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a chronic toxicity study *Daphnia magna* were exposed to BAS 560 F (metrafenone) for 21 days. The test was conducted under semi-static conditions (renewal every 2-3 days) with nominal test item concentrations of 89, 133, 200, 300, and 450 µg/L BAS 560 F. A negative control and solvent control (100 µg/L acetone) were tested in parallel. For each test concentration and the controls, ten test organisms (1 daphnid per replicate test vessel) were exposed.

Analytical verification of the test item concentrations demonstrated that recoveries at the different sampling days ranged from 101.2% to 98.2% of the nominal concentrations (freshly prepared and old test solutions). Hence, results were based on nominal test concentrations.

At the end of the test, parent mortality, body length, dry weight and reproductive performance were assessed. The first brood was observed on day 8 for the controls and all test concentrations. The number of offspring per parent varied between 186 and 197 for the controls and the test concentrations from 89 and 300 µg/L, but was significantly lower for the group exposed to 450 µg/L, where 158 offspring per parent were observed. At test end the body length of the adult daphnids ranged from 4.7 to 4.9 mm and the dry weight of the adult daphnids ranged from 0.92 to 1.06 mg. Significantly lower reproduction compared to the controls was observed at the nominal treatment 450 µg/L BAS 560 F. Body length of the parent daphnids was significantly affected at nominal concentration 450 µg/L BAS 560 F. No statistically significant effects on body dry weights of the parent daphnids could be observed in all test concentrations of BAS 560 F.

The toxic effect of the test item BAS 560 F to *Daphnia magna* was assessed in a semi-static reproduction test. The 21-day overall no-observed-effect concentration (NOEC) was determined to be 300 µg a.s./L (nominal). The lowest-observable-effect concentration (LOEC) was determined to be 450 µg a.s./L BAS 560 F and the MATC was 367 µg a.s./L, based on nominal test concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (Metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 89, 133, 200, 300 and 450 µg a.s./L (nominal)

3. Reference item: Potassium dichromate
(performed as a separate study, code no.: 346250_6; BASF DocID 2011/1122539)

4. Dilution water: Reconstituted water, M4 according to Elendt
Vehicle: Acetone

5. Test organism:
Species: *Daphnia magna* Straus
Age/life stage: Neonates >2 hours and < 24 hours old
Source: In-house culture
Diet: Fed *Desmodesmus subspicatus* algae at least 3 times per week. During the test the daphnids were fed with increasing amounts of algae until day 8 after which the amount of algae remained constant.
Test vessels: 100 mL glass vessels, containing 50 mL test solution.

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 20.2 – 21.4°C
pH: 7.69-8.02
Dissolved oxygen: 7.8-8.9 mg/L
Photoperiod: 16 hours light: 8 hours darkness (320-820 lux)

2. Animal assignment and treatment:
The experiment was initiated by adding the test item BAS 560 F (metrafenone) and the daphnid neonates to the synthetic water. Ten replicate test vessels were maintained in each treatment and control group with one parent *Daphnia* in each vessel containing 50 mL test volume. The following nominal test concentrations were tested: 0 (reconstituted water control and solvent control (100 µg/L acetone)), 89, 133, 200, 300 and 450 µg a.s./L . The test vessels were kept in a temperature-control incubator for 21 days.

3. Dose preparation:
To obtain the test concentrations a stock solution was prepared be adding 47.79 mg (taking the 94.2% purity into account) of the test item to 10 mL acetone. Appropriate amounts were taken from this stock solution (clear yellowish solution and no precipitation or undissolved particles were observed) to achieve the desired test concentrations. These amounts were topped with M4 water to a final volume of 1000 mL. Appropriate amounts of acetone were added in order to achieve an equal acetone concentration between all test concentrations and the solvent control. Renewal of the test solutions was performed on day 2, 5, 7, 9, 12, 14, 16 and 19.

4. Measurements and observations:
Assessments of parent mortality, numbers of live offspring, dead offspring, egg production and visual assessments of the daphnids were performed throughout the experiment (except days 0, 3 and 4). For calculation of reproduction, only living offspring of parent daphnids surviving until the end of the experiment were considered. Body length and weight of the parent animals were determined at test end.

Analytical samples were taken from all test item concentrations on day 0, day 2 (48 h old medium), day 5 (fresh medium), day 7 (48 h old medium), day 9 (fresh medium), day 12 (72 h old medium), day 14 (fresh medium), day 16 (48 h old medium), day 19 (fresh medium) and on day 21 (48 h old medium). Test concentration levels were analytically verified in each concentration at test initiation and termination, using HPLC with DAD detection. The analytical method was validated prior to the test.

5. Statistics:
Data were tested for homogeneity of variance and normal distribution. The determination of the NOEC was concluded via an analysis of variance (ANOVA) followed by a William's test (one sided). Control and solvent control were compared with a t-test. Since they were not significantly different ($p > 0.05$), pooled control and the solvent control data were used for statistical analyses. All calculations were carried out using the software package "ToxRatPro Version 2.10" (ToxRat Solutions GmbH, Alsdorf, Germany).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The first brood was observed in all concentrations on day 8 and the number of offspring varied between 158 and 197. At test end the body length of the adult daphnids ranged from 4.7 to 4.9 mm and the dry weight of the adult daphnids ranged from 0.92 to 1.06 mg. Significantly lower reproduction compared to both controls (control and solvent control) was observed in the highest treatment group, 450 µg/L BAS 560 F. No significant effects on body dry weight of the parent daphnids could be observed in all test concentrations of BAS 560 F. The biological results of the study are summarized in the following table.

Table 8.2.5.1-1: Summary of effects on adult survival, reproduction and growth of *Daphnia magna* exposed to BAS 560 F

Nominal concentration (µg/L BAS 560 F)	Mean percent adult survival	Mean total no. of offspring per daphnid	CV (%)	Day of first brood	Mean body length of daphnids (mm)	Mean dry weight of daphnids (mg)
Control	0	186	6.7	8-9	4.8	0.97
Solvent control	0	194	6.8	8-9	4.9	0.92
89	0	187	3.6	8-9	4.8	1.03
133	0	197	6.1	8-9	4.9	1.06
200	0	194	10.1	8-9	4.9	0.97
300	0	188	6.5	8-9	4.8	1.06
450	1	158*	13.7	8-11	4.7*	0.96

*Statistically significant as compared to the both controls (control and solvent control) (ANOVA followed by William's test, $p < 0.05$)

The study with the reference item (BASF Doc ID 2011/1122539) potassium dichromate resulted in a 24-hour EC_{50} = 1.38 mg/L and a NOEC = 1.13 mg/L.

B. ANALYSIS

For the analyzed experimental set-ups of BAS 560 F in M4-Medium the mean recoveries found for the freshly prepared samples were in the range of: 92.6-103.1% (day 0), 98.9-106.7% (day 5), 98.7-103.6% (day 9), 97.2-108.0% (day 14) and 101.7-111.0% (day 19), as shown in Table **8.2.5.1-2** below. The mean recoveries found for the aged samples were in the range of: 93.2-107.4% (day 2), 98.9-104.2% (day 7), 92.4-98.9% (day 12), 90.8-101.0% (day 16) and 96.3-103.1% (day 19).

Table 8.2.5.1-2: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration	Measured concentration (µg a.s./L)											% Nom-inal
	Day 0 (fresh)	Day 2 (aged)	Day 5 (new)	Day 7 (aged)	Day 9 (new)	Day 12 (aged)	Day 14 (fresh)	Day 16 (aged)	Day 19 (new)	Day 21 (aged)	Mean	
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
Solvent control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
89	91.7	94.7	94.9	91.3	88.7	82.6	90.8	83.9	98.8	90.8	90.8	102.0
133	123	143	138	139	138	132	144	134	146	137	137.4	103.3
200	186	199	198	199	197	185	194	182	214	203	195.7	97.8
300	283	300	299	297	297	284	299	281	310	292	294.2	98.1
450	432	420	451	446	445	418	459	418	458	434	438.1	97.3

C. DEFICIENCIES

None.

III. CONCLUSION

The toxic effect of the test item BAS 560 F (metrafenone) to *Daphnia magna* was assessed in a semi-static reproduction test. The 21-day overall no-observed-effect concentration (NOEC) was determined to be 300 µg a.s./L (nominal). The lowest-observable-effect concentration (LOEC) was determined to be 450 µg a.s./L BAS 560 F and the MATC was 367 µg a.s./L, based on nominal test concentrations.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Report: CA 8.2.5.2/1
Cafarella M.A., 2007a
BAS 560 F - Life-cycle toxicity test with mysids (Americamysis bahia)
2007/7009454
Guidelines: EPA 850.1350, FIFRA 72-4
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a life-cycle toxicity study mysids (*Americamysis bahia*) were exposed to BAS 560 F (metrafenone). The test was conducted under flow-through conditions over a period of 28 days with the following nominal test item concentrations: 3.1, 6.3, 13, 25, and 50 µg a.s./L. A control and solvent control (14 µL/L triethylene glycol) were tested in parallel. Initially 60 mysids (30 per replicate test vessel) were exposed to each test concentration and the controls. After reaching sexual maturity (day 14) mysids were redistributed within the test aquaria and mature male/female pairs were transferred to one of ten pairing chambers (one pair per chamber).

Analysis of the test solutions demonstrated that the expected concentration-gradient was maintained during the 28-day exposure. Mean measured concentrations ranged from 88% to 99% of nominal and defined the treatment levels tested as 2.9, 6.2, 12, 22 and 45 µg a.s./L.

Statistical analysis determined no significant difference in survival (male, female and combined) in any treatment levels tested as compared to the control (100%). A statistically significant difference in number of offspring per female and offspring per female per reproductive day was demonstrated for organisms exposed to the 45 µg a.s./L (mean measured) when compared to the control. Statistical analysis determined no significant difference in body length and weight (for both male and female mysids) in any treatment levels tested as compared to the control.

Based on statistical analysis of mysid reproduction the lowest-observable-effect concentration (LOEC) was determined to be 45 µg a.s./L and the no-observed-effect concentration (NOEC) was 22 µg a.s./L, based on mean measured concentrations. Therefore, the geometric mean Maximum-Acceptable-Toxicant (MATC) was estimated to be 31 ug a.s./L (mean measured). Since no concentration tested resulted in ≥ 50% reduction in survival, the 28-d LC_{50} value was empirically estimated to be > 45 µg a.s./L (mean measured), the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (Metrafenone; Reg.No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Not reported

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured: 0, 0.032, 0.066, 0.12, 0.21 and 0.33 mg a.s./L)

3. Reference item: None

4. Dilution water: Artificial seawater (salinity 20 ± 3‰)
Vehicle: Triethylene glycol

5. Test organism:
Species: *Americamysis bahia*
Age: ≤ 23 hours old
Source: In-house culture. Brood stock was originally obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado.
Diet: During the test, mysids were fed twice daily with brine shrimp (*Artemia salina*) nauplii ≤ 24 hours old. Prior to pairing at least one of the two feedings was with brine shrimp enriched with Selco®, a supplemental substance high in saturated fatty acids. During the period subsequent to pairing the Selco® enriched brine shrimp was fed to the test organisms every other day.
Test vessels: Glass test aquaria each measured 39 x 20 x 25 cm equipped with a glass self-starting siphon drain which allowed the solution volume within the aquarium to fluctuate between approximately 3.9 and 7.0 L. Each exposure aquarium contained two non-paired mysid retention chambers (glass petri dishes, 10 cm in diameter, 2 cm deep). Pairing chambers were 6-cm diameter petri dishes. Solution volume fluctuated from 390 to 710 mL and 100 to 180 mL in the non-paired mysid and pairing retention chambers, respectively.

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 25 – 27 °C
Salinity: 18 – 21‰
pH: 8.1 – 8.3
Dissolved oxygen: 5.5 – 7.3 mg/L
Photoperiod: 16 hours light: 8 hours darkness (550 - 1100 lux)

2. Animal assignment and treatment:
Mysids (≤ 23 hours old) were distributed among 28 beakers (15 mysids in each) containing culture water. Each group of 15 mysids was transferred to one of the 28 labeled retention chambers. The test was initiated when the retention chambers were placed in their respective test aquaria. Each aquarium contained two retention chambers yielding 30 mysids per replicate vessel and 60 organisms for each treatment and the controls. When the mysids reached sexual maturity (day 14) they were redistributed within the test aquaria. Mature male/female pairs within each exposure aquarium were transferred from the retention chambers to one of ten pairing chambers (one pair per chamber). The remaining mysids (after isolation of male-female pairs) were pooled and placed in one of the initial retention chambers within each aquarium where they were maintained for the duration of the chronic test. Male mysids from this pool were used to replace dead males from the paired (male/female) groups. Females which died in the pairing jars were not replaced.

3. Dose preparation:
To obtain the test concentrations, a 3.5 mg a.s./mL stock solution was prepared by adding 0.1861 g (taking the 94.2% purity into account) of BAS 560 F to 50 mL triethylene glycol. Appropriate amounts of the stock solution and dilution water were delivered in cycles into a diluter's chemical mixing chamber. The solution contained in the mixing chamber constituted the highest nominal test concentration (50 µg a.s./L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations. During each cycle of the diluter system, approximately 500 mL of exposure solution was delivered to each replicate test vessel. During the study, the diluter provided the exposure solutions to each test vessel at a rate of approximately 15 aquarium volume additions per day. In a similar way the solvent was mixed and delivered to the test vessels and the solvent concentration in the solvent control and each treatment level was 14 µL/L.

4. Measurements and observations:
After males and females had been paired (day 14), the number of dead males and females, the number of offspring produced by each individual female and any abnormal appearance or behavior was recorded daily throughout the study. At test termination, the individual body length to the nearest 0.1 mm and the total dry body weight to the nearest 0.01 mg of all mysids were determined and recorded separately for each replicate of each concentration and the controls. Reproduction was calculated for each replicate aquarium as the total number of offspring produced to the total number of females contained within each chamber. In addition, the number of reproductive females in each replicate of each treatment and the control was determined.

Water samples were taken from alternate replicate test solutions of each treatment level and the control on test days 0, 8, 14, 22 and 28 for analysis. All exposure solutions and QC samples were analyzed for BAS 560 F using high performance liquid chromatography with ultraviolet detection (HPLC/UV) based on validated methodology.

Temperature, dissolved oxygen concentration, pH and salinity were measured daily.

5. Statistics:

All statistical analyses were performed against the dilution water (negative) control data. Student's t-Test established no statistical differences between negative control and solvent control. Significant differences in the percent survival were determined following arcsine transformation. Shapiro-Wilk's Test and Bartlett's Test were used for normality and homogeneity of data, respectively. Williams' Test was used to determine treatment level effects. TOXSTAT® Version 3.5 was used to perform the statistical computations.

During this study, no concentration tested caused a reduction of 50% survival, therefore, the LC_{50} values were empirically estimated to be greater than the highest mean measured concentration tested and no statistical analyses were performed.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

At termination of the test, the mysids in the control and solvent control met the performance criteria of the OPPTS 850.1350 guideline (> 70% survival of F_0 mysids between pairing and test termination, > 75% of the females in the control released young and the controls produced > 3 offspring per female).

The biological results of the study i.e. male, female and combined mysid survival, a summary of first generation (F_0) reproductive success data and measurements of growth, as average total body length and average dry body weight, for all surviving adult mysids (F_0) at test termination are summarized in the table below.

Table 8.2.5.2-1: Summary of effects on adult survival, reproduction and growth of *Americamysis bahia* exposed to BAS 560 F

Mean measured concentration (µg/L BAS 560 F)	Mean % survival			% of females producing young	Mean number of offspring per female	Mean number of offspring per female per reproductive day	Mean body length of mysids (mm)		Mean dry weight of mysids (mg)	
	♂	♀	♂/♀				♂	♀	♂	♀
Control	100	100	100	100	5.8	0.44	7.4	7.6	0.87	1.06
Solvent control	95	96	96	100	4.9	0.36	7.8	7.8	0.90	0.99
2.9	84	83	85	89	5.8	0.43	8.0	8.2	0.90	1.14
6.2	100	100	100	95	6.5	0.47	7.4	7.4	0.90	1.05
12	96	100	98	85	3.1	0.22	7.3	7.2	0.85	1.00
22	96	89	93	85	3.4	0.27	8.1	7.9	0.90	1.06
45	95	93	95	25	1.4*	0.10*	7.5	7.5	0.90	1.05

*Statistically significant as compared to the negative (solvent-free) control based on William's test

B. ANALYSIS

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the 28-day study. Analysis of the stock used to prepare test solutions during the definitive test resulted in recoveries ranging from 94 to 120% of nominal concentration (3.5 mg a.s./mL) from artificial seawater. These analytical results and records of toxicant pump stock usage indicate that the appropriate amount of BAS 560 F was delivered to the exposure system during this study. The results of the analysis of the exposure solutions for BAS 560 F concentration during the in-life portion of the definitive test are presented in the following table. Analysis of the test solutions demonstrated that the expected concentration-gradient was generally maintained during the 28-day exposure.

Table 8.2.5.2-2: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Measured concentration (µg a.s./L)[a]						% Nominal[b]
	Day 0	Day 8	Day 14	Day 22	Day 28	Mean (SD)[b]	
Control	< 1.1	< 1.4	< 0.95	< 1.3	< 1.2	n.a.	n.a.
Solvent control	< 1.1	< 1.4	< 0.95	< 1.3	< 1.2	n.a.	n.a.
3.1	2.7	2.6	2.9	2.7	3.4	2.9 (0.33)	92
6.3	6.0	6.1	5.9	6.2	6.9	6.2 (0.42)	99
13	11	11	12	12	13	12 (0.94)	91
25	22	21	21	22	24	22 (1.1)	88
50	44	45	43	43	50	45 (2.9)	90

[a] Samples were alternated between replicate A and B. Day 0, 14 and 28 samples were taken from replicate A/ Day 8 and 22 samples were taken from replicate B.
[b] Mean measured values, standard deviations and % of nominal were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.
SD = standard deviation
n.a. = not applicable

C. DEFICIENCIES

None.

III. CONCLUSION

The Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC) for mysids (*Americamysis bahia*) exposed to metrafenone under flow-through conditions were determined to be 45 µg a.s./L and 22 µg a.s./L (mean measured), respectively, based on reproduction. Therefore, the geometric mean Maximum-Acceptable-Toxicant (MATC) was estimated to be 31 µg a.s./L (mean measured) and the 28-day LC_{50} value was determined to be > 45 µg a.s./L (mean measured), the highest concentration tested.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

Report:	CA 8.2.5.3/1 Pupp A.,Weltje L., 2007a Chronic toxicity of Metrafenone (BAS 560 F, Reg. No. 4037710) to the non-biting midge Chironomus riparius exposed via spiked water 2007/1018942
Guidelines:	OECD 219
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 28-day static laboratory study, non-biting midge larvae (*Chironomus riparius*) were exposed to metrafenone in the overlaying water. Nominal test concentrations were 125, 250, 500, 1000 and 2000 µg a.s./L. Additionally, a solvent (dimethylformamide (DMF, at 0.1 mL/L) control and a water control were tested. All test item concentrations and the water control had four replicates, whereas six replicates were tested for the solvent control. Each replicate test vessel contained twenty larvae.

Mean recoveries of metrafenone in the overlaying water were in the range of 91.8% - 119.4% of nominal concentrations at test initiation. The biological results were based on nominal concentrations. First emerged midges were observed fourteen days after exposure initiation. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the solvent control.

In the 28-day test with *Chironomus riparius* the no-observed-effect concentration (NOEC) for development rate and emergence rate was determined to be 2000 µg a.s./L based on nominal test conc

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 125, 250, 500, 1000 and 2000 µg a.s./L

3. Reference item: None

4. Dilution water: Reconstituted water, M4 according to Elendt
Vehicle: Dimethylformamide (DMF)
Artificial substrate: According to OECD 218, containing 5% sphagnum peat, 20% kaolin clay, 0.7% $CaCO_3$ and 75% quartz sand, and with a pH of 7.02 and a moisture content of 28.55%

5. Test organism:
Species: Non-biting midge (*Chironomus riparius*)
Age/life stage: First instar larvae, < 72 hours old
Source: In-house culture
Diet: Commercially available fish food TetraMin, 0.25 to 1.0 mg was added per larva each day
Test vessels: Glass beakers (600 mL), with 100 g wet artificial sediment and 400 mL dilution water

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 19.4 – 20.4 °C
pH: 7.46 – 8.53
Dissolved oxygen: 6.27 – 8.79 mg/L
Hardness: 3.115 – 3.293 mmol/L
Photoperiod: 16 hours light: 8 hours darkness (630 - 780 lux)
Aeration: Constant aeration

2. Animal assignment and treatment:
Five days prior to initiation of the exposure, fresh egg masses were collected from the culture and transferred to petri dishes with dilution water. About two days later the first larvae started hatching. One day before initiation of the exposure, the first instar larvae (< 72 hours old) were collected and added to test vessels randomly, until each test vessel contained twenty larvae. During addition of the larvae and until initiation of the exposure, the aeration was stopped to give the larvae the opportunity to settle into the sediment. The midges were exposed for 28 days, under static conditions, to nominal test item concentrations of 125, 250, 500, 1000 and 2000 µg a.s./L. A water control and solvent control (dimethylformamide (DMF) at a concentration of 0.1 mL/L) were tested in parallel. Each test item group and the water control group consisted of four replicates, and the solvent control contained six replicates.

3. Dose preparation:
First a stock solution was prepared by dissolving BAS 560 F in DMF at a nominal concentration of 20 mg a.s./mL. From this stock solution further dilutions were made in DMF to obtain the other four stock solutions. These five stock solutions were added to the test vessels containing the dilution water. The solvent control and all test item concentrations contained 0.1 mL DMF/L.

4. Measurements and observations:

Before emergence of the first midge, behavior and mortality were recorded at least three times a week. From the onset of emergence, behavior and mortality were determined daily, as well as the gender of the emerged adults.

Analytical verification of test item concentrations in the overlaying water (day 0, 2, 7, 14 and 28) and pore water (day 2 and 7) was conducted using a HPLC-method with MS detection. Sediment concentrations (Day 2, 7 and 28) were measured using a HPLC-method with MS/MS detection.

Temperature, dissolved oxygen concentration and pH were measured once a week.

5. Statistics:

Emergence rate and development rate were calculated using the appropriate equations and these data were statistically analyzed using ANOVA followed by Bonferroni test or William's-test (α = 0.05). Statistical analysis was performed using TOXSTAT Version 3.5 (2003).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

First emerged midges were observed fourteen days after exposure initiation. In both controls and all test item treatments fifteen to twenty midges emerged. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the solvent control. The results are summarized in the table below.

Table 8.2.5.3-1: Effects of BAS 560 F (metrafenone) exposure on emergence and development of *Chironomus riparius*

Concentration (nominal) (µg a.s./L)	**Control**	**Solvent control (DMSO)**	**125**	**250**	**500**	**1000**	**2000**
Emergence rate (ER)	0.8375	0.9083	0.8750	0.8625	0.8500	0.8625	0.8750
Development rate per day (DR)	0.0523	0.0506	0.0527	0.0515	0.0510	0.0530	0.0524
	Endpoints (µg a.s./L) (nominal)						
$EC_{50\ emergence}$	> 2000						
$NOEC_{emergence\ rate}$	2000						
$NOEC_{development\ rate}$	2000						

B. ANALYSIS

Mean recoveries of metrafenone in the overlaying water were in the range of 91.8% - 119.4% of nominal concentrations at test initiation, as shown in the table below. After 28 days, the measured concentration in the 500 µg a.s./L treatment was below the limit of quantification (1 µg a.s./L) and in the 2000 µg a.s./L 2.2% of the nominal concentration. The biological results were based on nominal water concentrations. Sediment concentrations in the 500 µg a.s./L treatment ranged from 1012 to 1647 µg a.s./kg sediment dry weight and in the 2000 µg a.s./L treatment from 3783 to 6539 µg a.s./kg sediment dry weight, respectively. The corresponding pore water concentrations were 21.8 and 5.86 µg a.s./L for the 500 µg a.s./L treatment and 33.5 and 89.3 µg a.s./L for the 2000 µg a.s./L treatment, respectively.

Table 8.2.5.3-2: Measured concentrations of BAS 560 F in the overlaying water

Nominal concentration (µg a.s./L)	Sampling time (days)	Measured concentration (µg a.s./L)	Mean measured as % of nominal
0 (negative control)	0	< LOQ	-
0 (solvent control)	0	< LOQ	-
125	0	149	119.4
250	0	290	116.0
500	0 2 7 14 28	539 342 167 7.50 < LOQ	107.8 68.4 33.5 1.5 -
1000	0	980	98.0
2000	0 2 7 14 28	1835 1263 594 66.5 44.6	91.8 63.1 29.7 3.3 2.2

LOQ = Limit of quantification (1 µg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

The no-observed-effect concentration (NOEC) for development rate and emergence rate of non-biting midges (*Chironomus riparius*) exposed to metrafenone for 28 days under static conditions was determined to be 2000 µg a.s./L, the highest nominal concentration tested. The lowest-observable effect concentration (LOEC) was > 2000 µg a.s./L (nominal).

CA 8.2.5.4 Sediment dwelling organisms

Report:	CA 8.2.5.4/1 Krueger H.O. et al., 2001a Toxicity of BAS 560 to Chironomus riparius during a prolonged sediment toxicity test 2001/7000462
Guidelines:	EEC 91/414 Annex II 8.2.7, EEC 96/12, Draft OECD Guideline for testing chemicals. Chironomid toxicity test using spiked water
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The no-observed-effect concentration (NOEC) for non-biting midges (*Chironomus riparius)* exposed to metrafenone for 40 days under static conditions was 1000 µg a.s./L, the highest nominal test concentration applied to the overlying water. The lowest-observable-effect concentration (LOEC) was determined to be > 1000 µg a.s./L (nominal). Please refer to Document M-II, Section 6, Point 8.2.5 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.5.4/2 Backfisch K.,Weltje L., 2011a Chronic toxicity of Reg.No. 4037710 (BAS 560 F; Metrafenone) to the non-biting midge Chironomus riparius - A spiked sediment study 2010/1145509
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BAS 560 F (metrafenone). The midges were exposed for 28 days to nominal test concentrations of 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw), under static conditions. Additionally, a solvent (acetone) control and a water control were tested. All test item concentrations and the water control had four replicates, whereas six replicates were tested for the solvent control.

Mean recoveries of metrafenone in sediment were in the range of 80.3% - 102.3% of nominal concentrations. The biological results were based on initial measured concentrations. First emerged midges were observed on day thirteen. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the water and solvent control.

In this 28-day spiked sediment test with *Chironomus riparius* exposed to metrafenone, the no-observed-effect concentration (NOEC) for development rate and emergence rate was determined to be 296.0 mg a.s./kg sediment dw (mean measured), the highest concentration tested. The lowest-observable-effect concentration (LOEC) and EC_{50} were both > 296.0 mg a.s./kg sediment dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw)

3. Reference item: None

4. Dilution water: Reconstituted water, M4 according to Elendt
Vehicle: Acetone
Artificial substrate: According to OECD 218; containing 5% sphagnum peat, 20% kaolin clay, 0.75% $CaCO_3$ and 75% quartz sand, and with a pH of 7.02

5. Test organism:
Species: Non-biting midge (*Chironomus riparius*)
Age/life stage: First instar larvae, < 48 hours old
Source: In-house culture
Diet: Commercially available fish food TetraMin, 0.25 to 1.0 mg was added per larva each day
Test vessels: 600 mL glass vessels with 100 g wet artificial sediment and 400 mL dilution water

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 20.1 – 21.5 °C
pH: 7.45 – 8.36
Dissolved oxygen: 7.74 – 8.93 mg/L
Hardness: 2.6 mmol/L
Photoperiod: 16 hours light: 8 hours darkness (535 - 850 lux)
Aeration: Constant aeration

2. Animal assignment and treatment:
Three days prior to initiation of the exposure, fresh egg masses were collected from the culture and transferred to petri dishes with dilution water. About two days later the first larvae started hatching. At initiation of the exposure, the first instar larvae (< 48 hours old) were collected and added to test vessels randomly, until each test vessel contained twenty larvae. During addition of the larvae and for 24 hours afterwards, the aeration was stopped to give the larvae the opportunity to settle into the sediment. The midges were exposed for 28 days, under static conditions, to nominal test item concentrations of 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw). A water control and solvent control (acetone) were tested in parallel. Each test item group and the water control group consisted of four replicates, and the solvent control contained six replicates.

3. Dose preparation:
First a primary stock solution was prepared by mixing BAS 560 F in acetone, at a nominal concentrations of 200 mg a.s./mL. The five different dosing stock solutions were obtained by further dilution of the primary stock solution in acetone. The dosing stock solutions were each mixed through an aliquot of the artificial sediment and these premixtures were placed in a fume hood for 1.5 hours for the acetone to partially evaporate. The premixtures were mixed through the rest of the artificial sediment. Each replicate received 100 g of sediment and then 400 mL of the dilution water was slowly added. The sediment/water mixtures were allowed to acclimate for two days prior to introduction of the test organisms.

4. Measurements and observations:

Before emergence of the first midge, behavior and mortality were recorded at least three times a week. From the onset of emergence, behavior and mortality were determined daily, as well as the gender of the emerged adults.

Analytical verification of test item concentrations in sediment, overlaying water and pore water samples, taken at exposure initiation and termination, was conducted using a HPLC-method with MS detection.

Temperature, dissolved oxygen concentration and pH were measured once a week.

5. Statistics:

Emergence rate and development rate were calculated using the appropriate equations and these data were statistically analyzed using ANOVA followed by Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$). Statistical analysis was performed using ToxRatPro Version 2.10.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

First emerged midges were observed on day thirteen. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the solvent control. The results are summarized in the table below.

Table 8.2.5.4-1: Effects of BAS 560 F (metrafenone) spiked sediment exposure on emergence and development of *Chironomus riparius*

Concentration (initial measured) (mg a.s./kg dry sediment)	**Control**	**Solvent control (acetone)**	**17.7**	**34.7**	**80.5**	**164.0**	**296.0**
Mean emergence rate, ER (SD)	0.8500 (0.0707)	0.8333 (0.0606)	0.8125 (0.0479)	0.8000 (0.0913)	0.7750 (0.0866)	0.7375 (0.1109)	0.7250 (0.1555)
Mean development rate per day, DR (SD)	0.0705 (0.0013)	0.0709 (0.0027)	0.0739 (0.0019)	0.0742 (0.0003)	0.0729 (0.0009)	0.0723 (0.0016)	0.0723 (0.0018)
	Endpoints (mg a.s./kg dry sediment) (initial measured)						
$EC_{50\ emergence}$	> 296.0						
$NOEC_{emergence\ rate}$	296.0						
$NOEC_{development\ rate}$	296.0						

SD = standard deviation

B. ANALYSIS

Mean recoveries of metrafenone in sediment were in the range of 80.3% - 102.3% of nominal concentrations. As recommended in OECD 218, the biological results were based on mean measured sediment concentrations at test initiation. Measured concentrations in overlaying water and pore water were quite similar and for nominal test concentrations of 80 mg a.s./kg sediment dw and higher the measured concentrations were close to the water solubility limit of metrafenone. In the overlaying water, the concentrations ranged from 0.056 to 0.624 mg a.s./L at test initiation and from 0.068 to 0.677 mg a.s./L at test termination. The pore water concentrations in the spiked sediment samples were between 0.039 and 0.901 mg a.s./L at test initiation and between 0.047 and 0.470 mg a.s./L at test termination.

Table 8.2.5.4-2: Measured concentrations of BAS 560 F in the overlaying water

Nominal concentration (mg a.s./kg sediment dw)	Sampling time (days)	Measured concentration (mg a.s./kg sediment dw)	Mean measured as % of nominal
0 (negative control)	0 28	< LOQ	-
0 (solvent control)	0 28	< LOQ	-
20	0 28	17.7 16.1	88.5 80.5
40	0 28	34.7 33.5	86.8 83.8
80	0 28	80.5 72.7	100.6 90.9
160	0 28	164 129	102.5 80.6
320	0 28	296 276	92.5 86.3

LOQ = Limit of quantification (0.01 mg a.s./kg)

C. DEFICIENCIES

None.

III. CONCLUSION

The no-observed-effect concentration (NOEC) and lowest-observable-effect concentration (LOEC) for development rate and emergence rate of non-biting midges (*Chironomus riparius*) exposed to metrafenone for 28 days under static conditions were determined to be 296.0 and > 296.0 mg a.s./kg sediment dw, respectively, the highest mean measured test concentration in the spiked sediment. The EC_{50} for emergence was > 296.0 mg a.s./kg sediment dw (mean measured).

Report: CA 8.2.5.4/3
Thomas S.T. et al., 2011b
BAS 560 F: A 10-day survival toxicity test with the marine amphipod (Leptocheirus plumulosus) using spiked sediment
2011/7000373
Guidelines: EPA 850.1740
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a spiked sediment toxicity study, marine amphipods (*Leptocheirus plumulosus*) were exposed to radio-labelled BAS 560 F (metrafenone) under static conditions. The amphipods were exposed for 10 days at nominal concentrations of 0.031, 0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./kg sediment dry weight (dw). A dilution water control and a solvent control (acetone; (partially) evaporated) were tested in parallel. Each group consisted of five replicates, containing 20 amphipods each. The amphipods were observed daily for mortality and abnormal behavior.

Measured concentrations of metrafenone in sediment samples ranged from 60% to 105% of nominal concentrations in the test system (pore water, sediment and overlying water). The biological results were based on mean measured concentrations in the sediment. At test termination, the mean survival of amphipods in the negative control, solvent control, 0.023, 0.049, 0.11, 0.16, 0.42, 0.76 and 1.7 mg a.s./kg sediment dw treatment groups were 96, 91, 79, 89, 90, 79, 76, 76 and 55%, respectively. Survival in the 1.7 mg a.s./kg treatment group was statistically significantly different when compared to the pooled control.

In this 10-day static sediment toxicity study with *Leptocheirus plumulosus*, the EC_{50} of metrafenone was > 1.7 mg a.s./kg sediment dw (mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.76 mg a.s./kg sediment dw, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Unlabeled test material: BAS 560 F (metrafenone)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

Labeled test material: ^{14}C-BAS 560 F (metrafenone)
Batch number: AC11662-17
Purity: 99.6%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.031, 0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./kg sediment dry weight (dw; nominal); (mean measured concentrations: 0, 0.023, 0.049, 0.11, 0.16, 0.42, 0.76 and 1.7 mg a.s./kg sediment dw)

3. Reference item: None

4. Dilution water: Natural seawater collected at Indian River Inlet, Delaware
Vehicle: Acetone
Artificial sediment: According to OECD 218, but with alpha cellulose (5%) instead of peat as source of organic matter. The other components of the sediment were 1% humic acid and dolomite, 14% silt and kaolin clay and 80% industrial quartz sand

5. Test organism:
Species: Marine amphipod *Leptocheirus plumulosus*
Age/life stage: Unknown
Length: 2 – 4 mm at test initiation
Source: Chesapeake Cultures, Virginia, USA
Acclimation period: Two days
Diet: No feeding during the exposure period
Test vessels: 1.0 L glass beakers, containing 175 mL sediment and 775 mL dilution water

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 24.8 – 25.9 °C
Salinity: 20 - 22‰
pH: 7.9 – 8.2
Dissolved oxygen: 6.1 – 8.4 mg/L
Photoperiod: Continuous light (648 lux)
Aeration: Constant aeration

2. Animal assignment and treatment:
At test initiation, the amphipods were collected from the culture and randomly assigned to test vessels, until each test vessel contained twenty amphipods. The amphipods were exposed for ten days to nominal test material concentrations of 0.031, 0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./kg sediment dry weight (dw), under static conditions. A dilution water control and a solvent control (acetone) were also included in the test. Each test item group and control group contained five replicate test vessels.

3. Dose preparation:
First primary stock solutions were prepared by mixing labelled and unlabeled BAS 560 F in acetone, at nominal concentrations of 0.200 and 0.25 mg a.s./mL, respectively. The dosing stock solutions for the three lowest test concentrations of 0.031, 0.063 and 0.13 mg a.s./kg sediment dw were obtained by further dilution of the labelled primary stock solution in acetone. To obtain the dosing stock solutions for the four higher test concentrations of 0.25, 0.50, 1.0 and 2.0 mg a.s./kg sediment dw, the labelled and unlabeled primary stock solution were mixed and diluted with acetone. The dosing stock solutions were mixed through an aliquot of the artificial sediment by hand with a glass stir rod. Then these premixtures were placed in a fume hood for one hour for the acetone to (partially) evaporate. The premixtures were added to the rest of the artificial sediment and mixed on a rotary mixer. Each replicate received 175 mL of sediment and then 775 mL of the dilution water was slowly added. The sediment/water mixtures were allowed to acclimate for 49 hours prior to introduction of the test organisms.

4. Measurements and observations:
Mortality and signs of toxic or abnormal behavior were recorded every day.

Sediment, pore water and overlying water samples were collected at test initiation and termination for analytical verification of test item concentrations, using a HPLC-method with UV- detection and liquid scintillation counting (LSC).

Temperature was recorded continuously throughout the exposure period. Dissolved oxygen was measured daily. Measurements of pH were made at test initiation, day 5 and test termination.

5. Statistics:
Mortality data were evaluated for normality and homogeneity of variances with Chi-Square and Bartlett's test, respectively. Statistical differences between treatment groups and the pooled control group were analyzed using Bonferroni t-test ($p < 0.05$).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

At test termination, the mean survival of amphipods in the negative control and solvent control were 96% and 91%, respectively. Survival in the 1.7 mg/kg treatment group was statistically significantly different when compared to the pooled control (Bonferroni t-test, $p < 0.05$). For results see the table below.

Table 8.2.5.4-3: Effects of metrafenone on marine amphipods (*Leptocheirus plumulosus*)

Concentration (mg/kg dry sediment) (nominal)	**Control**	**0.031**	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration (mg/kg dry sediment) (mean measured)	**Control**	**0.023**	**0.049**	**0.11**	**0.16**	**0.42**	**0.76**	**1.7**
Survival (10 d) (%)	91	79	89	90	79	76	76	55*
	Endpoints (mg a.s./kg dry sediment) (mean measured)							
EC_{50} (10 d)	> 1.7							
NOEC (10 d)	0.76							

* Statistically significant difference from pooled control (Bonferroni t-test, $p < 0.05$).

B. ANALYSIS

Measured concentrations of metrafenone in sediment samples ranged from 60% to 105% of nominal concentrations in the test system (pore water, sediment and overlying water), as is shown in the table below. Overlaying water concentrations were between 11% and 29% of nominal concentrations in test system and recoveries in the pore water samples ranged from 2% to 3% of nominal concentrations in test system. The biological results were based on mean measured concentrations in the sediment.

Table 8.2.5.4-4: Measured concentrations of BAS 560 F in the sediment

Nominal concentration (mg a.s./L)	**Sampling time (days)**	**Nominal BAS 560 F in test system (mg a.s.)**	**Measured BAS 560 F in test system* (mg a.s.)**	**Measured BAS 560 F in sediment (mg a.s.)**	**Measured BAS 560 F in sediment as % of nominal in test system**
0.031	0 10	0.006	0.006 0.005	0.005 0.004	86 62
0.063	0 10	0.012	0.011 0.013	0.009 0.010	73 81
0.13	0 10	0.024	0.027 0.024	0.023 0.017	95 71
0.25	0 10	0.047	0.035 0.044	0.028 0.032	60 67
0.50	0 10	0.094	0.101 0.094	0.088 0.069	94 72
1.0	0 10	0.188	0.197 0.171	0.169 0.116	90 62
2.0	0 10	0.374	0.446 0.379	0.393 0.263	105 69

* Test system = pore water, sediment and overlying water

C. DEFICIENCIES

None.

III. CONCLUSION

The EC_{50} for marine amphipods (*Leptocheirus plumulosus*) exposed to BAS 560 F (metrafenone) for ten days under static conditions was > 1.7 mg a.s./kg sediment dw (mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.76 mg a.s./kg sediment dw, based on mean measured concentrations.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Report:	CA 8.2.6.1/1 Barker C. et al., 2000c Effect of AC 375839 on growth of the green alga, Selenastrum capricornutum 2000/7000122
Guidelines:	EEC 91/414 Annex II 8.2.6, EEC 96/12, OECD 201
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The E_bC_{50} (biomass) and no-observed-effect concentration (NOEC) based on biomass for the green algae *Selenastrum capricornutum* exposed to metrafenone for 72 hours under static conditions were 711 µg a.s./L and 232 µg a.s./L, respectively, based on mean measured concentrations. The 72-hour E_rC_{50} (growth rate) and NOEC based on growth rate were > 870 µg a.s./L and 232 µg a.s./L, respectively. Please refer to Document M-II, Section 6, Point 8.2.6 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.6.1/2 Hoffmann F., 2012a Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the green alga Pseudokirchneriella subcapitata 2011/1254828
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour laboratory study. The study was conducted under static conditions with nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Additionally, a negative control and solvent control (0.1 mL/L acetone) were tested. Five replicates were tested for each concentration level and the negative control, and ten for the solvent control.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were 76.2% of nominal at test initiation and 74.1% of nominal at test termination. The test concentration of BAS 560 F in the centrifuged stock solution at test initiation was 0.342 mg/L which could be considered the functional solubility for these test conditions.

No morphological effects on the algae were observed at any concentration. The E_rC_{10}, E_rC_{50} (growth rate) and E_yC_{50} (yield) determined after 72 and 96 hours of exposure were all > 0.339 mg a.s./L (mean measured), the highest concentration tested. The E_yC_{10} was calculated to be 0.161 and 0.272 mg a.s./L (mean measured) after 72 and 96 hours of exposure, respectively. The no-observed-effect concentration (NOEC) was not reported in the study report, but was estimated to be 0.176 mg a.s./L (mean measured) for both growth rate and yield.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone, Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L (mean measured: 0, 0.022, 0.044, 0.085, 0.176 and 0.339 mg a.s./L)

3. Reference item: None

4. Test medium: Standard algal medium according to OECD 201
Vehicle: Acetone

5. Test organism:
Species: Green alga *Pseudokirchneriella subcapitata*
Source: Stock cultures are cultivated in-house. Fresh strains are obtained from the "UTEX Culture collection of Algae, University of Texas at Austin, USA"
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 100 mL Erlenmeyer dimple flask, containing 60 mL test medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 22 ± 1 °C
pH: 7.49 – 7.60 (at test termination)
Photoperiod: Continuous illumination (~8000 lux)
Shaking: Constant at 135 rpm

2. Assignment and treatment:
A static system was used with test duration of 96 hours. The following nominal test concentrations were used: 0 (negative and solvent control (0.1 mL/L acetone)), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Five replicates were tested for each concentration level and the negative control groups and ten for the solvent control group. Each test concentration and the control were inoculated to obtain initial algae density of 1 x 10^4 cells/mL. Each replicate consisted of 60 mL of test solution in a 100 mL flask.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving 4.82 mg of BAS 560 F in 1.0 mL acetone and then an aliquot (0.1 mL) of this solution was introduced to 1000 mL test medium. After centrifugation at 4000 rpm for 30 minutes to remove undissolved particles, the stock solution was colorless and clear. The different treatments were prepared by dilution of the stock solution with nutrient medium to reach the desired concentrations.

4. Measurements and observations:
Cell concentration in each flask was determined 24, 48, 72 and 96 hours after starting the experiment with a spectrophotometer at 623 nm, using 5 cm glass cuvettes (due to the high cell density a 1 cm glass cuvette was used for measurement at test termination). Algal medium without algae was used as a blank. The mean cell densities per treatment were used to calculate yield and growth rates. The percent inhibition values were calculated for each treatment group as the percent reduction in average yield and in average growth rate relative to the control replicates.

At the start and at the end of the test samples were taken out of pooled samples for verification of the test item concentrations. Samples were analyzed by an HPLC-method with MS-detection. At test termination, the pH of all individual samples (control as well as treated samples) was measured.

5. Statistics:
The mathematical determination of the EC_x was done by probit analysis. The calculations were conducted with a PC and the commercial software "TOXRAT Professional 2.10" (ToxRat Solutions GmbH, Alsdorf, Germany).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The following results (based on geometric mean concentrations) with respect to yield and growth rate were determined from the concentration-response relationship.

72 hour E_rC_{50} > 0.339 mg a.s./L (95% confidence limits: not calculated)
72 hour E_rC_{10} > 0.339 mg a.s./L (95% confidence limits: not calculated)
72 hour E_yC_{50} > 0.339 mg a.s./L (95% confidence limits: not calculated)
72 hour E_yC_{10} = 0.161 mg a.s./L (95% confidence limits: 0.132 – 0.185 mg a.s./L)

96 hour E_rC_{50} > 0.339 mg a.s./L (95% confidence limits: not calculated)
96 hour E_rC_{10} > 0.339 mg a.s./L (95% confidence limits: not calculated)
96 hour E_yC_{50} > 0.339 mg a.s./L (95% confidence limits: not calculated)
96 hour E_yC_{10} = 0.272 mg a.s./L (95% confidence limits: 0.225 – 0.292 mg a.s./L)

The table below summarizes the percentage of inhibition (of both growth rate and yield) observed in each test group.

Table 8.2.6.1-1: Percent inhibition of BAS 560 F on cell density, growth rate and yield of green algae *Pseudokirchneriella subcapitata*

Geometric mean measured concentration (mg a.s./L)	**0.022**	**0.044**	**0.085**	**0.176**	**0.339**
Inhibition at 96 h (yield) (%)	-3.1	-3.7	-0.8	-0.7	28.1
Inhibition at 96 h (growth rate) (%)	-0.5	-0.6	-0.1	-0.1	5.5

Negative values demonstrate an increase compared to the control

No morphological effects on the algae were observed at test termination.

B. ANALYSIS

Analytical verification of BAS 560 F was carried out in each tested concentration at the beginning and at the end of the test. Measured values for BAS 560 F at test initiation and at test termination are given in the table below.

Table 8.2.6.1-2: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	**0.0 (negative control)**		**0.0 (solvent control)**		**0.029**		**0.058**		**0.115**		**0.23**		**0.46**	
Sampling time (hours)	0	96	0	96	0	96	0	96	0	96	0	96	0	96
Measured concentration (mg a.s./L)	n.d.	n.d.	n.d.	n.d.	0.0220	0.0215	0.0454	0.0432	0.0866	0.0842	0.178	0.174	0.342	0.336
% nominal	--	--	--	--	75.9	74.0	78.2	74.4	75.3	73.2	77.2	75.6	74.4	73.1

n.d. = not detected

C. DEFICIENCIES

None.

III. CONCLUSION

The 96-hour exposure of BAS 560 F (metrafenone) to the freshwater green algae *Pseudokirchneriella subcapitata* under static conditions resulted in values for both E_rC_{50} (growth rate) and E_yC_{50} (yield) of > 0.339 mg a.s./L (mean measured), the highest test concentration. The E_rC_{10} was also > 0.339 mg a.s./L, based on mean measured concentrations. The E_yC_{10} was calculated to be 0.161 and 0.272 mg a.s./L (mean measured) after 72 and 96 hours of exposure, respectively. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.176 mg a.s./L (mean measured) for both growth rate and yield.

Report:	CA 8.2.6.1/3 Jatzek H.-J., 2002b CL 375 816 (metabolite of BAS 560 F, Benzophenone) - Determination of the inhibitory effect on the cell multiplication of unicellular green algae 2002/1004873
Guidelines:	EEC 92/69 A V C 3, OECD 201, EPA 850.5400
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The E_bC_{50} (biomass) and E_rC_{50} (growth rate) values for the green algae *Pseudokirchneriella subcapitata* exposed to CL 375816 for 72 hours under static conditions were both > 100 mg a.s./L, based on nominal concentrations. The no-observed-effect concentration (NOEC) for both biomass and growth rate was 100 mg a.s./L (nominal). Please refer to Document M-II, Section 6, Point 8.2.6 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.6.1/4 Jatzek H-.J., 2002b CL 4084 564 (metabolite of BAS 560 F) - Determination of the inhibitory effect on the cell multiplication of unicellular green algae 2002/1004872
Guidelines:	EEC 92/69 A V C 3, OECD 201, EPA 850.5400
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Conclusion

The E_bC_{50} (biomass) and E_rC_{50} (growth rate) values for the green algae *Pseudokirchneriella subcapitata* exposed to CL 4084564 for 72 hours under static conditions were 27.8 and 38.7 mg a.s./L, respectively, based on mean measured concentrations. The no-observed-effect concentration (NOEC) for both biomass and growth rate was 9.90 mg a.s./L (mean measured). Please refer to Document M-II, Section 6, Point 8.2.6 of the summary dossier dated 2002 for further details.

Report:	CA 8.2.6.1/5 Dabrunz A., 2015a S15-02612 (Algae growth inhibition test) and S15-02611 (Daphnia acute toxicity test) with CL3000402 / Reg.No. 4110838: Results of Non-GLP pre-tests regarding solubility and toxicity 2015/1177630
Guidelines:	<none>
GLP:	no (certified by <none>)

Conclusion

A laboratory study on the acute toxicity of CL 3000402 was attempted. It was not possible to obtain a clear solution, free of particles in Medium, as well as in several solvents (acetone, DMF and DMSO). Only by using a filtration approach it was possible to obtain a stock solution which was stable for 48 hours and showed a concentration of the dissolved test item of approximately 16 µg/L. When this filtrate was tested on algae for 72 hours in a range-finder, no effects on growth rate and yield were observed. However, this study does not accurately demonstrate the true toxicity of the metabolite, hence a definitive study has not been conducted.

CA 8.2.6.2 Effects on growth of an additional algal species

Report: CA 8.2.6.2/1
Desjardins D. et al., 2005b
BAS 560 F: A 96-hour toxicity test with the freshwater alga (Anabaena flos-aquae)
2005/7003441
Guidelines: OECD 201, EPA 850.5400, EEC 92/69 C 3
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the blue-green alga *Anabaena flos-aquae* was determined in a 96-hour laboratory study. The study was conducted under static conditions, with nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative control (medium only) and solvent control (0.1 mL/L dimethylformamide (DMF)) were tested in parallel. Three replicates were tested for each test substance concentration, the negative control and the solvent control.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were between 86% and 140% of nominal concentrations at test initiation, and between 37% and 45% of nominal concentrations at test termination. Results were based on initial mean measured concentrations.

No effects on area under the growth curve and growth rate were observed for *Anabaena flos-aquae* exposed to BAS 560 F for 96 hours. Therefore, E_bC_{50} (biomass) and E_rC_{50} (growth rate) values were determined to be > 0.86 mg a.s./L (initial mean measured), the highest test concentration. The no-observed-effect concentration (NOEC) for both biomass and growth rate was 0.86 mg a.s./L, based on initial mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (metrafenone)
   Batch number: AC12053-29
   Purity: 94.2%
   Description: Solid

2. **Test concentrations:** 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured at test initiation: 0, 0.088, 0.14, 0.22, 0.58 and 0.86 mg a.s./L)

3. **Reference item:** None

4. Test medium: Algal medium according to OECD 201
Vehicle: Dimethylformamide (DMF) (at 0.1 mL/L)

5. Test organism:
Species: Blue-green alga *Anabaena flos-aquae*
Source: Stock cultures were obtained from Wildlife International, Ltd., Maryland, USA. Original algal cultures were obtained from UTCC-University of Toronto
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 250 mL Erlenmeyer flask, containing 100 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions
Temperature: 22.5 – 23.4 °C
pH: 7.7 – 7.9
Photoperiod: Continuous illumination (2030 - 2230 lux)
Shaking: Constant at 100 rpm

2. Assignment and treatment:
At test initiation, algal cells were added to the test chambers, to achieve nominal cell densities of 1 x 10^4 cells/mL in each test chamber. Three replicate chambers were tested in each treatment and control group. The algae were exposed for 96 hours under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in dimethylformamide (DMF), at a nominal concentration of 10 mg a.s./mL. This solution was inverted at least 20 times to mix, and appeared clear and light yellow. Secondary stock solutions were prepared by diluting the primary stock solution in DMF to obtain nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./L, containing DMF at a concentration of 0.1 mL/L. The negative control group was exposed to test medium only and the solvent control was exposed to DMF at a concentration of 0.1 mL/L.

4. Measurements and observations:
Samples were collected at 24, 48, 72 and 96 hours, to determine cell densities. Cell densities were counted using a hemacytometer and microscope. In addition, subsamples from pooled samples per treatment were examined microscopically for atypical cell morphology. Also, each test vessel was examined for aggregations or flocculations of cells and adherence of the cells to the test vessel.

At the test initiation and termination, samples were taken for verification of the test item concentrations. Samples were analyzed by an HPLC-method using variable wavelength detection set at 220 nm.

Temperature was recorded twice a day. Light intensity was measured at test initiation. The pH was measured at test initiation and test termination.

5. Statistics:
The results from the cell density counts were used to calculate areas under the growth curve, growth rates and percent inhibition values. These calculations as well as all statistical analyses were conducted using the SAS System for Windows, Version 8.02 or TOXSTAT version 3.5. The negative and solvent controls were compared using Student's t-test. This showed that there were no statistically significant differences and therefore the two control groups were pooled. Non-linear regression or linear interpolation was used to calculate EC_{50} values and their corresponding 95% confidence intervals for cell density (EC_{50}), area under the growth curve (E_bC_{50}) and growth rate (E_rC_{50}). The data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. The Dunnett's test was used to compare the treatment groups to the pooled control group. The results of the statistical analyses as well as an evaluation of the concentration-response pattern were used to determine the NOEC relative to each parameter.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No statistically significant differences were observed for *Anabaena flos-aquae* exposed to BAS 560 F for 96 hours. The table below shows the effects of 96-hour exposure to cell density, area under the growth curve (biomass) and growth rate observed in each test group.

Table 8.2.6.2-1: Effects of BAS 560 F exposure on cell density, area under the growth curve and growth rate of *Anabaena flos-aquae*

Nominal concentration (mg a.s./L)	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Initial mean measured concentration (mg a.s./L)	**0.088**	**0.14**	**0.22**	**0.58**	**0.86**
Inhibition in cell density at 96 h (%)	-8.6	-36	-40	-19	-40
Inhibition in area under the growth curve at 96 h (%)	-17	-42	-35	-32	-32
Inhibition in growth rate at 96 h (%)	-1.8	-6.8	-7.4	-4.1	-7.4
	Endpoints [a] (mg a.s./L)				
EC_{50} (72 h)	>0.86				
EC_{50} (96 h)	>0.86				
NOEC (96 h)	0.86				
E_bC_{50} (72 h)	>0.86				
E_bC_{50} (96 h)	>0.86				
NOEC (96 h)	0.86				
E_rC_{50} (72 h)	>0.86				
E_rC_{50} (96 h)	>0.86				
NOEC (96 h)	0.86				

Negative values demonstrate an increase compared to the control
[a] Based on initial mean measured concentrations

Some signs of aggregation/flocculation of algae and changes in cell morphology (long chains) were observed in the control groups and each test substance group. As these were observed in all groups, including the controls, these changes were not considered to be treatment related.

B. ANALYSIS

Measured concentrations of BAS 560 F at test initiation ranged from 86% to 140% of nominal concentrations. At test termination the measured concentrations were between 37% and 45% of the nominal concentrations. Due to the decrease in test substance concentrations during the test, analyses of the results were based on the recoveries at test initiation. The results of the chemical analysis are given in the table below.

Table 8.2.6.2-2: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	% of nominal concentration
0.0 (negative control)	0 96	<LOQ	-
0.0 (solvent control)	0 96	<LOQ	-
0.063	0 96	0.088 0.025	140 39.9
0.13	0 96	0.139 0.048	107 36.7
0.25	0 96	0.217 0.107	87 42.9
0.50	0 96	0.580 0.217	116 43.4
1.0	0 96	0.862 0.445	86 44.5

LOQ = limit of quantification (0.040 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

The E_bC_{50} (biomass) and E_rC_{50} (growth rate) for blue-green alga *Anabaena flos-aquae* exposed to metrafenone for 96 hours under static conditions were both > 0.86 mg a.s./L (initial mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) for both biomass and growth rate was 0.86 mg a.s./L, based on initial mean measured concentrations.

Report:	CA 8.2.6.2/2 Hoffmann F., 2012b Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of blue-green alga Anabaena flos-aquae 2011/1254829
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 96-hour acute toxicity laboratory study, the effect of BAS 560 F (metrafenone) on the growth of the blue-green alga *Anabaena flos-aquae* was investigated. The algae were exposed to nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L, under static conditions. A negative control and a solvent control (acetone, at 0.1 mL/L) were tested in parallel. Assessment of growth was conducted 0, 24, 48, 72 and 96 h after test initiation. The percentage inhibition relative to the control was calculated for each test concentration from mean growth rates and yield.

Measured concentrations ranged from 91.3% to 103.3% of nominal concentrations. The following biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control group and at any of the concentrations tested.

The E_rC_{50} (growth rate), E_rC_{10} and the E_yC_{50} (yield) for *Anabaena flos-aquae* exposed to BAS 560 F (metrafenone) were all determined to be > 0.450 mg/L (mean measured), after 72 and 96 hours. The E_yC_{10} was 0.450 mg /L (mean measured) at 72 hours and > 0.450 mg/L (mean measured) at 96 hours. The no-observed-effect concentration (NOEC) was not reported in the study report, but was estimated to be 0.224 mg/L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone, Reg. no. 4037710)
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L; (geometric mean measured concentrations: 0, 0.027, 0.056, 0.119, 0.224 and 0.450 mg a.s./L)

3. Reference item: None

4. Test medium: Algal AAP-medium according to OECD 201
Vehicle: Acetone

5. Test organism
Species: Blue-green alga *Anabaena flos-aquae*
Strain: UTEX B 1444
Source: Stock obtained from UTEX – The Culture Centre Collection of Algae at the University of Texas, USA.
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 100 mL glass Erlenmeyer flask, containing 60 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions
Temperature: 24 ± 1 °C
pH: 7.80 – 7.87
Photoperiod: Continuous illumination (4300 lux)
Shaking: Constant at 170 rpm

2. Assignment and treatment:
At test initiation, algal cells were added to the test chambers, to achieve nominal cell densities of 1 x 10^4 cells/mL in each test chamber. Each treatment level and the negative control consisted of five replicate chambers, and the solvent control had ten replicates. The algae were exposed for 96 hours under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in acetone, at a nominal concentration of 4.55 mg a.s./mL. This solution was diluted in test medium, at a concentration of 0.1 mL/L, and was then centrifuged for 30 minutes at 4000 rpm to remove undissolved particles. Secondary stock solutions were prepared by further diluting the primary stock solution in the test medium to obtain nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. The negative control group was exposed to test medium only and the solvent control group was exposed to acetone at a concentration of 0.1 mL/L.

4. Measurements and observations:
Samples were collected at 24, 48, 72 and 96 hours, to determine cell densities with a spectrophotometer.

At the test initiation and termination, samples were taken for verification of the test item concentrations. Samples were analyzed by an HPLC-method with MS-detection.

The pH was measured at test initiation and test termination.

5. Statistics:
The results from the cell density counts were used to calculate growth rates and yield. These calculations as well as all statistical analyses were conducted using the TOXRAT Professional 2.10. EC_x values were determined using probit analysis.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No morphological effects on algae were observed in the control group and at any of the concentrations tested. Effects on algal growth rate and yield are summarized in **Table 8.2.6.2-3**.

Table 8.2.6.2-3: Effect of BAS 560 F (metrafenone) on the growth of the blue-green algae *Anabaena flos-aquae*

Concentration (mg a.s./L) (nominal)	**Control**	**0.029**	**0.058**	**0.115**	**0.230**	**0.460**
Concentration (mg a.s./L) (mean measured)	**Control**	**0.027**	**0.056**	**0.119**	**0.224**	**0.450**
Growth rate inhibition in 96 h (growth rate) (%)	--	0.1	0.1	0.0	0.0	1.2
Yield inhibition in 96 h (%)	--	0.4	0.6	0.2	-0.1 [a]	5.7
	Endpoints (mg a.s./L) (mean measured)					
E_rC_{50} (72 h)	> 0.450					
E_rC_{10} (72 h)	> 0.450					
E_yC_{50} (72 h)	> 0.450					
E_yC_{10} (72 h)	0.450					
E_rC_{50} (96 h)	> 0.450					
E_rC_{10} (96 h)	> 0.450					
E_yC_{50} (96 h)	> 0.450					
E_yC_{10} (96 h)	> 0.450					

[a] Negative values indicate stimulated growth.

B. ANALYSIS

Measured concentrations ranged from 96.2% to 103.3% of nominal at test initiation and from 91.3% to 103.1% of nominal at test termination. The biological results are based on geometric mean measured concentrations. The results of the chemical analysis are given in the table below.

Table 8.2.6.2-4: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Geomean measured concentration (mg a.s./L) [a]	% of nominal concentration	Geomean measured concentration (mg a.s./L) [b]
0.0 (negative control)	0 96	<LOQ	-	-
0.0 (solvent control)	0 96	<LOQ	-	-
0.029	0 96	0.0280 0.0265	96.4 91.3	0.027
0.058	0 96	0.0567 0.0559	97.7 96.4	0.056
0.115	0 96	0.119 0.119	103.3 103.1	0.119
0.230	0 96	0.224 0.224	97.6 97.4	0.224
0.460	0 96	0.443 0.458	96.2 99.5	0.450

LOQ = limit of quantification (0.001 mg a.s./L)
[a] Geomean measured concentrations of two replicates per sampling time
[b] Geomean measured concentrations for the two sampling times

C. DEFICIENCIES

None.

III. CONCLUSION

The E_rC_{50} (growth rate), E_rC_{10} and the E_yC_{50} (yield) for the blue-green algae *Anabaena flos-aquae* exposed to metrafenone for 96 hours under static conditions were all determined to be > 0.450 mg/L (mean measured), the highest concentration tested, after 72 and 96 hours. The E_yC_{10} was 0.450 mg /L (mean measured) at 72 hours and > 0.450 mg/L (mean measured) at 96 hours. The no-observed-effect concentration (NOEC) was not reported in the study report, but was estimated to be 0.224 mg/L, based on mean measured concentrations.

Report:	CA 8.2.6.2/3 Desjardins D. et al., 2005c BAS 560 F: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa) 2005/7003436
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the freshwater diatom *Navicula pelliculosa* was determined in a 96-hour laboratory study. The study was conducted under static conditions, with nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative control (medium only) and solvent control (0.1 mL/L dimethylformamide (DMF) were tested in parallel. Four replicates were tested for each test substance concentration, the negative control and the solvent control.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were between 86.4% and 121% of nominal concentrations at test initiation, and between 40.4% and 46.8% of nominal concentrations at test termination. Results were based on initial mean measured concentrations.

Statistically significant inhibition of area under the growth curve and growth rate was observed for *Navicula pelliculosa* exposed to BAS 560 F for 72 hours at the highest test concentration. After 96 hours of exposure, only the area under the growth curve was statistically significantly reduced at the highest test concentration.

E_bC_{50} (biomass) and E_rC_{50} (growth rate) values were > 0.91 mg a.s./L (initial mean measured), the highest test concentration. The no-observed-effect concentration (NOEC) for biomass was 0.43 mg a.s./L and for growth rate 0.91 mg a.s./L, based on initial mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured at test initiation: 0.076, 0.15, 0.28, 0.43 and 0.91 mg a.s./L)

3. Reference item: None

4. Test medium: US EPA medium AAP, also according to ASTM
Vehicle: Dimethylformamide (DMF) (at 0.1 mL/L)

5. Test organism:
Species: Diatom *Navicula pelliculosa*
Source: Stock cultures were cultivated at Wildlife International, Ltd., Maryland, USA. Original algal cultures were obtained from UTEX-The Culture Collection of Algae at the University of Texas at Austin, USA
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 250 mL Erlenmeyer flask, containing 100 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 22.2 – 23.6 ºC
pH: 7.6 – 7.9
Photoperiod: Continuous illumination (3890 - 4720 lux)
Shaking: Constant at 100 rpm

2. Assignment and treatment:
At test initiation, algal cells were added to the test chambers, to achieve nominal cell densities of 1 x 10^4 cells/mL in each test chamber. Four replicate chambers were tested in each treatment and control group. The algae were exposed for 96 hours under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in dimethylformamide (DMF), at a nominal concentration of 10 mg a.s./mL. This solution was inverted at least 20 times to mix, and appeared clear and light yellow. Secondary stock solutions were prepared by diluting the primary stock solution in DMF to obtain nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./L. The five different stock solutions were diluted with test medium to reach the nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, containing DMF at a concentration of 0.1 mL/L. The negative control group was exposed to test medium only and the solvent control was exposed to DMF at a concentration of 0.1 mL/L.

4. Measurements and observations:
Samples were collected at 24, 48, 72 and 96 hours, to determine cell densities. Cell densities were counted using a hemacytometer and microscope. In addition, subsamples from pooled samples per treatment were examined microscopically for atypical cell morphology. Also, each test vessel was examined for aggregations or flocculations of cells and adherence of the cells to the test vessel.

At test initiation and termination, samples were taken for verification of the test item concentrations. Samples were analyzed by an HPLC-method using variable wavelength detection set at 220 nm.

Temperature was recorded twice a day. Light intensity was measured at test initiation. The pH was measured at test initiation and test termination.

5. Statistics:
The results from the cell density counts were used to calculate areas under the growth curve, growth rates and percent inhibition values. These calculations as well as all statistical analyses were conducted using the SAS System for Windows Version 8.02. The negative and solvent controls were compared using Student's t-test. This showed that there were statistically significant differences at 24, 48 and 72 hours for cell density and growth rate, and at 24 and 48 hours for biomass. For these occasions, treatment groups were compared to the solvent control group, and when negative and solvent control groups were not statistically significantly different the two groups were pooled. Non-linear regression was used to calculate EC_{50} values for cell density (EC_{50}), area under the growth curve (E_bC_{50}) and growth rate (E_rC_{50}), when possible. The data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. The Dunnett's test was used to compare the treatment groups to the pooled control. The results of the statistical analyses as well as an evaluation of the concentration-response pattern were used to determine the NOEC for each parameter.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Navicula pelliculosa exposed to BAS 560 F at the highest test concentration (0.91 mg a.s./L; initial mean measured) for 72 hours showed a statistically significant decrease in cell density, area under the growth curve and growth rate. At test termination (96 hours), statistically significant difference to the control groups was only observed for area under the growth curve, at the highest test concentration of 0.91 mg a.s./L (initial mean measured). The table below shows the effects of 72-hour and 96-hour exposure to cell density, area under the growth curve (biomass) and growth rate observed in each test group.

Table 8.2.6.2-5: Effects of BAS 560 F exposure on cell density, area under the growth curve and growth rate of *Navicula pelliculosa*

Nominal concentration (mg a.s./L)	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Initial mean measured concentration (mg a.s./L)	**0.076**	**0.15**	**0.28**	**0.43**	**0.91**
Inhibition in cell density at 72 h (%)	0.14	0.99	-6.3	-5.9	11*
Inhibition in cell density at 96 h (%)	0.22	-0.44	-3.6	0.078	3.5
Inhibition in area under the growth curve at 72 h (%)	1.5	-4.2	-0.35	5.9	35*
Inhibition in area under the growth curve at 96 h (%)	-0.55	-3.4	-4.2	0.022	19*
Inhibition in growth rate at 72 h (%)	0.017	0.28	-1.3	-1.3	2.4*
Inhibition in growth rate at 96 h (%)	0.042	-0.096	0.74	0.014	0.75
	Endpoints [a] (mg a.s./L)				
EC_{50} (72 h)	>0.91				
EC_{50} (96 h)	>0.91				
NOEC (96 h)	0.91				
E_bC_{50} (72 h)	>0.91				
E_bC_{50} (96 h)	>0.91				
NOEC (96 h)	0.43				
E_rC_{50} (72 h)	>0.91				
E_rC_{50} (96 h)	>0.91				
NOEC (96 h)	0.91				

Negative values demonstrate an increase compared to the control
[a] Based on initial mean measured concentrations
*Statistically significantly different from the pooled control, Dunnett's test ($p<0.05$)

No signs of aggregation/flocculation of algae or changes in cell morphology were observed in the control groups or any test substance group.

B. ANALYSIS

Measured concentrations of BAS 560 F at test initiation ranged from 86.4% to 121% of nominal concentrations. At test termination the measured concentrations were between 40.4% and 46.8% of the nominal concentrations. Due to the decrease in test substance concentrations during the test, analyses of the results were based on the recoveries at test initiation. The results of the chemical analysis are given in the table below.

Table 8.2.6.2-6: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	% of nominal concentration
0.0 (negative control)	0 96	<LOQ	-
0.0 (solvent control)	0 96	<LOQ	-
0.063	0 96	0.076 0.029	121 45.5
0.13	0 96	0.154 0.053	118 40.4
0.25	0 96	0.276 0.103	110 41.4
0.50	0 96	0.432 0.234	86.4 46.8
1.0	0 96	0.914 0.467	91.4 46.7

LOQ = limit of quantification (0.040 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

The E_bC_{50} (biomass) and E_rC_{50} (growth rate) for the freshwater diatom *Navicula pelliculosa* exposed to metrafenone for 96 hours under static conditions were both > 0.91 mg a.s./L (initial mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) was 0.43 mg a.s./L for biomass and 0.91 mg a.s./L for growth rate, based on initial mean measured concentrations.

Report:	CA 8.2.6.2/4 Hoffmann F., 2012c Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the freshwater diatom Navicula pelliculosa 2011/1254831
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the freshwater diatom *Navicula pelliculosa* was determined in a 96-hour laboratory study. The study was conducted under static conditions, with nominal concentrations of 029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. A negative control (medium only) and solvent control (0.1 mL acetone) were tested in parallel. Five replicates were tested for each test substance concentration and the negative control and 10 replicates for the solvent control.
Assessment of growth was conducted daily. The percentage growth inhibition relative to the solvent control was calculated for each test concentration from mean growth rates and yield.

Chemical analysis of the test solutions showed that measured concentrations of metrafenone ranged from 77.1% to 80.4% of nominal concentrations. The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control groups and at any of the test item concentrations tested.

In this 96-hour toxicity test with *Navicula pelliculosa* exposed to metrafenone the E_rC_{50} (growth rate), E_rC_{10} and the E_yC_{50} (yield) were all determined to be > 0.357 mg/L (geometric mean measured), the highest concentration tested. The E_yC_{10} was calculated to be 0.299 mg a.s./L after 72 hours and 0.262 mg a.s./L after 96 hours, based on geometric mean measured concentrations. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.179 mg a.s./L (mean measured) for both growth rate and yield.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone, Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L; (geometric mean measured concentrations: 0, 0.023, 0.046, 0.090, 0.179 and 0.357 mg a.s./L)

3. Reference item: None

4. Test medium: OECD medium, according to OECD 201
Vehicle: Acetone

5. Test organism
Species: Diatom *Navicula pelliculosa*
Strain: UTEX 674
Source: Obtained from UTEX - The Culture Collection of Algae at the University of Texas, USA
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 100 mL Erlenmeyer flask, containing 60 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions
Temperature: 22 ± 1 ºC
pH: 7.70 – 7.83
Photoperiod: Continuous illumination (8000 lux)
Shaking: Constant at 135 rpm

2. Assignment and treatment:
At test initiation, algal cells were added to the test chambers, to achieve nominal cell densities of 1 x 10^4 cells/mL in each test chamber. Each treatment group and the negative control group consisted of five replicates and the solvent control contained ten replicates. The algae were exposed for 96 hours under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in acetone, at a nominal concentration of 4.56 mg a.s./mL. This solution was diluted in test medium, at a concentration of 0.1 mL/L, and was then centrifuged for 30 minutes at 4000 rpm to remove undissolved particles. Secondary stock solutions were prepared by further diluting the primary stock solution in the test medium to obtain nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. The negative control group was exposed to test medium only and the solvent control group was exposed to acetone at a concentration of 0.1 mL/L.

4. Measurements and observations:
Samples were collected at 24, 48, 72 and 96 hours, to determine cell densities with a spectrophotometer.

At the test initiation and termination, samples were taken for verification of the test item concentrations, by an HPLC-method with MS-detection.

The pH was measured at test initiation and test termination.

5. Statistics:
The results from the cell density counts were used to calculate growth rate and yield. EC_x values were determined using probit analysis. All calculations and statistical analyses were conducted using TOXRAT Professional 2.10.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No morphological effects on algae were observed in the control groups and at any of the test item concentrations tested. The effects on algal growth rate and yield are summarized in the table below.

Table 8.2.6.2-7: Effect of metrafenone on the growth of the freshwater diatom *Navicula pelliculosa* (72 h and 96 h)

Concentration (mg a.s./L) (nominal)	**Solvent control**	**Control**	**0.029**	**0.058**	**0.115**	**0.230**	**0.460**
Concentration (mg a.s./L) (geometric mean measured)	**--**	**--**	**0.023**	**0.046**	**0.090**	**0.179**	**0.357**
Growth rate inhibition in 72 h (%) [a]	--	-0.6	-0.6	-0.6	-0.3	-0.2	5.4
Yield inhibition in 72 h (%) [a]	--	2.2	-2.2	-2.2	-1.3	-0.9	19.1
Growth rate inhibition in 96 h (%) [a]	--	-0.1	-0.3	-0.1	0.4	0.3	6.1
Yield inhibition in 96 h (%) [a]	--	0.4	-1.7	-0.6	2.0	1.7	27.0
	Endpoints (mg a.s./L) (geometric mean measured)						
E_rC_{50} (72 h)	> 0.357						
E_rC_{10} (72 h)	> 0.357						
E_yC_{50} (72 h)	> 0.357						
E_yC_{10} (72 h)	0.299 (95% confidence limits: 0.276 - 0.312)						
E_rC_{50} (96 h)	> 0.357						
E_rC_{10} (96 h)	> 0.357						
E_yC_{50} (96 h)	> 0.357						
E_yC_{10} (96 h)	0.262 (95% confidence limits: 0.236 - 0.279)						

[a] Inhibition compared to the solvent control; negative values indicate stimulated growth

B. ANALYSIS

As shown in the table below, measured concentrations of metrafenone ranged from 77.9% to 79.0% of nominal at test initiation and from 77.1% to 80.4% of nominal at test termination. The biological results are based on geometric mean measured concentrations.

Table 8.2.6.2-8: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Geomean measured concentration (mg a.s./L) [a]	% of nominal concentration	Geomean measured concentration (mg a.s./L) [b]
0.0 (negative control)	0 96	<LOQ	-	-
0.0 (solvent control)	0 96	<LOQ	-	-
0.029	0 96	0.0229 0.0227	79.0 78.4	0.023
0.058	0 96	0.0454 0.0466	78.2 80.4	0.046
0.115	0 96	0.0907 0.0889	78.9 77.3	0.090
0.230	0 96	0.179 0.179	77.9 77.6	0.179
0.460	0 96	0.359 0.355	78.0 77.1	0.357

LOQ = limit of quantification (0.001 mg a.s./L)
[a] Geomean measured concentrations of two replicates per sampling time
[b] Geomean measured concentrations for the two sampling times

C. DEFICIENCIES

None.

III. CONCLUSION

The E_rC_{50} (growth rate), E_rC_{10} and the E_yC_{50} (yield) for the freshwater diatom *Navicula pelliculosa* exposed to metrafenone for 96 hours under static conditions were all determined to be > 0.357 mg a.s./L (geometric mean measured), the highest concentration tested. The E_yC_{10} was calculated to be 0.299 mg a.s./L at 72 hours and 0.262 mg a.s./L at 96 hours, based on geometric mean measured concentrations. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.179 mg a.s./L (geometric mean measured) for both growth rate and yield.

Report:	CA 8.2.6.2/5 Desjardins D. et al., 2005d BAS 560 F: A 96-hour toxicity test with the marine diatom (Skeletonema costatum) 2005/7003443
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the marine diatom *Skeletonema costatum* was determined in a 96-hour laboratory study. The study was conducted under static conditions, with nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative control (medium only) and solvent control (0.1 mL/L dimethylformamide (DMF)) were tested in parallel. Three replicates were tested for each test substance concentration, the negative control group and the solvent control group.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were between 68% and 85.4% of nominal concentrations at test initiation, and between 19.9% and 48.5% of nominal concentrations at test termination. Results were based on initial mean measured concentrations.

Statistically significant inhibition of area under the growth curve and growth rate was observed for *Skeletonema costatum* exposed to BAS 560 F for 72 and 96 hours. After 72 hours of exposure, an E_bC_{50} (biomass) value of 0.50 mg a.s./L (with confidence limits between 0.37 and 0.67) and an E_rC_{50} (growth rate) value of 0.67 mg a.s./L (with confidence limits of 0.56 and 0.67) were determined, based on initial mean measured concentrations. After 96 hours of exposure, the E_bC_{50} was 0.55 mg a.s./L (initial mean measured; with confidence limits between 0.40 and 0.75) and the E_rC_{50} was > 0.68 mg a.s./L (initial mean measured), the highest concentration tested. The no-observed-effect concentration (NOEC) for biomass and growth rate was 0.27 mg a.s./L, based on initial mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured at test initiation: 0, 0.051, 0.11, 0.21, 0.27 and 0.68 mg a.s./L)

3. Reference item: None

4. Test medium: Saltwater algal medium, according to ASTM
Vehicle: Dimethylformamide (DMF) (at 0.1 mL/L)

5. Test organism:
Species: Marine diatom *Skeletonema costatum*
Source: Stock cultures were obtained from Wildlife International, Ltd., Maryland, USA. Original algal cultures were obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP)
Initial cell density: 77 x 10^3 cells/mL
Test vessels: 250 mL Erlenmeyer flask, containing 100 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 20.4 – 20.7 ºC
pH: 8.0 – 8.4
Photoperiod: Continuous illumination (3970 - 4530 lux)
Shaking: Constant at 100 rpm

2. Assignment and treatment:
At test initiation, algal cells were added to the test chambers, to achieve nominal cell densities of 77 x 10^3 cells/mL in each test chamber. Three replicate chambers were tested in each treatment and control group. The algae were exposed for 96 hours under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in dimethylformamide (DMF), at a nominal concentration of 10 mg a.s./mL. This solution was inverted at least 20 times to mix, and appeared clear and light yellow. Secondary stock solutions were prepared by diluting the primary stock solution in DMF to obtain nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./L. The five different stock solutions were diluted with test medium to reach the nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, containing DMF at a concentration of 0.1 mL/L. The negative control group was exposed to test medium only and the solvent control was exposed to DMF at a concentration of 0.1 mL/L.

4. Measurements and observations:
Samples were collected at 24, 48, 72 and 96 hours, to determine cell densities. Cell densities were counted using an electronic particle counter. In addition, subsamples from pooled samples per treatment were examined microscopically for atypical cell morphology. Also, each test vessel was examined for aggregations or flocculations of cells and adherence of the cells to the test vessel.

At test initiation and termination, samples were taken for verification of the test item concentrations. Samples were analyzed by an HPLC-method using variable wavelength detection set at 220 nm.

Temperature was recorded twice a day. Light intensity was measured at test initiation. The pH was measured at test initiation and test termination.

5. Statistics:
The results from the cell density counts were used to calculate areas under the growth curve, growth rates and percent inhibition values. These calculations as well as all statistical analyses were conducted using the SAS System for Windows version 8.02 or TOXSTAT version 3.5. The negative and solvent controls were compared using Student's t-test. Since no differences between negative and solvent control groups were statistically significant, the two groups were pooled. Non-linear regression or linear interpolation was used to calculate EC_{50} values for cell density (EC_{50}), area under the growth curve (E_bC_{50}) and growth rate (E_rC_{50}), when possible. The data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. The Dunnett's test was used to compare the treatment groups to the pooled control. The results of the statistical analyses as well as an evaluation of the concentration-response pattern were used to determine the NOEC for each parameter.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Skeletonema costatum showed a statistically significant decrease in cell density, area under the growth curve and growth rate when exposed to initial mean measured concentrations of BAS 560 F of 0.11 and 0.68 mg a.s./L for 72 and 96 hours. Since the inhibition at the exposure of 0.11 mg a.s./L does not follow a dose response, this inhibition was not considered treatment related. The table below shows the effects of 72-hour and 96-hour exposure on cell density, area under the growth curve (biomass) and growth rate observed in each test group.

Table 8.2.6.2-9: Effects of BAS 560 F exposure on cell density, area under the growth curve and growth rate of *Skeletonema costatum*

Nominal concentration (mg a.s./L)	**0.063**	**0.13**	**0.25**	**0.50**	**1.0**
Initial mean measured concentration (mg a.s./L)	**0.051**	**0.11**	**0.21**	**0.27**	**0.68**
Inhibition in cell density at 72 h (%)	0.072	40*	-0.82	22	70*
Inhibition in cell density at 96 h (%)	-1.9	37*	-1.8	7.8	46*
Inhibition in area under the growth curve at 72 h (%)	7.0	43*	9.1	24	76*
Inhibition in area under the growth curve at 96 h (%)	3.5	43*	4.5	20	70*
Inhibition in growth rate at 72 h (%)	0.021	25*	-0.33	11	51*
Inhibition in growth rate at 96 h (%)	-0.69	21*	-0.69	4.1	27*
	Endpoints [a,b] (mg a.s./L)				
EC_{50} (72 h)	0.54 (0.40-0.73)				
EC_{50} (96 h)	>0.68				
NOEC (96 h)	0.27				
E_bC_{50} (72 h)	0.50 (0.37-0.67)				
E_bC_{50} (96 h)	0.55 (0.40-0.75)				
NOEC (96 h)	0.27				
E_rC_{50} (72 h)	0.67 (0.56-0.67)				
E_rC_{50} (96 h)	>0.68				
NOEC (96 h)	0.27				

Negative values demonstrate an increase compared to the control
[a] Based on initial mean measured concentrations
[b] EC_{50} values with 95% confidence limits, when it was possible to calculate these limits
*Statistically significantly different from the pooled control, Dunnett's test ($p<0.05$)

No signs of aggregation/flocculation of algae or changes in cell morphology were observed in the control groups or any test substance group. However, adherence to the test vessel was noted for all test substance groups and the control groups.

B. ANALYSIS

Measured concentrations of BAS 560 F at test initiation ranged from 68% to 85.4% of nominal concentrations. At test termination the measured concentrations were between 19.9% and 48.5% of the nominal concentrations. Due to the decrease in test substance concentrations during the test, analyses of the results were based on the recoveries at test initiation. The results of the chemical analysis are given in the table below.

Table 8.2.6.2-10: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	% of nominal concentration
0.0 (negative control)	0 96	<LOQ	-
0.0 (solvent control)	0 96	<LOQ	-
0.063	0 96	0.051 0.031	80.8 48.5
0.13	0 96	0.109 0.052	84.1 40.1
0.25	0 96	0.214 0.087	85.4 34.8
0.50	0 96	0.272 0.135	54.4 27.0
1.0	0 96	0.680 0.199	68.0 19.9

LOQ = limit of quantification (0.040 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

E_bC_{50} (biomass) values for the marine diatom *Skeletonema costatum* exposed to metrafenone for 72 and 96 hours under static conditions were calculated to be 0.50 mg a.s./L (with confidence limits of 0.37 to 0.67) and 0.55 mg a.s./L (with confidence limits of 0.40 to 0.75) respectively, based on initial mean measured concentrations. E_rC_{50} (growth rate) values of 0.67 mg a.s./L (with confidence limits between 0.56 and 0.67) and > 0.68 mg a.s./L (the highest initial mean measured test concentration) were determined, after 72 and 96 hours of exposure, respectively. The no-observed-effect concentration (NOEC) was 0.27 mg a.s./L for both biomass and growth rate, based on initial mean measured concentrations.

Report:	CA 8.2.6.2/6 Hoffmann F., 2012d Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the marine diatom Skeletonema costatum 2011/1254830
Guidelines:	OECD 201, EPA 850.5400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the marine diatom *Skeletonema costatum* was determined in a 96-hour laboratory study. The study was conducted under static conditions with nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Additionally, a negative control and a solvent control (acetone at 0.1 mL/L) were tested. Five replicates were tested for each concentration level and the negative control groups, and ten for the solvent control group.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were 77.6% of nominal at test initiation and 78.4% of nominal at test termination. The test concentration of BAS 560 F in the centrifuged stock solution at test initiation was 0.364 mg/L which could be considered the functional solubility for these test conditions.

No morphological effects on the algae were observed.

For *Skeletonema costatum* exposed to metrafenone for 96 hours, an E_rC_{50} (growth rate), E_rC_{10} and E_yC_{50} (yield) of all > 0.364 mg a.s./L (geometric mean measured), the highest concentration tested, were obtained. The E_yC_{10} was calculated to be 0.307 and 0.331 mg a.s./L after 72 and 96 hours respectively, based on geometric mean measured test concentrations. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.185 mg a.s./L (geometric mean measured) for both growth rate and yield.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	BAS 560 F (Metrafenone, Reg. No. 4037710)
	Batch number:	AC12053-29
	Purity:	94.2%
	Description:	Solid
2.	**Test concentrations:**	0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L (mean measured: 0, 0.021, 0.044, 0.094, 0.185 and 0.364 mg a.s./L)

3. Reference item: None

4. Test medium: "Enriched Salt Water" medium (according to ASTM 1218)
Vehicle: Acetone

5. Test organism:
Species: Marine diatom *Skeletonema costatum*
Source: Stock cultures are cultivated in-house. Fresh strains are obtained from the "UTEX Culture collection of Algae, University of Texas at Austin, USA"
Initial cell density: 1 x 10^4 cells/mL
Test vessels: 100 mL Erlenmeyer dimple flask, containing 60 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 20 ± 1 °C
pH: 7.63 – 7.76 (at test termination)
Salinity: 30 ‰
Photoperiod: 16 hours light: 8 hours darkness (~4300 lux)
Shaking: Constant at about 60 rpm

2. Assignment and treatment:
A static system was used with test duration of 96 hours. The following nominal test concentrations were used: 0 (negative and solvent controls (0.1 mL/L acetone)), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Five replicates were tested for each concentration level and the negative control groups and ten for the solvent control group. Each test concentration and the control were inoculated to obtain initial algae density of 1 x 10^4 cells/mL. Each replicate consisted of 60 mL of test solution in a 100 mL flask.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving 4.82 mg of BAS 560 F in 1.0 mL acetone and then an aliquot (0.1 mL) of the acetonic solution was given to 1000 mL test medium. After centrifugation at 4000 rpm for 30 minutes to remove undissolved particles, the stock solution was colorless and clear. The different treatments were prepared by dilution of the stock solution with nutrient medium to reach the desired concentrations.

4. Measurements and observations:
Cell concentrations in each flask was determined 24, 48, 72 and 96 hours after starting the experiment with a spectrophotometer at 445 nm, using 5 cm glass cuvettes. Algal medium without algae was used as a blank. The mean cell densities per treatment were used to calculate yield and growth rates. The percent inhibition values were calculated for each treatment group as the percent reduction in average yield and in average growth rate relative to the control replicates.

At the start and at the end of the test samples were taken out of pooled samples for verification of the test item concentrations. Samples were analyzed by HPLC with MS-detection. At test termination, the pH of all individual samples (control as well as treated samples) was measured.

5. Statistics:
The mathematical determination of the EC_x was done by probit analysis. The calculations were conducted with a PC and the commercial software "TOXRAT Professional 2.10" (ToxRat Solutions GmbH, Alsdorf, Germany).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The following results (based on geometric mean concentrations) with respect to yield and growth rate were determined from the concentration-response relationship.

72 hour E_rC_{50} > 0.364 mg a.s./L (95% confidence limits: not calculated)
72 hour E_rC_{10} > 0.364 mg a.s./L (95% confidence limits: not calculated)
72 hour E_yC_{50} > 0.364 mg a.s./L (95% confidence limits: not calculated)
72 hour E_yC_{10} = 0.331 mg a.s./L (95% confidence limits: 0.305 – 0.348 mg a.s./L)

96 hour E_rC_{50} > 0.364 mg a.s./L (95% confidence limits: not calculated)
96 hour E_rC_{10} > 0.364 mg a.s./L (95% confidence limits: not calculated)
96 hour E_yC_{50} > 0.364 mg a.s./L (95% confidence limits: not calculated)
96 hour E_yC_{10} = 0.307 mg a.s./L (95% confidence limits: 0.281 – 0.321 mg a.s./L)

The table below summarizes the percentage of inhibition (of both growth rate and yield) observed in each test group.

Table 8.2.6.2-11: Percent inhibition of BAS 560 F on cell density, growth rate and yield of marine diatom *Skeletonema costatum*

Geometric mean measured concentration (mg a.s./L)	**0.021**	**0.044**	**0.094**	**0.185**	**0.364**
Inhibition at 96 h (yield) (%)	-4.9	-2.3	-3.4	1.2	12.8
Inhibition at 96 h (growth rate) (%)	-1.2	-0.6	-0.8	0.3	3.5

Negative values demonstrate an increase compared to the control

No morphological effects on the algae were observed at test termination.

B. ANALYSIS

Measured values for BAS 560 F at test initiation and at test termination are given in the table below. Average measured recoveries for BAS 560 F were 77.6% of nominal at test initiation and 78.4% of nominal at test termination. The test concentration of BAS 560 F in the centrifuged stock solution at test initiation was 0.364 mg/L, which could be considered the functional solubility for these test conditions. The results were based on geometric mean measured concentrations.

Table 8.2.6.2-12: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (hours)	Geomean measured concentration (mg a.s./L) [a]	% of nominal concentration	Geomean measured concentration (mg a.s./L) [b]
0.0 (negative control)	0 96	<LOQ	-	-
0.0 (solvent control)	0 96	<LOQ	-	-
0.029	0 96	0.0207 0.0214	71.6 73.7	0.021
0.058	0 96	0.0441 0.0437	76.0 75.3	0.044
0.115	0 96	0.0939 0.0943	81.6 82.0	0.094
0.230	0 96	0.183 0.188	79.5 81.8	0.185
0.460	0 96	0.364 0.364	79.2 79.1	0.364

LOQ = limit of quantification (0.001 mg a.s./L)
[a] Geomean measured concentrations of two replicates per sampling time
[b] Geomean measured concentrations for the two sampling times

C. DEFICIENCIES

None.

III. CONCLUSION

The marine diatom *Skeletonema costatum* exposed to metrafenone under static conditions demonstrated an E_rC_{50} (growth rate), E_rC_{10} and E_yC_{50} (yield) of > 0.364 mg a.s./L, based on geometric mean measured concentrations, after 72 and 96 hours of exposure. The E_yC_{10} was calculated to be 0.307 mg a.s./L at 72 hours and 0.331 mg a.s./L at 96 hours, based on geometric mean measured test concentrations. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.185 mg a.s./L (geometric mean measured) for both growth rate and yield.

CA 8.2.7 Effects on aquatic macrophytes

Report: CA 8.2.7/1
Desjardins D. et al., 2005a
BAS 560 F: A 7-day static-renewal toxicity test with duckweed (Lemna gibba G3)
2005/7003440
Guidelines: EPA 850.4400, OECD 221
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the duckweed *Lemna gibba* was determined in a 7-day laboratory study. The study was conducted under static-renewal conditions, with renewal of the exposure solutions at day four and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative control (medium only) and solvent control (0.1 mL/L dimethylformamide (DMF)) were tested in parallel. Three replicates were tested for each test substance concentration, the negative control group and the solvent control group.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were between 67.6% and 98.9% of nominal concentrations for freshly made exposure solutions, and between 68.0% and 91.2% of nominal concentrations for old exposure solutions. Results were based on mean measured concentrations of the old and freshly made exposure solutions.

No statistically significant inhibition of frond number, biomass and growth rate based on frond number and biomass were observed for *Lemna gibba* exposed to BAS 560 F for seven days. Hence, the $EC_{50\ \text{frond number}}$, E_bC_{50} (biomass), $E_rC_{50\ \text{frond number}}$ (growth rate based on frond number) and $E_rC_{50\ \text{biomass}}$ (growth rate based on biomass) were all > 0.76 mg a.s./L (mean measured concentrations), the highest concentration tested. The no-observed-effect concentration (NOEC) was determined to be 0.76 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured at test initiation: 0, 0.057, 0.10, 0.21, 0.41 and 0.76 mg a.s./L)

3. Reference item: None

4. Test medium: 20x AAP medium
Vehicle: Dimethylformamide (DMF) (at 0.1 mL/L)

5. Test organism:
Species: Duckweed *Lemna gibba* G3
Source: Stock cultures were obtained from Wildlife International, Ltd., Maryland, USA. Original algal cultures were obtained from the United States Department of Agriculture
Initial frond numbers: Twelve fronds (on four plants)
Test vessels: 250 mL glass beakers, containing 100 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 24.4 – 25.0 ºC
pH: 7.8 – 8.9
Photoperiod: Continuous illumination (4520 - 5580 lux)

2. Assignment and treatment:
At test initiation, four plants totaling twelve fronds were added to the test vessels. Three replicate test vessels were tested in each treatment and control group. The duckweed was exposed for seven days under static-renewal conditions with exposure solution renewal at day four.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in dimethylformamide (DMF), at a nominal concentration of 10 mg a.s./mL. This solution was inverted at least 20 times to mix, and appeared clear and light yellow. Secondary stock solutions were prepared by diluting the primary stock solution in DMF to obtain nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./L. The five different stock solutions were diluted with test medium to reach the nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, containing DMF at a concentration of 0.1 mL/L. The negative control group was exposed to test medium only and the solvent control was exposed to DMF at a concentration of 0.1 mL/L.

4. Measurements and observations:
On test initiation and on day three, five and seven, frond numbers were counted and observations of effects (chlorosis, necrosis, dead fronds, root destruction and break-up of duckweed colonies) were performed in each replicate test vessel for all treatments. The total number of plants in each replicate test vessel was also determined at test termination (day seven). In addition, biomass was determined at test initiation with three samples (twelve fronds/four plants) of the inoculum culture and at test termination with samples from each replicate from each treatment.

Samples were taken for verification of the test item concentrations from freshly made test solutions at test initiation and at day four, and from old test solutions at day four and seven. Samples were analyzed by HPLC using variable wavelength detection set at 220 nm.

Temperature was recorded twice a day. Light intensity was measured at test initiation. The pH was measured at test initiation and on day four and seven.

5. Statistics:

The calculations of mean frond numbers, biomass, growth rates and percent inhibition values as well as percentages of necrotic, chlorotic and dead fronds were assessed using TOXSTAT version 3.5. The negative and solvent controls were compared using Student's t-test. Since no differences between the negative and solvent control groups were statistically significant, the two groups were pooled. Linear interpolation was used to calculate EC_{50} values for frond number, biomass and growth rate based on frond number and biomass, when possible. The percentages of dead, chlorotic and necrotic fronds were calculated relative to the total number of fronds present in each test vessel. The data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett's tests, respectively. The treatment groups were compared to the pooled control using analysis of variance (ANOVA) and Bonferroni's test. The results of the statistical analyses as well as an evaluation of the concentration-response pattern were used to determine the NOEC for each parameter.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Lemna gibba did not show a statistically significant increase in dead, chlorotic and necrotic fronds during the seven days of exposure to BAS 560 F. For frond number, biomass and growth rate (based on frond number and biomass), no statistically significant differences compared to the pooled control were observed either. The table below shows the effects of seven days of exposure on frond number, biomass and growth rate observed in each test group.

Table 8.2.7-1: Effects of BAS 560 F exposure on frond number, biomass and growth rate of *Lemna gibba*

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Day 7 frond number		Day 0 - day 7 biomass change		Day 0 - day 7 frond number growth rate		Day 0 - day 7 biomass growth rate	
		Mean	% [a]	Mean	% [a]	Mean	% [a]	Mean	% [a]
Negative control	0	187	-	29.0	-	0.392	-	0.482	-
Solvent control	0	173	-	28.3	-	0.380	-	0.476	-
Pooled control	0	180	-	28.7	-	0.386	-	0.479	-
0.063	0.057	162	9.8	27.9	2.7	0.372	3.7	0.476	0.61
0.13	0.10	173	3.7	27.9	2.7	0.381	1.2	0.476	0.59
0.25	0.21	175	3.0	27.7	3.4	0.383	0.92	0.475	0.78
0.50	0.41	171	5.0	25.9	9.7	0.379	1.7	0.465	2.8
1.0	0.76	162	10	24.4	15	0.372	3.8	0.457	4.4

[a] Percentage inhibition compared to the pooled control

B. ANALYSIS

Measured concentrations of BAS 560 F in freshly made solutions ranged from 67.6% to 98.9% of nominal concentrations, as shown in the table below. In old solutions the measured concentrations were between 68.0% and 91.2% of the nominal concentrations. Analyses of the results were based on mean measured concentrations of the freshly made and old test solutions at test initiation, day four and day seven.

Table 8.2.7-2: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (days)	Measured concentration (mg a.s./L)	% of nominal concentration	Average % of nominal concentration
0.0 (negative control)	0 (new) 4 (new) 4 (old) 7 (old)	< LOQ	-	-
0.0 (solvent control)	0 (new) 4 (new) 4 (old) 7 (old)	< LOQ	-	-
0.063	0 (new) 4 (new) 4 (old) 7 (old)	0.062 0.062 0.057 0.047	98.9 97.9 91.2 74.4	90
0.13	0 (new) 4 (new) 4 (old) 7 (old)	0.109 0.104 0.105 0.088	84.1 79.9 80.8 68.0	77
0.25	0 (new) 4 (new) 4 (old) 7 (old)	0.214 0.210 0.211 0.186	85.4 83.8 84.5 74.4	84
0.50	0 (new) 4 (new) 4 (old) 7 (old)	0.338 0.456 0.426 0.420	67.6 91.2 85.3 84.0	82
1.0	0 (new) 4 (new) 4 (old) 7 (old)	0.778 0.777 0.742 0.744	77.8 77.7 74.2 74.4	76

LOQ = limit of quantification (0.040 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

The EC_{50} values for frond number, biomass and growth rate (based on frond number and biomass) for the duckweed *Lemna gibba* exposed to metrafenone for seven days under static-renewal conditions were all determined to be > 0.76 mg a.s./L, which is the highest mean measured concentration tested. The no-observed-effect concentration (NOEC) was 0.76 mg a.s./L (mean measured) for frond number, biomass and growth rate.

Report:	CA 8.2.7/2 Hoffmann F., 2012e Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of Lemna gibba 2011/1254832
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 study, the effect of metrafenone on the growth of duckweed *Lemna gibba* was investigated. *Lemna gibba* was exposed to nominal concentrations of 0.029, 0.058, 0.115, 0.230, and 0.460 mg a.s./L. Additionally, a negative control and a solvent control (0.1 mL/L acetone) were tested. Three replicates were tested for each test substance concentration and the negative control group, and six replicates for the solvent control group. Assessment of growth and other effects was conducted on days three, five and seven after test initiation. The percentage growth inhibition, relative to the solvent control was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight (biomass).

Measured concentrations of metrafenone ranged from 80.3% to 93.5% of nominal concentrations at test initiation and from 57.2% to 68.4% of nominal concentrations at test termination. The biological results were based on geometric mean measured concentrations.

No morphological effects on *Lemna gibba* were observed in the control groups and at any of the test item concentrations tested.

In this 7-day toxicity test with *Lemna gibba*, the E_rC_{50} (growth rate) and E_yC_{50} (yield) of metrafenone were determined to be both > 0.327 mg a.s./L based on frond number and dry weight (geometric mean measured). The E_rC_{10} based on frond number and dry weight was also > 0.327 mg a.s./L (geometric mean measured). The E_yC_{10} based on frond number was calculated to be 0.280 mg a.s./L (geometric mean measured) and the E_yC_{10} based on dry weight was 0.240 mg a.s./L (geometric mean measured). The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.159 mg a.s./L for all endpoints.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 F (metrafenone; Reg. No. 40.37710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid

2. Test concentrations: 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L; (geometric mean measured concentrations: 0, 0, 0.021, 0.046, 0.089, 0.159 and 0.357 mg a.s./L)

3. Reference item: None

4. Test medium: 20x AAP medium
Vehicle: Acetone

5. Test organism:
Species: Duckweed *Lemna gibba* G3
Source: Cultures maintained in-house, stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany
Initial frond numbers: Eleven fronds (on three plants)
Test vessels: 400 mL glass beakers, containing 160 mL test or control medium

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 24.2 °C
pH: 7.5 at test initiation; 8.4 – 8.5 at test termination
Photoperiod: Continuous illumination (8100 lux)

2. Assignment and treatment:
At test initiation, three plants from a 10-day old culture were added to each vessel randomly; two plants with four fronds and one plant with three fronds. Three replicate test vessels were tested in each treatment group and the negative control group, and the solvent control included six replicates. The duckweed was exposed for seven days under static conditions.

3. Dose preparation:
A primary stock solvent solution was prepared by dissolving BAS 560 F in acetone, at a nominal concentration of 4.6 mg a.s./mL. This solution was diluted in test medium, at a concentration of 0.1 mL/L, and was then centrifuged for 30 minutes at 4000 rpm to remove undissolved particles. Secondary stock solutions were prepared by further diluting the primary stock solution in the test medium, to obtain nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. The negative control group was exposed to test medium only and the solvent control group was exposed to acetone diluted in test medium, at a concentration of 0.1 mL/L.

4. Measurements and observations:
Frond production and appearance (necrosis, chlorosis, changes in plant size or shape and root growth) were recorded on days three, five and seven. In addition, biomass was determined at test initiation with three samples of the inoculum culture and at test termination with the plant material from each replicate from each test concentration and the controls.

Samples were taken for verification of the test item concentrations at test initiation and termination. These samples were analyzed by HPLC with MS-detection.

Temperature was recorded continuously. The pH was measured at test initiation and test termination.

5. Statistics:
Calculations of growth rate and yield, based on frond number and dry weight, were made and analyzed by probit analysis to obtain EC_x values. These analyses were performed using TOXSTAT Professional 2.10.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The duckweed population in the control vessels showed exponential growth, increasing from eleven fronds per vessel to an average of 115 fronds per vessel in the control after 7 days (corresponding to a 10.4 x multiplication). The dry weight increased from 2.0 mg to an average of 22.5 mg per vessel in the control at test termination. No morphological effects on *Lemna gibba* were observed in the control groups and at any of the test item concentrations tested. The results are summarized in the table below.

Table 8.2.6.2-3: Effects of metrafenone on the growth of duckweed *Lemna gibba*

Concentration (mg a.s./L) (nominal)	**Solvent control**	**Control**	**0.029**	**0.058**	**0.115**	**0.230**	**0.460**
Concentration (mg a.s./L) (geometric mean measured)	--	--	**0.021**	**0.046**	**0.089**	**0.159**	**0.327**
Inhibition in 7 d (%) [a] (growth rate based on frond no.)	--	-0.3	-1.5	-0.7	0.3	-0.8	6.0
Inhibition in 7 d (%) [a] (yield based on frond no.)	--	0.9	-4.0	-1.8	0.8	-2.1	14.6
Inhibition in 7 d (%) (growth rate based on dry weight)	--	0.3	0.4	1.1	1.9	1.7	5.6
Inhibition in 7 d (%) (yield based on dry weight)	--	-1.5	1.2	3.0	4.9	4.5	14.0
	Endpoints (mg a.s./L) (geometric mean measured (95% confidence limits))						
E_rC_{50} / E_yC_{50} (7 d) based on frond no	> 0.327 (nd)						
E_rC_{50} / E_yC_{50} (7 d) based on dry weight	> 0.327 (nd)						
E_rC_{10} (7 d) based on frond no / dry weight	> 0.327 (nd)						
E_yC_{10} (7 d) based on frond no	0.280 (0.250 – 0.300)						
E_yC_{10} (7 d) based on dry weight	0.240 (0.150 – 0.390)						

[a] Inhibition compared to the solvent control; negative values indicate stimulated growth
nd = not determined

B. ANALYSIS

Measured concentrations of metrafenone ranged from 80.3% to 93.5% of nominal at test initiation and from 57.2% to 68.4% of nominal at test termination, as shown in the table below. The biological results are based on geometric mean measured concentrations.

Table 8.2.7-4: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (days)	Mean measured concentration (mg a.s./L)	% of nominal concentration	Geometric mean measured concentrations (mg a.s./L)
0.0 (negative control)	0 7	< LOQ	-	-
0.0 (solvent control)	0 7	< LOQ	-	-
0.029	0 7	0.0271 0.0166	93.3 57.2	0.021
0.058	0 7	0.0531 0.0397	91.6 68.4	0.046
0.115	0 7	0.107 0.0740	93.5 64.3	0.089
0.230	0 7	0.185 0.136	80.3 59.3	0.159
0.460	0 7	0.384 0.279	83.6 60.6	0.327

LOQ = limit of quantification (0.001 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

In this 7-day static toxicity test with the duckweed *Lemna gibba*, the E_rC_{50} (growth rate) and E_yC_{50} (yield) of metrafenone based on frond number and dry weight were determined to be both > 0.327 mg a.s./L (geometric mean measured), the highest concentration tested. The E_rC_{10} based on frond number and dry weight was also > 0.327 mg a.s./L (geometric mean measured). The E_yC_{10} based on frond number was calculated to be 0.280 mg a.s./L and the E_yC_{10} based on dry weight was 0.240 mg a.s./L, both based on geometric mean measured. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.159 mg a.s./L for all endpoints.

CA 8.2.8 Further testing on aquatic organisms

No further testing on aquatic organisms is required or submitted.

CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Acute oral and contact honey bee toxicity endpoints are available for the active substance, which were previously reviewed and considered acceptable for the first EU Review of metrafenone.

In accordance with the Commission Regulation (EU) No. 283/2013, a chronic bee and bee brood study have now been conducted. In addition, a semi-field tunnel study is available.

A summary of the available data for honey bees is presented in the following table.

Table 8.3.1-1: Toxicity of metrafenone and the formulated product BAS 560 02 F to honey bees

Test system (duration)	Test substance	Results	Reference
Laboratory studies			
Acute oral toxicity (48 hrs)	Metrafenone technical	$LD_{50\ oral}$ > 114 µg a.s/bee	KCA 8.3.1.1.1/1 2002/7004414
Acute contact toxicity (48 hrs)	Metrafenone technical	$LD_{50\ contact}$ > 100 µg a.s/bee	KCA 8.3.1.1.2/1 2002/7004414
Chronic oral toxicity (10 days)	BAS 560 02 F	10-d $LDD_{50\ oral}$ > 291 µg a.s./bee/day	KCA 8.3.1.2/1 2014/1093920
Bee brood (5 days)	BAS 560 02 F	5-d $NOED_{larvae}$ = 49.98 µg a.s./larvae	KCA 8.3.1.3/1 2014/1093921
Higher tier studies			
Semi-field (tunnel) (7 days exposure)	BAS 560 02 F	No adverse effects up to 1 x 660 mL product/ha (1 x 330 g a.s./ha) to actively foraging bees	KCA 8.3.1.4/1 2011/1078054

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 Strnad S.P.,Mulligan E., 2002a Acute toxicity of AC 375839 technical to the honey bee, Apis mellifera 2002/7004414
Guidelines:	EEC 91/414 Annex II 8.3.1.1, EEC 96/12, OECD 213, OECD 214
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The oral LD_{50} value for honey bees (*Apis mellifera*) exposed to metrafenone for 48 hours was > 114 µg a.s./bee, the highest concentration tested. Please refer to Document M-II, Section 6, Point 8.3.1 of the summary dossier dated 2002 for further details.

CA 8.3.1.1.2 Acute contact toxicity

Report:	CA 8.3.1.1.2/1 Strnad S.P.,Mulligan E., 2002a Acute toxicity of AC 375839 technical to the honey bee, Apis mellifera 2002/7004414
Guidelines:	EEC 91/414 Annex II 8.3.1.1, EEC 96/12, OECD 213, OECD 214
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The contact LD_{50} value for honey bees (*Apis mellifera*) exposed to metrafenone for 48 hours was > 100 µg a.s./bee, the highest concentration tested. Please refer to Document M-II, Section 6, Point 8.3.1 of the summary dossier dated 2002 for further details.

CA 8.3.1.2 Chronic toxicity to bees

Report: CA 8.3.1.2/1
Verge E., 2014a
BAS 560 02 F and BAS 560 AA F blank formulation - Assessment of effects on the adult honey bee, Apis mellifera L., in 10 days chronic feeding test under laboratory conditions
2014/1093920
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The toxicity of BAS 560 02 F to the honey bee *Apis mellifera* was determined in a chronic feeding study in the laboratory. Young adult worker bees were exposed for ten days to exposure solutions, which were replaced and weighted daily, with nominal concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food. The blank formulation BAS 560 AA F (at a nominal concentration of 7730 mg product/kg food), a negative control (feeding solution only) and a reference item (Perfekthion, at a nominal concentration of 0.9 mg dimethoate/kg food) were tested in parallel. Four replicates were tested for each test substance concentration, the blank formulation, the negative control and the reference item.

With the food intake measurements, the consumed concentrations were calculated. For the nominal concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food for BAS 560 02 F the measured uptake was 15.0, 32.6, 62.3, 128 and 291 µg a.s./bee/day. For BAS 560 AA F the measured uptake was 372 µg product/bee/day.

Mortality in the groups exposed to BAS 560 02 F ranged from 0 to 10% and was not statistically significantly different from the mortality in the control group (5%). No mortality was observed in the group exposed to the blank formulation BAS 560 AA F, indicating that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation. Some bees exposed to BAS 560 02 F at concentrations of 714.3 mg a.s./kg food and higher showed sub-lethal effects such as reduced coordination.

The 10-day LC_{50} value for *Apis mellifera* exposed to BAS 560 02 F was determined to be > 5714 mg a.s./kg food (nominal), which corresponds to a 10-day LDD_{50} > 291 µg a.s./bee/day (measured). The no-observed-effect concentration (NOEC) was determined to be 5714 mg a.s./kg food, which corresponds to a no-observed-effect daily dose (NOEDD) of 291 µg a.s./bee/day.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test material:**	BAS 560 02 F
	Batch number:	0008024566
	Purity:	501.7 g/L metrafenone (analyzed)
	Description:	Beige liquid
	Blank formulation:	BAS 560 AA F
	Batch number:	FD-140630-0026
	Purity:	Without metrafenone
	Description:	Beige liquid
2.	**Test concentrations:**	BAS 560 02 F: 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food (nominal; effective mean uptake: 15.0, 32.6, 62.3, 128 and 291 µg a.s./bee/day) BAS 560 AA F: 7730 mg product/kg food (nominal; effective mean uptake: 372 µg product/bee/day)
3.	**Reference item:**	Perfekthion (BAS 152 11 I, containing 400.9 g dimethoate/L), tested at a concentration of 0.9 mg a.s./kg food (nominal; effective mean uptake: 0.02 µg a.s./bee/day)
4.	**Vehicle:**	50% (w/v) aqueous sucrose solution
5.	**Test organism:**	
	Species:	Honey bee *Apis mellifera* L.
	Age/life stage:	Young adult worker bees
	Source:	Healthy colonies, descended from a breeding line of a beekeeper in Baden-Württemberg, Germany
	Diet:	Treated or untreated 50% (w/v) aqueous sucrose solution, *ad libitum*
	Test units:	Stainless steel cages (8 x 4 x 6 cm), with a transparent front side to enable observations and a perforated board at the bottom to guarantee sufficient air supply. The feeding solutions were offered in syringes

B. STUDY DESIGN

1.	**Environmental conditions:**	
	Temperature:	32.3 – 33.4°C
	Relative humidity:	53.2 – 62.0%
	Photoperiod:	Continuous darkness, except when the feeding solutions were replaced and the assessments were made

2. Assignment and treatment:
At test initiation, ten bees were added to each test unit. Four replicate test units were tested in each treatment, reference item and control group. The bees were continuously exposed to freshly prepared feeding solutions for ten days with treated (BAS 560 02 F, BAS 560 AA F or reference item) or untreated (control) 50% (w/w) aqueous sucrose solution.

3. Dose preparation:
A stock solvent solution was prepared (fresh daily) by dissolving BAS 560 02 F or BAS 560 AA F in tap water, at a concentration of 533 mg product/mL. This solution was diluted with 50% (w/v) aqueous sucrose solution to reach the nominal test concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food for BAS 560 02 F and 7730 mg product/kg food for BAS 560 AA F. The negative control group was exposed to untreated 50% (w/v) aqueous sucrose solution and the reference item group received a 50% (w/v) aqueous sucrose solution containing the reference item Perfekthion at a concentration of 0.9 mg a.s./kg food.

4. Measurements and observations:
Mortality and sub-lethal effects (moribund, affected, apathy, cramps or vomiting) were recorded daily during the exposure period of ten days. In addition, the weight of the syringes containing the feeding solutions was determined daily before and after feeding.

Temperature and relative humidity were recorded continuously throughout the test period of ten days.

5. Statistics:
Fisher's test (Bonferroni-Holms corrected) was used to evaluate differences between mortality data of bees treated with BAS 560 02 F and bees treated with BAS 560 AA F, and between these treated groups and the control group. These calculations were performed using ToxRat Professional 2.10.

II. RESULTS AND DISCUSSION

D. MORTALITY AND BEHAVIOUR

Apis mellifera in the control group showed a cumulative mortality of 5% after ten days (see table below) and no sub-lethal effects were observed for these bees during the test period. Mortality in the groups exposed to BAS 560 02 F and BAS 560 AA F was not statistically significantly different from the mortality in the control group. In the group exposed to the reference item Perfekthion 97.4% (corrected) of the bees died.

In the BAS 560 02 F treatment group, no behavioral abnormalities were observed at the lowest concentration. At the concentrations of 714.3 mg a.s./kg food one moribund bee (showing only very feeble movements) was observed at the assessment Day 9. Affected bees (reduced coordination) were recorded at the assessments Day 5 to Day 7 at the concentration of 1428.5 mg a.s./kg food, at the assessments Day 5 and Day 6 at the concentration of 2857 mg a.s./kg food and at the assessments Day 2 and Day 5 to Day 10 at the concentration of 5714 mg a.s./kg food. In the BAS 560 AA F treatment group, no behavioral abnormalities were observed during the entire 10-day test period.

The table below presents the percent mortality and sub-lethal effects.

Table 8.3.1.2-1: Mortality and sub-lethal effects for *Apis mellifera* exposed to BAS 560 02 F and the blank formulation BAS 560 AA F for ten days

Treatment	Concentration	Mean mortality		Cumulative number of affected honey bees[3]
	Nominal (mg/kg food)	(%)	Corrected (%)[1,2]	
Control	0.0	5.0	-	0
BAS 560 02 F	357.1	7.5	2.6	0
	714.3	10.0	5.3	1m
	1428.5	0.0	-5.3	5a
	2857	2.5	-2.6	3a
	5714	5.0	0.0	8a
BAS 560 AA F	7730	0.0	-5.3	0
Reference item Perfekthion	0.9	97.5	97.4	n.d.

[1] Corrected mortality according to Schneider-Orelli (1947)
[2] Negative values demonstrate an increase compared to the control
[3] Total number of observations of bees showing a sub-lethal effect, with the observations for replicate units and days cumulated; m = moribund and a = affected
n.d. = Not determined

E. FOOD CONSUMPTION

The mean measured food intake in the control group was 42.8 mg/bee/day. The table below shows the results from the food intake measurements for all test groups. With these food intake measurements the mean uptake of BAS 560 02 F, BAS 560 AA F and Perfekthion was calculated.

Table 8.3.1.2-2: Consumption rates for *Apis mellifera* exposed to BAS 560 02 F and the blank formulation BAS 560 AA F for ten days

Treatment	Concentration		
	Nominal (mg/kg food)	Mean consumption of feeding solution (mg/bee/day)	Mean daily uptake of test item (µg/bee/day)
Control	0.0	42.8	-
BAS 560 02 F	357.1	42.1	15.0
	714.3	45.7	32.6
	1428.5	43.6	62.3
	2857	44.8	128
	5714	51.0	291
BAS 560 AA F	7730	48.1	372
Reference item Perfekthion	0.9	27.5	0.02

F. DEFICIENCIES

None.

III. CONCLUSION

The 10-day LC_{50} value for honey bees (*Apis mellifera*) exposed to BAS 560 02 F was determined to be > 5714 mg a.s./kg food, the highest nominal concentration tested, which corresponds to a 10-day LDD_{50} > 291 µg a.s./bee/day (measured). The no-observed-effect concentration (NOEC) was determined to be 5714 mg a.s./kg food, which corresponds to a no-observed-effect daily dose (NOEDD) of 291 µg a.s./bee/day.

CA 8.3.1.3 Effects on honey bee development and other honey bee life stages

Report: CA 8.3.1.3/1
Eckert J., 2015a
BAS 560 02 F and BAS 560 AA F blank formulation - Honey bee (Apis mellifera L.) larval toxicity test (repeated feeding exposure)
2014/1093921

Guidelines: OECD Draft Test Guideline on Honey bee (Apis mellifera) Larval Toxicity Test Repeated Exposure (February 2014), OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure

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

Executive Summary

The toxicity of BAS 560 02 F to honey bee *Apis mellifera* larvae was determined in a repeated feeding study in the laboratory. First instar larvae were fed daily with diet containing BAS 560 02 F at nominal concentrations of 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg food (equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva), from exposure day three to day six. The blank formulation BAS 560 AA F (at a nominal concentration of 1758 mg product/kg food, corresponding to a cumulative dose of 270.73 µg product/larva), a negative control (feeding solution only) and the reference item dimethoate (at nominal concentration of 40 mg dimethoate/kg diet, equivalent to a cumulative dose of 6.16 µg a.s./larva) were tested in parallel. Bee larvae were obtained from three different colonies, and each colony represented a replicate in each test group, with twelve bee larvae per replicate. Mortality and food rejection were assessed after eight days (120 hours after the first feeding with treated diet).

Chemical analysis demonstrated that the measured concentrations of the BAS 560 02 F stock solutions from day three to day six were between 100 and 102% of the nominal concentrations.

The mortality of the bee larvae exposed to the two highest concentrations of BAS 560 02 F of 649 and 1298 mg a.s./kg diet was statistically significantly increased compared to the control. Hence, the 120-hour no-observed-effect concentration (NOEC) was determined to be 324.50 mg a.s./kg diet, which corresponds to a 120-hour no-observed-effect dose (NOED) of 49.98 µg a.s./larva. The 120-hour LC_{50} value for *Apis mellifera* larvae exposed to BAS 560 02 F was calculated to be 750.74 mg a.s./kg diet, which corresponds to a 120-hour LD_{50} value of 115.61 µg a.s./larva. The blank formulation BAS 560 AA F did not cause mortality, indicating that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 02 F
Batch number: 0008024566
Purity: 501.7 g/L metrafenone (analyzed)
Description: Beige liquid

Blank formulation: BAS 560 AA F
Batch number: FD-140630-0026
Purity: Without metrafenone
Description: Beige liquid

2. Test concentrations: BAS 560 02 F: 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg diet (nominal; equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva)
BAS 560 AA F: 1758 mg product/kg diet (nominal; equivalent to a cumulative dose of 270.73 µg product/larva)

3. Reference item: Dimethoate (BAS 152 I; purity: 99.8%), tested at 40 mg dimethoate/kg diet (equivalent to a cumulative dose of 6.16 µg a.s./larva)

4. Vehicle: Aqueous sugar and yeast solution mixed with fresh organic royal jelly (without antibiotics, pesticides and heavy metals (analyzed))

5. Test organism:
Species: Honey bee *Apis mellifera* L.
Age/life stage: First instar larvae (L1)
Source: Three healthy, adequately fed, disease-free and queen-right colonies, obtained from Eurofins Agroscience Services, EcoChem GmbH, Niefern-Öschelbronn, Germany
Diet: Daily feeding, except on day 2. Three different artificial diets (A, B and C), adapted to the needs of each larval stage, were prepared during the test. The cumulative feeding volume of treated diet was 140 µL diet/larva.

- Diet A (20 µL/larva, on day 1): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose
- Diet B (20 µL/larva, on day 3): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose
- Diet C (30 µL on day 4, 40 µL on day 5 and 50 µL on day 6): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

Test units: Crystal polysterene grafting cells with a diameter of 9 mm, which were sterilised by immersion in ethanol (70%) and then dried. Hereafter, the cells were placed into a 48-well plate, which was previously filled with a piece of dental roll wetted with 15% (w/v) glycerol. The plates were placed into a hermetically sealed Plexiglass desiccator, which was placed into an incubator with forced air circulation

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 33.3 – 34.4ºC
Relative humidity: 51.4 – 100.0%
Photoperiod: Continuous darkness, except during feeding and assessments

2. Assignment and treatment:

First instar (L1) honey bee larvae were selected from three different colonies and individually placed into cellular well-plates, using a grafting tool. The larvae were obtained from the three different colonies, with twelve larvae from the same colony in one replicate and hence three replicates per treatment group. At this day of grafting, the larvae were fed untreated diet A. At day three, four, five and six, the larvae were fed with a defined quantity of diet B or C, which contained the nominal BAS 560 02 F concentrations of 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg diet (nominal; equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva). Feeding occurred separately for each larva, using a sterile pipette. The food drop was placed next to the larvae. The blank formulation BAS 560 AA F (1758 mg product/kg diet (nominal; equivalent to a cumulative dose of 270.73 µg product/larva)), a control (diet only), and a reference product (dimethoate; at 40 mg dimethoate/kg diet (nominal), equivalent to a cumulative dose of 6.16 µg a.s./larva) were concurrently tested.

3. Dose preparation:

The stock solution of aqueous sugar solution was prepared daily. The treated solutions for BAS 560 02 F and BAS 560 AA F were prepared at each application day (day three, four, five and six), by dilution of the test items in deionised and autoclaved water. These solutions were mixed through the sugar solution. Thereafter, the royal jelly was added to each stock solution, to reach the final test concentrations. The control was exposed to the sugar solution only.

4. Measurements and observations:

Mortality was assessed daily from day four to day eight, which was 24 to 120 hours after the first feeding with treated diet. On day eight, the presence of uneaten food was also qualitatively assessed.

Samples of the stock solutions of BAS 560 02 F and the control solution were taken at each day of application and analysed for the concentration of the test item, using HPLC-PDA.

Temperature and humidity were recorded continuously.

5. Statistics:
Mortality data were corrected according to Abbott (1925). Fisher's exact test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used to assess whether there was a significant difference between mortality data of the treated groups and the control group. The LC_{50} value with 95% confidence limits was calculated using Probit analyses with linear maximum likelihood regression. All statistics were performed with ToxRat Professional 2.10.

II. RESULTS AND DISCUSSION

A. MORTALITY AND FOOD UPTAKE

No mortality occurred in the control group during the test period. Statistically significant difference from the control group was observed for honey bee larvae exposed to the two highest BAS 560 02 F concentrations of 649 and 1298 mg a.s./kg diet. No mortality was observed in the groups exposed to BAS 560 AA F and 97.2% bees died in the group exposed to the reference item dimethoate.

The measured concentrations of metrafenone ranged between 100 and 102% of the nominal concentrations.

The table below shows the mortality and food rejection for all the test groups at day eight (120 hours after the first feeding with treated diet).

Table 8.3.1.3-1: Mortality and food rejection of *Apis mellifera* larvae exposed to BAS 560 02 F and the blank formulation BAS 560 AA F

Treatment	Concentration (mg/kg diet)	Corrected cumulative mortality (%)[1,2]					Alive larvae with uneaten food[2]
		D4	D5	D6	D7	D8	
Control	0.0	0.0	0.0	0.0	0.0	0.0	0
BAS 560 02 F	81.125	0.0	0.0	0.0	0.0	0.0	0
	162.25	0.0	0.0	0.0	0.0	0.0	0
	324.5	0.0	0.0	5.6	5.6	5.6	2
	649	0.0	11.1	30.6*	38.9*	41.7*	15
	1298	0.0	47.2*	80.6*	83.3*	83.3*	6
BAS 560 AA F	1758	0.0	0.0	0.0	0.0	0.0	1
Reference item Perfekthion	40	25.0**	58.3**	91.7**	97.2**	97.2**	1

[1] Corrected mortality compared to the control group, according to Abbott (1925)
[2] With a total number of 36 larvae per group
* Significantly increased compared to the control (Fisher's Exact test with Bonferroni Correction)
** Significantly increased compared to the control (Fisher's Exact test)

B. DEFICIENCIES

None.

III. CONCLUSION

The 120-hour LC_{50} value for *Apis mellifera* larvae exposed to BAS 560 02 F was calculated to be 750.74 mg a.s./kg diet, which corresponds to a 120-hour LD_{50} value of 115.61 µg a.s./larva. The 120-hour no-observed-effect concentration (NOEC) was determined to be 324.50 mg a.s./kg diet, which corresponds to a 120-hour no-observed-effect dose (NOED) of 49.98 µg a.s./larva. The blank formulation BAS 560 AA F did not cause mortality, which indicates that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation.

CA 8.3.1.4 Sub-lethal effects

Report:	CA 8.3.1.4/1 Barth M., 2012a Effects of BAS 560 02 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on brood development 2011/1078054
Guidelines:	EPPO PP 1/170 (4) (2010), OECD 75 (2007)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The toxicity of BAS 560 02 F to honey bee *Apis mellifera* was determined in a semi-field (tunnel) test in flowering *Phacelia* (*Phacelia tanacetifolia*). One colony of bees per tunnel with 4 tunnels per treatment group were exposed to a single application of either 320 mL product/ha or 660 mL product/ha (equivalent to 160 g a.s./ha and 330 g a.s./ha, respectively), a control or reference item (Insegar 25 WG; 300 g a.s./ha). Application was carried out during daily bee flight. Assessments on mortality, foraging activity, behavior, colony health, colony strength, general and detailed brood development in marked cells were performed until day 28 post-application.

All tested colonies were determined to be comparable prior to application. On the day of application and thereafter, no biologically relevant increase in mortality was observed in the test item treatment groups compared to the pre-application period, the control treatment and between the two test concentrations. Up to Day 28, no relevant numbers of dead larvae or pupae were found in the control and in both test item treatment groups. Overall foraging activities were high and similar between the two treatment groups and comparable to the control or pre-application assessment at each observation period. There were no behavioral abnormalities compared to the control in either test item group throughout the exposure period. Colony strength in the test item groups was also comparable to the control. Overall total mean brood nest area, brood termination rate (%), brood index and brood compensation index for both test item groups were comparable to the control that showed continuous brood development.

Overall, BAS 560 02 F applied as a single application at rates of 320 mL product/ha (160 g a.s./ha) and 660 mL product/ha (330 g a.s./ha) to flowering *Phacelia tanacetifolia* under semi-field conditions during active honey bee foraging conditions does not cause adverse effects on honey bee colonies.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 02 F
Batch number: 0003477237
Purity: 500.0 g/L metrafenone (analyzed)
Description: Beige liquid

2. Test concentrations: 0 (control: 400 L tap water/ha), 320 mL BAS 560 02 F/ha (equivalent to 160 g a.s./ha) and 660 mL BAS 560 02 F/ha (equivalent to 330 g a.s./ha) in 400 L tap water/ha

3. Reference item: Insegar 25 WG /ha (batch: SM09B342, purity: 250 g fenoxycarb/kg (nominal)), tested at 1200 g Insegar 25 WG /ha (equivalent 300 g a.s./ha)

4. Vehicle: Tap water

5. Test organism:
Species: Honey bee *Apis mellifera carnica* P.
Number of bees: 3938-6525
Source: Healthy queen-right, full strength colonies in normal conditions from sister queens, obtained from a beekeeper (Mr. Kern) in Leipzig/Rehbach, Rechbacher Anger, Germany
Diet: Since the food status of all colonies was acceptable throughout the trial, no artificial food was offered.
Test units: Bee hives were made up of one polystyrene hive body. Each colony consisted of 11 combs, with 3-5 combs with brood and with all brood stages present and 5-9 combs containing honey and a sufficient amount of pollen.
Comb size: 37 cm x 22.3 cm = 825.1 cm^2 per comb side
Tunnel: Semi-circular metal (max height 2.5 m) covered with fine plastic gauze with a mesh size of 3 mm, which enclosed a *Phacelia* (*Phacelia tanacetifolia*, variety Balo) plot (18 x 6 m).The plant density was 10 kg seed/ha and application was carried out at BBCH crop stage 65 (full flowering).
Prior to installing the hives, to facilitate counting of dead bees the crop was removed from three 0.5 m wide paths along the front and head side of each tunnel and in the middle of the plot resulting in an effective crop area of 93.5 m^2 (total plot size covered by tunnel: 108 m^2). Gauze sheets (2x: 6 m x 0.5 m each and 1x: 18 m x 0.5 m) were spread out over the pathways to aid in collection and counting of dead bees on the ground.
Acclimation: 3 days under test conditions

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	7.8-31.8 °C
Relative humidity:	48.6-97.1%
Rainfall:	0-18.1 mm
Sun radiation:	38.6-331.4 W/m^2

2. Assignment and treatment:

The semi-field (tunnel) bee study was conducted in Cunnersdorf, Germany. The test consisted of 4 treatment groups: control (water treated), two concentrations of the test item (T1 and T2) and a reference item (containing fenoxycarb), with 4 replicates per test group).

Three days before application, (DAT -3) bee colonies were placed into the tunnels to enable the bees to become familiar with the environment. One day before application (DAT -1) the first colony and brood assessment was carried out to verify that all colonies were comparable. Shortly before application, the foraging activity of the honey bees was determined to ensure that the bees were sufficiently active during application. During application the hives were protected from direct spray residues by covering the colonies with plastic sheets. Seven days after application (DAT 7), the hives were removed from the tunnels in the evening (after bees stopped foraging) and transferred to a forest nearby (without flowering main crops or intensive agriculture), where they continued to be monitored up to day 28 post-application.

3. Dose preparation:

The spray solutions were prepared immediately before application, in the laboratory. The required amounts of test and reference items were diluted in tap water, to obtain the test concentrations of 320 and 660 mL 560 02 F/ha and 1200 g Insegar 25 WG/ha in 400 L/ha, respectively. These solutions were transported to the test field using a cooling box. The negative control consisted of 400 L/ha tap water only. The *Phacelia* plots of the different treatment groups were applied once using a calibrated plot-sprayer PL 1 with DG TEEJET 80015 VS nozzle. The spray volume which was needed for the application of the control, test item and reference item was calculated with a surplus factor of 1.6043 of the required spray volume per plot. Based on the spray volume of 400 L/ha and the plot size of 93.5 m^2, the target and prepared application volume was 3740 mL/plot and 6000 mL/plot, respectively. The remaining spray liquid after application was measured (by volume) and subsequently the actual amount applied was determined.

4. Measurements and observations:

Assessments of mortality and bee behavior were carried out for 2 days prior to application, on the day of application and on the following 28 days after application (with the first 7 days inside the tunnel). First mortality was observed, by collecting the dead bees found on the gauze sheets and by using dead bee traps attached to the test hives. This assessment was followed by foraging activity and behavior of bees. On the day of application additional foraging assessments were conducted in order to assess any immediate impact of the application; four times within the first hour after application, 2, 4 and 6 hours after application. On DAT 1, three foraging assessments were carried out during daily main bee flight activity.

Colony strength and brood assessments according to Imdorf et al. (1987) and Imdorf & Gerig (1999) were carried out on DAT -1 (BFD[1] 0), DAT 7 (BFD 8), DAT 10 (BFD 11), DAT 14 (BFD 15), DAT 21 (BFD 22) and DAT 28 (BFD 29). This included assessments of the pollen and nectar storage area, area containing eggs, larvae and capped cells as well as number of bees/colony and the presence of a healthy queen. For detailed brood assessment at least 400 single cells containing eggs were marked using high resolution digital images in combination with the Nextreat digital image analysis tool in each colony on DAT -1 (BFD 0). During the following colony assessments on BFD 8, BFD 11, BFD 15 and on BFD 22 the initially marked eggs were assessed with respect to further brood development.

During the test period the climatic data was recorded continuously.

5. Statistics (calculations):

The endpoints for statistical evaluation were mortality (number of dead bees/day), foraging activity (number of foraging bees/m^2) and brood termination rate (% terminated eggs/colony; the failure of individual eggs or larvae to develop), brood-index (indicator of brood development from egg to successful hatched) and brood compensation index (taking into account the refill of cleared cells). The arithmetic mean and the standard deviation per replicate and treatment group were calculated.

Pre-treatment data were statistically evaluated using the Tukey-test, comparing treatment means (control, test item and reference item) against each other. The post-treatment data were evaluated using the Student t-test (for variance homogeneous data) or the Welch t-test (for variance inhomogeneous data) for pair-wise comparison of treatments with the control (mortality and brood termination rate: one-sided greater; brood indices: one-sided smaller). For all statistical tests a significance level of $\alpha = 0.05$ was used. The %-value of the brood termination rate was arcsine-transformed to ensure the homogeneity (Cochran test) of the data before conducting the Student t-test. The statistical analysis was performed with the software Easy Assay 4.0 and ToxRat Professional 2.10.05.

II. RESULTS AND DISCUSSION

A. MORTALITY

Adult honey bees

On the days before application, the mortality of adult honey bees observed on the days before application in the control group was 19.6 bees/tunnel/day. The mortality in the test item treatment groups and the reference group was not statistically significantly different from the control (refer to the following table below).

[1] BFD = brood area fixing date

No statistically significant differences between the test item groups and the control group were observed directly after application, during exposure between DAT 0 to DAT 7, during the post-exposure period between DAT 7 and DAT 28 or at the overall comparison. Statistically significantly differences were only noted between control and test item treatment T2 on DAT 12 and 15, and between T1 and T2 on DAT 5, DAT 10 and DAT 12. However, these differences were not considered to be of biological relevance, given the low variability between and within the test groups and because no differences were observed between the test groups during the entire post application period.

The exposure of adult honey bees to the reference item did not result in an increased number of dead adult bees.

Larvae/pupae
On the days before application no increased mortality of honey bee larvae or pupae was observed in the control, test item groups or reference indicating comparable, healthy and well adapted colonies. On DAT 0 after application and in the further progress of the study between DAT 0aa to DAT 28 no relevant numbers of dead larvae or pupae were found in the control and test item treatment groups. In the reference item a distinct increase in the number of pupae was found between DAT 9 and DAT 19.

B. FORAGING ACTCIVITY

Before application, no statistically significant differences were observed in foraging activity between the control, the test item groups and references group (refer to the table below). The foraging activity was high and the bees were noted to continue to actively forage shortly before application, indicating that bees had adapted to the new environment that they were sufficiently exposed during the application.

On the day of application and at all following assessment days, a reduced foraging activity was observed for all test groups, showing an overall mean foraging activity of 73.8%, 73.9% and 74.2% compared with activity before application, for control, T1 and T2 test item groups, respectively. No statistically significant differences were observed between the control, test item groups and reference group during the exposure period (DAT 0 to DAT 7).

C. BEE BEHAVIOR

The exposure of bees to the test item did not result in any behavioral abnormalities, e.g. intoxication symptoms or differences compared to the control. Bees were calm and actively foraging in the treated crop.

D. COLONY STRENGTH

During colony strength assessment conducted one day before application (DAT -1), the estimated average number of bees per colony was similar in the control, T1, T2 and reference item. During the post-application period there was a comparable development of the colony strength in the control and test item groups in the course of the study. In contrast, the colony strength of the colonies in the reference item group remained on the pre-application level.

E. BROOD ASSESSMENT

Brood area
On DAT -1 honey bee queens were healthy and actively laying eggs, and colonies were generally producing brood in comparable amounts in all test colonies.

The mean comb area of the respective brood stages developed in a comparable way in the control and the T1 and the T2 treatment groups in the course of the study. For the T1 treatment, temporary differences were noted in the areas with cells filled with eggs on BFD 15 and 22 (DAT 14 and 21) and consequently also in the cells filled with pupae on BFD 22 and 29 (DAT 21 and 28). These differences were attributed to biological variability of egg laying rates, because they were not observed in the higher test rate group (T2). Accordingly, the overall total mean brood nest area (sum of comb area occupied by eggs, larvae and capped cells) in the control and the test item groups T1 and T2 increased during the study in a comparable way.

The total mean brood nest size in the reference item group remained constant in the range of the pre-application level and was therefore significantly lower between DAT 7 and DAT 28 compared to the control. The effect of the reference item on brood development showed that the test system was sensitive to detect possible effects on brood development.

Brood termination rate (%)
Twenty-two days after BFD (DAT 21) the mean brood termination rate was not statistically significantly different between the control, the T1 and the T2 test item groups. Thus neither a test item related effect nor a dose-response relationship of the test item on the brood development was detected.

The distinct increased termination rate of 55.2% in the reference item, together with the increased pupal mortality, indicated the suitability of the test system to detect potential effects of the test item on the brood development.

Brood index
No statistically significant differences were observed at any assessment day, between the control, the T1 and the T2 treatment groups.

In the reference treatment a strong effect with decreased brood-indices was noticed during the course of the study, which were statistically significant different from the control at BFD 8 to 22 (DAT 7 to 21).

Brood compensation index
The brood compensation-indices were not statistically significantly different between the control, the T1 and the T2 test item groups.

Despite a great loss of brood stages in the reference item group between BFD 0 and BFD 15 (DAT -1 and DAT 14) several of the emptied cells were refiled with eggs and this the brood-compensation indices were slightly higher than the corresponding brood indices at these assessment days.

A summary of the findings are presented in the following table.

Table 8.3.1.4-1: Summary of results obtained from the semi-field (tunnel) study with a single exposure of BAS 560 02 F to actively foraging honey bees

	Mean values ± SD per treatment group [1]			
	Control	**T1 (320 mL/ha)**	**T2 (660 mL/ha)**	**Reference item (1.2 kg/ha)**
Mortality of worker bees/colony/day [n] during:				
Pre-application phase [2]	19.6a ± 7.8	24.8ab ± 8.8	17.9a ± 7.8	25.3ab±10.1
Exposure phase in the tunnels [2]	19.8 ± 6.0	15.0 ± 6.8	18.0 ± 8.2	15.8 ± 7.5
Phase outside the tunnel [3]	17.7 ± 8.0	17.5 ± 9.0	17.7 ± 9.4	17.3 ± 3.8
Overall after application	18.3 ± 7.5	16.8 ± 8.5	17.8 ± 9.0	16.9 ± 2.0
Mortality of pupae/colony/day [n] during:				
Pre-application phase	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
Exposure phase in the tunnels	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.2	2.0 ± 3.5
Phase outside the tunnel [3]	0.1 ± 0.3	0.2 ± 0.7	0.1 ± 0.5	17.5 ± 25.5
Overall after application	0.1 ± 0.4	0.2 ± 0.6	0.1 ± 0.4	13.2 ± 22.9
Foraging activity/m²/colony/day [n] during:				
Pre-application phase	12.2ab ± 1.6	11.9a ± 1.0	13.2a ± 0.9	11.3a ± 1.5
Exposure phase in the tunnels	9.0 ± 0.5	8.8 ± 0.5	9.8 ± 1.1	8.9 ± 1.0
Colony strength (estimated average number of bees/colony):				
DAT -1 (BFD 0)	4219 ± 65	4838 ± 955	5456 ± 1008	4641 ± 605
DAT 7 (BFD 8)	6188 ± 832	5794 ± 436	6609 ± 598	4809 ± 665
DAT 10 (BFD 11)	5822 ± 591	5653 ± 463	6413 ± 243	5259 ± 192
DAT 14 (BFD 15)	6075 ± 636	5513 ± 390	5934 ± 249	4753 ± 539
DT 21 (BFD (22)	6525 ± 923	6019 ± 373	6638 ± 205	4472 ± 539
DAT 28 (BFD 29)	7791 ± 1084	6919 ± 570	8297 ± 702	4809 ± 834
Brood development (estimated mean area of bee brood/colony cm²/colony):				
Eggs:				
DAT -1 (BFD 0)	799 ± 176	825 ± 253	1109 ± 515	980 ± 273
DAT 7 (BFD 8)	1083 ± 197	285 ± 146	1418 ± 464	1135 ± 326
DAT 28 (BFD 29)	1882 ± 632	2166 ± 700	2217 ± 425	748 ± 351
Larvae:				
DAT -1 (BFD 0)	799 ± 296	980 ± 133	1006 ± 908	1006 ± 515
DAT 7 (BFD 8)	1289 ± 399	1083 ± 321	1650 ± 84	619 ± 304
DAT 28 (BFD 29)	1650 ± 223	1547 ± 223	2140 ± 351	1083 ± 362
Pupae:				
DAT -1 (BFD 0)	2217 ± 381	1315 ± 296	1470 ± 424	1238 ± 491
DAT 7 (BFD 8)	2243 ± 726	1985 ± 361	2707 ± 1811	1521 ± 969
DAT 28 (BFD 29)	3404 ± 533	2217 ± 610	3558 ± 717	2166 ± 1000

	Mean values ± SD per treatment group [1]			
	Control	**T1 (320 mL/ha)**	**T2 (660 mL/ha)**	**Reference item (1.2 kg/ha)**
Total brood in all stages:				
DAT -1 (BFD 0)	3816 ± 658	3120 ± 130	3584 ± 1524	3223 ± 464
DAT 7 (BFD 8)	4615 ± 1102	3893 ± 229	5776 ± 2135	3275 ± 1467
Compared to BFD 0	+21%	+25%	+61%	+2%
DAT 10 (BFD 11)	4693 ± 1174	4177 ± 342	5389 ± 1295	3197 ± 1422
Compared to BFD 0	+23%	+34%	+50%	-1%
DAT 14 (BFD 15)	5183 ± 1122	4177 ± 687	5982 ± 533	3146 ± 1529
Compared to BFD 0	+36%	+34%	+67%	-2%
DT 21 (BFD (22)	6214 ± 928	4744 ± 776	7116 ± 1007	3610 ± 1194
Compared to BFD 0	+63%	+52%	+99%	+12%
DAT 28 (BFD 29)	6936 ± 928	5930 ± 1080	916 ± 835	3997 ± 1445
Compared to BFD 0	+82%	+90%	+121%	+24%
Brood termination rate at BFD 22 (%)	23.4 ± 11.6	16.4 ± 9.8	12.2 ± 9.8	55.2* ± 11.6
Brood index at BFD 22 [n]	3.8 ± 0.6	4.2 ± 0.5	4.4 ± 0.4	2.2* ± 0.6
Brood compensation index at BFD 22 [n]	4.2 ± 0.4	4.6 ± 0.2	4.6 ± 0.3	3.2* ± 0.8

[1] Each with four tunnels (replicates)
[2] Sum of dead honey bees found in dead bee traps and on gauze strips in the tunnel
[3] Dead honey bees found in dead bee traps, only
a,b: same letters indicate that groups are not statistically significantly different at pre-application period
*: statistically significantly different at post-application period
DAT = Day after treatment
BFD = brood area fixing day

F. DEFICIENCIES

Colony and brood assessments scheduled for BFD5 ± 1 (DAT 4 ± 1) were shifted to BFD8 (DAT 7) because the weather conditions were unfavorable to perform colony brood assessments. This did not have an impact on the study, as the initially marked cells were already sealed during the second assessment.

III. CONCLUSION

Following a single application of BAS 560 02 F at a rates of 320 mL product/ha and 660 mL product/ha (equivalent to 160 g a.s./ha and 330 g a.s./ha, respectively) to flowering *Phacelia* under semi-field conditions during active foraging of honey bees (*Apis mellifera carnica*), there were no effects on mortality, foraging activity, behavior, colony development, colony strength and bee brood development observed.

Overall, based on the results from this study, BAS 560 02 F applied at a rate up to 660 mL product/ha does not cause any adverse effects on honey bee colonies.

CA 8.3.2 Effects on non-target arthropods other than bees

The effects of the formulated products (BAS 506 00 F and BAS 506 02 F) on non-target arthropods other than bees were investigated with the standard Tier I indicator species, the aphid parasitoid *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri*. Additional Tier I studies for *Aphidius rhopalosiphi* are available at higher test concentrations and are being submitted in support of this submission.

The referenced studies in the table below were summarised and considered acceptable during the initial EU review.

Table 8.3.2-1: Toxicity findings of formulated metrafenone to non-target arthropods

Species	Test item	Test substrate (dose)	Endpoint	Reference
First tier studies – inert substrate				
Aphidius rhopalosiphi (adult)	BAS 560 00 F	Glass-plate (150 & 300 g a.s./ha)	LR_{50} > 300 g a.s./ha	KCA 8.3.2.1/1 2001/7000254
Aphidius rhopalosiphi (adult)	BAS 560 00 F	Glass-plate (30, 150, 300 & 450 g a.s./ha)	LR_{50} > 450 g a.s./ha	KCA 8.3.2.1/2 2005/1025546
Aphidius rhopalosiphi (adult)	BAS 560 02 F	Glass-plate (10, 50, 75, 100, 150 & 300 g a.s./ha)	LR_{50} > 300 g a.s./ha	KCA 8.3.2.1/3 2001/7001677
Aphidius rhopalosiphi (adult)	BAS 560 02 F	Glass-plate (80, 160, 320, 480 & 550 g a.s./ha)	LR_{50} > 550 g a.s./ha	KCA 8.3.2.1/4 2006/1032567
Typhlodromus pyri (adult)	BAS 560 00 F	Glass-plate (150 & 300 g a.s./ha)	LR_{50} > 300 g a.s./ha	KCA 8.3.2.2/1 2001/7000253
Typhlodromus pyri (adult)	BAS 560 02 F	Glass-plate (100, 200, 300, 400 & 500 g a.s./ha)	LR_{50} > 500 g a.s./ha	KCA 8.3.2.2/2 2001/7000255

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

Report:	CA 8.3.2.1/1 Buttle V.L., 2001a A tier 1 laboratory study to estimate the LR50 of AC 375839 in a 300 g L-1 SC formulation (RLF 12359) on Aphidius rhopalosiphi (Hymenoptera: Braconidae) 2001/7000254
Guidelines:	EEC 91/414 Annex III 10.5.1, EEC 96/12
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Conclusion

The LR_{50} value for *Aphidius rhopalosiphi* exposed to glass plates treated with BAS 560 00 F was > 300 g a.s./ha, the highest concentration tested. The no-observed-effect concentration (NOEC), based on fecundity, was 300 g a.s./ha. Please refer to Document M-II, Section 6, Point 8.3.2 of the summary dossier dated 2002 for further details.

Report: CA 8.3.2.1/2
Vinall S., 2005a
A rate-response laboratory test to determine the effects of BAS 560 00 F on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae)
2005/1025546

Guidelines: Mead-Briggs M. et al. (2000) A laboratory test for evaluating the effects of plant protection products on the parasitic wasp Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera Braconidae), Barrett K.L. et al. (1994) Guidance Document on regulatory testing procedures for pesticides with non-target arthropods, Candolfi et al. 2001, EEC 96/12

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The toxicity of BAS 560 00 F to the parasitic wasp *Aphidius rhopalosiphi* was assessed in a laboratory study. Adults were exposed to air-dried residues on treated glass plates sprayed with nominal concentrations of 100, 500, 1000 and 1500 mL test material/ha, which corresponds to 30, 150, 300 and 450 g a.s./ha. Ten female adult wasps were introduced into each replicate test unit and there were four replicates per treatment. After 48 hours, mortality was assessed. Thereafter, surviving female wasps were obtained (fifteen per treatment) and individually confined over untreated barley plants infested with host cereal aphids for 24 hours. Their reproductive capacity (number of aphid mummies produced) was assessed after a further ten days. A negative control (purified water only) and reference item (at a concentration of 0.04 g dimethoate/ha) were tested in parallel.

Only the mortality for the highest test concentration of 1500 mL/ha showed a statistically significant difference to the control. The reproductive capacity was not assessed for the lowest test concentration of 100 mL/ha. Statistically significantly different from the control were observed for the lowest and the highest concentrations tested in the reproductive assessment of 500 and 1500 mL/ha, respectively.

Hence, the 48-hr LR_{50} value for *Aphidius rhopalosiphi* exposed to BAS 560 00 F was determined to be > 1500 mL/ha (corresponding to > 450 g a.s./ha), the highest rate tested. The no-observed-effect rate (NOER) based on mortality was 1000 mL/ha (corresponding to 300 g a.s./ha) and the NOER based on reproduction was lower than the lowest concentration tested in the reproductive assessment (500 mL/ha), which corresponds to < 150 g a.s./ha.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 00 F
Batch number: 3000
Purity: 300.0 g/L metrafenone (nominal; 292.6 g/L metrafenone analyzed)
Description: Beige opaque liquid

2. Test concentrations: 100, 500, 1000 and 1500 mL product/ha, corresponding to 30, 150, 300 and 450 g metrafenone/ha, respectively

3. Reference item: BAS 152 11 I (containing 400 g dimethoate/L), tested at a concentration of 0.10 mL BAS 152 11 I/200 L water/ha (equivalent to 0.04 g a.s./ha)

4. Vehicle: Purified water

5. Test organism
Species: Parasitic wasp *Aphidius rhopalosiphi*
Age/life stage: Adults, less than 48 hours old
Source: In-house culture, established using wasps from Katz Biotech AG, Germany
Acclimatization: Maximum 48 hours under test conditions in emergence chambers
Diet: 1:3 v/v solution of honey and water
Test units: Mortality assessment: Two treated glass plates (10 x 10 cm) fitted to a square frame made from metal casing, with holes covered with gauze to provide ventilation
Reproduction assessment: Pots containing ten to twenty untreated barley seedlings, which had been infested with host aphids (more than 100 adults and nymphs of a mixed cereal aphid culture). The wasps were confined over the pots of plants using clear acrylic cylinders (9 cm diameter and 20 cm high)

B. STUDY DESIGN

1. Environmental conditions
Temperature: 20 - 21°C (mortality assessment); 20°C (reproductive assessment)
Relative humidity: 65 - 71% (mortality assessment); 63 - 73% (reproductive assessment)
Photoperiod: 16 h light: 8 h darkness
Light intensity: 1700 - 2900 lux (mortality assessment); 5100 – 6060 lux (reproductive assessment)

2. Assignment and treatment:
One hour after the test units were treated, adult wasps (less than 48 hours old) were transferred to the test units using an aspirator. For the mortality assessment, there were four replicate test units per treatment, containing ten wasps each (with at least five females). For the subsequent reproduction assessment, including the control group and the groups exposed to the three highest test item concentrations, fifteen individually-confined female wasps (categorized as being alive) were used per treatment. The adult females were removed after 24 hours and the aphid-infested plants were kept for a further ten days under similar conditions.

3. Dose preparation:
First, a stock solution was prepared by dilution of the test material in purified water, at a concentration of 50 mL/L. The test concentrations of 100, 500, 1000 and 1500 mL/ha were prepared by further dilution of the stock solution in purified water. These solutions were applied using a spray tower at a deposition rate of 200 L/ha. A control (purified water only) and a reference item (BAS 152 11 I, tested at 0.04 g dimethoate/ha) were concurrently tested.

4. Measurements/observations:
For the mortality assessment, the condition of the wasps (live, affected, moribund or dead) was assessed two, 24 and 48 hours after test initiation. For the reproduction assessment, the number of mummies that developed on each test unit was recorded.
Temperature and humidity were recorded hourly.

5. Statistics:
Mortality data were corrected according to Abbott (1925). Fisher's exact test (α=0.05) was used to assess whether there was a significant difference between mortality data of the treated groups and the control group. For the reproductive assessment, the data were analyzed by one-way ANOVA and Dunnett's test. A square root transformation was carried out on the data prior to analysis. All statistics were performed with SPSS (2003).

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the control group during the test period and exposure to the reference item resulted in 100% mortality. For the test material, statistically significant difference from the control group was observed for the adults wasps exposed to the highest test concentration of 1500 mL/ha. Results are presented in the table below.

B. REPRODUCTION

In the control group, the mean number of mummies produced per surviving female was 68.3. For the test material, statistically significant differences from the control group were observed for the adults wasps exposed to 500 and 1500 mL/ha.

The table below shows the results of the mortality and the reproductive assessments.

Table 8.3.2.1-1: Mortality and reproduction of *Aphidius rhopalosiphi* exposed to BAS 560 00 F

Concentration of BAS 560 00 F (mL/ha)	Concentration of metrafenone (g/ha) [a]	Corrected mortality (%) [b]	Reproduction	
			Mean ± SD [c]	Reproduction rate (%) [d]
0.0 (Control)	0.0	-	68.3 ± 22.8	-
100	30	2.5	n.d.	n.d.
500	150	2.5	42.1 ± 12.1**	38.4
1000	300	10.0	54.7 ± 12.7	20.0
1500	450	15.0*	50.5 ± 12.98**	26.2
	Endpoint (L BAS 560 00 F/ha)			
LR_{50}	> 1.5			
Effects on reproduction	< 0.5			

[a] Based on nominal content of 300.0 g metrafenone/L BAS 560 00 F
[b] Corrected mortality compared to the control group, according to Abbott (1925)
[c] With 15, 14, 15 and 13 female wasps in the control group, 500, 1000 and 1500 mL/ha treatment group, respectively
[d] Compared to the control group
SD = Standard deviation
n.d. = not determined
*Significantly increased compared to the control (Fisher's Exact test)
**Significantly increased compared to the control (one-way ANOVA and Dunnett's test)

C. DEFICIENCIES

None.

III. CONCLUSION

The LR_{50} value for *Aphidius rhopalosiphi* exposed to BAS 560 00 F was determined to be higher than the highest test concentration of 1500 mL/ha, which is equivalent to > 450 g a.s./ha. The no-observed-effect rate (NOER) based on mortality was 1000 mL/ha (corresponding to 300 g a.s./ha) and the NOER based on reproduction was lower than the lowest concentration tested in the reproductive assessment (500 mL/ha), which corresponds to < 150 g a.s./ha.

Report:	CA 8.3.2.1/3 Walker H.M., 2001a A tier 1 laboratory study to estimate the LR50 of AC 375839 in a 500 g L-1 SC formulation (RLF 12360) on Aphidius rhopalosiphi (Hymenoptera: Braconidae) 2001/7001677
Guidelines:	EEC 91/414 Annex III 10.5.1, EEC 96/12, Mead-Briggs M. (1992) A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez). Aspects of Applied Biology 31 pp. 179-189
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Conclusion

The LR_{50} value for *Aphidius rhopalosiphi* exposed to glass plates treated with BAS 560 02 F was > 300 g a.s./ha, the highest concentration tested. In comparison to the controls, there was a statistically significant reduction in the number of mummies produced in the 300 g a.s./ha treatment only. Therefore, the no-observed-effect concentration (NOEC) for effects on fecundity was determine to be 150 g a.s./ha. Please refer to Document M-III, Section 10, Point 10.5.1 of the summary dossier dated 2002 for further details.

Report:	CA 8.3.2.1/4 McCormac A., 2006a A rate-response laboratory test to determine the effects of BAS 560 02 F on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae) 2006/1032567
Guidelines:	Mead-Briggs M. et al. (2000) A laboratory test for evaluating the effects of plant protection products on the parasitic wasp Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera Braconidae)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The toxicity of BAS 560 02 F to the parasitic wasp *Aphidius rhopalosiphi* was assessed in a laboratory study. Adults were exposed to air-dried residues on treated glass plates sprayed with nominal concentrations of 160, 320, 640, 960 and 1100 mL test material/ha, which corresponds to 80, 160, 320, 480 and 550 g a.s./ha. Ten female adult wasps were introduced into each replicate test unit and there were four replicates per treatment. After 48 hours, mortality was assessed. Thereafter, surviving female wasps were obtained (fifteen per treatment) and individually confined over untreated barley plants infested with host cereal aphids for 24 hours. Their reproductive capacity (number of aphid mummies produced) was assessed after a further ten days. A negative control (purified water only) and reference item (at a concentration of 0.04 g dimethoate/ha) were tested in parallel.

After 48 hours, mortalities of 0.0%, 5.0%, 0.0%, 2.5% and 0.0% were observed in the test item treatments of 160, 320, 640, 960, and 1100 mL BAS 560 02 F/ha in comparison to 0.0% in the control. The mean number of mummies produced per female was 30.2 in the control and 32.5, 27.3, 30.2, 30.1 and 31.4 in the test item treatments of 160, 320, 640, 960, and 1100 mL BAS 560 02 F/ha, respectively. No statistically significant differences compared to the control were observed neither for mortality nor for reproduction.

Hence, the 48-hr LR_{50} value for *Aphidius rhopalosiphi* exposed to BAS 560 02 F was determined to be > 1.1 L/ha (corresponding to > 550 g a.s./ha), the highest rate tested. The no-observed-effect rate (NOER) was 1.1 L BAS 560 02 F/ha (corresponding to 550 g a.s./ha).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 02 F
Batch number: 2003
Purity: 500.0 g/L metrafenone (nominal; 495.5 g/L metrafenone analyzed)
Description: White fluid

2. Test concentrations: 160, 320, 640, 960 and 1100 mL test material/ha, corresponding to 80, 160, 320, 480 and 550 g a.s./ha, respectively

3. Reference item: BAS 152 11 I (containing 400 g dimethoate/L), tested at a concentration of 0.10 mL BAS 152 11 I/200 L water/ha (equivalent to 0.04 g a.s./ha)

4. Vehicle: Purified water

5. Test organism
Species: Parasitic wasp *Aphidius rhopalosiphi*
Age/life stage: Adults, less than 48 hours old
Source: In-house culture, established using wasps from Katz Biotech AG, Germany
Acclimatization: Maximum 48 hours under test conditions in emergence chambers
Diet: 1:3 v/v solution of honey and water
Test units: Mortality assessment: Two treated glass plates (10 x 10 cm) fitted to a square frame made from metal casing, with holes covered with gauze to provide ventilation
Reproduction assessment: Pots containing approximately 15 untreated barley seedlings, which had been infested with host aphids (more than 100 adults and nymphs of a mixed cereal aphid culture). The wasps were confined over the pots of plants using clear acrylic cylinders (8-9 cm diameter and 20 cm high). Top covered with nylon netting

B. STUDY DESIGN

1. Environmental conditions
Temperature: 20°C - 21°C (mortality assessment); 19.0°C - 21.0°C (reproductive assessment)
Relative humidity: 69% - 78% (mortality assessment); 63% - 81% (reproductive assessment)
Photoperiod: 16 h light: 8 h darkness
Light intensity: 950 lux - 1050 lux (mortality assessment); 2400 lux (reproductive assessment)

2. Assignment and treatment:
One hour after the test units were treated, adult wasps (less than 48 hours old) were transferred to the test units using an aspirator. For the mortality assessment, there were four replicate test units per treatment, containing ten wasps each (with at least five females). For the subsequent reproduction assessment, fifteen individually-confined female wasps (categorized as being alive) per treatment were used. The adult females were removed after 24 hours and the aphid-infested plants were kept for a further ten days under similar conditions.

3. Dose preparation:
The dilution products were applied using a calibrated Potter Laboratory Spray Tower at a spray pressure of 0.7 bar and at a deposition rate of 200 L/ha. Treatments were applied in the order of control, test treatment (in ascending rate order) and then toxic reference.

4. Measurements/observations:
For the mortality assessment, the condition of the wasps (live, affected, moribund or dead) was assessed two, 24 and 48 hours after test initiation. For the reproduction assessment, the number of mummies that developed on each test unit was recorded.

Temperature and humidity were recorded hourly.

5. Statistics:
Mortality data were corrected according to Abbott (1925). Fisher's exact test (α=0.05) was used to assess whether there was a significant difference between mortality data of the treated groups and the control group. For the reproductive assessment, the data were analyzed by one-way ANOVA. A square root transformation was carried out on the data prior to analysis. All statistics were performed with SPSS (2003).

II. RESULTS AND DISCUSSION

A. MORTALITY

After 48 hours, mortalities of 0.0%, 5.0%, 0.0%, 2.5% and 0.0% were observed in the test item treatments of 160, 320, 640, 960, and 1100 mL BAS 560 02 F/ha in comparison to 0.0% in the control. No statistically significant differences compared to the control were observed. Exposure to the reference item resulted in 100% mortality. Results are presented in the table below.

B. REPRODUCTION

The mean number of mummies produced per female was 30.2 in the control and 32.5, 27.3, 30.2, 30.1 and 31.4 in the test item treatments of 160, 320, 640, 960, and 1100 mL BAS 560 02 F/ha, respectively. No statistically significant differences compared to the control were observed.

The table below shows the results of the mortality and the reproductive assessments.

Table 8.3.2.1-2: Effects on *Aphidius rhopalosiphi* exposed to BAS 560 02 F under worst-case laboratory conditions

Concentration of BAS 560 00 F (mL/ha) [a]	Concentration of metrafenone (g/ha)	Mortality (%) [b]	Reproduction	
			Reproduction [c] (eggs/female)	Reproduction rate (%) [d]
0.0 (Control)	0.0	0.0	30.2	--
160	80	0.0	32.5	-7.5
320	160	5.0	27.3	9.5
640	320	0.0	30.2	0.0
960	480	2.5	30.1	0.5
1100	550	100.0	31.4	-4.0
	Endpoint (L BAS 560 02 F/ha)			
LR_{50}	> 1.1			
Effects on reproduction	> 1.1			

[a] Application rate in 200 L water/ha.
[b] Mortality after 48 h of exposure to BAS 560 02 F on glass plates.
[c] Reproduction: mean number of parasitized aphids/female.
[d] A positive value indicates a decrease and a negative value an increase in reproduction, relative to the control.

C. DEFICIENCIES

None.

III. CONCLUSION

The LR_{50} value for *Aphidius rhopalosiphi* exposed to BAS 560 02 F was determined to be higher than the highest test concentration of 1.1 L/ha, which is equivalent to > 550 g a.s./ha. The no-observed-effect rate (NOER) based on mortality and reproduction was 1.1 L BAS 560 02 F/ha (corresponding to 550 g a.s./ha).

CA 8.3.2.2 Effects on *Typhlodromus pyri*

Report:	CA 8.3.2.2/1 Buttle V.L., 2001b A tier 1 laboratory study to estimate the LR50 of AC 375839 in a 300 g L-1 SC formulation (RLF 12359) on Typhlodromus pyri (Acari: Phytoseiidae) 2001/7000253
Guidelines:	EEC 91/414 Annex III 10.5.1, EEC 96/12
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Conclusion

The LR_{50} value for *Typhlodromus pyri* exposed to glass plates treated with BAS 560 00 F was > 300 g a.s./ha, the highest concentration tested. In comparison to the controls, there was a statistically significant reduction in the number of eggs produced in the 150 and 300 g a.s./ha treatments on assessment days ten and twelve, but not on day fourteen. Therefore, the no-observed-effect concentration (NOEC) for effects on fecundity was < 150 g a.s./ha. Please refer to Document M-II, Section 6, Point 8.3.2 of the summary dossier dated 2002 for further details.

Report:	CA 8.3.2.2/2 Walker H.M., 2001b A tier 1 laboratory study to estimate the LR50 of AC 375839 in a 500 g L-1 SC formulation (RLF 12360) on Typhlodromus pyri (Acari: Phytoseiidae) 2001/7000255
Guidelines:	EEC 91/414 Annex III 10.5.1, EEC 96/12, Louis F. Ufer A. (1995) Methodical improvements of standard laboratory tests for determining the side-effects of agrochemicals on predatory mites (Acari Phytoseiidae)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Conclusion

The LR_{50} value for *Typhlodromus pyri* exposed to glass plates treated with BAS 560 02 F was > 500 g a.s./ha, the highest concentration tested. In comparison to the controls, there was a statistically significant reduction in the number of eggs produced in the 500 and 400 g a.s./ha treatments. Therefore, the no-observed-effect concentration (NOEC) for effects on fecundity was < 400 g a.s./ha. Please refer to Document M-III, Section 10, Point 10.5.1 of the summary dossier dated 2002 for further details.

CA 8.4 Effects on non-target soil meso- and macrofauna

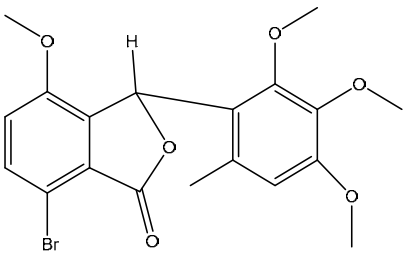
For the first EU review of metrafenone, acute toxicity studies on earthworms were performed with the active substance and the metabolite CL 377160. For the present submission, in accordance with the Commission Regulation (EU) No. 283/2013, chronic earthworm studies have been performed with the formulated products BAS 560 00 F and BAS 560 02 F which are summarized in the respective MCP-10 documents, Section 10.4.1, which also support the active substance. In addition, chronic earthworm studies have been performed with the major soil metabolites of metrafenone. The available data are summarized in the following table.

Table 8.4-1: Chronic toxicity of metrafenone soil metabolites to earthworms

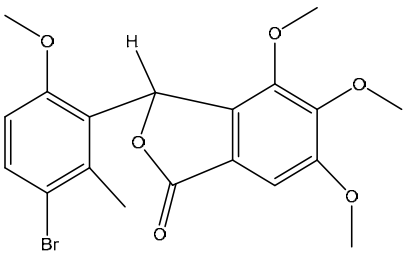
Test item	Test species	Duration	Endpoint	Reference
BAS 560 00 F	*Eisenia fetida*	Chronic, 56-days	NOEC = 400 mg product/kg soil dw [b, c] (**101.78** mg a.s./kg soil dw)	KCP 10.4.1.1/1 2013/1003202
BAS 560 02 F	*Eisenia fetida*	Chronic	56-d NOEC = 240 mg product/kg soil dw [a] (**101.61** mg a.s./kg soil dw)	KCP 10.4.1.1/1 2011/1000384
CL377160	*Eisenia andrei*	Chronic	56-d NOEC = 1000 mg/kg soil dw [a]	KCA 8.4.1/1 2014/1093923
CL3000402	*Eisenia andrei*	Chronic	56-d NOEC = 27.78 mg/kg soil dw [b, c]	KCA 8.4.1/2 2015/1041971

[a] Study conducted with 5% peat, therefore EPPO correction factor not required
[b] EPPO correction factor of 2 required as log Pow value < 2
[c] Based on reproduction

Data has not been generated for the potentially relevant soil metabolite CL 1500831 due to its structural similarities to CL 3000402 (figure below). Therefore the NOEC derived from the chronic earthworm study with CL 3000402 is considered acceptable to assess the potential risk to earthworms from CL 1500831 and further testing is deemed unnecessary.



CL 3000402



CL 1500831

CA 8.4.1 Earthworms – sub-lethal effects

No chronic earthworm studies with the active substance have been conducted. Chronic earthworm toxicity studies with the formulated products BAS 560 00 F and BAS 560 02 F have been performed, which also support the active substance. Summaries of these studies can be found in the respective MCP-10 documents, Section 10.4.1.

Report:	CA 8.4.1/1 McCormac A., 2014a CL377160 - Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2014/1093923
Guidelines:	OECD 222 (2004)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a chronic toxicity study, adults of *Eisenia andrei*, were exposed to CL377160 (metabolite of metrafenone). The test item was dissolved in acetone and then mixed into artificial soil (5% peat) at nominal concentrations of 204, 305, 458, 688 and 1032 mg test item/kg soil dry weight (dw); corresponding to 198, 296, 444, 667 and 1000 mg a.s./kg soil dw. In parallel an untreated and solvent control were tested. In the test, there were four replicate chambers of soil prepared for each variant concentration of the test item, each holding 10 adult worms. For controls, there were eight replicate chambers of soil, each holding 10 adult worms. Assessment of adult earthworm mortality, behavioral effects, 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, there were no significant effects on survival or change in biomass of earthworms exposed to the test item at levels up to and including 1000 mg a.s./kg soil dw. CL 377160 exposure had no statistically significant effects on the reproductive capacity of the confined worms at treatment concentrations up to and including 1000 mg a.s./kg soil dw.

Thus, the no-observed-effect concentration (NOEC) relating to mortality, change in biomass, behavior and reproductive capacity for CL377160 was found to be 1000 mg a.s./kg soil dw, the highest concentration tested. The lowest-observable-effect concentration (LOEC) was > 1000 mg a.s./kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** CL 377160 (metabolite of metrafenone)
Batch: AC12387-108
Purity: 96.9% w/w
Description: Light-brown crystalline solid

2. Test concentrations: 0 (Control and solvent control), 204, 305, 458, 688 and 1032 mg test item/kg soil dry weight (dw); corrected for purity 0, 198, 296, 444, 667 and 1000 mg a.s./kg soil dw

3. Vehicle: Acetone

4. Reference item: Delsene 50 Flo (500 g carbendazim/L), the effects were evaluated in a separate study

5. Artificial soil: Artificial soil according to OECD 222: 5% sphagnum peat, 20% kaolinite clay, 74.81% horticultural silver sand and 0.19% $CaCO_3$

Test units: Polystyrene boxes (6 cm deep with sides of 17.1 cm x 11.3 cm = 193.2 cm^2 surface area). Transparent perforated lids were used.

6. Test organism:

Species: Earthworm (*Eisenia andrei*) (Oligochaeta: Lumbricidae)
Age: Adult worms, with clitellum (5.5 months old), at test initiation
Source: In house culture
Weight: 300 mg to 600 mg (at test initiation)
Acclimation: Three days, in artificial soil, under test conditions.
Diet: Finely ground oats, weekly

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 20.4-28.7 °C (adults); 19.8-20.7 °C (juveniles)
pH of soil: 6.0 ± 0.5
Water content of soil: 50-55.0%
Photoperiod: 16 hours light: 8 hours darkness (490 to 755 lux)

2. Animal assignment and treatment

Different concentrations of the test item were mixed homogeneously into the soil after being dissolved in acetone, which was filled in the test units before the earthworms were introduced on top of the soil. In total, seven treatment groups were set up (five concentrations of the test item, water treated control, and solvent control) with four replicates for the test item treatment group and eight replicates for the controls and ten earthworms per replicate.

After 28 days adult earthworms were removed and the remaining soil was returned to the respective test units for an additional 28 days.

3. Dose preparation

The test item dissolved fully in the acetone and thorough mixing and agitation of each solution was carried out to ensure homogeneity. This test item solution was mixed through an aliquot of the artificial soil and the acetone was allowed to evaporate over a period of up to one hour. Once the treated sand was dry, it was mixed with the rest of the artificial soil to create the equivalent of 500 g dry weight of soil. Then, purified water was added to the individual batches of soil to achieve a final soil moisture content of 50% of its pre-determined maximum water-holding capacity (WHC_{max}).

4. Measurements and observations

Assessment of earthworm mortality, behavioral effects and biomass development was performed after 28 days of exposure. At the end of the test on day 56, the number of juveniles was counted to assess the reproduction rate.

Soil water content and pH was determined on the day of application and at study termination.

5. Statistics

Fisher's Exact Test ($\alpha = 0.05$) was performed on adult mortality data. One-way analysis of variance ($\alpha = 0.05$) was performed on percentage change in adult biomass. One-way analysis of variance ($\alpha = 0.05$) was performed on the number of juveniles.

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

After 28 days of exposure, adult mortality in the control and solvent control was 3.8 and 5.0%. The mortality in the individual test-item treatments was compared to that in the untreated control and none of the concentrations differed significantly (see table below). The LC_{50} was considered to be > 1000 mg/kg soil dw.

There was no loss in condition (open wounds, etc.) or change in behavior observed in worms treated with the test item at concentrations up to and including the maximum of 1000 mg/kg soil dw.

B. BODY WEIGHT

There were no significant changes in adult-worm biomass, relative to the pooled control data, in any of the test-item treatments.

C. REPRODUCTION CAPACITY

The mean number of juveniles produced per arena was 121.9 in the untreated control and 125.1 in the acetone control. There were no significant effects on reproduction, relative to the pooled control data, in any of the test-item treatments. The NOEC was therefore 1000 mg/kg soil dw.

The results are summarized in the table below.

Table 8.4.1-1: Effects on earthworms (*Eisenia Andrei*) exposed to CL377160 in a 56-day reproduction study

Test parameter	Test concentration (mg CL377160/kg soil dw)						
	Control	**Solvent control**	**198**	**296**	**444**	**667**	**1000**
Mortality (28 d) (%)	3.8	5.0	0.0	0.0	2.5	0.0	5.0
Corrected mortality (%)	-	1.3	0.0	0.0	0.0	0.0	1.3
Mean weight change (and standard deviation) (28 d) (%)	49.7 (14.9)	47.7 (10.6)	47.7 (9.3)	50.7 (14.4)	47.1 (13.5)	47.7 (10.0)	52.0 (13.2)
Number of juveniles / container (56 d)	121.9	125.1	120.5	123.0	122.8	105.5	121.3
Reproduction (% of control) (56 d) [a]	-	-3	1	-1	-1	13	1
	Endpoints (mg CL377160/kg soil dw)						
LC_{50}	> 1000						
NOEC	1000						
LOEC	> 1000						

[a] Positive values indicates a decrease, and negative values an increase in reproduction, relative to the control.

The toxic reference bioassay indicated a reproduction EC_{50} of 3.6 mg a.s. (carbendazim)/kg soil dw (95% CL 3.3 and 3.9 mg a.s./kg), i.e. the performance of the earthworm culture was consistent with the expectations of the OECD guidelines.

D. DEFICIENCIES

Due to a malfunction of the controlled-environment room in which the test was run, the upper temperature threshold was exceeded for a period of 65 hours during the first 28 days of the experiment, briefly reaching a maximum of 28.7 °C. However, as all treatments were subject to the same temperature regime and the validity criteria were met, it was considered that this deviation did not affect the integrity of the study.

III. CONCLUSION

In a 56-day reproduction study with the metabolite CL377160 exposure to earthworms (*Eisenia andrei*), the no-observed-effect concentration (NOEC) relating to mortality, change in biomass, behavior and reproductive capacity was found to be 1000 mg/kg soil dw, the highest test concentration. The lowest-observable-effect concentration (LOEC) was > 1000 mg /kg soil dw.

Report:	CA 8.4.1/2 McCormac A., 2015a CL 3000402 - Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2015/1041971
Guidelines:	OECD 222 - Earthworm reproduction Test (2004)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a chronic toxicity study, adults of *Eisenia andrei* were exposed to CL 3000402 (metabolite of metrafenone). The test item was mixed into artificial soil (10% peat) at nominal concentrations of 4.76, 8.57, 15.43, 27.78 and 50.0 mg a.s./kg soil dry weight (dw). In the test, there were four replicate chambers of soil prepared for each variant concentration of the test item, each holding 10 adult earthworms. In parallel an untreated control was tested, with eight replicate chambers of soil, each holding 10 adult earthworms. Assessment of adult earthworm mortality, behavioral effects, 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, the test item treatments up to and including 50.0 mg/kg soil dw did not have any significant effects on survival and change in biomass of the adult earthworms. CL 3000402 exposure had no statistically significant effects on the reproductive capacity of the earthworms, except for the earthworms exposed to the highest treatment concentration (50.0 mg/kg soil dw).

Thus, the no-observed-effect concentration (NOEC) relating to reproductive capacity for CL 3000402 was found to be 27.78 mg/kg soil dw. The lowest-observable-effect concentration (LOEC) based on reproduction was 50.00 mg/kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: CL 3000402 (metabolite of metrafenone)
Batch: L87-50
Purity: 97.1% w/w (analyzed)
Description: White powder

2. Test concentrations: 0 (Control), 4.76, 8.57, 15.43, 27.78 and 50.0 mg CL 3000402/kg soil dry weight (dw)

3. Vehicle: Purified water

4. Reference item: Effects of technical grade carbendazim were evaluated in a separate study

5. Artificial soil: Artificial soil according to OECD 222: 10% sphagnum peat, 20% kaolinite clay, 69.65% horticultural silver sand and 0.35% $CaCO_3$

Test units: Polystyrene boxes (6 cm deep with sides of 17.1 cm x 11.3 cm = 193.2 cm^2 surface area) with ventilated lids were used containing 500 g of dry soil.

6. Test organism:

Species: Earthworm (*Eisenia andrei*) (Oligochaeta: Lumbricidae)
Age: Adult worms, with clitellum (approximately 5 months old at test initiation)
Source: In-house culture
Weight: 341 mg to 474 mg (at test initiation)
Acclimation: One day, in artificial soil, under test conditions.
Diet: Finely ground oats, for the first 4 weeks of the test

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 20.4-21.7 °C (adults); 19.8-20.7 °C (juveniles)
pH of soil: 5.82-6.32
Water content of soil: 56.5-58.0%
Photoperiod: 16 hours light: 8 hours darkness (550 to 770 lux)

2. Animal assignment and treatment

One day prior to treatment application, earthworms were moved from the culture medium to the artificial soil medium, to acclimate to the different substrate. Immediately following preparation of the test soil, earthworms were randomly selected from this acclimatized population and distributed between treatments. The study design included four replicates of 10 earthworms per treatment concentration and 8 replicates of 10 worms for control treatment. After one day, food was provided and further food was supplied on a weekly basis.

At 28 days after treatment (DAT), the numbers of surviving earthworms and their fresh weights were recorded. Any apparent change in the behaviour or physical condition of the confined earthworms was noted. Adult earthworms were removed and the test soil with any cocoons or juvenile worms was returned to the test chambers. Food was provided on the soil surface. After a further 28 days (i.e. 56 DAT) the number of juvenile earthworms in each replicate arena was recorded.

3. Dose preparation

The test item was diluted in purified water shortly before application, at a concentration of 0.515g product/L purified water. This dilution was placed into a sonicating water-bath until the product was observed to have formed a fine homogeneous dispersion and was maintained on a magnetic stirrer whilst aliquots were removed for further dilutions, to obtain the four other required test dilutions. All dilutions were thoroughly agitated to ensure their homogeneity before taking aliquots to treat the test soil, to obtain the soil concentrations equivalent to 4.76, 8.57, 15.43, 27.78 and 50.00 mg CL 3000402/kg soil dw (based on the measured purity of the test item). The replicate arenas were treated individually. To make up each replicate batch of treated soil, the equivalent of 500 g dry soil was partially moistened with purified water, followed by the appropriate test item dilution. This resulted in bringing the total soil moisture content to 50% of the maximum water-holding capacity (WHC).

The treated soil was mixed well using an electrical mixer. No direct measurement was made of test item homogeneity in the soil, but care was taken to mix both the test item dilutions and the treated soil thoroughly. No measurement was made of the stability of the test item in the soil, but the earthworms were placed onto the soil immediately after the treatment of each arena, and all arenas had been treated within 1.5 hours of first making the test item dilutions.

4. Measurements and observations

Assessment of earthworm mortality, behavioral effects and biomass development was performed after 28 days of exposure. After an additional 28 days, at the end of the test on day 56, the number of juveniles was counted to assess the reproduction rate.

5. Statistics

Fisher's Exact Test ($\alpha = 0.05$) was performed on the adult mortality data and T-test for independent samples ($\alpha = 0.05$) or Mann-Whitney *U*-test ($\alpha = 0.05$) on percentage change were used to assess the adult biomass data. One-way analysis of variance (one-sided, $\alpha = 0.05$) and Probit analysis were used to evaluate the number of juveniles. All statistical analyses were carried out using SPSS (2013).

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

After 28 days of exposure, adult mortality in the control was 0.0%. The mortality in the test item treatment groups did not differ statistically significantly from the control group (see table below). Hence, the LC_{50} was considered to be > 50.0 mg/kg soil dw.

There was no loss in condition (open wounds, etc.) or change in behavior observed in earthworms treated with the test item at concentrations up to and including the maximum of 50.0 mg/kg soil dw.

B. BODY WEIGHT

There were no statistically significant changes in the biomass of the adult earthworms in any of the treatment groups, relative to the control group (see table below).

C. REPRODUCTION CAPACITY

The mean number of juveniles produced per arena was 129.0 in the control and the coefficient of variation for the reproduction in the control group was 25.5%. In terms of numbers of offspring found at 56 days, statistically significant effects compared to the control were only observed at the treatment concentration of 50.00 mg/kg soil dw.

Thus the LOEC for reproductive effects was 50.00 mg/kg soil dw, and the NOEC was 27.78 mg /kg soil dw. The EC_{50} was > 50.00 mg CL 3000402/kg soil dw and the EC_{20} and EC_{10} for effects on reproduction were calculated to be 39.2 and 33.4 mg/kg soil dw, respectively.

The results are summarized in the table below.

Table 8.4.1-2: Effects on earthworms (*Eisenia Andrei*) exposed to CL 3000402 in a 56-day reproduction study

Test parameter	Test concentration [a] (mg CL 3000402/kg soil dw)					
	Control	**4.76**	**8.57**	**15.43**	**27.78**	**50.0**
Mortality (28 d) (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean fresh weight change (and standard deviation) (28 d) (%) [b]	23.8 (13.4)	27.7 (26.3)	29.1 (7.5)	35.4 (14.7)	38.6 (3.0)	18.6 (8.0)
Number of juveniles / container (56 d) [c]	129.0	132.8	141.8	149.0	162.5	74.0*
Reproduction (% of control) (56 d) [d]	-	-2.9	-9.9	-15.5	-26.0	42.6
	Endpoints (mg CL 3000402/kg soil dw)					
LC_{50}	> 50.00					
EC_{50} [e]	> 50.00					
EC_{20} (95% CL) [e]	39.2 (30.2 and 45.4)					
EC_{10} (95% CL) [e]	33.4 (21.4 and 39.1)					
NOEC	27.78					
LOEC	50.00					

[a] Application concentration in terms of mg CL 3000402/kg soil dw, based on measured purity.
[b] The mean change in adult worm weight in each arena between 0 and 28 DAT. A positive value indicates an increase in fresh weight. Standard Deviation given in parentheses. Individual treatments compared to the control by t-test for independent samples ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$). There were no significant differences.
[c] Mean number of juvenile worms per replicate arena. The results for the individual treatments were compared to the control by one-way ANOVA and Dunnett's t-test (one-sided, $\alpha = 0.05$). An asterisk indicates a significant difference.
[d] Positive values indicates a decrease, and negative values an increase in reproduction, relative to the control.
[e] Values for the reproduction EC_{50}, EC_{20} and EC_{10} effect concentrations and their 95% confidence limits (where applicable). Only data from the active part of the response curve (shaded values) were included in the Probit regression analysis used to calculate EC20 and EC10 values.

The toxic reference bioassay indicated a reproduction EC_{50} of 2.4 mg a.s. (carbendazim)/kg soil dw (95% CL 1.9 and 3.0 mg a.s./kg), i.e. the performance of the earthworm culture was consistent with the expectations of the OECD guidelines.

D. DEFICIENCIES

None.

III. CONCLUSION

In a 56-day reproduction study with the metabolite CL 3000402 exposure to earthworms (*Eisenia andrei*), statistically significant effects were noted for reproductive capacity at the highest test concentration, 50 mg/kg soil dw. Thus the no-observed-effect concentration (NOEC) relating to reproductive capacity was found to be 27.78 mg a.s./kg soil dw. The lowest-observable-effect concentration (LOEC) was 50.00 mg a.s./kg soil dw.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

No studies with other non-target soil meso- or marcofauna have been performed for the active substance. However, chronic toxicity studies with collembola, *Folsomia candida,* exposed to the formulated products BAS 560 00 F and BAS 560 02 F are available and are submitted in support of this submission. In addition, two litter bag studies were performed with BAS 560 02 F. Summaries of these studies can be found in the respective MCP-10 documents, Section 10.4.2.1. No additional data is being submitted. A summary of the available data is presented in Table 8.4.2-1.

Table 8.4.2-1: Chronic toxicity of BAS 560 00 F and BAS 560 02 F to other non-target soil meso- and macrofauna (other than earthworms)

Test item	Test species	Study type	Endpoint	Reference
BAS 560 00 F	*Folsomia candida*	Chronic, 28-days	NOEC = 250 mg product/kg soil dw [a] (75 mg a.s./kg soil dw; $NOEC_{corr}$ = 37.5 mg a.s./kg soil dw)	KCP 10.4.2/1 2002/1006160
BAS 560 02 F	*Folsomia candida*	Chronic, 28-days	NOEC = 1000 mg product/kg soil dw [b] (422.65 mg a.s./kg soil dw;	KCP 10.4.2.1/1 2013/1003203 ?
BAS 560 02 F	*Litter bag*	6 months	3 applications of 0.1 kg a.s./ha resulted in no adverse effects	KCP 10.4.2.1/2 2002/7005131
BAS 560 02 F	*Litter bag*	6 months	Applied up 1.10 kg a.s./ha resulted in no adverse effects	KCP 10.4.2.1/3 2004/1004371

a EPPO correction factor applied as log Pow value > 2; $NOEC_{corr}$ = corrected NOEC
b Study conducted with 5% peat, therefore EPPO correction factor not required

No tests have been performed with *Hypoaspis aculeifer*,. Also, data have not been generated for potentially relevant soil metabolites. These data are not considered necessary considering the generally low toxicity of metrafenone to soil organisms (based on earthworm, collembolan and nitrogen transformation studies).

CA 8.4.2.1 Species level testing

Please refer to point CA 8.4.2.

CA 8.5 Effects on nitrogen transformation

No data are available for the active substance. However, to support the first EU evaluation of metrafenone, nitrogen transformation studies with the formulation BAS 560 00 F and the soil metabolite CL377160 were submitted and were considered acceptable. For the current submission, nitrogen transformation studies with the product BAS 560 02 F and the soil metabolite CL3000402 are now also available and are submitted.

Although no studies with the active substance have been performed, nitrogen transformation studies with the formulated products BAS 560 00 F and BAS 560 02 F are available which are summarized in the respective MCP-10 documents, Section 10.5. The available data for metrafenone products and metabolites on effects to soil micro-organisms are summarized in the following table.

Table 8.5-1: Summary of effects of metrafenone metabolites to soil micro-organisms

Test	Test substance	Endpoint	Reference
Nitrogen transformation	BAS 560 00 F	< 25% after 28 days at **2.00** mg a.s./kg soil dw (equivalent to 8.19 mg product/ha)	KCP 10.5/1 2001/7000264
Nitrogen transformation	BAS 560 02 F	< 25% after 28 days at **8.8 mg a.s./kg soil dw** (equivalent to 13.2 L product/ha)	KCP 10.5/1 2011/1000401
Nitrogen transformation	CL377160	< 25% after 28 days at 0.31 mg a.s./kg soil dw	KCA 8.5/1 2001/7001052
Nitrogen transformation	CL3000402	< 25% after 28 days at 1.00 mg a.s./kg soil dw	KCA 8.5/2 2015/1132053

Report:	CA 8.5/1 Kolk J. van der, 2001a CL 377160: Determination of effects on nitrogen transformation of soil microflora 2001/7001052
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Eidgenoessisches Departement fuer Umwelt, Verkehr, Energie und Kommunikation, Bern, Schweiz)

Conclusion

The results from this study indicate that incorporation of the metabolite CL 377160 into soil at concentrations as high as 0.31 mg a.s./kg of dry soil does not have adverse effects (deviation from control < 25%) on nitrogen transformation by the soil microflora after 28 days. Please refer to Document M-II, Section 6, Point 8.5 of the summary dossier dated 2002 for further details.

Report: CA 8.5/2
Schoebinger U., 2015a
CL3000402: Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation)
2015/1132053
Guidelines: OECD 216 (2000)
GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The effect of CL3000402 on nitrogen transformation by soil microflora was determined in a medium silty sand soil (DIN 4220) in the laboratory over the course of 28 days. CL3000402 was applied to samples of the soil at nominal test concentrations of 0.200 and 1.00 mg/kg soil dry weight (dw). A control and a solvent control (0.3 mL acetone/kg soil wet weight) were tested in parallel. Nitrogen transformation (measured as NO_3-N production) was determined 0, 7, 14 and 28 days after application.

There were no effects above 25 % deviation to the solvent control on the nitrogen transformation for the application of 0.200 and 1.00 mg test item/kg soil dw at the end of the 28-day incubation period.

CL 3000402 applied to a field soil up to a test concentration of 1.00 mg/kg soil dw caused no adverse effects (deviation from the solvent control < 25 %) on soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CL 3000402
Description: White solid
Lot/Batch: L87-50
Purity: 97.1% w/w

2. Test concentrations: Water control (untreated quartz sand), solvent control (solvent treated quartz sand), 0.200 and 1.00 mg CL3000402 /kg soil dw

3. Vehicle: Acetone

4. Reference item: Sodium Chloride. The reference item was tested in a separate study at a rate of 20 g a.s./kg soil dw

5. Test soil:

Source:	Offenbach, Rhineland-Palatinate, Germany, latitude E 439713, longitude N 5449850 (no plant protection products had been applied for at least 4 years)
Type:	Medium silty sand soil (DIN 4220)
pH:	7.0
Total organic carbon:	0.58%
Nitrate content:	13.9 mg/kg soil dw
Total nitrogen:	0.08%
Cation exchange capacity:	6.8 meq/100 g
Maximum water holding capacity:	36.61%
Microbial biomass:	42.3 mg C/100 g soil dw
Test vessels:	Glass vessels loosely closed with screw caps

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	19.8 - 21.3 °C
pH of soil:	6.8-7.1
Water content:	Approximately 42% of maximum water holding capacity. Measured water content: 13.4 – 15.6 g/100 g soil
Photoperiod:	Continuous darkness

2. Preparation of soil:

The test soil was sieved to a particle size of 2 mm. Prior to the initiation of the study, the moisture content of the soil and the amount of water needed to adjust the soil moisture content to 42 % WHCmax (maximum water holding capacity) were calculated. The test item was applied at a concentration of 0.20 and 1.00 mg/kg soil dw, respectively. Untreated control and solvent control (0.3 mL acetone/kg soil wet weight) groups were tested in parallel.

For the nitrogen turnover the soil and finely ground lucerne meal were thoroughly mixed before application by hand. The final concentration of the dried lucerne meal was 0.5 % of the soil dw.

After treating the soil and mixing thoroughly, three equally sized soil sub-samples from each treatment group (approx. 1100 g) were placed in appropriate glass bottles. The bottles were closed loosely with screw caps and weighed for the determination of the starting weight.

3. Dose preparation:

The test item solution T2 was prepared by diluting the necessary amount of the test item with acetone. An aliquot of T2 was further diluted with acetone to obtain test item solution T1. The test item solutions were homogenized by shaking. For application, 1 mL of the test item solutions were added to quartz sand and mixed thoroughly with the test soil. To achieve 42 % of WHC_{max}, 77.2 g deionized water were added to the respective treatment groups. Each treatment group (soil with the test item added at two different concentrations and untreated controls) contained 3300 g pre-moistened soil for the nitrogen turnover.

4. Measurements and observations:

Samples of the treated soil were taken from each replicate on days 0, 7, 14 and 28 after treatment. Approximately 50 g was used for determination of nitrogen content. Soil nitrification was determined by measuring the NO_3^- contents of aqueous soil extracts by means of Segmented Flow Analysis (SFA) using photometric measurement of nitrate. The concentrations of NO_3-N in the soil were then calculated from the measured values.

At each sampling data, pH and water content were determined. The amount of moisture loss was determined and water was added when required to adjust vessels to the starting weight.

5. Statistics:

Descriptive statistics.

II. RESULTS AND DISCUSSION

A. NITRIFICATION

No adverse effects of CL3000402 on nitrogen transformation in soil could be observed in both test concentrations (0.200 and 1.00 mg/kg soil dw) after 28 days of exposure. The results are summarized in the table below.

Table 8.5-2: Summary of CL 3000402 effects on nitrogen transformation

Interval [a] Sampling days	Solvent Control	Water Control		0.200 mg CL3000402 / kg soil dw		1.00 mg CL3000402 / kg soil dw	
	Mean formation rate [b]						
	mg NO_3-N/ kg soil dw /day	mg NO_3-N/ kg soil dw /day	Dev. % [c]	mg NO_3-N/ kg soil dw /day	Dev. % [c]	mg NO_3-N/ kg soil dw /day	Dev. % [c]
0-7	-1.05	-1.07	-1.60	-1.29	-22.0	-1.48	-40.4
0-14	1.02	0.994	-2.52	0.764	-25.1	0.671	-34.2
0-28	1.19	1.17	-2.01	1.01	-15.1	0.987	-17.2

[a] time interval from test start (day 0) until measurement
[b] calculated from the mean values of NO_3^--N content between day 0 and the sampling date
[c] deviation from solvent control
- = inhibition; + = stimulation

The toxic reference item (sodium chloride), tested in a separate study, had significant effects on the soil nitrogen turnover (decrease of the nitrate transformation rate for the interval of 0 to 28 days of 96.7 %, decrease of the nitrate transformation rate for the interval of 14 to 28 days of 85.1 %) and the short-term respiration (89.5 % inhibition) in a field soil tested at a concentration of 20.0 g/kg soil dry weight.

B. DEFICIENCIES

CaO and MgO had been applied to the field soil within 6 months prior to sampling to increase the soil pH in the field. It is considered that this has not impacted on the validity of the study.

III. CONCLUSION

CL 3000402 applied to a field soil up to a test concentration of 1.00 mg/kg soil dry weight caused no adverse effects (deviation from the solvent control < 25 %) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

CA 8.6 Effects on terrestrial non-target higher plants

For the first EU review of metrafenone a vegetative vigor study with six non-target plants performed with the formulation BAS 560 00 F was evaluated and considered acceptable. In addition, a seedling emergence study and a vegetative vigor study each evaluating ten non-target plant species were performed with the formulation BAS 560 02 F. These studies were also evaluated during the first EU review. An additional vegetative vigor and seeding emergence study with BAS 560 02 F, each evaluating six non-target plant species, are now also available and are submitted in support of this application.

A summary of the results are presented in the following table (only the endpoint for the most sensitive species tested is listed for simplicity).

Table 8.6-1: Effects of products containing metrafenone non-target terrestrial plants

Test substance	Study type	Most sensitive test species (& parameter)	ER_{50}	Reference
BAS 560 00 F	Vegetative vigor	All species were equivalent (all test parameters equivalent)	> 300 g a.s./ha	KCA 8.6.2/1 2002/1004862
BAS 560 02 F	Vegetative vigor	All species were equivalent (all test parameters equivalent)	> 300 g a.s./ha	KCA 8.6.2/2 2001/7000266
BAS 560 02 F	Vegetative vigor	Oats (*Avena sativa*) (plant height)	> 1500 g a.s./ha	KCA 8.6.2/3 2011/1000441
BAS 560 02 F	Seedling emergence	All species were equivalent (all test parameters equivalent)	> 300 g a.s./ha	KCA 8.6.2/4 2001/7000265
BAS 560 02 F	Seedling emergence	Peas (*Pisum sativum*) (emergence)	> 3000 g a.s./ha	KCA 8.6.2/5 2004/1025687

CA 8.6.1 Summary of screening data

Screening studies for the active substance have not been conducted.

CA 8.6.2 Testing on non-target plants

Report: CA 8.6.2/1
Oberwalder C.,Schmidt O., 2002a
BAS 560 00 F: Effects on non-target plants in the greenhouse - A limit test
2002/1004862
Guidelines: OECD 208 Revision
GLP: no
(certified by none)

Conclusion

Based on the results of this study it can be concluded that BAS 560 00 F has no phytotoxic potential to non-target plants if applied at a rate of 0.5 or 1.0 L BAS 560 00 F/ha (equivalent to 150 and 300 g a.s./ha) post-emergence at growth stage BBCH 12-14 to oats (*Avena sativa*), onion (*Allium cepa*), cabbage (*Brassica oleracea*), pea (*Pisum sativum*), carrot (*Daucus carota*) and sunflower (*Helianthus annuus*). For all plant species the plant weight was at the same level as the weight of the control plants. No statistically significant differences were observed.

The ER_{50} for terrestrial non-target plants based on plant weight and visual damage was determined to be > 300 g a.s./ha, the highest concentration tested, after 14 days of exposure. The NOER was determined to be 300 g a.s./ha. Please refer to Document M-III, Section 10, Point 10.8 of the summary dossier dated 2003 for further details.

Report:	CA 8.6.2/2 Porch J.R.,Krueger H.O., 2001a Effect of BAS 560 F 500 g/L SC (RLF12380) on vegetative vigor of ten species of plants 2001/7000266
Guidelines:	OECD 208, EPA 850.4150
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 8.6.2/3 Porch J.,Krueger H.O., 2001a Effect of BAS 560 F 500 g/L SC (RLF12380) on vegetative vigor of ten species of plants BN-123-038
Guidelines:	OECD 208 Revision, EPA 850.4150
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

In a vegetative vigor study ten terrestrial non-target plants (corn (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*), ryegrass (*Lolium perenne*), cucumber (*Cucumis sativa*), lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugarbeet (*Beta vulgaris*), and tomato (*Lycopersicon esculentum*)) were treated with BAS 560 02 F at rates of 100 or 300 g a.s./ha. There were no statistically significant treatment-related effects in any of the test species at both treatment rates.

The ER_{50} for terrestrial non-target plants based on plant height and dry weight was determined to be > 300 g a.s./ha, the highest concentration tested, after 21 days of exposure. NOER was determined to be 300 g a.s./ha. Please refer to Document M-III, Section 10, Point 10.8 of the summary dossier dated 2003 for further details.

Report:	CA 8.6.2/4 Minarski A., 2011a BAS 560 02 F: A test to determine the effects on non-target plants 2011/1000441
Guidelines:	OECD 227 July 2006, EPA 850.4250 Tier II
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a vegetative vigor test, three species of dicotyledonous plants (oilseed rape, pea and sunflower) and three species of monocotyledonous plants (onion, oats, corn) were exposed to BAS 560 02 F for 21 days under glasshouse conditions. The test item was applied post-emergence at growth stage BBCH 12 – 13 at nominal rates of 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 L/ha (equivalent to 46.88, 93.75, 187.5, 375, 750 and 1500 g a.s./ha). A negative control consisting of deionised water only was tested in parallel. Assessments for phytotoxicity and plant height were done 7, 14 and 21 days after application (DAA). Plant dry weight, of the plant biomass above ground, was determined at study termination (21 DAA).

After treatment with BAS 560 02 F, slight visible damages were only found in sunflower, oats and pea. Application of the highest tested treatment rate of 3.0 L BAS 560 02 F resulted in mean visible damages of 3%, 5% and 8% for sunflower, oats and pea, respectively. However, these damages only occurred in some replicates and not at all test concentrations (at 0.1875, 0.375, 0.750 and 3.0 L/ha for sunflower; at 0.375, 0.750 and 3.0 L/ha for oats). In addition, effects were below the average phytotoxicity score of 5% for sunflower (all concentrations) and oats (at 0.375 and 0.750 L/ha). No statistically significant reductions in plant weight were observed. Adverse effects to plant height were only observed for oats. The plant height of oats in the test rates of 0.75 and 3.0 L/ha BAS 560 02 F was statistically significantly reduced compared to the control, but less than 25%.

Based on the results of this study conducted under worst-case glasshouse conditions, it can be concluded that applications of BAS 560 02 F of up to 3.0 L/ha do not cause unacceptable effects on non-target terrestrial plants. All ER_{50} values were > 3.0 L BAS 560 02 F/ha (equivalent to > 1500 g a.s./ha), the highest concentration tested. The lowest no-observed-effect rate (NOER) was observed for the plant height of oats and was determined to be 1500 mL BAS 560 02 F/ha (equivalent to 750 g a.s./ha).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 02 F
Batch: 0002643758
Purity: 500.0 g metrafenone/L (nominal); 490.0 g/L (analyzed)
Description: Beige liquid

2. Test concentrations: 0 (control), 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 L/ha BAS 560 02 F (equivalent to 0, 46.88, 93.75, 187.5, 375, 750 and 1500 g a.s./ha)

3. Vehicle: Deionized water

4. Test soil: Sandy silt soil, with 5.1% clay, 35.1% silt, 59.8% sand, 0.93% organic carbon, a pH of 7.6 and a silt content of 1.32 g KCl/L
Test units: Plastic pots (13 cm diameter) with bottom watering

5. Test species:

Class	Family	Species	English Name	Variety
Dicotyledonae	*Brassicaceae*	*Brassica napus*	Oilseed rape	Prima
	Fabaceae	*Pisum sativum*	Pea	Norli
	Asteraceae	*Helianthus annuus*	Sunflower	Salut RM
Monocotyledonae	*Liliaceae*	*Allium cepa*	Onion	Pal
	Poaceae	*Avena sativa*	Oats	Aragon
	Poaceae	*Zea mays*	Corn	Ronaldinio

B. STUDY DESIGN

1. Environmental conditions:
Temperature: 18.8 – 32.1°C
Air humidity: 33 – 77%
pH of soil: 7.6
Photoperiod: 16 hours light: 8 hours darkness (8800 - 17500 lux)

2. Design and treatment

The seeds were sown into the pots at different density and depth at different time points, depending on the time required for the plant species to reach the two to four leaf growth stage. Each treatment included six replicates and each replicate pot contained six plants. The plants were exposed for 21 days.

3. Dose preparation

The treatment solutions were prepared just before application. First, a stock solution was prepared, by diluting the test item in deionized water, at a concentration of 17.72 g/L. This stock solution was further diluted with deionized water to obtain the required concentration range of 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 L/ha BAS 560 02 F (equivalent to 0, 46.88, 93.75, 187.5, 375, 750 and 1500 g a.s./ha). The control consisted of deionized water only. The treatment solutions (at 200 L water/ha) were sprayed upon the plants done by one application run of the nozzle (1.0 mm), with a spraying pressure of two bar.

4. Measurements and observations

The growth stage (BBCH code), mean plant height and degree of damage and phytotoxic symptoms were assessed for each replicate at 7, 14 and 21 days after application. At test termination, the plant dry weight was determined, by drying of the plants in a drying cupboard at 60ºC for at least 48 hours.

Temperature, air humidity and lighting conditions were recorded daily.

5. Statistics

Data of phytotoxicity and growth stage were not subjected to further evaluations. The data of plant dry weight and height were analyzed using Kolmogoroff-Smirnoff test for evaluation of normal distribution of data set ($\alpha = 0.05$) and one-way analysis of variance ($\alpha = 0.05$) was performed in case of variance homogeneity. Dunnett's t-test and Bonferroni-Welch-t test ($\alpha = 0.05$) were applied to determine statistically significant differences between control and treatment group.

II. RESULTS AND DISCUSSION

E. BIOLOGICAL EFFECTS

The mortality in the control group was 0%, the plants in this group did not show phytotoxicity throughout the test period and the rate of seedling emergence was >70% for all tested plant species in the control group.

After treatment with BAS 560 02 F slight visible damages were only found in sunflower, oats and pea. Application of the highest tested treatment rate of 3.0 L BAS 560 02 F resulted in mean visible damages of 3%, 5% and 8% for sunflower, oats and pea, respectively. However, these damages only occurred in some replicates and not at all test concentrations (at 0.1875, 0.375, 0.750 and 3.0 ml/ha for sunflower; at 0.375, 0.750 and 3.0 L/ha for oats). In addition, effects were below the average phytotoxicity score of 5% for sunflower (all concentrations) and oats (at 0.375 and 0.750 L/ha).

The plant dry weight was not affected for any of the tested plant species. No statistically significant reductions in plant weight were observed.

Adverse effects to plant height were only observed for oats. The plant height in the test rates of 0.75 and 3.0 L/ha BAS 560 02 F was statistically significantly reduced compared to the control, but less than 25%. The results are summarized in the table below.

Table 8.6.2-1: Effect of BAS 560 02 F on plant biomass and plant condition at 21 DAA

Treatment (L/ha)	Onion	Oats	Pea	Corn	Oilseed rape	Sunflower
Mean plant dry weight (% of control[a])						
control	100	100	100	100	100	100
0.0938	95	97	112	91	109	95
0.1875	91	89	90	88	105	104
0.375	93	99	89	87	103	101
0.75	93	100	92	95	102	98
1.5	89	96	73	101	95	101
3.0	94	87	92	90	100	100
Mean plant height (% of control[a])						
control	100	100	100	100	100	100
0.0938	103	98	103	100	105	100
0.1875	98	94	90	96	108	98
0.375	102	98	89	97	105	101
0.75	99	**91 ***	81	98	101	100
1.5	98	92	78	100	110	100
3.0	97	**90 ***	94	97	106	100
Mean visible damage (% damage compared to control[b])						
control	0	0	0	0	0	0
0.0938	0	0	0	0	0	0
0.1875	0	0	8	0	0	3
0.375	0	1	15	0	0	3
0.75	0	2	20	0	0	1
1.5	0	0	23	0	0	0
3.0	0	5	8	0	0	3

* Statistically significant different compared to the control (Dunnett's t-test, $\alpha = 0.05$).
[a] Compared to the control (control = 100%).
[b] Compared to the control (control = 0%).

F. DEFICIENCIES

None.

III. CONCLUSION

Applications of BAS 560 02 F up to 3.0 L/ha did not cause adverse effects on six species of non-target terrestrial plants, when exposed for 21 days. All ER_{50} values were > 3.0 L BAS 560 02 F/ha (equivalent to > 1500 g a.s./ha), the highest concentration tested. The lowest no-observed-effect rate (NOER) was observed for the plant height of oats and was determined to be 1500 mL BAS 560 02 F/ha (equivalent to 750 g a.s./ha).

Report:	CA 8.6.2/5 Porch J.R. et al., 2001a Effect of BAS 560 F 500 g/L SC (RLF 12380) on seedling emergence of ten species of plants 2001/7000265
Guidelines:	OECD 208, EPA 850.4100
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 8.6.2/6 Porch J.R. et al., 2001b Effect of bas 560 F 500 g/L SC (RLF12380) on seedling emergence of ten species of plants BN-123-037
Guidelines:	OECD 208 Revision, EPA 850.4100
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

In a seedling emergence study ten terrestrial non-target plants (corn (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*), ryegrass (*Lolium perenne*), cucumber (*Cucumbis sativa*), lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugarbeet (*Beta vulgaris*) and tomato (*Lycopersicon esculentum*)) were treated with BAS 560 02 F at rates of 100 or 300 g a.s./ha. There were no statistically significant treatment-related effects in any of the test species at both treatment rates.

The ER_{50} for ten terrestrial non-target plants based on emergence, plant height and dry weight was determined to be > 300 g a.s./ha, the highest concentration tested, after 21 days of exposure. The NOER was determined to be 300 g a.s./ha. Please refer to Document M-III, Section 10, Point 10.8 of the summary dossier dated 2003 for further details.

Report: CA 8.6.2/7
Stroemel C.,Teresiak H., 2004a
Effect of BAS 560 02 F on seedling emergence of six species of terrestrial plants
2004/1025687
Guidelines: OECD 208 A (Draft 2000)
GLP: yes
(certified by Land Brandenburg Ministerium fuer Landwirtschaft, Umweltschutz und Raumordnung, Potsdam, Germany)

Executive Summary

In a dose response test the phytotoxic potential of BAS 560 02 F on six terrestrial plant species (oilseed rape, pea, sunflower, onion, oats and corn) was evaluated. BAS 560 02 F was applied pre-emergence at test rates of 0.2, 0.6, 2.0 and 6.0 L/ha (equivalent to 100, 300, 1000 and 3000 g a.s./ha). Following the application the plants were cultivated under glasshouse conditions for 21 days. Assessments for seedling emergence and plant damage were done 7, 14 and 21 days after application (DAA). Shoot fresh weight was determined at study termination (21 DAA).

None of the tested plant species' seedling emergence, plant survival or biomass were adversely affected by the application of BAS 560 02 F at rates of up to 6.0 L/ha, except for pea plants. For pea, a slight but statistically significant reduction of 10% in seedling emergence was observed at the highest test rate of 6.0 L/ha. No statistically significant differences compared to the control were observed for any of the other tested species.

No unacceptable effects on seedling emergence, plant survival and plant biomass were observed 21 days after application of up to 6.0 L BAS 560 02 F/ha on six different plant species under glasshouse conditions. Therefore, the ER_{50} was > 6.0 L BAS 560 02 F/ha (equivalent to > 3000 g a.s./ha) for all tested plant species. The NOER was observed for the emergence of peas and was determined to be 6.0 L BAS 560 02 F/ha (equivalent to 3000 g a.s./ha).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: BAS 560 02 F
Batch: 2002
Purity: 500.0 g metrafenone/L (nominal); 504.0 g/L (analyzed)
Description: Liquid

2. Test concentrations: 0 (control), 0.2, 0.6, 2.0 and 6.0 L BAS 560 02 F/ha (equivalent to 0, 100, 300, 1000 and 3000 g a.s./ha)

3. Vehicle: Water

4. Test soil: Silty sand soil, mixed with peat-culture substrate, with 0.93 to 1.12% organic carbon.

Test units: Plastic pots (11 or 12 cm diameter) with bottom watering

5. Test species:

Class	Family	Species	English Name	Variety
Dicotyledonae	*Brassicaceae*	*Brassica napus*	Oilseed rape	Oase
	Fabaceae	*Pisum sativum*	Pea	Livioletta
	Asteraceae	*Helianthus annuus*	Sunflower	Barolo
Monocotyledonae	*Liliaceae*	*Allium cepa*	Onion	Red Baron
	Poaceae	*Avena sativa*	Oats	Fläming-profi
	Poaceae	*Zea mays*	Corn	Center

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 15.2 – 39.1 °C
Air humidity: 23.3 – 93.4%
pH of soil: 7.3 - 7.4
Photoperiod: ≥ 12 h light: ≤ 10 h darkness (additional light supply automatically when outdoor illumination was less than 10 klux for 12 h/day)

2. Design and treatment

Test plant species were seeded immediately before application. The test included four to seven replicates per treatment group. Each replicate comprised of one pot with six to ten seeds each. During the test, the pots were kept weed free by hand weeding. Fertilizer was added to each pot on a weekly basis, with universal fertilizer and N-fertilizer for all plant species, and additional P-fertilizer for corn. The study was terminated 21 days after application.

3. Dose preparation

The treatment solutions were prepared just before application. The test item was weighed for each treatment separately and then dissolved in the respective water volume to achieve the target dose based on a mean output volume of sprayer at 233 L/ha. The test included five treatment groups; with exposure to 0.200, 0.600, 2.0 and 6.0 L BAS 560 02 F/ha or water only (control). Applications were applied via a calibrated spray chamber “Spraylab 210/110-SPS”.

4. Measurements and observations

Plant emergence was determined for each plant species one week after application. At this time at least 50% of the seeds in the control group had emerged, thus all other assessment were counted as total days from that point (days after application = DAA). Assessments included signs of phytotoxicity on a weekly bases (days 7, 14 and 21), plant survival and fresh weight on 21 DAA.

Samples of the solution were taken from each treatment before application and stored frozen. Samples from the control and of the lowest and highest test item rates were analyzed using a HPLC method with UV detection.

Temperature, air humidity and lighting conditions were recorded daily.

5. Statistics

Data of phytotoxicity was not subjected to further evaluations. Weight data were analyzed for outliers using Statistika for Windowns of StatSoft Inc (USA) and outliers were excluded from the analysis. Data for plant mortality and biomass were statistically analyzed using Barlett's test (α = 0.05) for homogeneity. Differences between treatment groups were analyzed using analysis of variance, followed by the Dunnett's t-test (α = 0.05). In case of non-homogenous variance the Welch-t test with Bonferroni adjustment was used. Statistical analysis was performed using ToxRat Standard (version 2.09).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

All emerged control plants remained healthy throughout the study. No control mortality was observed and the rate of emergence in the controls was > 65%. The only observed effect was for pea plants, which showed a statistically significant reduction of 10% in seedling emergence at the highest test rate of 6.0 L/ha. No statistically significant differences compared to the control were observed for seedling emergence, plant survival, or biomass of any of the other tested species, after the application of BAS 560 02 F at rates of up to 6.0 L/ha.

The results are summarized in the table below.

Table 8.6.2-2: Effects of BAS 560 02 F on seedling emergence, phytotoxicity and plant biomass at 21 DAA

Treatment (L/ha)	Pea	Oilseed rape	Onion	Oat	Sunflower	Corn
Seedling emergence (%[a])						
control	100	100	100	100	100	100
0.2	95	95	103	105	100	100
0.6	93	98	97	103	102	100
2.0	95	100	92	100	100	98
6.0	**90 ***	100	100	100	98	100
Visible phytotoxicity (%[b])						
control	0	0	0	0	0	0
0.2	2 #	0	0	0	0	0
0.6	1 #	0	0	0	0	0
2.0	0	0	0	0	0	0
6.0	6 #	0	0	0	0	0
Mean shoot fresh weight (%[a])						
control	100	100	100	100	100	100
0.2	100	104	104	105	99	100
0.6	93	102	85	105	104	103
2.0	90	105	94	103	97	99
6.0	90	101	99	102	103	100

* Statistically significant different compared to the control (Dunnett's t-test, α = 0.05).
# Symptom: stunting.
[a] Compared to the control (control = 100%).
[b] Compared to the control (control = 0%).

B. DEFICIENCIES

None.

III. CONCLUSION

No unacceptable effects on seedling emergence, plant survival, and plant biomass were observed 21 days after application of up to 6.0 L BAS 560 02 F/ha on six different plant species under glasshouse conditions. Therefore, the ER_{50} was > 6.0 L BAS 560 02 F/ha (equivalent to > 3000 g a.s./ha) for all tested plant species. The NOER was observed for the emergence of peas and was determined to be 6.0 L BAS 560 02 F/ha (equivalent to 3000 g a.s./ha).

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No further data are being submitted. The information provided in the preceding annex points is considered sufficient to assess the potential toxicity of metrafenone to non-target organisms.

CA 8.8 Effects on biological methods for sewage treatment

One study with activated sludge exposed to the active substance (metrafenone) is available, which was previously reviewed and considered acceptable for the first EU Review of metrafenone.

The available data for activated sludge are summarized in the table below.

Table 8.8-1: Summary of the toxicity of metrafenone to activated sludge

Test material	Species	Time scale, study type	End-point	Result (mg a.s./L)	Reference
Metrafenone technical	Activated sludge	3 hrs	EC_{50}	> 600	KCA 8.8/1 2002/7004605

Report:	CA 8.8/1 Hicks S.,Canez V., 2002a BAS 560 F (AC 375839): Activated sludge, respiration inhibition test 2002/7004605
Guidelines:	EEC 91/414 Annex II 8.7, EEC 96/12, OECD 209
GLP:	yes (certified by United States Environmental Protection Agency)

Conclusion

The test results indicated that metrafenone did not have significant inhibitory effects on the respiration rate of activated sludge collected from a wastewater treatment plant up to a test concentration of 600 mg a.s./L. Hence, the EC_{50} value for BAS 560 F is > 600 mg a.s/L. Please refer to Document M-II, Section 6, Point 8.7 of the summary dossier dated 2002 for further details.

CA 8.9 Monitoring data

No data submitted.

We create chemistry

Metrafenone

Document M-CA, Section 9

LITERATURE DATA

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 9 LITERATURE DATA

A review of the published literature on metrafenone, its impurities and degradation products of interest impurities and formulations has been performed by BASF Group Information Center. The literature Search Report on metrafenone describes the general search methodology and search terms as well as details on search profiles, search histories and tables providing summaries of the results.

The complete search report is provided in K-CA 9 (BASF Doc ID 2015/1215510)

The search identified a number of published papers (records) which were then analysed in two step process to assess whether records were relevant and reliable. The analysis was performed by experts in the relevant fields.

The first step was based on a consideration of the title and abstract of each record found. In many cases, "records" were identified as clearly irrelevant (or "ballast") on the basis of their titles and / or abstracts. Many of these publications concerned analytical methods or the effectiveness of plant protection products. In most other cases, records were categorised as not relevant on the basis of the abstract.

For the remaining "records", the full articles were obtained and reviewed and then an assessment of relevance and if appropriate their reliability was then made. The reliability assessment for relevant studies was carried out according to Klimisch *et al.* (1997)[1]

Published papers which should be included in the dossier were selected on the following basis:

> Records providing information about additional/new/unknown/potentially contradictory effects or data which might impact the hazard assessment endpoints or the risk assessments parameters and which in addition have a high grade of reliability of grade 1 or 2 based on the 'Klimisch' scoring system

The process is documented in EXCEL files which are appended to the search report in K-CA 9.

Conclusion

In a comprehensive review of the open literature, only one reference was considered to be both relevant and reliable (Reliability 2: reliable with restrictions, according to Klimisch *et al.*, 1997.

> Fernandez, D., Vermeirssen, E. L. M. and Bandow, N. Calibration and field application of passive sampling for episodic exposure to polar organic pesticides in streams. Environmental Pollution (Oxford, United Kingdom) (2014), 194, 196-202 CODEN: ENPOEK; ISSN: 0269-7491

[1] Klimisch, H-J., Andreae, M. & Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology **25** pp 1-5

A summary of this paper is presented in Document M-CA, Section 7, Point CA 7.5.

No references were identified that could have a significant effect on the regulatory assessment of human health, animal health or the environment for the active substance, metrafenone.

We create chemistry

Metrafenone

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O Box 120
67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
e-mail

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

Table of Contents

CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Classification and labelling of the active substance in accordance with the provisions of Regulation (EC) No 1272/2008 of the European Parliament and Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures[1] (health and environment effects).

Classification proposed

Hazard Category	Aquatic Acute 1 Aquatic Chronic 2

Labelling proposed

GHS Pictogram:	
Signal Word:	Warning
Hazard Statement:	H400: Very toxic to aquatic life H411: Toxic to aquatic life with long lasting effects
Supplemental Hazard Information:	EUH401: To avoid risks to human health and the environment, comply with the instructions for use
Precautionary Statement: Prevention	P273: Avoid release to the environment.
Precautionary Statement Response	P391: Collect spillage
Precautionary Statement Storage	
Precautionary Statement Disposal	P501: Dispose of contents/container to hazardous or special waste collection point

[1] OJL 353, 31.12.2008, p. 1.