Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

metaflumizone (ISO); (*EZ*)-2'-[2-(4-cyanophenyl)-1-(α,α,α -trifluoro-m-tolyl)ethylidene]-[4-(trifluoromethoxy)phenyl]carbanilohydrazide [*E*-isomer ≥ 90%, *Z*-isomer ≤10% relative content] [1]

(*E*)-2'-[2-(4-cyanophenyl)-1-(α,α,α -trifluoro-m-tolyl)ethylidene]-[4-(trifluoromethoxy)phenyl]carbanilohydrazide [2]

EC Number: -
CAS Number: [1] 139968-49-3; [2] 852403-68-0

CLH-O-0000001412-86-179/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Metaflumizone

EC Number: Not yet assigned

CAS Number: 139968-49-3

Index Number: Not yet assigned

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Version number: **1** **Date:** **March 2016**

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Part A.

7 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

7.1 Substance

Table 1: Substance identity

Substance name:	***Metaflumizone***
EC number:	***Not yet assigned***
CAS number:	***139968-49-3****
Annex VI Index number:	***Not yet assigned***
Degree of purity:	***94.5% (85-100% E-isomer and 0-10 % Z-isomer)***
Impurities:	***Relevant impurities identified as isocyanate*, hydrazine and toluene. There are a number of other process impurities, these have been taken into account and are not considered to impact on the proposed classification and labelling.***

* *As included in the DAR and EFSA conclusion*

7.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	STOT-RE 2: H373 - May cause damage to organs through prolonged or repeated oral or inhalation exposure Repr. Tox. 2: H361d - Suspected of damaging the unborn child Lact.: H362 – May cause harm to breast fed children
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	STOT-RE-2: H373 - May cause damage to organs through prolonged or repeated oral or inhalation exposure Repr. Tox. 2: H361d - Suspected of damaging the unborn child Lact.: H362 – May cause harm to breast fed children

7.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	**Repr. Tox. 2: H361d (Suspected of damaging the unborn child)** **Lact.: H362 (May cause harm to breast-fed children)**	None	Not classified	-
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	**STOT-RE 2: H373 (May cause damage to organs through prolonged or repeated oral or inhalation exposure)**	**None**	Not classified	-
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

4.1.	Hazardous to the aquatic environment	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s): **GHS08**

Signal word: **Warning**

Hazard statements: **H373 - May cause damage to organs through prolonged or repeated oral or inhalation exposure**
H361d - Suspected of damaging the unborn child
H362 - May cause harm to breast-fed children

Precautionary statements: **Not included in Annex VI**

8 BACKGROUND TO THE CLH PROPOSAL

8.1 History of the previous classification and labelling

Metaflumizone is a new pesticide active substance and has been reviewed in accordance with Directive 91/414/EEC with the UK as the Rapporteur Member State (RMS). There is no existing entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance. In accordance with Article 36(2) of the CLP Regulation, metaflumizone should now be considered for harmonised classification and labelling. Therefore, this CLH proposal considers all human health and environmental endpoints with the aim of achieving such.

At the time of submission, the substance is not registered under REACH.

8.2 Short summary of the scientific justification for the CLH proposal

Metaflumizone is an insecticide used on potatoes, tomatoes and peppers. Following peer-review of the Draft Assessment Report (DAR), EFSA concluded (EFSA Journal 2013; 11(10):3373) that metaflumizone was of low acute oral toxicity in rats and mice, and low dermal and inhalation toxicity in rats. It was not considered a skin irritant or sensitiser; however, it was a slight eye irritant in rabbits, although not sufficient for classification. These conclusions are supported in the CLH report and it is not proposed to classify metaflumizone for these hazard classes.

Repeated exposure to metaflumizone via the oral, dermal and inhalation routes was characterised primarily by effects on body weight, body weight gain and food consumption. Target organs were considered to be the spleen and liver in dogs. Concern for classification with STOT-RE 2 (H373) was raised in the EFSA conclusion based on premature sacrifices in dogs at doses ≥ 30 mg/kg bw/day. **Classification with STOT-RE 2; H373 – May cause damage to organs through prolonged or repeated oral or inhalation exposure,** is proposed in this report based on severe and consistent effects on body weight and premature sacrifices or death in dogs, mice and rats.

Metaflumizone was considered not genotoxic in humans and there was no evidence of carcinogenic potential in rat and mouse studies. Therefore it is not proposed to classify metaflumizone for mutagenicity or carcinogenicty.

Metaflumizone caused adverse effects during the lactation period in a rat multi-generation study and was shown to be excreted in milk; therefore, EFSA raised concern for classification with Lact.; H362 – May cause harm to breast-fed children. In developmental toxicity studies there was an increased incidence of absent subclavian artery in rabbit fetuses leading EFSA to suggest classification with Repr. 2; H361d. This report agrees with these conclusions and proposes classification with both **Repr. 2; H361d – Suspected of damaging the unborn child and Lact.; H362 – May cause harm to breast-fed children.**

Metaflumizone is considered not rapidly degradable for the purpose of classification and labelling and both the Log Kow and representative fish BCFs are above the trigger values intended to identify a substance with a potential to bioaccumulate (i.e., ≥ 4 and ≥ 500 respectively). Aquatic acute toxicity data on metaflumizone are available for fish, invertebrates, algae and aquatic plants. No acute/short-term $L(E)C_{50}$ endpoints were observed for fish, invertebrates or algae/aquatic plants up to the quoted limit of water solubility using metaflumizone (0.00181 mg/l at 20ºC and pH 7). **Overall, metaflumizone should not be classified for Aquatic Acute classification**.

Chronic toxicity data on metaflumizone are available for fish, invertebrates, algae and aquatic plants using standard test species. There are no valid chronic endpoints below the quoted limit of water solubility of metaflumizone. Metaflumizone meets the following criteria for classification as Aquatic Chronic 4:

- no acute toxicity recorded at levels up to the water solubility (poorly soluble substance with water solubility < 1 mg/l)
- not rapidly degradable
- experimentally determined BCF ≥ 500

However, valid chronic NOECs > 1 mg/l or > the quoted limit of water solubility are available for standard hazard classification surrogate test species. On this basis the eMS is of the opinion that Aquatic Chronic 4 is not applicable based on the data available at the time of submission.

Overall, it is proposed that metaflumizone should not be classified for Aquatic Chronic hazard.

8.3 Current harmonised classification and labelling

8.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Metaflumizone is not currently listed in Annex VI to the CLP Regulation.

8.4 Current self-classification and labelling

8.4.1 Current self-classification and labelling

At the time of submission there are a number of self-classification entries for metaflumizone in the C&L inventory. These are tabulated below:

Classification		Labelling	
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Stot RE 2 Aquatic Acute 1 Aquatic chronic 1	H373 (not specified) H400 H410	H373 H400 H410	GHS09 GHS08 Warning
Stot RE 2 Aquatic Acute 1 Aquatic chronic 1	H373 (not specified) H400 H410	H373 H410	GHS07 GHS08 Warning
Aquatic Acute 1	H400	H400	GHS09 Warning
Not classified			

NB. While the basis of environmental self-classification is unclear it is assumed to relate to the proposed classification of N;R50/53 (under the Dangerous Substances Directive 67/548/EEC) in the DAR Vol.1 (2012) which would have been translated under CLP to Aquatic Acute 1, Aquatic Chronic 1. The basis for the proposal under EU Regulation 1107/2009 is quoted as 'Toxicity to *Oncorhynchus mykiss* (Rainbow trout) <1mg/l'.

The Regulation 1107/2009 review concluded the *Oncorhynchus mykiss* 96-h LC_{50} for the active substance was >0.0378 mg a.s./l based on the highest mean measured concentration at which the active substance appeared to be dissolved [60]. At this concentration 5% mortality was recorded.

An acute toxicity study on *Oncorhynchus mykiss* with the preparation BAS 320 00I (2004) [88] (suspension concentrate containing 240 g metaflumizone/L) resulted in a 96-h LC_{50} of 0.732 mg a.s./l based on mean measured concentrations. In the study effects were only observed at the highest test concentration where 75% mortality was observed. Under review for Regulation 1107/2009, it was noted that undissolved material was present in higher concentration exposure solutions. The highest test concentration where test material was considered dissolved was 0.228 mg/l a.s./l (based on mean measured concentrations). At this concentration zero mortality was observed. On this basis the 96-h LC_{50} was considered >0.228 mg.a.s./l.

From the DAR Vol. 3, B.9.2.7 (2012), it appears the preparation study forms the basis of the earlier R50 classification proposal although it is not clear how the quoted LC_{50} met the R50 criteria. This approach using the preparation study is not supported in this CLH proposal possibly resulting in the difference between self-classification and that proposed for harmonisation.

RAC general comment
There is no existing entry in Annex VI of CLP for metaflumizone. Therefore, all human health and environmental endpoints have been evaluated. Metaflumizone consists of two stereoisomers: in the substance evaluated the E and Z isomer content was 91% and 6.35%, on average respectively. With regard to impurities, there is no concern for classification at the proposed levels. Individual impurities are discussed in the DAR in detail.

9 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Metaflumizone is a pesticide active substance in the scope of Reg 1107/2009. In accordance with Article 36 (2) of the CLP Regulation metaflumizone should be subject to harmonised classification and labelling, taking into consideration all human health and environmental endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

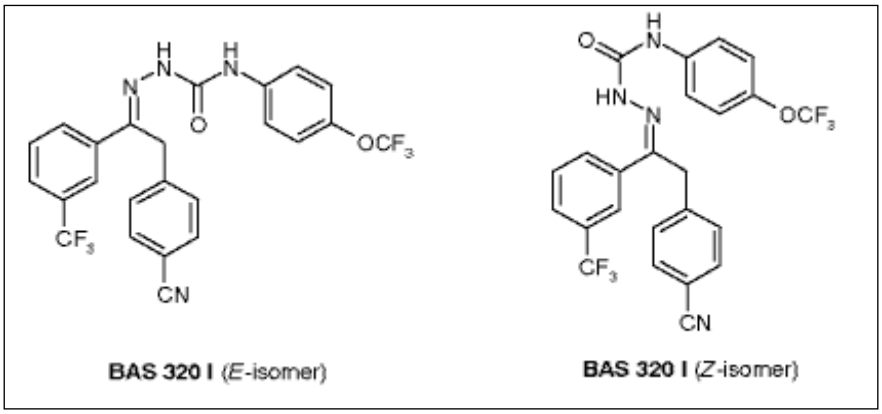
1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not assigned
EC name:	Not assigned
CAS number (EC inventory):	Not assigned
CAS number:	139968-49-3*
CAS name:	Hydrazinecarboxamide, 2-[2-4-cyanophenyl)-1-[3-(trifluoromethyl)phenyl]ethylidene]-N-[4-(trifluoromethoxy)phenyl]-
IUPAC name:	(*EZ*)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-*m*-tolyl)ethylidene]-[4-(trifluoromethoxy)phenyl]carbanilohydrazide*
CLP Annex VI Index number:	Not yet assigned
Molecular formula:	$C_{24}H_{16}N_4O_2F_6$
Molecular weight range:	506.4

As included in the DAR and EFSA conclusion

Structural formula:



1.2 Composition of the substance

Metaflumizone consists of two stereoisomers: the *E* and the *Z* as shown above. In the evaluated substance the *E*/*Z* ratio was approximately 90:10.

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Metaflumizone	97.3%	94.5 - 100%	
E-isomer	91.0%	85 - 100%	
Z-isomer	6.35%	0 - 9.5%	

The ISO name metaflumizone is associated with the IUPAC name (*EZ*)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-*m*-tolyl)ethylidene]-[4-(trifluoromethoxy)phenyl]carbanilohydrazide. However, it is noted that the E-isomer (CAS 852403-68-0) is the main constituent, present at concentration levels > 80%. Therefore, the substance should be identified as the E-isomer; (*E*)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-*m*-tolyl)ethylidene]-[4-(trifluoromethoxy)phenyl]carbanilohydrazide (CAS 852403-68-0). However, for reasons of clarity and consistency with the active substance identification, it is proposed that both terms are included in the Annex VI entry.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
4-(trifluoromethoxy)phenyl isocyanate *	-	≤ 100 mg/kg	No concern for classification at the proposed levels
Hydrazine*	-	≤ 1 mg/kg	No toxicological concern for classification at the proposed levels
Toluene*	-	≤ 2 g/kg	No toxicological concern for classification at the proposed levels

Impurities listed as toxicologically relevant in the EFSA conclusion
* As listed in the EFSA conclusion. Full details are provided in the technical dossier.

There are a number of other process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further

information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Current Annex VI entry:

Table 6a Hydrazine:

Classification		Labelling		Specific Concentration limits, M-Factors
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**	
Flam. Liq. 3	H226	H226	GHS02	Skin Irrit. 2; H315: 3% ≤ C < 10%
Acute Tox. 3 *	H301	H301	GHS06	Skin Corr. 1B; H314: C ≥ 10%
Acute Tox. 3 *	H311	H311	GHS09	Eye Irrit. 2; H319: 3% ≤ C < 10%
Skin Corr. 1B	H314	H314	GHS05	
Skin Sens. 1	H317	H317	GHS08	
Acute Tox. 3 *	H331	H331	Dgr	
Carc. 1B	H350	H350		
Aquatic Acute 1	H400			
Aquatic Chronic 1	H410	H410		

Table 6b Toluene:

Classification		Labelling		Specific Concentration limits, M-Factors
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**	
Flam. Liq. 2	H225	H225	GHS07	
Asp. Tox. 1	H304	H304	GHS02	
Skin Irrit. 2	H315	H315	GHS08	
STOT SE 3	H336	H336	Dgr	
Repr. 2	H361d ***	H361d ***		
STOT RE 2 *	H373 **	H373 **		

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The substance used in the physico-chemical, toxicology and environmental studies was considered to be equivalent to that described above.

1.3 Physico-chemical properties

The physic-chemical properties of metaflumizone are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.2; Physical and Chemical properties – May 2012.

All studies were conducted to appropriate quality standards and were considered adequate during the peer review.

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid powder	Kaestel R (2003a and 2003 b) [1][2]	Visual inspection Purity: 97.8 % DAR (B.2.1.7)
Melting/freezing point	Two melting peaks: 133 °C and 188 °C	Kaestel R (2003 b)[2]	EEC method A1 (DSC) Purity: 97.8 % DAR (B.2.1.1)
Boiling point	Metaflumizone decomposes prior to boiling (decomposition temperature ~ 232 °C)	Kaestel R (2003 b)[2]	EEC method A1 (DSC) Purity 97.8 % DAR (B.2.1.2)
Relative density	Mixture of E/Z (unspecified ratio): 1.433 E isomer: 1.446 Z isomer: 1.461 at 20 °C	Kaestel R (2005a and 2005 b)[1][2]	EEC method A3 1.4.3 (pycnometer) Purity: 98.7 % (E, Z 98.7 – 99.4 %) DAR (B.2.1.4)
Vapour pressure	Mixture of E/Z (unspecified ratio): 1.24×10^{-8} Pa at 20 °C 3.41×10^{-8} Pa at 25 °C E isomer: 7.94×10^{-10} Pa at 20 °C 2.46×10^{-9} Pa at 25 °C Z isomer: 2.42×10^{-7} Pa at 20 °C 5.82×10^{-7} Pa at 25 °C Very slightly volatile	Yacoub R. (2004a)[3]	OECD 104 (Thermogravimetric analyser. Vp at 20 and 25 °C calculated by extrapolation) Purity: 100 % (E 98.7 %, Z 96.9 %) DAR (B.2.1.5)

Surface tension	70.5 mN/m (0.01 %) 70.1 mN/m (0.1 %) at 20 °C Not surface active	Kaestel R. (2003a)[1]	EEC method A5 1.6.1 (plate method) DAR (B.1.24)
Water solubility	Mixture of E/Z (92.2:7.8): pH 5 – 1.35 µg/l pH 7 – 1.81 µg/l pH 9 – 1.73 µg/l Deionized water – 1.79 µg/l E isomer: 1.43 µg/l Z isomer: 2.03 µg/l Determined in deionized water at 20 °C (pH 8.1 – 8.7)	Yan Z. (2001)[4]	EEC method A6 1.4.1 (column elution method) Purity: 100 % (E:Z), 98.7 % (E), 99.4 % (Z) DAR (B.2.1.11)
Partition coefficient n-octanol/water	Mixture of E/Z [90 % (min):10 % (max)] $LogP_{ow}$ at pH 5 and 30 °C: Z isomer: 4.4 E isomer: 5.1 E and Z isomers: $LogP_{ow}$ at pH 7 and 20 °C: Z isomer: 4.2 E isomer: 4.9 $LogP_{ow}$ at pH 3 and 20 °C: Z isomer: 3.8 E isomer: 4.4	Holman J.C., Petry A.S. (2001)[5] Class T. (2006a and b)[6][7]	EEC method A8 (HPLC method) Purity: 96.3 % EEC method A8 (HPLC method) Purity: 98.7 % (E), 99.7 % (Z) DAR (B.2.1.13)
Flash point	Not tested or required as the melting point is above 40 °C		DAR (B.2.1.21)
Flammability	Test substance did not burn under conditions of the test. Experience in handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with air.	Loeffler U. 2003[8]	EEC method A10 – flammability Purity: 96.3 % DAR (B.2.1.20)

Explosive properties	Exothermic decomposition energy < 500 J/g 1st exothermic reaction – energy release = 240 J/g 2nd exothermic reaction – energy release > 130 J/g (part of 2nd reaction was outside the measuring range – measured up to ~490 °C) Not explosive.	Loeffler U. 2003[8]	Exothermic decomposition energy calculation Purity: 96.3% DAR (B.2.1.22)
Self-ignition temperature	No self-heating was observed up to the melting point.	Loeffler U. 2003[8]	EEC method A16 – auto-flammability Purity: 96.3 % DAR (B.2.1.20)
Oxidising properties	Not tested. The chemical structure does not contain any structural alerts indicative of oxidising properties. Not oxidising	Loeffler U. 2003[8]	DAR (B.2.1.23)
Dissociation constant	Mixture of E/Z [90 % (min):10 % (max)], E isomer, Z isomer: Over the pH range of 2 – 12a dissociation constant does not exist for all forms of metaflumizone tested (no significant spectral shifts were observed)	Petry A.S. (2001)[9]	OECD TG 112 Purity: Mixture of E/Z (97.8 %), E (98.7 %), Z (99.4 %) DAR (B.2.1.18)

2 MANUFACTURE AND USES

2.1 Manufacture

Metaflumizone is manufactured within the EU for use as a pesticidal active substance.

2.2 Identified uses

The representative uses of metaflumizone are foliar spraying of potato crops against the Colorado beetle and also against chewing insect pests on tomatoes and peppers.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Summary of relevant physico-chemical studies

Please refer to table 8

3.1 Physico-chemical properties

3.1.1 Summary and discussion of physico-chemical properties

In a standard study (EEC Method A10), metaflumizone did not burn under the standard test conditions therefore, it does not meet the criteria for classification as a flammable solid. No self-ignition was observed up to the temperature of the melting point. Further, experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.

The exothermic decomposition energy of metaflumizone was measured and determined to be less than 500 J/g; based on this result, EEC Method A14 was not conducted. Metaflumizone was therefore shown to not have explosive properties.

Finally, metaflumizone was not considered an oxidiser based on analysis of the chemical structure, oxygen and fluorine atoms are chemically bonded to carbon or hydrogen atoms only.

3.1.2 Comparison with criteria

Metaflumizone did not meet the criteria for classification for physico-chemical hazards.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Information provided in the CLH report summarised that metaflumizone is not flammable and not self-ignitable and does not evolve flammable gases in contact with water. Metaflumizone has no explosive properties.

The dossier submitter (DS) proposed no classification for physical hazards.

Comments received during public consultation

No comments on physical hazards were received during the public consultation.

Assessment and comparison with the classification criteria

Metaflumizone does not meet the relevant CLP criteria and therefore RAC supports the proposal of the DS not to classify metaflumizone for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

References are taken from the Draft Assessment Report – DAR – Metaflumizone – Volume 3, Annex B.6; toxicology and metabolism – May 2012.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The absorption, distribution, metabolism and elimination of radiolabelled metaflumizone has been investigated in Sprague-Dawley rats in a number of experiments in which animals were administered test material as a suspension in 0.5 % aqueous carboxymethylcellulose (CMC) at doses of 30 and 1000 mg/kg bw. A number of additional bioavailability studies were also performed in Wistar rats to investigate the effects of dose, vehicle and type of administration (gavage versus dietary). The experiments were performed with ^{14}C-labelled metaflumizone, labelled either in the benzonitrile ring (B-label) or in the trifluoromethoxyphenyl ring (T-label).

Absorption

Metaflumizone was found to be relatively rapidly absorbed following single oral doses (gavage) to cannulated rats. Kinetics differed depending on whether a low or high dose was administered and the extent of oral absorption was inversely proportional to the dose level. The type of vehicle used had an effect on absorption, with the addition of a surfactant causing increased bioavailability. Bioavailability following administration in CMC was 16.8 % and with cremaphor it was 33.4 %. Investigations into the effects of dietary administration versus gavage showed that following dietary administration the absorption of metaflumizone may be higher. The difference in absorption could be due to a function of metaflumizone's high lipophilicity (E isomer LogP = 4.2, Z isomer Log P = 4.9).

Distribution

Tissue and organ distribution experiments demonstrated that [^{14}C]-metaflumizone was rapidly distributed to the muscle, liver, kidney and fat within 0.5 – 2 hours. Total radioactive residues reached a maximal level at or near the blood T_{max} in both male and female rats, irrespective of dose. At or near the T_{max}, levels of radioactivity were greatest in the fat or liver, followed by the kidney and blood/plasma/muscle. The volume of distribution was high, indicating extensive tissue binding or association.

In an accumulation study, [^{14}C]-metaflumizone was administered to rats for 14 days (30 mg/kg bw/day). Levels of radioactivity in tissues were increased compared to after a single oral dose. Levels were 43 times higher in fat, 26 times higher in muscle and plasma and 13 times higher in liver and kidney. Other tissues noted to have high levels of radioactivity were adrenals, pancreas, liver and female sex organs.

Metabolism

Metaflumizone was metabolized in the rat *via* hydroxylation of the aniline or benzonitrile ring and hydrolysis of the central hydrazine carboxamide group to yield the aniline derivatives and phenacylbenzoylnitrile derivatives. The trifluoromethoxyaniline was shown to conjugate with malonic and oxalic acids. The ring-hydroxylated derivatives were readily conjugated with sulphate or glucuronic acid. Glycine conjugation occurred at the carboxyl group of the cyanobenzoic acid, whereas glutathione conjugation occurred by displacement of one of the fluorine atoms of the

trifluoromethyl or trifluoromethoxy group to form S-(N-(N-γ-glutamyl)-cysteinyl-, glycyl-conjugate. Analysis of tissues after a single oral dose of 30 or 1000 mg/kg bw metaflumizone revealed that the major residue present was unchanged parent. Other components were minimal.

Excretion

Excretion was found to be predominantly via the faeces. Biliary excretion accounted for > 5 % of the administered dose: excretion of the B-label was greater than the T-label, and was proportionally higher at the high dose level than at the low dose level. Urinary excretion accounted for ≤ 0.5 % of the administered dose. Recovery of radioactivity in tissues and excreta at 168 hours after administration in the excretion balance study confirmed that excretion was predominantly faecal, and a similarly low urinary excretion (< 2 %) was demonstrated.

4.1.2 Human information

There are no data on human toxicokinetics of metaflumizone.

4.1.3 Summary and discussion on toxicokinetics

Metaflumizone has been shown to be relatively rapidly absorbed and excreted in a number of toxicokinetic studies in rats. Levels of metaflumizone were highest in fat, muscle, liver, kidney and plasma, with a clear potential for accumulation in fat. Mainly excreted as unchanged parent in faeces, metaflumizone has limited metabolism. The extent of oral absorption was found to be highly variable, influenced by the mode of administration (dietary > gavage), the dose level (low dose > high dose) and the dosing vehicle (surfactant > aqueous). The agreed oral absorption value was 16.8 % and the calculated lipophilicity was 4.2-4.9.

4.2 Acute toxicity

Table 9: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
Rat, Sprague-Dawley (5/sex) (2000) [10] 0 and 5000 mg/kg bw 0.5 % Carboxymethylcellulose (CMC) (aq.) OECD 401 (1987) GLP Purity 96.3 % DAR (B.6.2.1a)	> 5000 mg/kg bw	No mortality. Animals appeared healthy and there were no gross internal findings.
Mouse, CD-1 (5/sex) (2001) [11] 0 & 5000 mg/kg bw 0.5 % CMC (aq.) OECD 401 (1987) GLP Purity 96.3 % DAR (B.6.2.1b)	> 5000 mg/kg bw	No mortality. Animals appeared healthy and there were no gross internal findings.
Acute Inhalation		
Method	**LC_{50}**	**Observations and remarks**
Rat, Wistar (5/sex) (2002) [12] O & 5.3 mg/L (MMAD 3.6 ± 3.2 µm) 4 h, nose only (dust) OECD 403 GLP Purity 96.9 % DAR (B.6.2.3)	> 5.3 mg/L	No mortality. Accelerated breathing and attempts to escape. Squatting posture and smeared fur on the exposure day until day 6. Necropsy: Red discolouration of the lobes of the lungs was observed in all animals.

Acute Dermal		
Method	**LD_{50}**	**Observations and remarks**
Rat, Sprague-Dawley (5/sex) (2001) [13] 5000 mg/kg bw Occlusive, 24 h OECD 402 (1987) GLP Purity 96.3 % DAR (B.6.2.2)	> 5000 mg/kg bw	One animal died, 15 min post-dosing (not treatment-related) Animals appeared healthy and there were no gross internal findings.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Two guideline acute toxicity experiments by the oral route are available, one in rats and one in mice [10, 11]. Sprague-Dawley rats (five/sex) and CD-1 mice (five/sex) were administered a single oral limit dose of metaflumizone (5000 mg/kg bw) in aqueous carboxymethylcellulose and were observed for fourteen days.

All animals survived, gained weight and appeared healthy throughout the study and there were no gross internal findings at necropsy. The LD_{50} was > 5000 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

One guideline acute toxicity study by the inhalation route is available [12]. Wistar rats (5/sex) were exposed (nose only) to metaflumizone at an analytically determined concentration of 5.3 mg/L (MMAD 3.6 ± 3.2 µm) for four hours. The test substance was prepared with 2 % Aerosil 200 in order to improve dust formation. Animals were observed for fourteen days.

All animals survived the study; however there were signs of toxicity. These included accelerated respiration and attempts to escape. Also noted were squatting posture and smeared fur which began on the exposure day and persisted until day six for both sexes. Mean body weights of the males increased during the study whereas females lost weight during the first week but gained weight during the second, their initial body weight being exceeded by the end of the study. Red discolouration of the lungs was observed in all animals at necropsy. The LC_{50} was > 5.3 mg/L.

4.2.1.3 Acute toxicity: dermal

One guideline acute toxicity study by the dermal route is available, in rats [13]. Sprague-Dawley rats (5/sex) were exposed to a single application of metaflumizone at 5000 mg/kg bw. The test material was applied to the shorn dorsal skin and covered with an occlusive dressing for 24 hours. Animals were then observed for fourteen days.

One male was found dead, fifteen minutes post-dosing. The study report stated that the death did not appear to be treatment-related because there were no clinical signs of toxicity or mortality observed in the remaining animals during the fourteen day study. All surviving animals gained weight and

appeared healthy throughout the study and there were no gross internal findings at necropsy. The LD_{50} was > 5000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

There are no human data available.

4.2.3 Summary and discussion of acute toxicity

Metaflumizone was found to be of low toxicity by the oral, inhalation and dermal routes following a single exposure in rats and mice, with LD_{50} > 5000 mg/kg bw for oral and dermal routes and LC_{50} > 5.3 mg/L following inhalation exposure.

4.2.4 Comparison with criteria

The oral LD_{50} value in a mouse and rat study was found to be > 5000 mg/kg bw for males and females combined. In order to be classified with acute toxicity category 4 (oral), the LD_{50} should be between $300 < LD_{50} \leq 2000$. Therefore, metaflumizone should not be classified for acute toxicity by the oral route.

The inhalation LC_{50} value in a 4 hour rat study (nose-only exposure) was found to be > 5.2 mg/L for males and females combined. In order to be classified with acute toxicity category 4 (inhalation), the LC_{50} should be between $1.0 < LC_{50} \leq 5.0$ (dusts and mists). Therefore, metaflumizone should not be classified for acute toxicity by the inhalation route.

The dermal LD_{50} value in a rat study was found to be > 5000 mg/kg bw for males and females combined. In order to be classified with acute toxicity category four (dermal), the LD_{50} should fall between $1000 < LD_{50} \leq 2000$. Therefore, no classification for acute toxicity via the dermal route is proposed for metaflumizone.

4.2.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The CLH dossier presented acute toxicity OECD Test Guideline studies on all routes of exposure in rats. In addition, a guideline-compliant acute oral toxicity study in mice is available. The oral LD_{50} values in a mouse and rat study and the dermal LD_{50} value in a rat study were found to be > 5000 mg/kg bw for males and females combined. The inhalation LC_{50} value in a 4-hour rat study (nose-only exposure) was found to be above the tested concentration of 5.3 mg/L (mass median aerodynamic diameters 3.6 ± 3.2 µm) for males and females combined.

These values are all above the guidance values for classification for acute toxicity. Therefore, the DS proposed no classification for acute toxicity for all routes.

Comments received during public consultation

One MSCA asked for the exact concentration tested in the acute inhalation study. No other comments were received.

Assessment and comparison with the classification criteria

The oral LD_{50} values in a mouse and rat study of above 5000 mg/kg bw does not justify acute toxicity category 4 (oral; 300 < LD_{50} ≤ 2000 mg/kg bw). The LC_{50} value in a 4-h rat study (nose-only exposure) of 5.3 mg/L does not justify acute toxicity category 4 (inhalation; 1.0 < LC_{50} ≤ 5.0 mg/L for dusts and mists). The dermal LD_{50} value in a rat study of above > 5000 mg/kg bw does not justify acute toxicity 4 (dermal; 1000 < LD_{50} ≤ 2000 mg/kg bw). Apart from one animal dying 15 min after dermal application, no other mortalities were seen in the available acute toxicity studies in rats and mice.

RAC agrees with the conclusions of the DS that all LD_{50} and LC_{50} estimates were above the guidance values for classification and **no classification is warranted for acute toxicity for the oral, inhalation and dermal routes.**

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Four guideline studies investigating the effects of metaflumizone after a single dose via oral, dermal and inhalation routes were reported. In the acute inhalation study (nose-only exposure) accelerated respiration was observed and red discolouration of the lobes of the lungs of all animals at necropsy [12]. In a 28-day repeated dose inhalation study in rats, also using nose-only exposure, there were signs of damage to the lungs [14]. Minimal hypertrophy of the respiratory epithelium was noted in all animals at a dose of 0.7 mg/L and in 2/5 males and females at 0.1 mg/L. Minimal to slight multifocal hyperplasia of type II cells was present in animals from a dose of 0.1 mg/L. Animals in these groups showed a higher incidence of alveolar macrophages which accumulated in clusters. These effects were not observed in a 28-day repeated dose inhalation study using whole-body exposure.

4.3.2 Comparison with criteria

Substances that have produced significant non-lethal toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following single exposure are classified as STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a constant and identifiable effect. In the acute oral, inhalation and dermal studies available, there were no signs of specific target organ toxicity. Therefore, classification with STOT-SE 1 or 2 is not warranted.

Category 3 for specific target organ toxicity following a single dose is reserved for substances or mixtures causing transient target organ effects (in the absence of lethality). This category includes narcotic effects and upper respiratory tract irritation. In an acute inhalation study and a 28-day repeated study there were signs of lung damage, however, no upper respiratory tract irritation was observed. Therefore, metaflumizone should not be classified with STOT-SE 3.

4.3.3 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS concluded that classification as STOT SE 1 or 2 is not warranted since no signs of specific target organ toxicity were identified in the acute oral, inhalation and dermal toxicity studies.

Category 3 for specific target organ toxicity following a single dose is reserved for substances or mixtures causing transient target organ effects such as narcotic effects and upper respiratory irritation. The DS concluded that STOT SE 3 classification is not warranted based on the observations in the acute inhalation study where rats showed accelerated respiration, squatting posture and red discolouration of the lobes of the lungs. In a 28-d repeated dose inhalation study in rats, minimal hypertrophy of the respiratory epithelium was noted in all animals at a dose of 0.7 mg/L and in 2/5 males and females at 0.1 mg/L. Minimal to slight multifocal hyperplasia of type II cells and higher incidence of alveolar macrophages was observed in animals from a dose of 0.1 mg/L.

Comments received during public consultation

No comments on STOT SE were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS that STOT SE 1 and 2 are not warranted. No specific target organ toxicity after single exposure was identified.

Regarding STOT SE 3, no evidence of narcotic effects and no unequivocal evidence of transient irritation of the upper or lower respiratory tract were observed. Accelerated respiration was observed during exposure and could either be considered as signs of toxicity or as signs of irritation. Red discoloration of the lung lobes was noted at the end of the 14-d observation period and is not considered to indicate a transient (acute) hyperaemia. Hypertrophy of the respiratory epithelium and hyperplasia of type II alveolar cells are effects that need repeated exposure and thus are to be considered under STOT RE. As no specific evidence on the transient nature and the sensory/cytotoxic irritation is given and animals were tested at relatively high test concentrations, RAC agrees with the Dossier Submitter on **no classification** for STOT SE.

4.4 Irritation

4.4.1 Skin irritation

Table 10: Summary table of relevant skin irritation studies

Method	Results
New Zealand White Rabbits (3 males) (2000) [15] 500 mg Distilled water (0.5 mL) Semi-occlusive (4 h) OECD 404 (1992) GLP Purity 96.3 % DAR (B.6.2.4)	Mean individual animal scores over 24 – 72 h: Erythema: 0 – 0 – 0 Oedema: 0 – 0 – 0

4.4.1.1 Non-human information

In a guideline study, three male New Zealand white rabbits were exposed to metaflumizone (500 mg) in distilled water (0.5 mL) under semi-occlusive conditions for four hours [15]. Animals were then observed for seventy-two hours and dermal irritation was scored according to the Draize scale. There were no observations of erythema or oedema at any time-point during the study.

4.4.1.2 Human information

There are no human data on skin irritancy available.

4.4.1.3 Summary and discussion of skin irritation

In a single guideline study in rabbits, there were no signs of dermal irritation in any rabbit following treatment with metaflumizone. There were no deaths or signs of toxicity observed during the study.

4.4.1.4 Comparison with criteria

There were no signs of oedema or erythema reported in a guideline skin irritation study, therefore metaflumizone does not meet the criteria for classification as a skin irritant.

4.4.1.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

An OECD TG study with three male New Zealand white rabbits did not reveal observations of erythema or oedema at any time-point during the study after exposure to metaflumizone (500 mg) in distilled water (0.5 mL) under semi-occlusive conditions for four hours. The DS concluded that classification of metaflumizone as a skin irritant is not warranted.

Comments received during public consultation

No comments on skin corrosion/irritation were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS's proposal that **classification for skin irritation/corrosion is not warranted** based on the available study (using water as a vehicle).

4.4.2 Eye irritation

Table 11: Summary table of relevant eye irritation studies

Method	Results
New Zealand White Rabbits (3 males) (2002) [16] 0.1 mL (0.038 g) 24 h rinse with water OECD 405 (1987) GLP Purity 96.3 % DAR (B.6.2.5)	Mean individual animal scores over 24 – 72 h: Corneal opacity: 0 – 0 – 0 Iritis; 0 – 0 – 0 Conjunctival erythema: 0.33 – 0.33 – 0.33 All effects were fully reversible by 48 h.

4.4.2.1 Non-human information

In a guideline study metaflumizone (0.038 g, equivalent of 0.1 mL) was instilled into one eye of three male New Zealand White rabbits [16]. After twenty-four hours the treated eyes were rinsed with warm water. Animals were observed at 1, 24, 48 and 72 hours post-instillation and irritation was scored according to the Draize scale.

No corneal opacity or iritis was observed during the study period. One hour after instillation, two out of three rabbits exhibited conjunctival erythema (grade 1). At 24 hours, the treated eye of the third rabbit exhibited conjunctival erythema (grade 1) also. All animals were free of ocular irritation by 48 hours.

4.4.2.2 Human information

There are no data for eye irritation in humans available.

4.4.2.3 Summary and discussion of eye irritation

Metaflumizone was shown to cause minimal irritation in the form of conjunctival erythema in three rabbits (grade 1) at 24 hours, which was cleared by 48 hours post-instillation. There was no evidence of corneal opacity or iritis in any rabbits.

4.4.2.4 Comparison with criteria

According to the CLP criteria, classification of eye irritation category two is necessary when the average score (24 – 72 hours) for conjunctival erythema is ≥ 2 in at least two out of three animals. The average score for conjunctival erythema for each rabbit was 0.33 and there was no corneal opacity or iritis. Therefore, metaflumizone does not meet the criteria for classification for eye irritation.

4.4.2.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Metaflumizone tested in a OECD TG study was shown to cause minimal irritation in the form of conjunctival erythema in three rabbits (grade 1) at 24 hours, which was cleared by 48 hours post-instillation. There was no evidence of corneal opacity or iritis in any of the rabbits. The DS considered that metaflumizone does not meet the criteria for classification as an eye irritant.

Comments received during public consultation

No comments on serious eye damage/irritation were received.

Assessment and comparison with the classification criteria

The average score for conjunctival erythema for each rabbit was 0.33 and there was no corneal opacity or iritis. Regarding classification for eye irritation, this is below the average score for conjunctival erythema specified in the CLP criteria of ≥ 2 (24-72 h) in at least two out of three animals.

RAC agrees that **no classification for serious eye damage/irritation is warranted**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

Refer to section 4.3.

4.4.3.2 Human information

There are no human data available for respiratory tract irritation.

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

4.5 Corrosivity

Table 12: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Refer to table 10.			

4.5.1 Non-human information

Metaflumizone was shown not to be irritating to the skin of rabbits in a guideline study (section 4.4).

4.5.2 Human information

There are no human data available for skin irritation.

4.5.3 Summary and discussion of corrosivity

Metaflumizone has been shown to be non-irritating to the skin of rabbits. There were no signs of corrosivity in a guideline study.

4.5.4 Comparison with criteria

Metaflumizone is not corrosive to skin.

4.5.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 13: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result
Guinea Pig Maximisation Test (2002) [17] DH Guinea Pigs (20 females/test group, 10 females/controls) 1 % CMC (aq.) OECD 406 (1992) GLP Purity 96.9 % DAR (B.6.2.6)	***Induction:*** Intradermal - 5 % Topical – 50 % ***Challenge:*** Topical – 25 %	No dermal reactions were noted at any site in any of the control of test animals following challenge.	***Negative***

4.6.1.1 Non-human information

Metaflumizone was tested for skin sensitisation in a guideline Maximisation test carried out in DH guinea pigs (20 females in the test group, 10 females/control groups) [17]. During a preliminary testing phase, appropriate concentrations of the test substance were determined for the intradermal induction (5 %), topical induction (50 %) and topical challenge phases (25 %).

During the intradermal induction phase, moderate to intense erythema and swelling was observed in all test animals. During the topical induction phase, encrustation, partially open and intense erythema and swelling were observed at all dose sites. Following challenge, no dermal reactions were noted at any site in any of the control or test animals.

4.6.1.2 Human information

There are no data on skin sensitisation in humans.

4.6.1.3 Summary and discussion of skin sensitisation

In a guinea pig Magnusson and Kligman assay, no skin sensitisation was observed following a challenge dose of 25 %. However, it must be noted that the induction doses used were above those recommended in the OECD 406 test guideline. In the test guideline it states that the concentration of the test substance used for each induction exposure should be well tolerated systemically and should be the highest to cause mild – moderate skin irritation. During both the intradermal and the topical induction phases moderate to intense erythema were observed. As no irritation was noted after challenge, this deviation was thought not to affect the results of the study.

4.6.1.4 Comparison with criteria

Metaflumizone did not cause skin sensitisation in a guideline maximisation test in guinea pigs; therefore it should not be classified for this end point.

4.6.1.5 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Metaflumizone did not cause skin sensitisation in a OECD TG maximisation test in Guinea pigs. According to the DS's proposal, no classification is justified for this hazard class.

The CLH dossier informs about intradermal and topical induction doses that caused moderate to intense erythema. The test guideline recommends that selected concentrations should cause mild to moderate skin irritation.

Comments received during public consultation

No comments on skin sensitisation were received.

Assessment and comparison with the classification criteria

Metaflumizone did not cause a positive response in a guideline-compliant maximisation test in Guinea pigs. RAC agrees with the DS's proposal that **classification for skin sensitisation is not warranted**.

4.6.2 Respiratory sensitisation

Table 14: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
Not applicable			

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

Not classified - data lacking

4.7 Repeated dose toxicity

The repeated dose toxicity of metaflumizone has been investigated in a number of studies in the rat, mouse and dog. Of the studies available, 3/4 of the oral dosing experiments carried out in rats and 2/2 studies in mice could not be considered guideline due to a number of deviations. However, despite the limitations, they still provide useful information and will be considered towards the classification of metaflumizone.

The tables below contain the most significant toxicological effects observed after repeated dosing with metaflumizone in animals. Further information on repeated dosing can be found in the carcinogenicity and reproductive toxicity sections 4.10 (table 18) and 4.11 (table 19).

Table 15a: Summary table of relevant repeated dose toxicity studies via the oral route

†All figures quoted as reduced by/increased by [denoted by↓ and ↑ (respectively)] are relative to controls.
‡NOAELS have been copied from the DAR for information only.
*PALS = peri-arteriolar lymphatic sheath.

Oral Studies		
Method	**Dose Levels**	**Observations and Remarks**
28-Day Oral Study (1998) [18] Rats, Sprague-Dawley CD (5/sex/group) Dietary Non-guideline Non-GLP Purity unknown DAR (B.6.3.1a) *Guideline value for classification: ≤ 300 mg/kg bw/day (28 day rat study)*	0, 250, 1000, 5000, 10000 ppm (Equivalent to 0, 27.2, 86.4, 418, 798 mg/kg bw/day in males and 0, 23.8, 83.2, 361 and 890 mg/kg bw/day in females)	**<u>10000 ppm (798/890 mg/kg bw/day)</u>** ***Observations:*** ↓ BW: 43 % (males) and 38 % (females) relative to controls at the end of the study † ↓ BW gain: 69 % (males) and 95 % (females) ↓ Food consumption: 53 % (males) and 49 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 52 % (males and females) ↑ Bilirubin: 200 % (males) and 400 % (females) ***Organ weights:*** ↓ Ovary weight: 65 % (abs.), 44 % (rel.) ↓ Uterus weight: 86 % (abs.), 76 % (rel.) ***Histopathology:*** Spleen - atrophy: 4/5 males and 5/5 females - ↓ leucocytes: 4/5 males and 5/5 females Uterus - atrophy: 5/5 females - ↓number corpora lutea: 5/5 females **<u>5000 ppm (418/361 mg/kg bw/day)</u>** ***Observations:*** ↓ BW: 41% (males) and 42 % (females) ↓ BW gain: 68% (males) and 102 % (females) ↓ Food consumption: 51 % (males) and 54 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 47 % (males) and 43 % (females) ↑ Bilirubin: 200 % (males) and 300 % (females) ***Organ weights:*** ↓ Ovary weight: 65 % (abs.), 41 % (rel.) ↓ Uterus weight: 86 % (abs.), 75 % (rel.)

		Histopathology: Spleen - atrophy: 2/5 males and 5/5 females - ↓ leucocytes: 5/5 females Uterus - atrophy: 5/5 females - ↓corpora lutea: 5/5 females **<u>1000 ppm (86.4/83.2 mg/kg bw/day)</u>** ***Observations:*** ↓ BW: 39 % (males) and 29 % (females) ↓ BW gain: 65% (males) and 67 % (females) ↓ Food consumption: 47 % (males) and 40 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 33 % (males) and 22 % (females) ***Organ weights:*** ↓ Ovary weight: 60 % (abs), 43 % (rel.) ↓ Uterus weight: 74 % (abs), 64 % (rel.) ***Histopathology:*** Uterus – atrophy: 5/5 females - corpora lutea: 5/5 females **<u>250 ppm (27.2/23.8 mg/kg bw/day)</u>** ***Observations:*** ↓ BW: 16 % (males) ↓ BW gain: 25% (males) ↓ Food consumption: 18 % (males) and 13 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 26 % (females) ***Organ weights:*** ↓ Ovary weight – 23 % (abs), 20 % (rel.) ↓ Uterus weight: 39 % (abs), 36 % (rel.) ***A NOAEL could not be established from this study due to effects on body weight gain and food consumption observed in all treated groups*** ‡

28-Day Oral Study (1999) [19] Rats, Strain not specified (5/sex/group) Dietary Non-guideline Non-GLP Purity 97.7 % DAR (B.6.3.1c) *Guideline value for classification: ≤ 300 mg/kg bw/day (28 day rat study)*	0, 10, 20, 40 ppm (Equivalent to 0, 1.1, 2.2, 4.3 mg/kg bw/day in males and females)	**There were no toxicologically significant findings in this study.** ***A NOAEL of > 40 ppm (4.3 mg/kg bw/day) was determined for males and females.***
90-Day Oral Study (1998) [20] Rats, Sprague-Dawley (15/sex/group) (5 animals/sex/group were sacrificed at 28 days) Dietary Non-guideline Non-GLP Purity not specified DAR (B.6.3.1b) *Guideline value for classification: ≤ 100 mg/kg bw/day (90 day rat study)*	0, 50, 100, 200, 400 ppm (Equivalent to 0, 3.65, 7.16, 13.7, 25.9 mg/kg bw/day in males and 0, 4.03, 7.24, 13.7 and 26.7 mg/kg bw/day in females)	**400 ppm (25.9/26.7 mg/kg bw/day)** ***Observations:*** ↓ BW gain: 57 % (males) and 55 % (females) ↓ BW: 43 % (males) and 33 % (females) ↓ Food consumption: 37 % (males) and 36 % (females) ***Organ Weights:*** ↓ Ovary weight ***Histopathology:*** Ovary: ↓ corpora lutea Uterus: Visibly small and hypoplastic, with no evidence of cyclic activity **200 ppm (13.7 mg/kg bw/day)** ***Observations:*** ↓ BW gain: 36% (for both males and females) ↓ BW: 27 % (males) and 22 % (females) ↓ Food consumption: 22 % (males) and 26 % (females) **100 ppm (7.16/7.24 mg/kg bw/day)** ***Observations:*** ↓ BW gain: 27 (males) and 33 % (females) ↓ BW: 21 % (males) and 20 % (females) ↓ Food consumption: 17 % (males) and 21 % (females) **50 ppm (3.65/4.03 mg/kg bw/day)** ***Observations:*** ↓ BW gain: 11 % (males) and 15.4 % (females) ***A NOAEL could not be established from this study due to effects on body weight gain observed in all treated groups.***

28- and 90-Day Studies (2000) [21] Rats, Sprague-Dawley (5/sex/group) Oral, gavage Vehicle: 0.5 % CMC (aq.) OECD 408 Non-GLP Purity 95.1 % DAR (B.6.3.1d) *Guideline value for classification: ≤ 300 mg/kg bw/day (28 day rat study)*	0, 100, 500, 1000 mg/kg bw/day (28 days)	<u>28-days:</u> <u>1000 mg/kg</u> ***Observations:*** ↓ BW gain: 51 % (males) and 43 % (females) ↓ BW: 28 % (males) and 23 % (females) ↓ Food consumption: 29 % (males) and 40 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 41 % (males) and 37 % (females) ***Organ Weights:*** ↑ Liver weight: 19 % (males) ***Histopathology:*** Hepatocyte hypertrophy: 4/5 males (versus 0 in controls) Extramedullary haematopoiesis: 3/5 males, 5/5 females (versus 0 in controls) <u>500 mg/kg</u> ***Observations:*** ↓ BW gain: 36 % (males) and 46 % (females) ↓ BW: 20 % (for both males and females) ↓ Food consumption: 32 % (males) and 37 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 47 % (females) ***Organ Weights:*** ↑ Liver weight: 15 % (males) ***Histopathology:*** Hepatocyte hypertrophy: 2/5 males Extramedullary haematopoiesis: 2/5 males, 3/5 females <u>100 mg/kg</u> ***Observations:*** ↓ BW gain: 23 % (males) and 12 % (females) ↓ Food consumption: 17 % (males) ***Clinical Chemistry:*** ↑ Cholesterol: 36 % (females) ***A NOAEL could not be established from this study due to effects on body weight gain observed in all treated groups.***

Guideline value for classification: ≤ 100 mg/kg bw/day (90 day rat study)	0, 100 mg/kg bw/day (90 days) (Note – Dose started at 1000 mg/kg bw/day but was reduced to 100 mg/kg bw/day during week 1 due to marked body weight loss)	<u>90-days:</u> <u>100 mg/kg</u> ***Observations:*** ↓ BW gain: 13 % (females) ↓ Food consumption (males and females) ***A NOAEL could not be established from this study due to effects on body weight gain observed in all treated groups.***
28-Day Oral Study (1999) [22] Mouse, CD-1 (5/sex/group) Dietary Non-guideline Non-GLP Purity not stated DAR (B.6.3.2a) *Guideline value for classification: ≤ 300 mg/kg bw/day (based on a 28 day rat study)*	0, 50, 200, 800 ppm (equivalent to 0, 10, 42, 101 mg/kg bw/day in males and females)	<u>800 ppm (101 mg/kg bw/day)</u> ***Observations:*** 5/5 Males and 4/5 females died during weeks 1-2 ↓ BW gain: males lost 7.8 g (versus a gain of 1.22 in controls), females lost 4.9 g (versus a gain of 0.82 in controls) during week 1 ↓ BW: 26 % (males) and 20 % (females) during week 1 ↓ Food consumption: 55 % (males) and 37 % (females) during week 1 ***Histopathology:*** Spleen: atrophy 5/5 males and 5/5 females <u>200ppm (42 mg/kg bw/day)</u> ***Observations:*** ↓ BW gain: 91 % (males) and 45 % (females) ***A NOAEL of 50 ppm (10 mg/kg bw/day) for males and females was determined for this study, based on effects on food consumption and body weight gain at higher doses.***

28-Day Oral Study (1999) [23] Mouse, CD-1 (5/sex/group) Dietary Non-guideline Non-GLP Purity not stated DAR (B.6.3.2b) *Guideline value for classification: ≤ 300 mg/kg bw/day (based on a 28 day rat study)*	0, 10, 20, 40 ppm (equivalent to 0, 2.0, 4.3, 8.2 mg/kg bw/day in males and females) Recovery period = 28 days (0, 40 ppm)	<u>40 ppm (8.2 mg/kg bw/day)</u> ***Observations:*** ↓ BW gain: 21 % (males) ***A NOAEL of 20 ppm (8.2 mg/kg bw/day) was determined for males based on effects on body weight gain at higher doses and a NOAEL of > 40 ppm was determined for females (top dose).***
12-Month Oral Study (2003) [24] Dog, Beagle (5/sex/group) Capsule OECD 409 (1998) and OECD 452 (1981) GLP Purity 96.9 % DAR (B.6.3.3a)	0, 6, 12, 30, 60/40/30 mg/kg bw/day NB. 60 mg/kg bw/day was reduced to 40 mg/kg bw/day on day 49 and then to 30 mg/kg bw/day on day 245.	<u>60/40/30 mg/kg bw/day</u> ***Observations:*** 3/5 females and 2/5 males were sacrificed in extremis (days 57 – 250) ↓ BW gain: 39 % week 52 (females) ↑ Hypochromasia: 2/3 males ***Organ Weights:*** ↑ Spleen weight: (abs.): 40 % and 40 %, (rel.): 27 % and 57 % (males and females respectively) ***Histopathology:*** ↑ Liver haemosiderosis: 4/5 males and 4/5 females <u>30 mg/kg bw/day</u> ***Observations:*** 2/5 females were sacrificed in extremis (days 215 and 237) ↓ BW gain: 25 % (males), 21 % (females) ↑ Hypochromasia: 3/5 males ***Organ Weights:*** ↑ Spleen weight: 50 % and 20 % (abs.), 56 % and 23 % (rel.) (males and females respectively) ***Histopathology:*** ↑ Liver haemosiderosis: 5/5 males and 5/5 females <u>12 mg/kg bw/day</u> ***Observations:*** ↓ BW gain: 19 % in week 13, 33 % week 52 (females) ***Organ Weights:*** ↑ Spleen weight: 29 % and 10 % (abs.), 22 % and 24 % (rel.) (males and females respectively) ***Histopathology:*** ↑ Liver haemosiderosis: 3/5 males and 5/5 females ***A NOAEL of 6 mg/kg bw/day for males and females was determined, based on an increase in absolute and relative spleen weight and effects on body weight gain at higher doses.***

Table 15b: Summary table of relevant repeated dose toxicity studies via the inhalation route

†All figures quoted as reduced by/increased by [denoted by↓ and ↑ (respectively)] are relative to controls.
‡NOAELS have been copied from the DAR for information only.
*PALS = peri-arteriolar lymphatic sheath.

Inhalation Studies		
Method	**Dose Levels**	**Observations and Remarks**
28-Day Inhalation Study (2002) [14] Rats, Wistar (5/sex/group) Nose-only (dust aerosol), 6 h/day (20 exposures over 28 days) MMAD 1.7 – 3.3 µm OECD 412 and 413 (1981) GLP Purity 96.9 % DAR (B.6.3.5.1) *Guideline value for classification: ≤ 0.6 mg/L (based on a 28 day rat study)*	0, 0.03, 0.1 and 0.7 mg/L	<u>0.7 mg/L</u> ***Observations:*** 1/5 males died (day 17), 3/5 females were sacrificed in extremis (days 9 and 10) ↓ BW: 23 % (males), 31 % (females) ↓ Food consumption: 29-44 % (males) and 43-66 % (females) (weeks 1 – 4) ***Organ Weights:*** ↑ Lung weight: 17 % (abs) (males), 54 % and 42 % (rel) (males and females respectively) ↓ Thymus weight: 54 % and 74 % (abs), 42 % and 61 % (rel) (males and females respectively) ↓ Ovaries weight: 38 % (abs), 11 % (rel) ↓ Uterus weight: 72 % (abs), 60 % (rel) ***Clinical Chemistry:*** ↓ Prothrombin time: 13 % (males) ↓ Urea: 19 % (males) ↑ Cholesterol: 155 % (males) and 63 % (females) ***Histopathology: (see table below)*** <u>0.1 mg/L</u> ***Observations:*** ↓ BW: 14 % (females) ↓ Food consumption: 15-33 % (females) (weeks 1 – 4) ***Organ Weights:*** ↑ Lung weight: 10 % (abs) (males), 13 % (rel.) (males) ↓ Thymus weight: 12 % and 42 % (abs), 10 % and 33 % (rel.) (males and females respectively) ↓ Ovaries weight: 27 % (abs), 15 % (rel.) ↓ Uterus weight: 52 % (abs), 44 % (rel.) ***Clinical Chemistry:*** ↑ Cholesterol: 22 % (males) and 38 % (females)

<table>
<tr>
<td></td>
<td></td>
<td>
Histopathology: (see table below)

<u>0.03 mg/L</u>
Organ Weights:
↓ Thymus weight: 25 % (abs), 22 % (rel) (females)

Histopathology Table:
<table>
<tr><th></th><th colspan="4">Males (mg/l)</th><th colspan="4">Females (mg/l)</th></tr>
<tr><th></th><th>0</th><th>0.03</th><th>0.1</th><th>0.7</th><th>0</th><th>0.03</th><th>0.1</th><th>0.7</th></tr>
<tr><td>Lungs</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Type II hyperplasia</td><td>-</td><td>-</td><td>2/5</td><td>4/5</td><td>-</td><td>-</td><td>3/5</td><td>3/5</td></tr>
<tr><td>Alveolar inflammation</td><td>-</td><td>-</td><td>1/5</td><td>5/5</td><td>-</td><td>-</td><td>3/5</td><td>3/5</td></tr>
<tr><td>Alveolar histiocytosis</td><td>-</td><td>1/-/-</td><td>4/1/-</td><td>-/2/3</td><td>2/-</td><td>2/-/-</td><td>2/3/-</td><td>3/2/-</td></tr>
<tr><td>Macrophage cluster</td><td>-</td><td>-</td><td>4/5</td><td>5/5</td><td>-</td><td>1/5</td><td>5/5</td><td>3/5</td></tr>
<tr><td>BALT: granulomas</td><td>-</td><td>-</td><td>-</td><td>1/2/1</td><td>-</td><td>-</td><td>-</td><td>-/1/-</td></tr>
</table>
A NOAEL of 0.03 mg/L for males and females can be determined for this study, based on effects on body weight and reduced food consumption at 0.1 mg/L in females and damage to the lungs in both sexes at 0.1 mg/L.
</td>
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<tr>
<td>28-Day Inhalation Study (2004) [25]

Rats, Wistar (10/sex/group)

Whole-body (dust aerosol), 6 h/day (20 exposures over 28 days)

MMAD 1.6 – 2.2 µm

OECD 412 (1981) GLP

Purity 95.8 %

DAR (B.6.3.5.2)

Guideline value for classification: ≤ 0.6 mg/L (based on a 28 day rat study)</td>
<td>0, 0.03 mg/L</td>
<td>
<u>0.03 mg/L</u>
Observations:
↓ BW gain: 39-58% (males) and 57-112% (females) (days 7-28)
↓ Food consumption: 17-23% (females) (days 14-28)

Organ weights:
↑ Adrenals weight: 8 % (abs.) and 20 % (rel.) (females)

Histopathology:
↑ incidence of adrenal cortex cytoplasmic vacuolation (females) 9/10 versus 2/10 in controls

A NOAEL could not be determined from this study due to reduced body weight gain and food consumption in males and females at 0.03 mg/L.
</td>
</tr>
</table>

Table 15c: Summary table of relevant repeated dose toxicity studies via the dermal route

†All figures quoted as reduced by/increased by [denoted by↓ and ↑ (respectively)] are relative to controls.
‡NOAELS have been copied from the DAR for information only.
*PALS = peri-arteriolar lymphatic sheath.

Dermal Studies		
Method	**Dose Levels**	**Observations and Remarks**
90-Day Dermal Study (2002) [26] Rats, Wistar (10/sex/group) Vehicle: 0.5 % CMC (aq.) Semi-occlusive 6 h/day, 5 days/week OECD 411 GLP Purity 96.9 % DAR (B.6.3.4) *Guideline value for classification: ≤ 200 mg/kg bw/day (90 day rat study)*	0, 100, 300, 1000 mg/kg bw/day	<u>1000 mg/kg bw/day</u> ***Observations:*** ↓ BW: 19 % (females) ↓ BW gain: 16 % (males) and 53 % (females) ↓ Food consumption: 9 % (males) and 30 % (females) ***Organ weights:*** ↑ Liver weight: 10 % (rel) (females) ***Clinical Chemistry:*** ↑ Cholesterol: 57 % (females) ***Histopathology:*** Spleen - ↓ cells in PALS*: 3/10 females versus 0 in controls Spleen - haemosiderin deposition: 3/10 females versus 0 in controls Thymus - starry sky cells: 9/10 females versus 5/10 in controls Mesenteric lymph node - lymphocyte necrosis/apoptosis: 4/10 females versus 0 in controls Mesenteric lymph node - atrophy, diffuse: 1/10 females versus 0 in controls Adrenals - vacuolation of zona fasciculate: 6/10 females versus 0 in controls <u>300 mg/kg bw/day</u> ***Observations:*** ↓ BW: 11 % (females) ↓ BW gain: 13 % (males) and 32 % (females) ↓ Food consumption: 13 % (females) ***Clinical Chemistry:*** ↑ Cholesterol: 25 % (females) ***Histopathology:*** Thymus - starry sky cells: 8/10 females versus 5/10 in controls Mesenteric lymph node - lymphocyte necrosis/apoptosis: 3/10 females versus 0 in controls ***A NOAEL of 100 mg/kg bw/day for males and females can be determined for this study, based on body weight gain in males and females and decreased body weight, food consumption and increased serum cholesterol and thymus and lymph node effects in females.***

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Studies in Rats

Four oral studies are available in rats, all of which were considered limited in their reporting. Also available are a 2-year chronic carcinogenicity study and a 2-generation reproductive toxicity study, both carried out in rats.

28-Day oral dietary study[18]

In this 28-day study, the doses relevant for classification were 250 and 1000 ppm (~25 and 85 mg/kg bw/day) (STOT RE 1: C < 30 mg/kg bw/day and STOT RE 2: 30 < C ≤ 300 mg/kg bw/day).

Each animal (5/sex/group) received 0, 250, 1000, 5000 or 10000 ppm of metaflumizone in their diet (equivalent to 0, 27.2, 86.4, 418 and 798 mg/kg bw/day in males and 0, 23.8, 83.2, 361 and 890 mg/kg bw/day in females) for 28 days. At the end of the experiment, gross necropsy was performed on all animals and weights of organs were recorded. Histopathology was limited to the adrenals, kidneys, liver, ovaries, spleen, testes and uterus.

No deaths occurred during the study period however mean bodyweight and bodyweight gain of all treated groups were much lower than controls throughout the study period. At 28 days, males treated with 250 ppm were found to have bodyweight 16 % less than control animals and males and females in the 1000 ppm treatment group were observed to be 29-39 % less than controls. Bodyweight gain was similarly much lower than controls with males at 250 ppm having reduced bodyweight gain of 25 % and males and females at 1000 ppm reduced by 65-67 %. These effects were also seen to a greater extent at doses of ≥ 5000 ppm. In all groups, food consumption was reduced and food scatter increased.

Cholesterol concentrations were found to be significantly increased in males at doses ≥ 1000 ppm and females in all treatment groups [(males: 33, 47 and 52 % at 1000, 5000 and 10000 ppm respectively) and (females: 26, 22, 43 and 52 % at 250, 1000, 5000 and 10000 ppm respectively)]. Bilirubin levels were also significantly elevated from doses of ≥ 5000 ppm.

Effects on red blood cell parameters were noted in all groups of treated males and females at doses of ≥ 1000 ppm, however a dose-response was not apparent.

Interpretation of organ weights was hampered by the effects on bodyweight observed in all treatment groups. The absolute weight of the majority of organs was significantly lower; however with the exception of the uterus and ovaries, the relative weights did not indicate an effect of treatment. Ovary and uterus weight were significantly reduced compared to controls in females at 250 and 1000 ppm. Ovaries: (absolute weights: 23 and 60 % and relative weights: 20 and 43 % respectively). Uterus: (absolute weights: 39 and 74 % and relative weights: 36 and 64 % respectively). The reductions in absolute and relative weights continued at doses of ≥ 5000 ppm. Histopathology revealed atrophic/undeveloped uterus and cervix and reduced numbers of corpora lutea in females fed with doses ≥ 1000 ppm only (5/5 females versus 0 in controls at 1000, 5000 and 10000 ppm).

Histopathology also revealed splenic effects which were increased incidences of atrophy of the white pulp and reduced leucocytes in red pulp effects at doses of ≥ 5000 ppm in males and females.

28-Day oral dietary study [19]

In this 28-day study, all doses were relevant for classification (STOT RE 1: C ≤ 30 mg/kg bw/day).

Rats (strain unspecified) (5/sex/group) received metaflumizone in their diet for 28 days (0, 10, 20, 40 ppm, equivalent to 0, 1.1, 2.2 and 4.3 mg/kg bw/day in males and females).

Mean bodyweights and bodyweight gain in top dose group animals were slightly lower throughout the study period, but values did not attain statistical significance. Haematology findings were not reported and clinical chemistry revealed a slight tendency to increased plasma cholesterol in top dose females only. Organ weights were unaffected by treatment with metaflumizone and necropsy did not reveal any treatment-related findings.

90-Day oral dietary study [20]

In this 90-day study, all doses were relevant for classification (STOT RE 1: C ≤ 10; STOT RE 2: 10 < C ≤ 100 mg/kg bw/day).

Rats (15/sex/group) received metaflumizone in their diet (0, 50, 100, 200 or 400 ppm) (equivalent to 0, 3.65, 7.16, 13.7, 25.9 mg/kg bw/day in males and 0, 4.03, 7.24, 13.7 and 26.7 mg/kg bw/day in females) for up to 90 days. In the intermediate dose group, histopathology was limited to the ovaries and uterus only.

The main effects observed in this study were on bodyweight and bodyweight gain. Mean bodyweights of all treated groups were significantly lower than controls throughout the study period, as a result of initial weight loss and subsequent reduced bodyweight gain (reduced body weight: 20-21, 22-27 and 33-43 % in males and females at 100, 200 and 400 ppm respectively) and reduced body weight gain: 11-15, 27-33, 36 and 55-57 % in males and females at 50, 100, 200 and 400 ppm respectively). The observed weight loss was accompanied by reduced food consumption. Hepatocyte vacuolisation was reduced at ≥ 50 ppm which was considered to be a consequence of the reduced bodyweight and food consumption seen in this study.

The report in the DAR stated that mean ovary weight was reduced at 400 ppm, however this was not quantified. Treatment-related findings were apparent in the ovaries and uterus at this dose (visibly smaller ovaries with reduced number of corpora lutea and visibly small and hypoplastic uterus with no evidence of cyclic activity). These findings were considered by the study author to be consistent with delayed sexual maturation caused by the reduction in bodyweight.

28 and 90-Day oral dietary studies [21]

For both the 28-day and the 90-day studies, only the 100 mg/kg bw/day dose was relevant for classification [STOT-RE 2: 30 < C ≤ 300 mg/kg bw/day (28-days) and 10 < C ≤ 100 mg/kg bw/day (90-days)].

In this guideline study, rats (5/sex/group) were administered metaflumizone in 0.5 % aqueous carboxymethylcellulose (CMC) by gavage at dose levels of 0, 100, 500, 1000 mg/kg bw/day for 28 days or 0, 100 mg/kg bw/day for 90 days. For the 90-day phase, animals were initially dosed with 1000 mg/kg bw/day but after one week, this was reduced to 100 mg/kg bw/day on account of marked weight loss observed.

Survival was found to be unaffected by treatment with metaflumizone.

After 28-days, effects on bodyweight gain, associated with reduced food consumption, were observed in the presence of a dose response at all treatment levels in both males and females. At 100 mg/kg bw/day bodyweight was reduced by 23 % in males and 12 % in females (compared to controls). Bodyweight gain was reduced from doses of 500 mg/kg bw/day and above. After 90-days, a reduction in body weight gain of 13 % was observed in females only and food consumption was reduced in males and females. It was postulated that the reduction in bodyweight gain and food consumption was due to the initial treatment with 1000 mg/kg bw/day, however it is thought that these parameters would have shown recovery over the duration of the study and so are likely to be treatment-related.

Cholesterol concentration was statistically significantly increased in males at 1000 mg/kg bw/day and in females of all treatment groups; however no dose-response was evident. There were no treatment-related clinical chemistry changes noted in either males or females at study termination.

At 28-days, relative liver weights were significantly higher in males dosed with ≥ 500 mg/kg bw/day metaflumizone. The histopathological correlate to this was hepatocellular hypertrophy in males only (2/5 males at 500 mg/kg bw/day and 4/5 males at 1000 mg/kg bw/day versus 0 in controls). Splenic effects were also observed, notably, extramedullary haematopoiesis in males at doses ≥ 500 mg/kg bw/day. Relative ovary weights were found to be lower in all treated groups of females with a statistical reduction in weight in females of the top dose treatment group. There were no treatment-related organ weight changes in animals of the 90-day phase.

Two-year carcinogenicity gavage study [27] (see table 18)

All doses were outside of those relevant for classification for a 2-year study (STOT-RE 2: 1.2 < C ≤ 12 mg/kg bw/day).

In a 2-year carcinogenicity study in Sprague-Dawley rats (80/sex/group), animals were administered metaflumizone in carboxymethylcellulose (0, 30, 60 and 200/300 mg/kg bw/day) by gavage The dose level of 300 mg/kg bw/day was reduced to 200 mg/kg bw/day in females during week 3 due to effects on bodyweight. No other toxicologically relevant effects were observed.

Two-generation reproductive toxicity gavage study [28] (see table 19)

The study length was equivalent to that of a 90-day study, and so all doses used in this study were relevant for classification (STOT RE 2: 10 < C ≤ 100 mg/kg) bw/day).

Wistar rats (25/sex/group) received metaflumizone in carboxymethylcellulose (0, 12, 30, 75 mg/kg bw/day, days 0-125 and 0, 12, 20 and 50 mg/kg bw/day from day 126 onwards) orally by gavage. Reductions in bodyweight and bodyweight gain were observed at 75 mg/kg bw/day (14 % and 22 % respectively) and there was a slight, but statistically significant reduction in bodyweight at 50 mg/kg bw/day (7 %).

Studies in Mice

Two non-guideline, non-GLP, 28-day studies are available in mice; both of these studies are limited in their analysis and reporting. Also available is an 18-month carcinogenicity study in mice.

28-Day dietary study [22]

All doses used in this study were relevant for classification (STOT RE 2: 30 < C ≤ 300 mg/kg bw/day, STOT RE 1: C ≤ 30 mg/kg bw/day for a 28 day study).

Mice (5/sex/group) received 0, 50, 200 or 800 ppm metaflumizone in their diet (equivalent to 0, 10, 42 and 101 mg/kg bw/day for both males and females) for 28 days.

Two males and three females dosed with 800 ppm metaflumizone died during week 1, the remaining males and females of this dose group died during week 2. Ataxia and convulsions were observed in some of these animals prior to death. Marked initial weight loss was observed in this dose group, a decrease of 26 % in males and 20 % in females during week 1. This was associated with reduced food consumption. Gross necropsy of the decedents revealed splenic atrophy and also reduced numbers of corpora lutea and uterine hyperplasia. These findings were considered secondary to the effects on body weight. Weight loss was also observed in males dosed with 200 ppm (13 % of controls) and reduced weight gain was noted in males and females of the 200 ppm dose group (males: 91 % and females: 45 %). No toxicologically significant findings were observed at 50 ppm.

28-Day dietary study [23]

All doses used in this study were relevant for classification (STOT RE 1: C ≤ 30 mg/kg for a 28 day study).

Mice were administered metaflumizone through their diet at doses of 0, 10, 20 and 40 ppm (equivalent to 0, 2.0, 4.3 and 8.2 mg/kg bw/day in males and females) (5/sex/group). A 28 day recovery period was employed for satellite groups of control animals and those treated with 40 ppm.

No deaths occurred and no signs of toxicity were observed during this study. Reduced bodyweight gain occurred during week 1 in 40 ppm males, which led to consistently lower body weights during the course of the study, however values did not reach statistical significance. Gross necropsy and limited histopathology did not reveal any treatment-related findings.

18-Month carcinogenicity gavage study [11]

All doses used in this study were above those relevant for classification (for an 18-month study STOT RE 2: 1.6 < C ≤ 16 mg/kg bw/day).

Mice (65/sex/group) received an oral dose of metaflumizone (0, 100, 250 and 1000 mg/kg bw/day) by oral gavage (Kelly CM, 2003a). Effects were observed at the top dose only; these included a decrease in bodyweight gain in males (17 %), an increased reticulocyte count (112 % in males and 48 % in females), a statistically significant decreased mean cell haematocrit (6.4 % females) and an increase in incidence of brown pigmentation of the spleen [19/65 males (versus 0 in controls) and 27/65 females (versus 3/65 in controls)].

Studies in Dogs

One 12-month study, carried out to OECD guidelines (with deviations) and GLP, was available in dogs.

12-Month oral capsule study [24]

Beagle dogs (5/sex/group) were administered gelatine capsules containing metaflumizone at doses of 0, 6, 12, 30 or 60/40/30 mg/kg bw/day for up to 12 months. Due to pronounced clinical findings in the high dose group, the dose level was reduced from 60 mg/kg bw/day to 40 mg/kg bw/day on day 49 and subsequently to 30 mg/kg bw/day on day 245.

Two females and one male from the top dose level were sacrificed *in extremis* on day 57 due to reduced general state, drastic bodyweight loss and reduced food consumption. An additional male from this group was sacrificed on day 250 (vomitus, ataxia, reduced general state and lateral position) and one female was sacrificed on day 226 (severe salivation and lateral position). Two females of the 30 mg/kg bw/day dose group were also sacrificed prematurely on days 215 and 237 showing similar observations.

Bodyweight gain was reduced in females treated with ≥ 12 mg/kg bw/day (33, 21 and 39 % reduction at doses of 12, 30 and 60/40/30 mg/kg bw/day respectively). This reduction in bodyweight gain was associated with reduced food consumption.

No toxicologically significant effects were noted on haematological parameters. There was an increase in hypochromasia seen in some males and females at doses ≥ 30 mg/kg bw/day, however there was no clear dose-response. It is possible this finding was a treatment-related effect on red blood cells; however in the absence of a dose-response and with the dose groups affected being outside those relevant for classification, this effect will not be further considered for classification purposes.

Absolute and relative spleen weights were increased in both males and females at all dose levels [absolute weights: (males: 16, 29, 50 and 40 % and females: 6, 10, 20 and 40 % at 6, 12, 30 and 60/40/30 mg/kg bw/day respectively); relative weights: (males: 6, 22, 56 and 27 % and females: 9, 24, 23 and 57 % at 6, 12, 30 and 60/40/30 mg/kg bw/day respectively)]. There were no histopathological correlates to the increased spleen weight; however the magnitude of the increase was such that the effect was considered toxicologically significant.

Histopathology revealed a tendency towards haemosiderosis in liver Kuppfer cells in both males and females (5/5 and 4/5 males affected at doses of 30 and 60/40/30 mg/kg bw/day respectively and 5/5, 5/5 and 4/5 females affected at doses of 12, 30 and 60/40/30 mg/kg bw/day respectively versus 2/5 in controls).

4.7.1.2 Repeated dose toxicity: inhalation

Studies in Rats

Two guideline inhalation studies, carried out according to GLP are available in rats. The doses relevant for classification are: 0.03 and 0.1 mg/l (STOT RE 2 (dusts and mists): $0.06 < C \leq 0.6$ mg/l; STOT RE 1 (dusts and mists): $C \leq 0.06$ mg/l).

28-Day nose-only study [14]

Wistar rats (5/sex/group) were exposed to metaflumizone by nose-only exposure for 6h/day for 28 days (20 exposures in total) (0, 0.03, 0.1 and 0.7 mg/l).

No mortality occurred in the mid and low dose groups. There was reduced survival in both males and females of the top dose group with 1 male dying during exposure on day 17 and three females being sacrificed moribund on days 9 and 10. These animals displayed severe ataxia, apathy, lateral or abdominal position and reduced general condition. Tremor and splayed limbs were also observed and surviving animals displayed similar clinical signs with one male additionally showing visually accelerated respiration.

Females of the mid dose group (0.1 mg/l) also had a significant reduction in terminal body weight (14 %) associated with reduced food consumption, males were unaffected. This reduction in bodyweight was observed, to a greater extent, in males and females of the top dose group also.

There were no treatment related effects on haematological parameters. Clinical chemistry revealed an increase in cholesterol concentrations in males at 0.7 mg/l. Necropsy revealed an increased incidence of erosion/ulceration of the glandular stomach at 0.7 mg/l. This was considered treatment-related but the study author attributed it to stress/inanition.

An increase in both absolute and relative lung weight was noted in males at 0.1 and 0.7mg/l (absolute 17 and 10 % respectively and relative 54 and 13 % respectively), while relative lung weight only was increased in females at 0.7mg/l (42 %). Minimal hypertrophy of the respiratory epithelium (the most caudal level) was noted in all animals at 0.7mg/l and in two males and females at 0.1 mg/l; minimal to slight multifocal hyperplasia of alveolar type II cells was present in animals at the mid and top doses (0.1 mg/l: 2/5 males and 3/5 females; 0.7 mg/l: 4/5 males and 3/5 females). Animals in these groups showed a higher incidence of alveolar macrophages: these accumulated in clusters at these doses, but were present as clusters in only one animal at 0.03 mg/l and were absent in controls.

A dose-related decrease in relative and absolute thymus weight was observed in males and females [males: (abs. 12 and 54 % and rel. 10 and 42 % at 0.1 and 0.7 mg/l respectively); females: (abs. 25, 42 and 74 % and rel. 22, 33 and 61 % at 0.03, 0.1 and 0.7 mg/l respectively)]. The microscopic correlate of the decreased thymus weight was a slight to severe decrease in cellularity at 0.1 and 0.7 mg/l, with the effect being more pronounced in females. Starry sky appearance (single cell necrosis) of the cortex was seen in all groups, but increased in severity at 0.1 and 0.7 mg/l. Cellularity of the medulla was decreased in males at 0.7 mg/l and females at 0.1 and 0.7 mg/l. Thymic architecture was lost in one male and three females at 0.1 mg/l, and the cortex and medulla could not be differentiated. Thymic histopathology was most prominent in the top dose male that died and the females that were prematurely sacrificed.

A dose-related decrease in absolute ovary and uterus weight was noted at the mid and top doses (ovary: 27 and 38 % and uterus: 52 and 72 % at 0.1 and 0.7 mg/l respectively), and at 0.1 and 0.7

mg/l relative uterus weight was also decreased (44 and 60 % at 0.1 and 0.7 mg/l respectively). Increased apoptosis of granulosa cells in antral follicles was found in the ovaries of females at 0.1 and 0.7 mg/l.

A number of other treatment-related histopathological findings were observed. The cellularity of the splenic peri-arteriolar lymphatic sheath (PALS) was decreased in females at 0.1 and 0.7 mg/l. This effect was most pronounced in the females that were sacrificed prematurely. However, slight to moderate effects were noted in two control males and one control female. Similar decreases in the cellularity of the marginal zone were observed; with a more severe effect in females at 0.7 mg/l. Haemosiderin was demonstrated in the spleen of all animals, with slightly increased severity in top dose animals. This was apparent in female survivors, but not the animals sacrificed early. There were no effects on spleen weight. Reduced cellularity of the mesenteric lymph node was noted in all females at 0.7 mg/l and two females at 0.1 mg/l. Paracortical lymphocyte necrosis was observed in all female treatment groups (0 at 0 mg/l, 3 at 0.03 mg/l, 2 at 0.1 mg/l and 3 at 0.7 mg/l). Reduced cellularity and lymphocyte necrosis were of greater severity in decedents. A minimal to slight diffuse vacuolation of the zona fasciculate in the adrenal cortex was observed in most animals at 0.7 mg/l and in one male and one female at 0.1 mg/l.

28-Day whole-body study [25]

Wistar rats (10/sex/group) were administered metaflumizone via whole body exposure at concentrations of 0 and 0.03 mg/l for 6 hours per working day for a total of 20 exposures over 28 days.

No deaths occurred during the course of this study. Bodyweight gain was significantly reduced in both males and females treated with 0.03 mg/l (males: 39 – 58 % and females: 57 – 112 % days 7-28). In females, this was associated with reduced food consumption during days 14 – 28 (17 – 23 %).

In treated females, relative and absolute adrenal weights were increased (abs. 8 % and rel. 20 %). This finding was accompanied by mild/slight vacuolation of the adrenal cortex in 9/10 females (absent in the controls). The study director attributed this effect secondary to stress. In females, there was also an increase in absolute thymus weight (14 %) but this was not statistically significant and was not accompanied by any histopathology.

4.7.1.3 Repeated dose toxicity: dermal

Studies in Rats

One guideline, 90-day dermal study, carried out to GLP standards was available in rats. Only the 100 mg/kg bw/day dose level was relevant for classification: (STOT RE 2: 20 < C ≤ 200 mg/kg bw/day).

In this study [26]; Wistar rats (10/sex/group) were topically treated with metaflumizone (0, 100, 300 or 1000 mg/kg bw/day) in 0.5 % aq. CMC, under semi-occlusive conditions, for 6 hours/day, 5 days/week for 90 days.

No deaths occurred during the study duration and there were no adverse effects at 100 mg/kg bw/day.

Decreases in bodyweight were observed in females at doses of ≥ 300 mg/kg bw/day and a reduction in body weight gain was noted in both males and females at the same doses, with females affected to a greater extent.

Increased cholesterol concentrations were observed in females at doses ≥ 300 mg/kg bw/day. The increase at 1000 mg/kg bw/day was determined to be within the historical control range, however the magnitude of the increase coupled with an apparent dose-response rendered the effect to be considered toxicologically significant.

Absolute spleen and brain weights were reduced in males at ≥ 300 mg/kg bw/day. However, as no effect on relative weights were noted, these findings were not considered toxicologically significant. Additionally, changes in absolute organ weights were seen in several organs (adrenal glands, liver, kidney, ovaries, uterus, heart, spleen, thymus, brain) were seen in females mainly at the high dose level, but only in liver and brain relative weight were increased. Thus, the changes in absolute values were attributed to the markedly decreased bodyweights and were considered unrelated to treatment. There were no microscopic findings that were related to the relative weight changes in brain and liver, therefore these findings were considered unrelated to treatment.

Histopathological evaluation revealed a reduction in haemosiderin deposition in the spleen in females at 1000 mg/kg bw/day. A dose-dependent increase in ‘starry sky’ cells in the thymus was seen in females at ≥ 300 mg/kg bw/day and this, combined with a reduction of cells in the periarteriolar lymphoid sheath at 1000 mg/kg bw, was considered to be toxicologically significant. Increased incidence of lymphocyte necrosis/apoptosis in the mesenteric lymph node was seen at 300 and 1000 mg/kg bw/day, while the incidence of diffuse atrophy of the mandibular lymph node was increased at 1000 mg/kg bw/day, both effects were considered to be toxicologically significant. Increased incidence of vacuolation of the adrenal cortex *zona fasciculata* was seen at 1000 mg/kg bw/day. No treatment-related histopathological findings were noted in males.

4.7.1.4 Repeated dose toxicity: other routes

There was no data provided for repeated dose toxicity by other routes of administration.

4.7.1.5 Human information

There was no human data.

4.7.1.6 Other relevant information

Three further toxicological studies were carried out to assess the effect of metaflumizone in the diet on palatability. In the first study, male Sprague-Dawley rats (5/group) were offered a choice between two jars containing control diet (control group); or a choice between two jars containing control diet or diet containing metaflumizone (400 ppm) (test group). The results of the study showed that the test group showed a marked preference for the diet containing control diet.

In the second study, two male Sprague-Dawley rats were fed either control diet (on days 1 and 3) or diet containing 400 ppm metaflumizone (on days 2 and 4). Food consumption and body weights were measured daily. On days 2 and 4 food consumption was decreased and this was accompanied by weight loss. Increased food consumption and weight gain was observed on days 1 and 3, when the control diet was offered.

The third study was a 7-day repeated dosing study in male Sprague-Dawley rats (5/group) using doses to mirror those in the 28/90 day study. Animals were dosed by gavage and the effects observed compared to those following dietary administration. This study provided limited information; however, there were no effects on bodyweights, weight gain or food consumption during the course of this study.

4.8 Specific target organ toxicity – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

In a number of short and longer term repeated dose studies with metaflumizone there was no evidence of any consistent effects to a particular tissue. The text below summarises the adverse effects observed at doses relevant for classification.

Oral studies

The oral repeated dose toxicity of metaflumizone has been investigated in rats, mice and dogs. In rats there are three 28-day studies (two dietary, one gavage), two 90-day studies (one dietary and one gavage) and one multi-generation study (gavage) (Section 4.11). In mice, there are two 28-day studies available (dietary) and in dogs, there is one 12-month study (capsule). Effects discussed below pertain only to the dose levels relevant for classification.

Mortality

Mortality was observed in one mouse study and in a dog study. In a 28-day dose-ranging mouse study, all 5 males and 4/5 females died in weeks 1-2 after a dose of 101 mg/kg bw/day (800 ppm) [22]. Marked initial weight loss was described in this group with significantly reduced food consumption. The increased mortality in this group was not considered to be an acute effect on the basis that in an acute study in mice, there was no mortality up to a dose of 5000 mg/kg bw. In the dog study, 2/5

females dosed with 30 mg/kg bw/day were sacrificed prematurely showing reduced general state, vomitus, ataxia and lateral position [24].

Body weight

Effects on body weight and body weight gain were observed in all oral studies. The most pronounced findings occurred when metaflumizone was administered via the diet to rats and mice. Mice appeared more sensitive than rats; in rats treated for 28 days effects on bodyweight became apparent from a dose of approximately 25 mg/kg bw/day (250 ppm) [18] and in mice, treated for the same period, bodyweight was affected from a dose of 8.2 mg/kg bw/day (40 ppm) [23]. In the 28-day dietary study [22] administering doses of 0-101 mg/kg bw/day (800 ppm), mice actually suffered body weight *loss* when dosed at the top dose of 101 mg/kg bw/day. The bodyweight loss observed was attributed to the cause of increased mortality in this group. When rats were given metaflumizone in the diet for 90 days, effects on bodyweight were observed from just 4 mg/kg bw/day (50 ppm) [20]. In most cases, the effects on bodyweight were observed in conjunction with reduced food consumption and were often related to palatability of the treated food.

In dogs, treated with metaflumizone in capsule-form, body weight gain was reduced from doses of 12 mg/kg bw/day and above.

Effects on bodyweight were also observed when metaflumizone was administered by gavage (rats), albeit to a lesser extent and occurred at higher doses. In a 28-day and 90-day gavage study [21], effects on bodyweight gain were observed at 100 mg/kg and in a multi-generation reproductive toxicity study, effects on bodyweight and bodyweight gain in dams, observed at 75 mg/kg bw/day, were so severe, the dose level had to be reduced to 50 mg/kg bw/day [28]. Food consumption in these groups was also reduced.

Effects of metaflumizone on palatability were investigated and the results of these studies provide some evidence that the effects observed on bodyweight and food consumption and those effects considered secondary to these could be a result of the test substance causing an adverse effect on the palatability of the diet. However, reduced weight gain and food consumption were identified as critical effects of metaflumizone toxicity in the subsequent gavage toxicity studies and also similar effects on food consumption and weight gain were observed in studies using inhalation and dermal exposure (although in the dermal study, effects were observed at doses above the guidance values for classification). As effects were seen at lower dose levels following dietary administration, the possibility therefore exists that the effects on food consumption and bodyweight gain in the dietary range-finding studies may be due (at least in part) to the toxicity of metaflumizone, rather than being entirely due to an effect on palatability

Metaflumizone is highly lipophilic (LogP 4.2 – 4.9) and the oral absorption following gavage administration is known to be highly variable, with values of between < 1 % and 33.4 %. Therefore, it is possible that the apparently greater toxicity of metaflumizone following dietary administration may be a consequence of more extensive oral absorption of metaflumizone from the diet, compared to the oral absorption when administered by capsule or by gavage as an aqueous suspension (Section 4.1).

Organ weight and pathology

Effects on the spleen were noted in studies in mice and dogs. In dogs, spleen weight was increased from doses of 12 mg/kg bw/day and above and in mice splenic atrophy was revealed at gross necropsy. The ovaries and uterus were often affected in rodent dietary studies, with increased weight

noted in rats (absolute and relative) from doses of 23.2 mg/kg bw/day and atrophy and reduced numbers of corpora lutea observed. Uterine hyperplasia was noted in a 28-day study in mice and in a 90-day study in rats, the uterus was often described as small and hypoplastic. The effects to organs observed were often described as being secondary to stress and the severe bodyweight loss described in these studies. In the gavage studies, where bodyweight effects were less severe there were no such effects to spleen, uterus or ovaries.

Inhalation studies

There are two 28-day inhalation studies available in rats, one nose-only and one whole-body study. Effects discussed below pertain only to the dose levels relevant for classification.

Mortality

In the nose-only study [14], 1/5 males died on day 17 and 3/5 females from the top dose level of 0.7 mg/l were sacrificed in extremis on days 9 and 10. The females died due to severe ataxia, apathy, lateral or abdominal position and reduced general condition. The male was described as having slight-moderate smeared anogenital region and urinous odour from day 8 until death. All surviving animals of this dose level displayed comparable clinical signs.

Body weight

Bodyweight was affected in both inhalation studies performed. In the nose-only study, bodyweight was reduced by 14 – 31 % in females exposed to 0.1 and 0.7 mg/l and in males exposed to 0.7 mg/l only (23 %). In the whole-body study [25]; only one dose of 0.03 mg/l was tested. At this level, there was a severe reduction in bodyweight gain in males (39-58 %) and in females (57-112 %). In females, this was associated with reduced food consumption.

Organ weight and pathology

Organs affected after nose-only exposure were the lungs, thymus, ovaries and uterus. Increased lung weight was observed at doses of 0.1 mg/L and above in both males and females. Histopathology revealed an increased incidence of type II hyperplasia, alveolar inflammation. Histiocytic granulomas in the BALT of the lung and in the mediastinal lymph nodes at 0.7 mg/l were attributed to phagocytosis of the inhaled test material. Thymus weight was increased from a dose of 0.03 mg/l and above in the nose-only study. Histopathology was most prominent in the male and females that died before the end of the study. Reduced ovary and uterus weight was noted at dose of 0.1 mg/l and above. The effects to the thymus, uterus and ovaries were considered secondary to the significant bodyweight effects observed. There were no such effects in the whole-body study (carried out using a single dose level of 0.03 mg/l). However, in this study adrenal weight was increased in females and there was an increased incidence of adrenal cortex cytoplasmic vacuolation. This was attributed to an effect secondary to reduced bodyweight gain and stress.

Dermal studies

There is one 90-day dermal study available in rats [26]. There were no effects at doses relevant for classification in this study.

A summary of the mortality and body weigh effects observed following repeat dosing of metaflumizone are shown in table 16 for ease of reference.

Table 16: Summary of mortality and body weight effects observed following repeat dosing of metaflumizone

Study	Doses (mg/kg bw/day	Doses Relevant for STOT-RE 2 (mg/kg bw/day)	Effects at doses rel. for STOT-RE 2 (mg/kg bw/day)	Doses Rel. for STOT-RE 1 (mg/kg bw/day)	Effects at doses Rel. for STOT-RE 1 (mg/kg bw/day)
Oral Studies					
Rat, 28-day (dietary) [18]	0, 27.2, 86.4, 418 and 798 (males) 0, 23.8, 83.2, 361 and 890 (females)	86.4 83.2 **(30 < C ≤ 300)**	↓ bw gain (65-67 %) ♀ and ♂ ↓ bw (29-39 %) ♀ and ♂ ↓ FC (40- 47 %) ♀ and ♂	27.2/23.8 **(C < 30)**	↓ bw gain (25 %) ♂ ↓ bw (16 %) ♂ ↓ FC (18 - 13%) ♀ and ♂
Rat, 28 day (gavage) [21]	0, 100, 500 and 1000	100 **(30 < C ≤ 300)**	↓ bw gain (12-23 %) ♀ and ♂ ↓ FC (17 %) ♂	 **(C < 30)**	N/A
Mouse, 28 day (dietary) [22]	0, 10, 42 and 101	42 and 101 **(30 < C ≤ 300)**	101 mg/kg **Mortality in wks 1-2 (5/5 ♂ and 4/5 ♀)** bw loss (7.8 g ♂ and 4.9 g ♀) ↓ bw wk 1 (20-26 %) ♀ and ♂ 42 mg/kg ↓ bw gain (65-67 %) ♀ and ♂	10 **(C < 30)**	No adverse effects
Mouse, 28 day (dietary) [23]	0, 2, 4.3 and 8.2	 **(30 < C ≤ 300)**	N/A	2, 4.3 and 8.2 **(C < 30)**	↓ bw gain at 8.2 mg/kg (21 %) ♂
Rat, 90-day (dietary) [20]	0, 4, 7, 14 and 26	14 and 26 **(10 < C ≤ 100)**	26 mg/kg ↓ bw gain (55-57 %) ♀ and ♂ ↓ bw (33-43 %) ♀ and ♂ ↓ FC (36-37 %) ♀ and ♂ 14 mg/kg ↓ bw gain (36 %) ♀ and ♂ ↓ bw (22-27 %) ♀ and ♂ ↓ FC (22-26 %) ♀ and ♂	4 and 7 **(C < 10)**	7 mg/kg ↓ bw gain (27-33 %) ♀ and ♂ ↓ bw (20-21 %) ♀ and ♂ ↓ FC (17-21 %) ♀ and ♂ 4 mg/kg ↓ bw gain (11-15 %) ♀ and ♂

Rat, 90-day (gavage) [21]	0 and 100	100 **(10 < C ≤ 100)**	↓ bw gain (13 %) ♀ ↓ FC ♀ and ♂	 **(C < 10)**	N/A
Rat, 2-generation (gavage) [28]	0, 12, 20/30 and 50/75	12, 20/30, 50/75 **(10 < C ≤ 100)**	75 mg/kg ↓ bw gain (22 %) ♀ ↓ bw (14 %) ♀ 50 mg/kg ↓ bw (7 %) ♀	 **(C < 10)**	N/A
Dog, 365-day (capsule) [24]	0, 6, 12, 30, 60/40/30	6, 12, 30	30 mg/kg **Mortality 2/5** ♂ ↓ bw gain (21-25%) ♀ and ♂ 12 mg/kg ↓ bw gain (33 %)		N/A
Inhalation studies					
Study	**Doses (mg/l**	**Doses Relevant for Stot-RE 2 (mg/l)**	**Effects at doses rel. for STOT-RE 2 (mg/l)**	**Doses Rel. for STOT-RE 1 (mg/l)**	**Effects at doses Rel. for STOT-RE 1 (mg/l)**
Rat, 28-day (nose-only) [14]	0, 0.03, 0.1 and 0.7	0.7 **(0.2 < C ≤ 1)**	**Mortality (1/5 ♂, 3/5 ♀)** ↓ bw (23-31 %) ♀ and ♂ ↓ FC (29-66 %) ♀ and ♂	0.03 and 0.1 **(C < 0.2)**	0.1 mg/l ↓ bw (14 %) ♀ ↓ FC (15-33 %) ♀
Rat, 28-day (whole-body) [25]	0, 0.03	 **(0.2 < C ≤ 1)**	N/A	0.03 **(C < 0.2)**	↓ bw gain (58 %) ♂ bw loss (2.2 g) ♀ ↓ FC (17-23%) ♀
Dermal studies					
Study	**Doses (mg/kg bw/day**	**Doses Relevant for Stot-RE 2 (mg/kg bw/day)**	**Effects at doses rel. for STOT-RE 2 (mg/kg bw/day)**	**Doses Rel. for STOT-RE 1 (mg/kg bw/day)**	**Effects at doses Rel. for STOT-RE 1 (mg/kg bw/day)**
Rat, 90-day (Dermal) [26]	0, 100, 300 and 1000	100 **(2 < C ≤ 200)**	No adverse effects	 **(C < 2)**	N/A

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

In the available repeated dose studies, an increase in mortality was noted in animals exposed to metaflumizone. This was not observed in every study, but was observed in studies conducted via both the oral (mouse and dog) and inhalation (rat) routes of exposure at doses relevant for classification (refer to table 16). A clear basis for this has not been established, but animals were described as having reduced general condition and often marked weight loss. Significant effects on bodyweight and bodyweight gain were observed consistently in the available studies at doses relevant for classification (refer to table 16). Effects to organs such as the spleen, thymus, uterus and ovaries were prevalent, however these were considered secondary to the marked weight loss and weight gain effects.

Whilst the underlying cause of the increased mortality and the bodyweight effects is unclear, the severity of the findings indicates that classification is warranted. In general, the effects occurred at doses relevant for classification with STOT RE 2; however in some cases, bodyweight was markedly reduced at doses much lower than this and relevant for STOT RE 1. In these cases, the severity of the effect was less and varied between sexes and therefore, classification with STOT RE 2 is considered more appropriate. As the cause of mortality has not been established, no tissue has been specified as the target organ. These effects were observed via the oral and inhalation routes of exposure, no effects of relevance to classification were observed via the dermal route. It is therefore considered appropriate to specify the oral and inhalation routes of exposure in the hazard statement.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings

STOT RE 2; H373 – May cause damage to organs through prolonged or repeated oral or inhalation exposure

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

In the repeated dose studies included in the CLH report, an increase in mortality was noted in animals exposed to metaflumizone. This was not observed in every study, but was observed in studies conducted via both the oral (mouse and dog) and inhalation (rat) routes of exposure at doses relevant for classification (see table 16 of the Background Document (BD)). A clear basis for the increase in mortality has not been established, but animals were described as having reduced general condition and often marked weight loss. Significant effects on bodyweight and bodyweight gain were observed consistently in the available studies at doses relevant for classification (table 16 of the BD). Effects on organs such as the spleen, thymus, uterus and ovaries were prevalent; however, these were considered secondary to the marked weight loss and weight gain effects. Whilst the underlying cause of the increased mortality and

the bodyweight effects is unclear, the severity of the findings indicates that classification is warranted. In general, the effects occurred at doses relevant for classification as STOT RE 2; however, in some cases, bodyweight was markedly reduced at doses much lower than this and relevant for STOT RE 1. In these cases, the severity of the effect was lower and varied between sexes and therefore, classification as STOT RE 2 was considered more appropriate by the DS. As the cause of mortality has not been established, no tissue/organ was been proposed as the target organ. These effects were observed via the oral and inhalation routes of exposure, no effects of relevance to classification were observed via the dermal route. Therefore, the DS considered it appropriate to specify the oral and inhalation routes of exposure in the hazard statement.

Comments received during public consultation

Three MSCAs supported the proposed classification for STOT RE, two in agreement with Category 2, another tending towards Category 1.

One MSCA questioned whether palatability could have contributed to the effects on body weight since the toxicity was observed at lower doses in the diet than by gavage administration. Another MSCA proposed to consider the 90-d study with 15 rats/sex/group (study no. 20, table 16 BD) as the most reliable study considering that the available 28-d studies included only 5 animals/sex/group.

Assessment and comparison with the classification criteria

Please note that table 16 of the BD summarises the effects on mortality and body weights that were seen below the guidance values for STOT RE 2 and STOT RE 1. The order of the studies reflects the relative importance of arguments supporting the classification proposal.

Regarding the effects at doses with relevance for STOT RE 1:

In all rat studies (oral and inhalation) that showed lower body weight gain (BWG) and lower (absolute) body weight at doses relevant for STOT RE 1 (studies no. 18, 20, 14 and 25, in the right-hand column of table 16 in the BD), a lower food consumption (FC) was also indicated. For the oral studies (no. 18 and 20) the level of reduction in FC was at a similar range as the body weight effects.

In the two inhalation studies (no. 14 and 25), rats showed either lower BWG or, in the case of the female rats in study 25, even body weight loss. Food consumption was lower (-15 to –33%) in female rats in both studies, while lower FC (up to -8%) was observed in the male rats of study 25 from week 1 to 3 (except the last week) (see table 16 of the BD).

In the mouse study (no. 23) lower BWG was observed at 8.2 mg/kg bw/d (-21%) which was in the same range as the reduction of the food consumption at this dose (-16%) (this information is in addition to table 16 of the BD). However, the reduction in FC in these groups of male mice did not show a clear dose response; the effect on BWG and FC were even stronger in males at 4.3 mg/kg bw/d (FC – 20%, BWG -47% in comparison to control values). In female mice, the food consumption was lower in all dose groups than in the controls, gaining significance in the low and high dose groups without a dose-response relationship. These effects did not match the BWG which was highest in the low dose group.

In conclusion, the effects on the BWG and absolute body weight were reflected by the FC or in the case of the mouse study, both BWG and FC were reduced, but did not show a clear

dose-response relationship. Overall, the effects observed at doses relevant for STOT RE 1 are not considered to be serious effects that warrant classification per se. However, some uncertainties remain as the lower food consumption in the rat inhalation study appears unrelated to palatability.

Regarding the effects at doses relevant for STOT RE 2:

- *12-month (gavage) dog study (no. 24)*

Beagle dogs (5/sex/group) were administered capsules containing metaflumizone at doses of 0, 6, 12, 30 or 60/40/30 mg/kg bw/d.

Premature sacrifices of 2/5 female dogs (receiving 30 mg/kg bw/d by gavage administration) due to their reduced general state, vomiting, ataxia and lateral position warrant classification with STOT RE 2.

This dose, 30 mg/kg bw/d, is below the guidance values for STOT RE 2 (≤ 100 mg/kg bw/d for 90 days corresponds to ≤ 43 mg/kg bw/d for 215 days, the date at which one of the two females were sacrificed (one at day 215 and the second at day 237)).

While the sacrifice of two females and one male of the top dose on day 57 should be attributed to the dose of 60 mg/kg bw/d that was administered until day 49, sacrifice of one additional male and female on day 250 and 226 of this dose group could be attributed to the dose level of 40 mg/kg bw/d (which was administered after day 49) which is still at a dose that warrants STOT RE 2. The high dose was further reduced to 30 mg/kg bw/d on day 245.

One male given 12 mg/kg bw/d was found dead on day 317. The study report indicated that this death was not treatment-related, but did not explain the reasons. These additional sacrifices/deaths support classification as STOT RE 2.

According to the report, lower body weights, reduced BWG and/or body weight loss and reduced FC in treated dogs were noted at ≥ 30 mg/kg bw/d. Lower BWG in female dogs (-33%, -21% and -39%, respectively) were observed at 12, 30 and 60/40/30 mg/kg bw/d (low, mid and high doses). No information on the percentage of reduced FC was, however, given. Thus reduced BGW after gavage administration of metaflumizone, with no information on the contribution of reduced FC on the impaired growth, is considered as supporting evidence of non-specific toxicity of metaflumizone ≥ 12 mg/kg bw/d.

A higher incidence rate of haemosiderosis in Kupffer cells of the liver at ≥ 12 mg/kg bw/d and lower MCHC at ≥ 30 mg/kg bw/d may indicate an (intravascular) haemolytic, hypochromic anaemia. Treatment-related effects on other blood parameters were not seen (not fully documented in detail, the DAR indicated a tendency to lower Hb and higher total bilirubin from day 173 at ≥ 30 mg/kg bw/d). No data on the grading of haemosiderosis was reported. Spleen weights increased up to 57% in both sexes at all doses without a clear dose-response relationship. In male animals, due to the magnitude of the increase, it was considered as toxicologically relevant in the study report (Table B.6.24 in the DAR). The statement in the study report that there was no histopathological correlate to this increase in spleen weight seems not plausible, or the study was limited in the methods to identify the composition in the spleen compartments. No cause of death or obvious mode of action was identified.

Although no clear picture of the severity of the anaemic effects was obvious, haemosiderosis and hypochromasia at dose levels of ≥ 12 mg/kg bw/d are adverse effects that are not sufficient in themselves to support classification, but could be interpreted as supporting the proposed classification. It should be noted that table 16 of the BD indicates mortality in 2/5 male dogs, which deviates from the original summary in the DAR. It can also be noted that an

increased spleen weight may be due to different effects that may occur simultaneously such as increased (haemolytic) erythrocyte degradation and macrophage activation in the red pulpa, haemosiderin deposition and/or hyperaemia.

- *28-d (inhalation, nose-only) rat study (no. 14)*

Wistar rats (5/sex/group) were exposed to metaflumizone at 0, 0.03, 0.1 and 0.7 mg/L.

One male rat died on day 17 at 0.7 mg/L, three female rats were sacrificed on days 9-10. Severe ataxia, apathy, lateral/abdominal position, tremor, splayed limbs and reduced general condition were reported for these animals and comparable signs were seen for surviving animals at this dose. These adverse effects at 0.7 mg/L occurred at the upper range of the guidance value for classification ($0.06 < C \leq 0.6$ mg/L for a dust aerosol). Due to their delayed occurrence during the study, the mortalities/moribundities could not be attributed to acute inhalation toxicity and are considered to support classification as STOT RE 2.

Lower BWG at 0.1 and 0.7 mg/L in male and female rats and even body weight loss at 0.7 mg/L (final body weight was 20/30% lower compared to day 0 in the male/female rats) was linked to lower FC (-40% and –64% in males and females, respectively). Unlike in the discussion of the diet studies in rats, reduced food consumption throughout the inhalation study cannot be explained by palatability problems and should therefore be considered as an effect secondary to the poor general health status and/or erosions/ulcer observed in the glandular stomach at 0.7 mg/L. However, no data on the incidences/severity of erosions/ulcer were given in the study summary in the DAR or the CLH report.

Besides the observed systemic toxic effects, minimum to marked alveolar inflammation and histiocytosis, minimum to slight multifocal hyperplasia of type II pneumocytes at 0.1 and 0.7 mg/L and histiocytic granulomas in BALT/mediastinal lymph nodes at 0.7 mg/L indicated that metaflumizone is accumulated in the lungs, induces granulomatous alveolar and lymph node inflammation and alveolar hyperplasia, and is transported to local lymph nodes.

In the nose, hypertrophy of respiratory epithelium was seen in all animals at 0.7 mg/L and in two males and females at 0.1 mg/L. Although most of the effects on the respiratory tract were not reported to be of marked severity, they are considered as adverse and their occurrence started at 0.1 mg/L, a concentration which is close to STOT RE 1, these effects are considered to support the proposal for STOT RE 2.

Lesions in the thymus, lymph nodes and spleen, indicating an immunosuppressive/cytotoxic effect on T-lymphocytes at 0.7 mg/L and with lower severity/incidences at 0.1 mg/L, support the need for classification. The DS's view that the effect on the immune system is secondary to the reduced BWG and stress may justified. However, an independent immunotoxic effect cannot be ruled out.

Please note that the guidance value range of $0.2 < C \leq 1$ mg/L referred to in table 16 of the BD refers to vapour.

- *28-d (inhalation, whole body) rat study (no. 25)*

In the second 28-d inhalation study, Wistar rats (10/sex/group) were exposed to 0 or 0.03 mg/L only (i.e. control and the low concentration of the study mentioned above). Even at this low concentration, lower body weight, BWG and FC were observed. A slight increase in the severity of adrenal cortical cytoplasmic vacuolation in female rats and an increase in the grade of haemosiderin deposition in male and female rats were also seen.

- *28-d (diet) mouse study (no. 22)*

Mice (5/sex/group) received 0, 50, 200 or 800 ppm metaflumizone in their diet (equivalent to 0, 10, 42 and 101 mg/kg bw/d for both males and females).

Mortalities at 800 ppm during week 1 and 2 in 5/5 male and 4/5 female mice and clinical signs of toxicity (ataxia, convulsions) support the proposal for classification as STOT RE 2 based on the guidance values of 30 < C ≤ 300 mg/kg bw/d for this type and duration of study. Body weight loss, lower BWG and reduced FC, splenic atrophy in males and females at 800 ppm and lower mean BWG in females at 200 ppm (42 mg/kg bw/d) were additional effects supporting classification as STOT RE 2.

- *28-d/90-d (gavage) range-finding rat study (no. 21)*

Rats (5/sex/group) were administered metaflumizone in 0.5% aqueous carboxymethylcellulose (CMC) by gavage at dose levels of 0, 100, 500, 1000 mg/kg bw/d for 28 days or 0, 100 mg/kg bw/d for 90 days. In the 90-d part after one week, the initial dose of 1000 mg/kg bw/d was reduced to 100 mg/kg bw/d because of the marked weight loss observed.

At doses with relevance for classification with STOT RE; lower final bw (-7% in males, -9% in females) and lower (mean) BWG (-12% in males, -23% in females) were noted at 100 mg/kg bw/d after 28 days. Mean food consumption was lower than in controls (- 7% in males, - 17% in females) after 28 days. Body weight gain was reduced in female only (-13%) at 1000 (initial)/100 mg/kg bw/d after 90 days. Effects were much stronger at 500 and 1000 mg/kg bw/d (outside the guidance values of relevance for STOT RE). This gavage study showed that the effects on growth were severe, but much less than after diet administration at comparable or lower doses.

- *28-d, 90-d (dietary) and two-generation (gavage) rat studies (no. 18, 20, 28)*

Lower BWG and final body weight at doses with relevance for classification with STOT RE 2 were seen in rats of the 28-d study (no. 18), 90-d study (no. 20) and two-generation study (no. 28) (see table 16 of the BD). Reduction in growth at doses relevant for classification could be considered as consistent with the effects seen after gavage administration and in the inhalation studies where the palatability could be ruled out as the cause. In comparison to the gavage studies, the effects on body weight seem to occur at lower doses in the diet studies. As the FC was reduced at similar ranges as the reduction in BWG in the oral diet studies in the rat, these effects may at least in part be due to lower FC. Mortalities/moribundities were not seen in the diet studies in rats.

Thus, these studies and the observed findings at doses relevant for classification are less crucial for the classification with STOT RE 2.

Carcinogenicity study

Sprague-Dawley rats (80/sex/group), animals were administered metaflumizone in carboxymethylcellulose (0, 30, 60 and 200/300 mg/kg bw/d) by gavage.

The effect on the body weight was most prominent in the diet studies and were discussed as being due to the palatability. A lower body weight (-11% until week 48) was noted in the carcinogenicity study in female rats after oral administration of 300 mg/kg bw/d which lead to a reduction of the high dose to 200 mg/kg bw/d during week three.

In conclusion, RAC agrees with the DS's proposal that classification with STOT RE 2 is warranted based on the observation of mortality/moribund condition in dogs, mice and rats

from gavage, diet and inhalation studies and severe clinical signs of toxicity and reduced bw/BWG at doses with relevance for STOT RE 2 observed in these studies. Additional supporting evidence consisting of haemolytic effects (dogs), chronic granulomatous lung inflammatory/granulomatous responses and indications of immunotoxic effects on T-lymphocytes (in rats, following inhalation) were given.

RAC agrees with the DS's proposal not to specify a given organ systems as non-specific toxicity is predominant. The specification of the routes (oral and inhalation) seems acceptable based on the lack of effects in the dermal rat study at doses within the guidance value range for classification. However, 'starry sky[1]' cells in the thymus, increased lymphocyte necrosis/apoptosis in the mesenteric lymph nodes at ≥ 300 mg/kg bw/d and reduced (T-cell) cellularity in the perioarteriolar lymphoid sheath of the spleen, and diffuse atrophy of the mandibular lymph nodes at 1000 mg/kg bw/d were observed and thus indicated that – similar to the immunotoxic effects in the 28-d inhalation study – immunotoxic effects were seen after repeated dermal exposure.

Moreover, as the gavage/capsule administration studies indicated that dogs could be more sensitive than rats and no information on dermal route in dogs is given, RAC prefers not to indicate specific routes of exposure.

Overall, RAC agrees to classify metaflumizone as **STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure), with no indication of specific organs or routes of exposure**.

[1] The 'starry sky' appearance of the thymus, observed in the rat dermal study (no. 26), indicates an increased (apoptotic) necrosis of thymus cells which are phagocytised by numerous macrophages containing cellular/nuclear debris of the apoptotic lymphocytes in their cytoplasm. Apoptosis of thymic lymphocytes may occur at a low extent as a physiological process in healthy individuals (without the 'starry sky' appearance).

4.9 Germ cell mutagenicity (Mutagenicity)

Table 17: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

<table>
<tr><th colspan="4">In Vitro Data</th></tr>
<tr><th>Method</th><th>Organism/
strain</th><th>Concentrations tested</th><th>Result</th></tr>
<tr><td>Bacterial reverse mutation assay (Ames Test) (2001) [30]

OECD 471 (1997)
GLP

Purity 96.3 %

DAR: B. 6.4.1a</td><td>S. typhimurium: TA98, TA100, TA1535 and TA 1537

E-coli: WP2 uvrA</td><td>0, 15, 50, 150, 500, 1500 and 5000 µg/plate (in DMSO)</td><td>±S9: Negative

Precipitation was observed at ≥ 150 µg/plate</td></tr>
<tr><td>Gene mutation test in CHO cells (HPRT locus Assay) (2002) [31]

OECD 476 (1997)
GLP

Purity 96.9 %

DAR: B.6.4.1b</td><td>Chinese hamster V79 cells</td><td>First Assay (±S9): 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/ml

Second Assay (-S9): 0, 25, 50, 100, 200, 400 and 800 230g/ml (+S9): 0, 50, 100, 200, 400, 800 and 1200 µg/ml

(in DMSO)</td><td>±S9: Negative

Precipitation was observed in the absence of S9 at ALL concentrations tested</td></tr>
<tr><td>Chromosome aberration assay in CHO cells (2005) [32]

OECD 473
GLP

Purity 96.9 %

Engelhardt G and Liebold E 2002

DAR: B.6.4.1c</td><td>Chinese hamster V79 cells</td><td>-S9

First Assay: 0, 12.5, 25 and 50 µg/ml

Second Assay: 0, 3.125, 6.25, 12.5, 25 and 50 µg/ml

+S9

0, 25, 50 and 100 µg/ml</td><td>+S9: Negative

-S9: Positive:

<table>
<tr><th>Treatment (µg/ml)</th><th>Exchanges</th><th>Aberrant metaphases (%)</th></tr>
<tr><td colspan="3">Assay 1 (-S9)</td></tr>
<tr><td>0</td><td>2</td><td>1.5</td></tr>
<tr><td>12.5</td><td>22*</td><td>13*</td></tr>
<tr><td>25</td><td>3</td><td>2.5</td></tr>
<tr><td>50</td><td>20*</td><td>12.5*</td></tr>
<tr><td>EMS (350)</td><td>11*</td><td>19*</td></tr>
<tr><td colspan="3">Assay 2 (-S9)</td></tr>
<tr><td>0</td><td>1</td><td>2</td></tr>
<tr><td>3.125</td><td>3</td><td>4.5</td></tr>
<tr><td>6.25</td><td>6</td><td>5.5</td></tr>
<tr><td>12.5</td><td>10*</td><td>15*</td></tr>
<tr><td>25</td><td>14*</td><td>15*</td></tr>
<tr><td>50</td><td>17*</td><td>21*</td></tr>
<tr><td>EMS (350)</td><td>8</td><td>15*</td></tr>
</table>
* significantly different to controls (p<0.05)

Cytotoxicity observed at 100 µg/ml</td></tr>
</table>

In vivo Data			
Method	**Organism/ strain**	**Concentrations tested**	**Result**
Mouse bone marrow micronucleus assay (2002) [33] Intraperitoneal (i.p.) Vehicle: 0.5 % aq. CMC OECD 474 (1997) GLP Purity 96.9 % DAR: B.6.4.2a	NMR1 mice (5 males/group)	0, 500, 1000 or 2000 mg/kg bw	**Negative** ***Clinical signs:*** Squatting posture (all doses) Piloerection and poor general state (at 2000 mg/kg bw)
Unscheduled DNA synthesis assay in rat hepatocytes (2003) [34] Oral (gavage) Vehicle: 0.5 % aq. CMC OECD 486 (1997) GLP Purity 96.9 % DAR: B.6.4.2b	Wistar rats (3 males/group)	0, 1000 or 2000 mg/kg bw	**Negative**

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Three *in vitro* studies are available to assess the mutagenic potential of metaflumizone. In a guideline Ames test [30] and a mammalian cell mutation assay [31] no evidence of mutagenicity was observed under the conditions of the assays. In a study to investigate *in vitro* chromosome aberrations in Chinese hamster V79 cells, there was no evidence of clastogenicity when metaflumizone was tested in the presence of metabolic activation [32]. When tested in the absence of metabolic activation however, there was a statistically significant increase in the number of cells with aberrations. Two assays were carried out in the absence of S9 metabolising mix. In the first, aberrations were noted at 12.5 µg/ml and 50 µg/ml, but there was no increase at 25 µg/ml and the results at 100 µg/ml revealed an insufficient number of well spread metaphases due to cytotoxicity. In the second assay, employing five concentrations ranging from 3.125 – 50 µg/ml, an increase in the proportion of aberrant cells was quite clearly observed, with a dose-response relationship, at concentrations ≥ 12.5 µg/ml. Therefore, metaflumizone was shown to have clasotogenic activity *in vitro* in Chinese hamster V79 cells, in the absence of metabolic activation.

4.9.1.2 *In vivo* data

Metaflumizone was tested in a guideline *in vivo* micronucleus assay in mice [33] and a guideline unscheduled DNA synthesis assay with rat hepatocytes [34]. When tested for chromosomal damage and for the ability to induce spindle poison effects in NMR1 mice using the micronucleus test method, metaflumizone showed no increase in the number of polychromatic erythrocytes containing either small or large micronuclei, following intraperitoneal injection. Clinical signs included squatting posture, piloerection and general poor state of condition. In an unscheduled DNA synthesis assay, there was no increase in net nuclear grain count over the negative control value observed with any test concentration of metaflumizone. There was no increase in the percentage of cells in repair, up to the highest dose tested (2000 mg/kg bw). In this study, there were no signs of clinical toxicity.

4.9.2 Human information

There was no human information available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

The potential mutagenicity of metaflumizone has been well investigated. *In vitro*, negative results were obtained with and without S9 in bacterial and mammalian cell gene mutation tests. Similarly, no increases in chromosome aberrations were seen in CHO V79 cells with S9, but a reproducible dose-related increase was seen in the absence of any exogenous metabolic activation system. *In vivo*, well conducted tests for micronuclei in the bone marrow of mice and UDS in rat liver cells both gave negative results. Overall, it can be concluded that metaflumizone lacks mutagenic potential.

4.9.5 Comparison with criteria

Although a positive result was observed in an *in vitro* chromosome aberration test (-S9), a well conduced *in vivo* bone marrow micronucleus test involving intra-peritoneal administration of metaflumizone to mice gave a negative result. This indicates that the aberrations observed in the vitro test do not indicate potential for in vivo mutagenic activity. Further reassurance that metaflumizone lacks mutagenic potential was provided by the in vitro gene mutation tests in bacteria and mammalian cells and a rat liver UDS assay. As metaflumizone lacks mutagenic potential, no classification is required for this endpoint.

4.9.6 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Based on the available data, the DS did not propose to classify metaflumizone for germ cell mutagenicity.

***In vitro* tests**

A bacterial gene mutation test (Ames test, OECD TG 471, GLP) and a mammalian cell gene mutation test (HPRT test, Chinese hamster V79 cells, OECD TG 476, GLP) were negative with and without metabolic activation. A mammalian chromosomal aberration test with V79 cells showed negative results when metaflumizone was tested in the presence of metabolic activation. When tested in the absence of metabolic activation there was a statistically significant increase of chromosomal aberrations (OECD TG 473, GLP).

***In vivo* tests**

A micronucleus test (OECD TG 474, GLP) in mice was negative up to the highest tested dose of 2000 mg/kg bw after i.p. injection. Clinical signs were observed at all tested doses. No information was given on cytotoxic effects (PCE/NCE).

Furthermore, an unscheduled DNA synthesis test (indicator test, OECD TG 486, GLP) in rats was negative after oral administration (gavage) of doses up to 2000 mg/kg bw. No signs of clinical toxicity were observed.

In summary, the induction of clastogenic effects in a positive *in vitro* chromosomal aberration test was not confirmed in a negative *in vivo* mutagenicity test (micronucleus test) in mice. Overall, the DS concluded that metaflumizone does not have mutagenic potential *in vivo*.

Comments received during public consultation

No comments on germ cell mutagenicity were submitted.

Assessment and comparison with the classification criteria

RAC concluded in agreement with the DS's proposal that no classification for germ cell mutagenicity is warranted.

Metaflumizone did not induce gene mutations in bacteria or in a mammalian cell culture (V79 cells) *in vitro* but showed clastogenic activity in a mammalian cell culture (V79 cells) *in vitro* only in the absence of metabolic activation. Based on the negative *in vivo* micronucleus test no mutagenicity was induced in somatic cells (criterion for classification as Category 2). Information on the induction of germ cell mutagenicity (criterion for classification as Category 1B) is not available.

RAC considers that metaflumizone does not meet the classification criteria for germ cell mutagenicity, as defined in CLP, and **no classification as a germ cell mutagen** is therefore proposed.

4.10 Carcinogenicity

No information on the carcinogenicity of metaflumizone in humans is available. One carcinogenicity study in the rat (oral route 2003(b)[27]) and one study in the mouse (oral route (2003a[29])) are summarised in Table 18.

Table 18: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
2-Year Carcinogenicity study in rats (Sprague-Dawley) (2003b) [27] (80/sex/group) Oral (gavage) 0.5 % aq. CMC OECD 453 GLP Purity 96.3 % DAR: B.6.5.1	0, 30, 60 and 200/300 mg/kg bw/day (The dose level of 300 mg/kg bw/day was reduced to 200 mg/kg bw/day in females during wk 3 due to effects on body weight)	**There were no toxicologically significant findings in this study (neoplastic or non-neoplastic).**
18-Month Carcinogenicity study in mice (CD-1) (2003a) [29] (65/sex/group) Oral (gavage) 0.5 % aq. CMC OECD 451 GLP Purity 96.3 % DAR: B.6.5.2	0, 100, 250 and 1000 mg/kg bw/day	**There were no neoplastic findings in this study.** **Non-neoplastic findings:** **1000mg/kg bw/day:** ↓ BW gain: 17 % (males only) ↑ Reticulocyte count: 112 % (males) and 48 % (females) ↓ Mean Cell Haematocrit: 6.4 % (females) ↑ Spleen – brown pigmentation: 19/65 males (versus 0 in controls) and 27/65 females (versus 3/65 in controls)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Two oral chronic toxicity and carcinogenicity studies are available, one in rats and one in mice.

The rat study was carried out according to guidelines and GLP but with a number of deviations, however these were not considered to have affected the results [27]. Sprague-Dawley rats

(80/sex/group) were administered metaflumizone orally by gavage in 0.5 % aqueous CMC (0, 30, 60 and 300 mg/kg bw/day) for 24 months (the final time-point for males was 23 months). During week 3, the dose level of 300 mg/kg bw/day was reduced to 200 mg/kg bw/day (in females only) due to effects on body weight (15 % reduction in body weight compared to controls).

Mortality was observed over the duration of the study but was not attributable to treatment with metaflumizone, and in general survival rates of test animals were comparable to or greater than controls. Bodyweight was decreased in the top dose group of females up until week 48 (11 – 15 %) after which time there was recovery to levels comparable with controls. Bodyweight gain was reduced in weeks 0-3 for males and females (males: 10 % and females: 52 %) but by the end of the study weight gain was comparable or greater than that of controls.

Gross necropsy did not reveal any treatment-related findings in this study. Microscopic analysis revealed an increased incidence of centrilobular hepatocellular hypertrophy in males at doses ≥ 60 mg/kg bw/day (14/60 and 32/60 males affected at 60 mg/kg bw/day and 300 mg/kg bw/day respectively) and in females at 300 mg/kg bw/day only (13/60 females affected). The incidence of this in controls was 0. There was a slight increase in the incidence of basophilic hepatocellular alteration in males at doses ≥ 60 mg/kg bw/day (15/60 and 16/60 versus 7/60 in controls at 60 and 300 mg/kg bw/day respectively). These hepatic histopathological findings were not considered toxicologically significant, as they were not supported by any other findings such as clinical chemistry, and instead represented adaptive changes to treatment with metaflumizone.

There were no neoplastic findings in this study.

An 18-month oral chronic toxicity and carcinogenicity study was carried out in mice [29]. CD-1 mice (65/sex/dose) received metaflumizone suspended in 0.5 % aqueous CMC by gavage (0, 100, 250 or 1000 mg/kg bw/day) for up to 18 months.

There were no treatment-related effects on mortality in this study and body weights remained within 6 % of control values for both males and females. In males, there was an overall reduction in weight gain of 10.4 % in the top dose group only.

There were some effects noted on haematological parameters. At 12 months males showed a statistically significantly higher reticulocyte count at a dose of 1000 mg/kg bw/day (24 %) and at 18 months the count was still higher than controls (12 %), but not statistically significant. In females, reticulocyte count was statistically significantly higher at the top dose of 1000 mg/kg bw/day at 18 months (48 %). Mean cell haematocrit (MCH) was reduced in females at 1000 mg/kg bw/day (10 % and 6 % at 12 and 18 months respectively). A microscopic correlate to these findings was the presence of an increased incidence of haemosiderin in the spleen (19/65 males, versus 0 in controls and 27/65 females, versus 3/65 in controls).

There were no neoplastic findings that were considered related to treatment.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

There is no other relevant information.

4.10.4 Summary and discussion of carcinogenicity

Daily treatment of rats and mice with metaflumizone, orally by gavage, for up to 24 months resulted in no test-substance-related neoplastic findings.

In rats, reduced body weight and body weight gain were observed in females at 300 mg/kg bw/day, resulting in the dose being reduced to 200 mg/kg bw/day during week 3 of the study. Body weight and body weight gain was comparable to controls by the end of the study. There were increased incidences of centrilobular hepatocyte hypertrophy in males and females and basophilic hepatocellular alteration in males at doses of 60 mg/kg bw/day and above. These responses were considered adaptive and not toxicologically significant.

In mice, there was a decrease in cumulative weight gain in males of the top dose group only. Indications of increased erythrocyte turnover, including increased haemosiderin in the spleen and haematological changes (increased mean reticulocyte count and decreased MCH) were observed at 1000 mg/kg bw/day in females. These findings were at a dose not relevant for classification with specific target organ toxicity.

4.10.5 Comparison with criteria

There were no neoplastic findings attributable to treatment with metaflumizone in a 24-month rat study or an 18-month mouse carcinogenicity study. Therefore, classification with carcinogenicity is not required.

4.10.6 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS concluded, based on two carcinogenicity studies by gavage that were included in the CLH report, that no metaflumizone-related increase in neoplastic findings were observed in rats at 30, 60, and 300/200 mg/kg bw/d (24 months, OECD TG 453, GLP), or in mice at 100,

250, and 1000 mg/kg bw/d (18 months, OECD TG 451, GLP) and thus no classification was proposed.

A slightly increased mortality in females at 300/200 mg/kg bw/d was noted during the first 12 months; however, the pattern of mortality (2 animals at 4 and 5 months and 1 animal at months 6, 7, 9 and 11, respectively) was considered by the DS, and in the DAR, as not indicating a relationship to treatment.

Non-neoplastic findings in rats were reduced bw and bw gain in females at 300 mg/kg bw/d that resulted in lowering of the dose to 200 mg/kg bw/d during week 3 of the study. Increased incidences of hepatocellular hypertrophy in males at ≥ 60 mg/kg bw/d and females at 200 mg/kg bw/d as well as basophilic hepatocellular alteration in males at 60 mg/kg bw/d were seen.

In mice, lower final bw was seen in males at 1000 mg/kg bw/d. In addition, increased erythrocyte turnover through haemolysis (increased red blood cell; RBC), decreased mean corpuscular hemoglobin concentration (MCHC, in males, also at 250 mg/kg bw/d), decreased mean cell haematocrit (MCH, females at 12 and 18 months), increased reticulocytes (at 18 months) increased splenic haemosiderosis in males at 12 and 18 months and females at 18 months) were seen.

Comments received during public consultation

No specific comments regarding carcinogenicity were received.

Assessment and comparison with the classification criteria

RAC does not agree with the interpretation that the slight increase in the incidence of basophilic hepatocellular alterations in male rats at doses ≥ 60 mg/kg bw/d was of an adaptive nature. Basophilic alterations could be linked to liver tumorigenesis. However, these lesions were also observed spontaneously and might or might not progress to liver tumours or also regress spontaneously. In the rat study on metaflumizone, the incidences of basophilic hepatocellular alterations in males were 25% (15/60), 26.7% (16/60) at 60 and 300 mg/kg bw/d, respectively, versus 12% (7/60) in the controls.

No increase in liver tumours was seen in treated male and female rats and no increase of basophilic foci was seen in treated female rats. A summary table on the tumour incidences was not included in either the CLH report or in the DAR study summary; thus any further details of these studies could not be evaluated by RAC.

As no treatment-related increase in tumour responses were reported and no concern was identified for somatic cell mutagenicity, RAC supports the DS's proposal that **no classification for carcinogenicity is warranted**.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Table 19: Summary table of relevant reproductive toxicity studies – Fertility

≠ **All figures quoted as reduced /increased by [denoted by↓ and ↑ (respectively)] are relative to controls.**
Statistically different from controls, *p<0.05, **p<0.01
† Male fertility index = Number of males proving fertility/Number of males placed with females x 100
‡ Female fertility index = Number of females pregnant/Number of females mated x 100
∞ Lactation index = (Survival at PND 21/Survival at PND 4) x 100
$ Pup viability index = (Number of live pups on day 4 (before standardisation) after birth/Number of live pups on the day of birth) x 100
£ Live birth index = (Number of live offspring/Number of offspring delivered) x 100
¥NOAELS have been copied from the DAR for information only.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two-generation study in rats (Wistar) (2003) [28] (25/sex/group) Oral (gavage) 0.5 % aq. CMC OECD 416 GLP Purity 96.9 % DAR: B.6.6.1	F0 Generation: 0, 12, 30, 75 mg/kg bw/day (days 0-125) Dose was reduced due to "excessive maternal and developmental toxicity" 0, 12, 20 50 mg/kg bw/day (from day 126) F1 Generation: 0, 12, 20 50 mg/kg bw/day	***General toxicity:F0 generation - mating A:*** 75 mg/kg bw/day ↓ BW, week 10: 14 %≠ (females)** ↓ BW gain, weeks 0-10: 27 % (females)** ↓ Food consumption, weeks 0-10: 13 % (females) 30 mg/kg bw/day: No treatment-related effects 12 mg/kg bw/day: No treatment-related effects ***F0 generation - mating B:*** 50 mg/kg bw/day: ↓ BW, week 10: 7 % (females)** 20 mg/kg bw/day: No treatment-related effects 12 mg/kg bw/day: No treatment-related effects ***F1 generation:*** No treatment-related effects ***Reproductive effects:*** ***F0 generation - mating A:*** 75 mg/kg bw/day: Fertility index: 72 % (males)† and 75 % (females)‡ [versus 96 % in controls (both males and females)]* Improper nursing behaviour: 4/18 dams

		30 mg/kg bw/day: Improper nursing behaviour: 1/21 dams 12 mg/kg bw/day: No treatment-related effects ***F0 generation - mating B:*** 50 mg/kg bw/day: Improper nursing behaviour: 2/20 dams 20 mg/kg bw/day: No treatment-related effects 12 mg/kg bw/day: No treatment-related effects ***F1 generation:*** No treatment-related effects ***Offspring effects:*** ***F_{1A} generation:*** 75/50 mg/kg bw/day: Complete litter losses: 5/18 dams lost all their pups due to either improper nursing behaviour or cannibalisation (4 dams lost all pups shortly after birth and 1 dam lost all pups by PND 11) ↓ Total no. of live fetuses at birth: 37 % (163 versus 257 in controls) Total death rate (Days 0-4): 7.4 % (versus 0.4 % in controls), (Days 4-21): 23.6 % (versus 0 % in controls) Pup viability index = 93 % (versus 99 % in controls)$^{**\$}$ Lactation index = 70 % (versus 100 % in controls)$^{**\infty}$ ↓ BW gain (group mean), PND 1-4: 29 % and PND 4-7: 25 % 30/20 mg/kg bw/day: Complete litter loss: 1/21 dams had no pups alive by PND 3 ↓ Total no. of live fetuses at birth: 23 % (197 versus 257 in controls) 12 mg/kg bw/day: No treatment-related effects ***F_{1B} generation:*** 50 mg/kg bw/day: Complete litter loss: 3/20 dams had no pups alive (PND 1, 4 and 6) ↓ Total no. of live fetuses at birth: 21% (179 versus 227 in controls)* Total no. of dead fetuses at birth: 12 versus 5 in controls (6.3 %)* Live birth index: 94 % (versus 98 % in controls)$^{*£}$ Total death rate (Days 0-4): 8.4 % (versus 0.4 % in controls), (Days 4-21): 6.2 % (versus 0 % in controls) Pup viability index: 92 % (versus 100 % in controls)$^{*\$}$ Lactation index: 92 % (versus 100 % in controls)*† 20 mg/kg bw/day: ↓ Total no. of live fetuses at birth: 15 % (194 versus 227 in controls) 12 mg/kg bw/day: No treatment-related effects ***F_2 generation:*** No treatment-related effects *Parental, reproductive and offspring NOAELs of 20 mg/kg bw/day were determined.*$^{¥}$

4.11.1.1 Non-human information

A multi-generation reproductive toxicity study was carried out in Wistar rats (F_0 parental generation: 25/sex/dose) [28]. Parental animals received a daily dose of metaflumizone by oral gavage (0, 12, 30 or 75 mg/kg bw/day). Animals were treated for at least 75 days before F_0 males and females were mated to produce a first litter F_{1A} – mating A. Treatment was continued throughout gestation and the *post-natal* lactation period (PND 0 – 21).

At the top dose of 75 mg/kg bw/day excessive maternal and developmental toxicity were described, characterised as poor general state, reduced body weight gain (27% decrease compared to controls), reduced food consumption in females and high pup mortality (pup deaths increased on PND 0-4: 7.4 % and PND 4-21: 23.6 %, compared to 0.4% and 0% in controls on PND 0-4 and 4-21 respectively). All surviving F_{1A} pups were killed on Day 21 *post-partum* (PND 21) and examined microscopically at necropsy. From Day 126 the F_0 generation parental animals received the test substance at partially reduced doses (0, 12, 20 or 50 mg/kg bw/day) until one day before sacrifice. F_0 rats were then treated for a 10-week period (pre-mating) and were mated with the same partner as for mating A to produce a second litter (F_{1B}) – mating B. Females were allowed to litter and rear the F_{1B} generation pups until Day 4 (standardisation) (PND 4) or PND 21. Animals (25/sex/dose) were selected from the F_{1B} generation pups and were treated at 0, 12, 20 and 50 mg/kg bw/day post weaning and the breeding programme was repeated to produce the F_2 generation pups.

Parental effects

Female rats of the top dose (mating A) showed statistically significantly reduced body weight in comparison to controls during week 10 (prior to mating) (14 %) and reduced body weight gain in comparison to controls during weeks 0 – 10 (pre-mating) (27 %) associated with reduced food consumption (13 %). The reduced dose of 50 mg/kg bw/day used in mating B led to statistically significantly reduced body weight in females (compared to controls), however this was below 10 % and so not considered toxicologically significant. No effects on body weight gain were observed in this group. Poor general condition was noted for top dose animals of both mating A and B during pre-mating, gestation and lactation periods.

Reproductive function and performance

Fertility index was decreased in comparison to controls in both males and females of the top dose group from mating A (males: 72 % and females: 75 % versus 96 % in controls). These values were outside of the laboratory historical control data range (HCD) of 84-100 % and were found to be statistically significant.

In males, fertility index was defined as the number of males proving fertility (defined by a female giving birth to a litter or with pups/implantations in utero) over the number of males placed with females. Female fertility index was defined as the number of females pregnant (defined by the number of females giving birth to a litter or with pups/implantations in utero) over the number of females mated. Therefore, the number of females giving birth to litters affects both male and female fertility indices.

When the same pairs of animals were re-mated (mating B), after being administered the lowered dose of 50 mg/kg bw/day, the fertility index was 88 % (for both males and females). Whilst this still represented a decrease from the fertility index of the control animals (100 %) it was within the laboratory historical control data and indicated that many of the previously infertile animals had gone on to successfully mate (with the exception of two pairs of animals who remained infertile).

A decrease in fertility index was also observed in mid-dose males and females of mating A and B (84 % in males and females at the first mating and 88 % in males and females in the re-mating); this was not statistically significant and was within the HCD.

No conclusive adverse histopathological findings were observed to account for the observed impaired fertility. Analysis of sperm parameters from control and high dose males revealed no treatment-related effects and no differences in sperm motility were noted across groups. There were no treatment-related effects in the F1 parents.

Table 19a: Fertility Index in males and females

Fertility Index (%) F0 (Mating A)				
Dose Leve (mg/kg bw/d)	**0**	**12**	**30**	**75**
Males	96	100	84	72*
Females	96	100	84	75*
Fertility Index (%) F0 (Mating B)				
Dose level (mg/kg bw/d)	**0**	**12**	**20**	**50**
Males	100	100	88	88
Females	100	100	88	88
Fertility Index (%) F1				
Dose level (mg/kg bw/d)	**0**	**12**	**20**	**50**
Males	96	96	92	96
Females	100	96	92	96

**significantly different to control, p<0.05; ** p<0.01.*

Offspring effects

A decrease in the number of live fetuses at birth was observed in both the mid and high dose groups of the F_{1A} and F_{1B} generations (refer to table 19b). In the F_{1A} generation, the number of live fetuses born in the top and mid dose groups respectively was 37 % and 23 % lower compared to control In the top and mid dose F_{1B} generation the number of live fetuses at birth was 21 % and 15 % (respectively) lower compared to controls. The reduction in the live birth index only reached statistical significance in the high dose group of the F_{1B} generation (94 % versus 98 % in controls). There were no effects on numbers of live fetuses or live birth index in the F_2 generation.

There was an increased rate of pup death and/or cannibalisation following treatment with metaflumizone with complete litter loss observed for 5/18 dams receiving 75 mg/kg bw/day (Mating A). Of these 5 dams, 4 lost their pups shortly after birth and were noted to exhibit improper nursing behaviour (confirmed at necropsy by increased incidences of empty stomachs). The fifth dam showed normal nursing behaviour. The total pup death between PND 0-4 was 7.4 % (versus 0.4 % in controls). One dam of the mid dose group of mating A also lost the complete litter, no improper nursing behaviour was identified. Following the second mating (Mating B), complete litter loss was observed for 3/20 dams receiving 50 mg/kg bw/day. Of these, 2 showed improper nursing behaviour. The total death rate between PND 0-4 was 8.4 % (versus 0.4 % in controls). There were no such effects observed in the pups of the F_2 generation.

During the lactation period, PND 4-21 the total death rate in the F_{1A} generation was increased (23.6 % versus 0 in controls). This gave rise to a statistically significantly decreased lactation index (survival at PND 21 over survival at PND 4) 70 % versus 100 % in controls). This was also observed in the F_{1B} generation to a lesser extent (PND 4-21 total death rate of 6.2 % versus 0 in controls) leading to a reduced lactation index of 92 % (versus 100 % in controls). The lactation index in the F_2 generation was unaffected.

Table 19b: Litter parameters for the F1A, F1B and F2 generations

F1A Generation				
Dose level (mg/kg bw/d)	**0**	**12**	**30**	**75**
Live fetuses	257	279*	197	163
Dead fetuses	0	5*	2	2
Deaths: Days 0-4 (%)	1 (0.4)	3 (1.1)	3 (1.5)	12 (7.4)
Deaths: Days 4-21 (%)	0 (0)	0 (0)	1 (0.5)	38 (23.6)
Live birth index (%)	100	98	99	99
Viability index (%)	99	99	98	93**
Lactation index (%)	100	100	99	70**
F1B generation				
Dose level (mg/kg bw/d)	Control	12	20	50
Live fetuses	227	232	194	179*
Dead fetuses	5	4	1	12*
Deaths: Days 0-4 (%)	1 (0.4)	1 (0.4)	3 (1.5)	15 (8.4)
Deaths: Days 4-21 (%)	0 (0)	2 (0.8)	0 (0)	11 (6.2)
Gestation index (%)	96	96	100	95
Live birth index (%)	98	98	99	94*
Viability index (%)	100	100	98	92*
Lactation index (%)	100	99	100	92*
F2 generation				
Dose level (mg/kg bw/d)	Control	12	20	50
Live fetuses	249	262	244	234
Dead fetuses	4	1	3	5
Deaths: Days 0-4 (%)	14 (5.6)	5 (1.9)	6 (2.4)	8 (3.4)
Deaths: Days 4-21 (%)	1 (0.4)	0 (0)	1 (0.4)	3 (1.3)
Gestation index (%)	100	100	100	100
Live birth index (%)	98	100	99	98
Viability index (%)	94	98	98	97
Lactation index (%)	99	100	99	98

** significantly different to controls, p<0.05; ** p<0.01*

Body weight gain was significantly reduced in comparison to controls in the top dose-treated F_{1A} generation. Following treatment with 75 mg/kg bw/day, body weight gain during PND 0-4 was reduced by 29 % and during PND 4-7 by 25 %. Bodyweights and body weight gain were similar to controls after PND 7. Necropsy revealed an increased incidence of an empty stomach resulting from the improper nursing behaviour in dams. Body weight and body weight gain was unaffected in F_{1B} and F_2 pups. There were no other treatment-related findings at necropsy

4.11.1.2 Human information

There is no human data available.

4.11.2 Developmental toxicity

Table 20: Summary table of relevant reproductive toxicity studies - Development

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks

(effects of major toxicological significance)</th></tr>
<tr><td>Developmental toxicity study in the rat (Wistar) (2003) [35]

(25/females/group)

Oral (gavage)
0.5 % aq. CMC

OECD 414 (2001)
GLP

Purity 96.9 %

DAR: B.6.6.2</td><td>0, 15, 40 and 120 mg/kg bw/day

(Days 6-19 of gestation)</td><td>Maternal toxicity:

<u>120 mg/kg bw/day:</u>
↓ BW gain: 22 %

<u>≤ 40 mg/kg bw/day:</u>
No treatment-related effects

A NOAEL of 40 mg/kg bw/day was determined.

Fetal findings:

There were no treatment-related external variations, skeletal variations or visceral effects observed.</td></tr>
<tr><td>Developmental toxicity study in the rabbit (Himalayan) (2003) [36]

(25/females/group)

Oral (gavage)
0.5 % aq. CMC

OECD 414 (2001)
GLP

Purity 96.9 %

DAR: B.6.6.3</td><td>0, 30, 100 and 300 mg/kg bw/day

(Days 6-28 of gestation)</td><td>Maternal toxicity:

<u>300 mg/kg bw/day:</u>
3/25 dams were sacrificed before schedule due to moribund condition
4/25 exhibited signs of toxicity including lateral position, ataxia, poor general state, no defecation and blood in bedding.

Fetal findings:

<u>300 mg/kg bw/day:</u>
↑ Runts: 13.8 % versus 3.3 % in controls

Developmental toxicity:

<table>
<tr><th colspan="3" rowspan="2">Parameter</th><th colspan="4">Dose level (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>30</th><th>100</th><th>300</th></tr>
<tr><td rowspan="3">Absent subclavian artery</td><td>Fetal incidence</td><td>(#)
(%)</td><td>0/121
0</td><td>1/129
0.8</td><td>1/131
0.8</td><td>3/131
2.3</td></tr>
<tr><td>Litter incidence</td><td>(#)
%</td><td>0/20
0</td><td>1/23
4.3</td><td>1/19
5.3</td><td>3/20
15</td></tr>
<tr><td>Affected litters</td><td>(%)</td><td>0
0</td><td>0.6
±3.0</td><td>0.7
±2.9</td><td>3.1*
±8.4</td></tr>
<tr><td rowspan="3">Incomplete ossification of sternebra</td><td>Fetal incidence</td><td>(#)
(%)</td><td>42/121
35</td><td>45/129
35</td><td>66/131
50</td><td>58/131
46</td></tr>
<tr><td>Litter incidence</td><td>(#)
(%)</td><td>15/20
75</td><td>18/23
78</td><td>18/19
95</td><td>18/20
90</td></tr>
<tr><td>Affected litters</td><td>(%)</td><td>29.7
±24.4</td><td>32.3
±27.3</td><td>47.5*
±28.2</td><td>46.5**
±23.4</td></tr>
</table>
*significantly different (p< 0.05) to controls; ** (p< 0.01)

NOAELs of 100 mg/kg bw/day were determined for both dams and pup development.</td></tr>
</table>

4.11.2.1 Non-human information

Two developmental toxicity studies are available, one in rats and one in rabbits, both following OECD guidelines and carried out to GLP.

In the rat study [35]; time-mated female Wistar rats (25/dose) were treated with metaflumizone suspended in 0.5 % carboxymethylcellulose (0, 15, 40 or 120 mg/kg bw/day) via oral gavage on days 6-19 of gestation.

There was no mortality or clinical signs in dams. Bodyweight gain was significantly reduced between gestational days 6-8 (54 %) and days 6-19 (12 %) resulting in an overall reduction in bodyweight gain of 22 %.

There were no treatment-related external or skeletal variations or visceral effects observed in fetuses.

In the rabbit study [36]; artificially inseminated female Himalayan rabbits (25/dose) were administered metaflumizone suspended in carboxymethylcellulose (0, 30, 100 or 300 mg/kg bw/day) daily by gavage on Days 6-28 of gestation.

Two rabbits of the top dose group were intentionally not gavaged on gestational days 25-28 due to very poor general state. One of these was sacrificed on schedule whilst the other was sacrificed before schedule; both aborted their litters. A third rabbit of this dose group was also sacrificed in moribund condition. Signs of toxicity in 4/25 rabbits at 300 mg/kg bw/day included lateral position, ataxia, poor general state, no defecation or blood in the bedding. There were no clinical signs at lower doses and no effects on body weight, body weight gain or food consumption.

Fetal findings included an increase in the number of runts from 3.3 % in controls to 13.8 % at 300 mg/kg bw/day. External examination of fetuses revealed no treatment-related findings. Visceral examination revealed an increase in the fetal and litter incidence of the malformation absent subclavian artery at 300 mg/kg bw/day [fetal incidence in controls: 0, and at 300 mg/kg bw/day 3/131 (2.3 %), 3/20 litters affected]. Historical control data provided by the applicant revealed only 1 study out of a total of 34 (dating from 1995 – 2008) whereby 3 control fetuses were affected by this malformation (Table 21). The incidence was 3/146 fetuses affected (3/22 litters), giving a HCD range of 0 – 2.1 %, just short of the percentage obtained in this study.

Table 21: Historical control data for litter incidences of absent subclavian artery in Himalayan rabbits between 1995 – 2008 (provided by the Applicant)

Study	Number of fetuses with absent subclavian artery (Number of litters)			
Start date	**Controls**	**Low**	**Mid**	**High**
January 2006	0/144 (23 litters)	0	0	NA
April 2002	0/119 (21 litters)	0	0	0
September 2002	0	ND	ND	ND
August 2004	0/145 (23 litters)	1/150 (23 litters)	0/129 (23 litters)	2/125 (20 litters)
March 2003	0/121 (20 litters)	1/129 (23 litters)	1/131 (19 litters)	3/120 (20 litters)
November 2003*	1/155 (24 litters)	0/142 (19 litters)	0/125 (21 litters)	1/135 (23 litters)
February 2005	0/157 (25 litters)	0	0	0
January 2007	0/163 (24 litters)	0	0	1
May 2005	0/154 (23 litters)	0	0	0
January 2006	0/150 (25 litters)	ND	ND	0
November 2006	1/172 (25 litters)	0/130 (23 litters)	1/168 (25 litters)	2/158 (25 litters)
June 2008	0/144 (24 litters)	0	0	0
September 2008	0/155 (23 litters)	0/155 (24 litters)	1/134 (21 litters)	1/144 (23 litters)
January 2008	0/163 (23 litters)	0	0	0
August 2007	0/144 (23 litters)	1/168 (24 litters)	1/148 (24 litters)	0/83 (14 litters)
August 2008	0/153 (23 litters)	0/117 (19 litters)	1/146 (24 litters)	0/168 (24 litters)
November 2007	0	ND	ND	ND
April 2008	0/171 (25 litters)	0	0	0
June 2008	0/172 (25 litters)	0	0	0
May 1995	0/121 (20 litters)	ND	ND	0/129 (18 litters)
December 1995	0/114 (17 litters)	0/93 (14 litters)	0/101 (15 litters)	0/109 (18 litters)
January 1995	0/96 (15 litters)	0	0	0
June 1996	0	ND	ND	ND
February 1997	0/114 (17 litters)	0	0	0
April 1997	0/121 (17 litters)	0	0	0
April 1999	0/164 (24 litters)	0	0	0
May 1997	0/92 (14 litters)	0	0	0
May 2004	0/128 (23 litters)	0	0	0
October 1999	0/161 (24 litters)	0	0	0
April 2001	3/146 (22 litters)	1/144 (24 litters)	1/151 (25 litters)	3/173 (24 litters)
November 2003	0/38 (5 litters)	0	0	0
November 2005	0/74 (10 litters)	1/57 (9 litters)	0/69 (9 litters)	0/60 (8 litters)
July 2001	0/146 (24 litters)	0	0	0
January 2000	0/188 (24 litters)	0	0	0

*Metaflumizone study
ND = No data – due to only raw data being available or the study being confidential.

Skeletal examination also revealed an increased fetal and litter incidence of incomplete ossification of the sternbra at 100 and 300 mg/kg bw/day [fetal incidence: 66/131 (50 %) and 58/131 (46 %); litter incidence: 18/19 (95 %) and 18/20 (90 %) respectively] which exceeded the historical control data for this effect [fetal incidence: 17.1-29.5 %; litter incidence 70.8-87.5 %). It was noted that the concurrent control data was also above the HCD and that the effect was considered to be minor and occurring in the absence of a dose-response. Also noted was the number of live fetuses/dam were much higher in dose groups as compared to concurrent control. This effect is therefore not considered further for classification.

4.11.2.2 Human information

There are no human data available.

4.11.3 Other relevant information

Residues in milk and plasma

A range-finding study was carried out to determine the residues of metaflumizone in milk and plasma of Wistar rats [37]. In brief, pregnant Wistar rats (20/dose) were administered metaflumizone daily by oral gavage (0, 50, 80 or 120 mg/kg bw/day) from Day 6 *post-coitum* to Day 10 *post-partum* (Group A) or from Day 6 *post-coitum* to Day 21 *post-partum* (Group B). Samples of milk were taken from the dams and plasma levels in pups were analysed.

Poor general state of the dams was observed and insufficient nursing (reduced/no milk in the stomach) and subsequent litter loss was noted in two dams. There were no treatment-related effects in pups.

The results of the study showed that whilst levels of metaflumizone in milk showed considerable variation between individuals, the average levels showed an almost linear relationship to dose at the beginning of the sampling period (5.9-14.6 mg/kg in Group A and 5.2 – 11.4 mg/kg in Group B). At the end of dosing, levels in milk declined rapidly, as see in Group A. When administration of metaflumizone was continued throughout the study (Group B), only a moderate decline was observed. Average plasma levels in the pups were much lower than the levels in milk with levels of 0.26-0.5 mg/kg after birth and a peak of 4.0 mg/kg in Group B only.

Effects on reproductive organs from repeated dose studies

Effects to the uterus and ovaries were noted in a 28-day and 90-day oral (dietary) study in rats (section 4.8) following dosing with metaflumizone. In both studies, decreased absolute and relative ovary and uterus weights were observed alongside reduced numbers of corpora lutea. In a 28-day nose-only inhalation study, also in rats, decreased relative and absolute ovary and uterus weights were also seen at doses of 0.1-0.7 mg/L/day. The study authors concluded that these were due to the significant body weight effects observed in these studies. In the reproduction and fertility studies such organ effects were not noted and as there were no alterations to the female or male reproductive system, no adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, parturition, pregnancy outcomes, premature reproductive senescence or modifications on other functions that are dependent on the integrity of the reproductive systems; they are not deemed relevant for classification. The decrease in fertility index was considered to be due to the high dose administered causing effects on body weight and general ill-health and this was not observed in most animals once the dose was reduced. According to the guidance on the application of the CLP criteria

"adverse effects on fertility and reproductive performance seen only at dose levels causing marked system toxicity (e.g. dramatic reduction in absolute body weight) are not relevant for classification purposes".

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility and offspring

In a two-generation reproductive toxicity study in rats a decrease in the male and female fertility indices was noted in animals of the F_0 generation at the top dose (75 mg/kg bw/day) (mating A). This was observed in the presence of maternal toxicity, characterised by reduced body weight and body weight gain and general poor state. When the same animals were administered a reduced dose of 50 mg/kg bw/day and re-mated, signs of toxicity were observed (poor general condition) but body weight was only 7 % lower than controls at week 10. Fertility index was found to be lower than controls at week 10, but to a lesser extent and remained within the HCD. As animals were able to go on and mate successfully during mating B and there were no effects in the F_1 generation, it is not proposed to classify for effects on fertility.

There was some evidence of toxicity occurring during gestation indicated by a reduction in total numbers of live fetuses at birth. This was observed in mid- and high-dose groups of both generations in a dose-dependent-manner. Statistical significance was only achieved in the top-dose group of the F_{1B} generation, although the more marked magnitude was observed in the F_{1A} generation at 75 mg/kg bw/day. Despite the statistical significance, the numbers presented indicate that the reduction in live fetuses was due to maternal toxicity and lowering the dose from 75 mg/kg bw/day to 50 mg/kg bw/day led to an increase in number of live fetuses. No effects on the number of live fetuses were observed in the low dose groups or in any dose groups of the F_2 generation.

Complete litter loss occurred in the F_{1A} generation dosed with 75 mg/kg bw/day (the only group to have significant effects on bodyweight gain) and to a lesser extent the top-dose F_{1B} generation. Complete litter loss was also noted in one dam of the F_{1A} mid-dose group. All dams that lost litters also exhibited inadequate nursing behaviour and/or increased cannibalisation, which was attributed to be the cause. The lack of maternal care, inadequate nursing and cannibalisation (leading to increased litter loss) were considered to be a non-specific secondary consequence of maternal toxicity and stress. It is proposed that these findings are not relevant to humans and therefore, it is not considered appropriate to classify for these effects.

Effects on offspring were apparent during lactation. During PND 0-4 and 4-21 the total death rate was significantly increased in comparison with controls. This effect was also observed, to a lesser extent, in F_{1B} generation pups of the top-dose group. Body weight gain was also significantly reduced in F_{1A} pups of the top-dose group between *post-natal* days 1 – 7 indicating a clear adverse effect on health. Necropsy of F_{1A} pups revealed an increased incidence of an empty stomach.

Metaflumizone is a lipophilic substance, as evidenced by its physical and chemical properties (e.g. high lipophillicity), and as such might be expected to be transferred to milk. An additional study was carried out to assess this [Section 4.11.3 [37]]. In this study, residues of metaflumizone were found in milk at levels approximately 10 times *less* than the dose administered (peaking at 14.6 mg/kg). The amount transferred to pups was 3 – 28 times less than that found in breast milk (peaking at 4.0 mg/kg when dosing of the dam continued throughout the *post-natal* period). These findings suggest that metaflumizone *can* enter the breast milk and to levels that have been shown to cause body weight effects in repeated dose dietary studies in rats [20]. It is difficult to ascertain whether metaflumizone

caused an effect on pups directly through the milk or as a secondary effect due to impaired maternal care from dams suffering with ill-health. However, these effects on pups occurred during the period of time when the mother provided the sole means of nutrition; therefore it is proposed to classify for effects on lactation.

Developmental effects

In a developmental study in rats, there were no treatment-related external variations, skeletal variations or visceral effects observed. The number of live fetuses at birth remained constant across the control and dose groups (8.4 – 9.6 fetuses/dam) and there was no effect on post-implantation loss.

In a developmental study in rabbits, serious maternal toxicity occurred leading to premature sacrifice of a number of the dams. However, there was an increased incidence of absent subclavian artery in fetuses of the top dose group (3/131 (2.3%) versus 0 in controls; 3/20 litters). Whilst this finding has been observed in controls, it is rare, occurring in only one other study with an incidence of 3/145 fetuses (2.1%) in 3/22 litters; an increased incidence was also observed in the high dose group of this study (with an unspecified substance) 3/173 fetuses (1.7%) (Table 21). Whilst it is plausible that the malformation arose spontaneously, in the absence of any incidences in the concurrent control and given the rare nature of this finding, it is difficult to dismiss it as not being treatment-related.

4.11.5 Comparison with criteria

In a two generation study in rats, metaflumizone was shown to cause a reduced fertility index in top dose-treated males and females of the F_0 generation. This was observed in the presence of maternal toxicity (reduced bodyweight gain and poor general health). On a subsequent mating using a lower dose of metaflumizone this effect was reduced and it was not observed in the F1 generation at all. Similarly, there was a reduction in the number of live fetuses at the mid and top dose in F_{1A} generation but these numbers decreased in the F_{1B} generation when the dose of metaflumizone was lowered. All dams that lost litters also exhibited inadequate nursing behaviour and/or increased cannibalisation, which was attributed to be the cause. The lack of maternal care, inadequate nursing and cannibalisation (leading to increased litter loss) are considered to be a non-specific secondary consequence of maternal toxicity and stress. There were no effects on reproductive organs in this study. Therefore, it is not proposed to classify metaflumizone for effects on fertility.

In a developmental study with metaflumizone there was an increased incidence of absent subclavian artery in rabbits of the top dose group, whilst it is plausible that this malformation arose spontaneously, in the absence of any incidence in the concurrent control and given the rare nature of this finding, it is considered to be treatment related and classification for developmental effects should be considered.

In accordance with the criteria, classification with category 2 is reserved for substances where there is *some* evidence from experimental animals; however the evidence is not sufficiently convincing for category 1. Classification with category 1A is reserved for known human toxicants and category 1B should be considered when there is *clear* evidence of an adverse effect occurring in the absence of other toxic effects.

The incidence of absent subclavian artery occurred in one fetus from each of the low and mid-dose groups and 3 fetuses affected in the top-dose group. This led to an incidence that was marginally above the historical control data. It is also noted that this finding was rare in the historical controls, occurring only in 3 additional studies out of 34 and only in 1 of those studies at a rate comparable to the high dose group in the metaflumizone study. The clinical relevance of this malformation is

unclear and because of this and the fact it occurred in the presence of maternal toxicity, in one species only, classification with category 2 for developmental toxicity is deemed appropriate.

In addition to the classification for developmental toxicity, there were also effects occurring in a two-generation study in rats during lactation. One possibility is that metaflumizone caused direct toxicity to the neonates as both the physico-chemical properties of the molecule together with a supporting study suggest that the substance is able to pass into the breast milk at levels that could cause effects on body weight. However, there was clear evidence in the two-generation study that maternal toxicity led to impaired nursing behaviour and if the impaired nursing behaviour is proven to be a substance-related specific effect on behaviour, then classification for effects on or via lactation may be appropriate. Therefore, it is proposed that metaflumizone is also classified for effects on or via lactation.

4.11.6 Conclusions on classification and labelling

Repr. 2; H361d - Suspected of damaging the unborn child

Lact.; H362 - May cause harm to breast-fed children

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on fertility and offspring

The DS summarised the effects on fertility and offspring seen in a two-generation reproductive toxicity study in rats (no. 28, OECD TG 416, GLP) as a decrease in the male and female fertility indices in animals in the F0 generation at the top dose (75 mg/kg bw/d, mating A). This was observed in the presence of maternal toxicity, characterised by reduced body weight and BWG and general poor state. When the same animals were administered a reduced dose of 50 mg/kg bw/d and re-mated, signs of toxicity were observed (poor general condition) but body weight was only reduced by 7%. Fertility index was again decreased in comparison to controls, but to a lesser extent and remained within the historical control data (HCD). As animals were able to mate successfully during mating B and there were no effects in the F1 generation, it was not proposed by the DS to classify for effects on fertility.

Some evidence of reproductive toxicity occurring during gestation was indicated by a reduction in total numbers of live foetuses at birth. This was observed in mid- and high-dose groups of both generations in a dose-dependent manner. Statistical significance was only observed in the top-dose group of the F1B generation, although it was observed at a higher magnitude in the F1A generation at 75 mg/kg bw/d.

Complete litter loss occurred in the F1A generation dosed with 75 mg/kg bw/d (the only group having significant body weight effects) and to a lesser extent in the top-dose F1B generation. Complete litter loss was also noted in one dam of the F1A mid-dose group. All dams that lost litters also showed inadequate nursing behaviour and/or increased cannibalisation; this latter was considered to be the cause of the litter loss. Increased litter losses, increased cannibalisation and inadequate nursing are responses that are very commonly observed when

dams are stressed due to ill-health. The DS regarded these findings as not relevant to humans and therefore, did not considered it appropriate to classify for these effects.

The tables below contain a summary (from the DAR) of the results from this two-generation study in rats by gavage:

Table: Pre-mating bodyweight and food consumption in F0 animals

Observations/study week	**Dose level (mating A/mating B)** (mg/kg bw/d)			
	0/0	**12/12**	**30/20**	**75/50**
	Parental Generation (Mating A)			
	Males			
Body weight (g): Week 10	336.9	335.3	348.5	338.2
Weight gain (g): Weeks 0-10	221.0	221.0	234.5	224.6
Food consumption (g/d): Weeks 0-10	21.1	21.1	21.5	20.7
	Females			
Body weight (g): Week 10	204.8	205.3	200.6	176.1**
Weight gain (g): Weeks 0-10	105.9	105.4	101.3	76.9**
Food consumption (g/d): Weeks 0-10	15.3	15.5	15.8	13.3
	Parental Generation (Mating B)			
	Males			
Body weight (g): Week 10	413.2	412.4	429.3	423.5
Weight gain (g): Weeks 0-10	31.5	31.5	33.25	32.7
Food consumption (g/d): Weeks 0-10	20.2	20.6	20.5	19.8
	Females			
Body weight (g): Week 10	239.2	241.5	237.0	221.9**
Weight gain (g): Weeks 0-10	10.3	13.4	10.5	9.9
Food consumption (g/d): Weeks 0-10	15.0	15.7	16.1	15.1

* significantly different to controls, $p < 0.05$; ** $p < 0.01$

Table: Reproductive performance of F0 and F1 parents

Observation	**Dose level** (mg/kg bw/d)			
F0 (Mating A)	**0**	**12**	**30**	**75**
Pre-coital interval (d)	2.3 ± 1.1	2.7 ± 1.1	2.6 ± 1.0	3.0 ± 1.2
	Males			
Placed with females (#)	24	25	25	25
Successfully mated (#)	24	25	25	24
Mating index (%)	100	100	100	96
Fertility index (%)	96	100	84	72*
Intercurrent deaths (#)	1	-	-	-
	Females			
Placed with males (#)	25	25	25	25
Successfully mated (#)	25	25	25	24
Mating index (%)	100	100	100	96
Fertility index (%)	96	100	84	75*
Intercurrent deaths (#)	-	-	-	-
Gestation interval (d)	22.1 ± 0.3	22.1 ± 0.3	22.2 ± 0.5	22.2 ± 0.4
Litters (#)	24	25	21	18
F0 (Mating B)				
Dose level (mg/kg bw/d)	**0**	**12**	**20**	**50**
Pre-coital interval (d)	2.4 ± 1.1	2.8 ± 1.2	2.8 ± 0.9	2.4 ± 1.0
	Males			
Placed with females (#)	24	25	24	24
Successfully mated (#)	24	25	24	24
Mating index (%)	100	100	100	100

Fertility index (%)	100	100	88	88
Intercurrent deaths (#)	-	-	-	-
		Females		
Placed with males (#)	25	25	24	24
Successfully mated (#)	25	25	24	24
Mating index (%)	100	100	100	100
Fertility index (%)	100	100	88	88
Intercurrent deaths (#)	0	0	0	0
Gestation interval (d)	22.3 ± 0.6	22.4 ± 0.6	22.4 ± 0.5	22.4 ± 0.5
Litters (#)	25	25	21	20
F1				
Dose level (mg/kg bw/d)	**0**	**12**	**20**	**50**
		Males		
Placed with females (#)	24	24	25	25
Successfully mated (#)	23	24	25	25
Mating index (%)	96	100	100	100
Fertility index (%)	96	96	92	96
		Females		
Placed with males (#)	24	25	25	25
Successfully mated (#)	23	25	25	25
Mating index (%)	96	100	100	100
Fertility index (%)	100	96	92	96
Gestation interval (d)	22.1 ± 0.29	22.0 ± 0.46	22.1 ± 0.34	22.2 ± 0.41

* significantly different to controls, $p < 0.05$; ** $p < 0.01$

Table: Litter parameters for F1A, F1B and F2 generations

Parameter	**Dose level** (F1A/F1B and F2) (mg/kg bw/d)			
Dose level (mg/kg bw/d)	**-**	**12/12**	**30/20**	**75/50**
		F1A Generation		
Live foetuses (#)	257	279*	197	163
Dead foetuses	0	5*	2	2
Sex ratio Day 0 (% males)	44.4	46.6	57.9	44.2
Deaths: Days 0-4 (%)	1 (0.4)	3 (1.1)	3 (1.5)	12 (7.4)
Deaths: Days 4-21 (%)	0 (0)	0 (0)	1 (0.5)	38 (23.6)
Litter size: PND 0	10.7 ± 1.9	11.2 ± 2.4	9.4 ± 3.0	9.1 ± 2.1
PND 4 (pre-cull)	10.6 ± 2.0	11.0 ± 2.4	9.2 ± 3.1	8.4 ± 2.7
PND 4 (post-cull)	7.8 ± 0.6	7.8 ± 0.8	7.2 ± 1.9	7.1 ± 1.9
PND 7	7.8 ± 0.6	7.8 ± 0.8	7.2 ± 1.9	5.8 ± 3.0
PND 14	7.8 ± 0.6	7.8 ± 0.8	7.2 ± 1.9	5.0 ± 3.5
PND 21	7.8 ± 0.6	7.8 ± 0.8	7.2 ± 1.9	5.0 ± 3.5
Gestation index (%)	100	100	100	100
Live birth index (%)	100	98	99	99
Viability index (%)	99	99	98	93**
Lactation index (%)	100	100	99	70**
		F1B generation		
Dose level (mg/kg bw/d)	Control	12	20	50
Live foetuses (#)	227	232	194	179*
Dead foetuses	5	4	1	12*
Sex ratio Day 0 (% males)	48.0	51.3	44.3	46.9
Deaths: Days 0-4 (%)	1 (0.4)	1 (0.4)	3 (1.5)	15 (8.4)
Deaths: Days 4-21 (%)	0 (0)	2 (0.8)	0 (0)	11 (6.2)
Litter size: PND 0	9.1 ± 2.8	9.3 ± 3.6	9.2 ± 2.7	8.9 ± 2.5
PND 4 (pre-cull)	9.0 ± 2.8	9.2 ± 3.6	9.1 ± 2.6	8.2 ± 3.4
PND 4 (post-cull)	7.3 ± 1.8	7.0 ± 2.2	7.4 ± 1.4	6.9 ± 2.4
PND 7	7.3 ± 1.8	6.9 ± 2.2	7.4 ± 1.4	6.5 ± 2.9
PND 14	7.3 ± 1.8	6.9 ± 2.2	7.4 ± 1.4	6.3 ± 2.9
PND 21	7.3 ± 1.8	6.9 ± 2.2	7.4 ± 1.4	6.3 ± 2.9
Gestation index (%)	96	96	100	95
Live birth index (%)	98	98	99	94*

Viability index (%)	100	100	98	92*
Lactation index (%)	100	99	100	92*
		F2 generation		
Implantation sites (#)	269 ± 11.7	279 ± 11.6	261 ± 11.3	262 ± 10.9
Live foetuses (#)		262	244	234
Dead foetuses	249	1	3	5
Sex ratio Day 0 (% males)	46.6	47.3	47.5	50.0
Deaths: Days 0-4 (%)	14 (5.6)	5 (1.9)	6 (2.4)	8 (3.4)
Deaths: Days 4-21 (%)	1 (0.4)	0 (0)	1 (0.4)	3 (1.3)
Litter size: PND 0	10.8 ± 1.8	10.9 ± 2.2	10.6 ± 2.1	9.8 ± 2.9
PND 4 (pre-cull)	10.2 ± 2.9	10.7 ± 2.5	10.3 ± 2.1	9.4 ± 3.2
PND 4 (post-cull)	7.7 ± 1.7	7.8 ± 1.0	7.9 ± 0.5	7.3 ± 1.9
PND 7	7.6 ± 1.7	7.8 ± 1.0	7.8 ± 0.5	7.2 ± 1.9
PND 14	7.6 ± 1.7	7.8 ± 1.0	7.8 ± 0.5	7.2 ± 2.0
PND 21	7.6 ± 1.7	7.8 ± 1.0	7.8 ± 0.5	7.2 ± 2.0
Gestation index (%)	100	100	100	100
Live birth index (%)	98	100	99	98
Viability index (%)	94	98	98	97
Lactation index (%)	99	100	99	98

* significantly different to controls, $p < 0.05$; ** $p < 0.01$

Key elements from the DAR on the developmental toxicity study in <u>rabbits</u>:

Table: Foetal findings

Observation	**Dose level** (mg/kg bw/d)			
	0	**30**	**100**	**300**
Mated (#)	25	25	25	25
Pregnant (#)	21	23	19	23
Non-pregnant (#)	4	2	6	2
Died/sacrificed (#)	1	-	-	3
Died/sacrificed pregnant (#)	-	-	-	1
Died/sacrificed non-pregnant (#)	1	-	-	2
Aborted (#)	1	-	-	2
Premature delivery (#)	-	-	-	-
Litters (#)	20	23	19	20
Corpora lutea (#)	152	171	165	155
(#/dam)	(7.6 ± 1.5)	(7.4 ± 1.4)	(8.7 ± 1.3)	(7.8 ± 1.6)
Implantations (#)	129	146	148	138
(#/dam)	(6.4 ± 2.2)	(6.3 ± 2.0)	(7.8 ± 1.4)	(6.9 ± 2.1)
Live foetuses (#)	121	129	131	130
(#/dam)	(6.1 ± 2.4)	(5.6 ± 1.8)	(6.9 ± 1.8)	(6.5 ± 2.1)
Dead foetuses	0	0	0	1
(#/dam)	0	0	0	0.1 ± 0.2
Resorptions (#)	8	17	17	7
Early	8	8	14	5
Late	0	9	3	2
Resorptions/dam (#)	0.4 ± 0.6	0.7 ± 1.0	0.9 ± 0.9	0.3 ± 0.5
Early	0.4 ± 0.6	0.3 ± 0.7	0.7 ± 0.9	0.3 ± 0.4
Late	0.0 ± 0.0	0.4 ± 0.7 *	0.2 ± 0.4	0.1 ± 0.3
Total resorptions (#)	7	10	11	7
Foetal weight (g)	36.3 ± 3.7	38.1 ± 3.7	34.3 ± 3.9	33.6 ± 5.5
Males	36.2 ± 4.3	37.4 ± 4.6	34.0 ± 3.6	33.6 ± 5.8
Females	36.5 ± 3.9	38.3 ± 4.3	34.3 ± 4.0	33.5 ± 5.9
Litter weight (g)	214.2 ± 75.3	209.5 ± 55.4	232.1 ± 48.2	212.4 ± 66.7
Runts (#)[a]	4 (3.3)	2 (1.6)	7 (5.3)	18 (13.8)
Sex ratio (% male)	47.1	47.3	48.9	42.3
Pre-implantation loss (%)	15.6 ± 21.9	15.0 ± 20.4	10.3 ± 11.7	11.4 ± 19.6
Post-implantation loss (%)	8.4 ± 14.4	10.2 ± 12.7	11.9 ± 13.1	6.1 ± 9.5

Gravid uterine weight (g)	297.8 ± 98.2	297.3 ± 73.3	321.0 ± 63.4	302.2 ± 86.8

[a]foetuses with a body weight ≤ 75% of control mean, * significantly different to controls, $p < 0.05$; ** $p < 0.01$

Table: Developmental toxicity

Parameter			**Dose level** (mg/kg bw/d)				
			Historical control	**0**	**30**	**100**	**300**
Absent subclavian artery	**Foetal incidence**	(#) (%)	3/805 0.4 (0-2.1)	0/121 0	1/129 0.8	1/131 0.8	3/131 2.3
	Litter incidence	(#) %	3/118 2.5 (0-13.6)	0/20 0	1/23 4.3	1/19 5.3	3/20 15
	Affected litters	(%)	0.4 (0-1.9)	0 0	0.6 ±3.0	0.7 ±2.9	3.1* ±8.4
Incomplete ossification of sternebra	**Foetal incidence**	(#) (%)	197/805 24.5 (17.1-29.5)	42/121 35	45/129 35	66/131 50	58/131 46
	Litter incidence	(#) (%)	92/118 78.0 (70.8-87.5)	15/20 75	18/23 78	18/19 95	18/20 90
	Affected litters	(%)	24.2 (16.6-31.6)	29.7 ±24.4	32.3 ±27.3	47.5* ±28.2	46.5** ±23.4

* significantly different to controls, $p < 0.05$; ** $p < 0.01$

Developmental effects

In a developmental study in rats (OECD TG 414, GLP), there were no treatment-related external variations, skeletal variations or visceral effects observed. In this study (no. 35) time-mated female Wistar rats (25/dose) were treated with metaflumizone suspended in 0.5% carboxymethylcellulose (0, 15, 40 or 120 mg/kg bw/d) via oral gavage on days 6-19 of gestation. There was no mortality or clinical signs in dams. Bodyweight gain was significantly reduced between gestational days 6 and 8 (54%) and days 6 and 19 (12%) resulting in an overall bodyweight loss of 22%. According to the DAR, a 6% reduction on food consumption was noted at 120 mg/kg bw/d on GD 6-19. There were no treatment-related external or skeletal variations or visceral effects observed in foetuses.

In a developmental study in rabbits (OECD 414, GLP), serious maternal toxicity occurred leading to premature sacrifice of a number of the dams. However, there was an increased incidence of absent subclavian artery in foetuses of the high-dose group (3/131 (2.3%) versus 0 in controls; 3/20 litters). Whilst this finding has been observed in historical controls, it is rare, occurring in only one other study of the 33 conducted in the same period in the same laboratory, with an incidence of 3/145 foetuses (2.1%) in 3/22 litter. Whilst it is plausible that the malformation arose spontaneously, in the absence of any incidences in the concurrent control and given the rare nature of this finding, it is difficult to dismiss it as not being treatment-related. The DS considered that the absence of subclavian arteria seen constituted some evidence and hence does not justify Category 1B, but proposed classification with Category 2. The incidence of absent subclavian artery occurred in one foetus from each of the low- and mid-dose groups and three foetuses in the top-dose group. This led to an incidence that was marginally above the HCD.

The DS interpreted the clinical relevance of this malformation as unclear and because of this and the fact that it occurred in the presence of maternal toxicity, and in one species only,

classification in Category 2 for developmental toxicity (Repr. 2, H361d) was deemed appropriate.

Effects on lactation

The DS proposed to classify for effects on lactation based on the increased incidence of pup deaths during PND 0-4 and PND 4-21 in F1A pups and to a lesser extent in the F1B generation. Lower pup body weight during PND 1-7 and empty stomach at necropsy was considered as supporting evidence.

Metaflumizone is a lipophilic substance, as evidenced by its physical and chemical properties, and as such might be expected to be transferred to milk. An additional study was carried out to assess this (no. 37). In this study, residues of metaflumizone were found in milk at levels approximately 10 times less than the dose administered (peaking at 14.6 mg/kg bw/d). The amount transferred to pups was 3-28 times less than that found in breast milk (peaking at 4.0 mg/kg bw/d when dosing of the dam continued throughout the post-natal period). These findings suggested that metaflumizone can enter the breast milk and in levels that have been shown to cause body weight effects in repeated dose dietary studies in rats (no. 20). It is difficult to ascertain whether metaflumizone caused an effect on pups directly through the milk or if it was a secondary effect due to impaired maternal care from dams suffering from ill-health. However, these effects on pups occurred during the time period when the mother provided the sole means of nutrition; therefore the DS proposed to classify for effects on lactation.

Comments received during public consultation

Three MSCAs agreed with the DS's view on no classification for fertility effects and two MSCAs supported classification for lactation. One MSCA expressed its general agreement with the proposed classification proposals for human health.

Two MSCAs supported the classification for effects on development.

In total four MSCAs agreed with the classification proposal for Repr. 2 H361d; another MSCA proposed Category 1B. One MSCA supported the Category 2 classification for developmental effects based on the incidence of absent clavicola which is a rare finding but has been observed in control animals previously. Another MSCA agreed based on the incidence of absent subclavian artery outside the historical control incidence. One MSCA addressed the weaknesses of the available HCD on absent clavian artery which lack information on median and mean values, and considered also the incidences in the low- and mid-dose groups as above the median/mean historical control incidences.

The manufacturer disagreed with the proposed classification as Repr. 2 H361d. As their main evidence, they proposed to disregard one case of absent clavian artery at 300 mg/kg bw/d as it was observed in a dead pup and they required a correction of the final incidence at the top dose to 1.5% instead of 2.3%. It was highlighted that the arterial branching patterns vary considerably in humans and rabbits and that there was no evidence of malformation or atrophy in the upper limbs of the subjected rabbit foetuses. Thus the effect should be considered as a variation. Based on insufficient severity of the effect and that the incidences were considered within the HCD, the manufacturer disagreed with the proposal. It was suggested to allocate

the absent clavian artery in one foetus at 100 mg/kg bw/d to a syndrome as also a right sided aortic arch and a ventricular septal defect were seen.

Assessment and comparison with the classification criteria

Effects on fertility and offspring

Systemic toxicity in the high-dose F0 parental generation (75 or 50 mg/kg bw/d) was reported as an increased occurrence of a poor general state, statistically significant decreased food consumption, statistically significant lower body weights and impaired body weight gain. There were no indications of treatment-related organ weight changes, gross lesions, or microscopic findings. At 75 mg/kg bw/d, lower bw (-14%) and BWG (-27%) during weeks 0 to 10 (premating), associated with lower food consumption (-13%) in comparison with controls, were seen. However, no evidence on other clinical signs of toxicity is given in the DAR or the CLH report. RAC considers it unlikely that the slight difference in the final bw is responsible for increased oestrus cycle length, lower fertility index or complete litter losses shortly after birth in 4/18 dams (the latter may be more relevant for developmental toxicity), perinatal pup deaths and lower viability of pups at PND 0-4. Due to missing information, it is questioned whether the parental animals were severely affected by general toxicity as they were able to mate at similar rates as controls. It cannot be ruled out that the cause of prolonged cycle length in two dams was a metaflumizone-related effect rather than a sign of non-specific toxicity.

Moreover, it is uncertain whether the reduced pup viability can only be attributed to improper nursing behaviour, as a non-specific maternal effect, as one litter loss occurred in a dam with normal nursing behaviour.

When comparing the effects in these parental animals at 75 mg/kg bw/d with the effects observed in the 28/90-d gavage study in rats (no. 21) it can be concluded that marked weight loss was observed only at 1000 mg/kg bw/d and led to a dose reduction during week 1. No mortalities and no clinical signs were observed at 500 mg/kg bw/d. Body weights, BWG and FC were significantly lower at 500 mg/kg bw/d and non-significantly reduced at 100 mg/kg bw/d after 28 days. A slight decrease in FC and BWG (-13%) was observed in females at 1000/100 mg/kg bw/d after 90 days of treatment. The observation that gavage doses of 500 mg/kg bw/d were tolerated for 28 days without any clinical signs of toxicity, and only slight effects on BWG and FC observed at 100 mg/kg bw/d after 28 days and 90 days of treatment questions whether the slight effects on body weight at 75 and 50 mg/kg bw/d – in the absence of any clinical sign of toxicity, and otherwise consistent with observations in repeated dose gavage administration of 100 mg/kg bw/d – should be considered as severe maternal effects.

Comparing the effects in rats seen in gavage studies with dose-responses seen in diet studies, indicated that effects of comparable magnitude (lower BWG of about 12-23%, lower FC of about 7-17% seen in the 28/90-d study, no. 21) at 100 mg/kg bw/d correspond to effects seen in diet studies at 4-7 mg/kg bw/d in the 90-d study (no. 20) or at 23.8/27.2 mg/kg bw/d in the 28-d study (no. 18). It is assumed that absorption after gavage administration (possible due to lipophilicity of metaflumizone) is markedly lower than after diet administration (factor of more than 10 for the 90-d study part, factor of more than 3 for the 28-d study design).

The outcome of the second mating (mating B) at 50 mg/kg bw/d may also be relevant for classification. In this part of the study, animals from study day 126 received metaflumizone at reduced doses of 0, 12, 20 or 50 mg/kg bw/d by oral gavage, and parental rats were treated for a 10-week period (pre-mating) and were mated with the same partner as for their first

mating to produce a second litter (F1B). Treatment resulted in a significantly lower body weight (–7%) in F0-females at week 10 in comparison with the controls. Food consumption was unchanged, and the BWG was slightly (non-significantly) lower (-4%). No effects on body weight and FC were seen in F0 males. The DAR summary stated that F0 high-dose females were in poor general state, but neither the DAR nor the CLH report reported details on the nature of the signs and their incidences.

Compared with 75 mg/kg bw/d, the dose of 50 mg/kg bw/d resulted in a slightly lower body weight (-7%) and minor effects on the BWG (-4%) that could not explain the increased number of dead pups at 50 mg/kg bw/d. In contrast to 75 mg/kg, 50 mg/kg bw/d did not result in a prolongation of the oestrus cycle. The mating index was unaffected at both 75 and 50 mg/kg bw/d in F0 and F1 females indicating that the general health status at 50 mg/kg bw/d was sufficient for mating performance and conception. The fertility index was slightly (non-significantly) lower at 20 and 50 mg/kg bw/d in both parental generations.

The number of stillborn foetuses was increased at 50 mg/kg bw/d in the F0 generation (12 versus 5 in controls) but not in the pups from F1 dams. While the viability during PND 0-4 in the F1B pups was significantly lower (92% versus 100% in controls), no such effect was seen in the F2 pups (5 at 50 mg/kg bw/d versus 4 in controls). As the $LogK_{OW}$ values for metaflumizone's isomers are rather high, a potential for accumulation could not be ruled out, in particular as high levels of radioactivity were seen in female sex organs in the accumulation study. This may indicate that the internal doses at mating B might have been higher than just 50 mg/kg bw/d. Regarding the impaired nursing behaviours as a sign of poor general state, it is noted that the DAR reported that two females at 50 mg/kg bw/d showed improper nursing behaviour, losing all of their pups shortly after birth, as did a third female which showed normal nursing behaviour.

RAC questioned whether the dosing was high enough for the chosen gavage administration, whether the increased number of stillborn pups and reduced pup viability should be regarded as a fertility effect, and indicated that ovary effects were also seen in repeated dose studies in rats. The Industry representative emphasised the palatability problems in the diet studies as rats selectively had chosen food without the test substance instead of food with the test substance. The prolonged oestrus cycle was in their view only attributable to two dams, one that died later on with severe chronic nephropathy and the other that showed normalised oestrus cycle after receiving 50 mg/kg bw/d in mating B.

According to the CLP criteria, adverse effects on fertility and reproductive performance are normally not relevant for classification purposes if the adverse effects were seen at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma). In the absence of marked toxicity at 50 and at 75 mg/kg bw/d the effects on fertility and offspring could not considered to be secondary effects to the slightly reduced body weight/BWG. Therefore RAC agreed (in contrast to the original proposal for no classification by the DS) **to classify metaflumizone as Repr. 2; H361f for fertility**.

Lactation

Increased incidences/rates of pup death at PND 4 and PND 21 and significantly lower lactation indices were observed in the F1A generation (in dams receiving 75 mg/kg bw/d) and F1B generation (in dams receiving 50 mg/kg bw/d). While a stronger effect was seen at 75 mg/kg bw/d during PND 4-21, pups from dams following gavage exposure to 50 mg/kg bw/d showed a higher death rate during PND 0-4.

The number of dams showing improved nursing behaviour, after the second mating with lower doses, was not available to RAC. The DAR indicated only 2 dams at 50 mg/kg bw/d, thus the contribution of the nursing behaviour remains unclear. As the bw was (non-significantly) lower (-21%) in comparison with controls in F1A pups and less severe in F1B and F2 pup generations (-9% and -8%), this effect points either to a direct effect that was still ongoing during the first PND or to effect via lactation.

No treatment-related effect on the lactation index was observed in the F2 generation.

According to the CLP criteria, clear evidence on the offspring due to transfer to the milk or adverse effect on the quality of the milk or indications that the substance is present at potentially toxic levels in breast milk justifies classification for lactation effects. For RAC, the lower lactation index and the reduced survival at PND 4-21 where milk is the only nutrition source justifies classification for lactation effects. As pointed out in the CLH report, additional studies have found residues of metaflumizone in the milk after birth and during the lactation period. As BWG effects were seen at lower doses in diet studies compared to gavage studies, it seems plausible that even low doses in milk can have effects on the growth.

In conclusion, RAC agrees with the DS to classify metaflumizone for **effects on or via lactation, Lact.; H362**.

Developmental effects

RAC agrees with the DS's view that no treatment-related external variations, skeletal variations or visceral effects were observed (see summary tables B.6.48 and B.49 in the DAR).

The crucial effect seen in the developmental toxicity study in rabbits was an increased incidence of absent subclavian artery in foetuses at the high dose compared to controls. In addition, a significant increase of incomplete ossification of sternebra (a minor lesion) was observed at mid and high dose.

There were clinical signs of toxicity and sacrifices due to moribundity that demonstrated maternal toxicity in 4/25 rabbits at 300 mg/kg bw/d. Two non-pregnant and one pregnant animal at the top dose were sacrificed. Two litters were aborted at the top dose versus one in controls. The number of live foetuses was not affected in any of the dose groups, while the foetal weight was slightly (non-significantly) lower at the top dose in comparison with controls.

Neither the CLP dossier nor the DAR summary allow a conclusion on whether the malformation in 3/20 litters was observed in those dams that were sacrificed (one pregnant dam) or demonstrated clinical signs of toxicity. Although it is likely to assume, it remains unclear from the summary table and reporting whether the clinical signs reported for 4/25 animals at 300 mg/kg bw/d (lateral position, ataxia, poor general state, no defecation or blood in the bedding) were seen in the two non-pregnant animals and the pregnant animal that were sacrificed. Some more information is found in the RCOM (DS response to comment 7): prior to abortion one of two dams that aborted exhibited clinical signs from day 26 of gestation. Another dam was sacrificed in moribund status on day 28 after clinical signs seen from day 24 of gestation (RAC assumed that the information given in the RCOM that all foetuses found to be stunted relates to this animal). Obviously, visceral effects were not assessed in the two aborted litters and hence may be underestimated. Unequivocally, maternal toxicity is observed at the gavage dose of 300 mg/kg bw/d, the number of sacrificed rabbits (2 non-pregnant, 1 pregnant) is in the 10% range above which data should not be considered for classification. Based on the assumption that the malformations observed related to those 20 dams that were not in poor

general condition, these effects may be of relevance for classification. This is supported by the lack of effects on the bw/BWG, FC or corrected uterus weight.

The CLP criteria states in such cases that developmental effects that occur in the presence of maternal toxicity are considered to be evidence of developmental toxicity unless it can be unequivocally demonstrated that the developmental effect are secondary to maternal toxicity. Classification (in this case in category 2) should be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant postnatal functional deficiencies.

For metaflumizone the observed malformations were not unequivocally demonstrated to be (only) secondary to maternal toxicity. Absent subclavian artery is a rare malformation in control animals, was absent in the study control group and its incidence increased with dose (0/20, 1/23, 1/19, 3/20 litters at 0, 30, 100, 300 mg/kg bw/d, respectively). Comparison with the provided HCD indicated that the incidence (2.3%) at the high dose was slightly above the upper limit of historical control incidences (HCD range of 0-2.1%, from 1995-2008). The study on metaflumizone was finalised in 2004.

The DS indicated that the clinical relevance of this malformation is unclear and pointed out in their response to the comment from the MSCA that the effect of the absent subclavian artery on the rabbit development is unknown as it is not known whether the arterial branching pattern would compensate in some way. The study outcome remains unclear as information on whether other branches were compensating or alterations in other branches have been seen is not available. The manufacturer in his comment (no. 8) stated that no evidence of malformation or atrophy of the upper limbs were seen in the affected rabbit foetuses.

Although no skeletal deficiencies were identified in the treated rabbits and compensation by other vascular structures must be assumed, uncertainties remain about the clinical relevance in rabbits as no follow-up information on living foetuses with absent subclavian artery is available. With regard to the situation in humans, RAC was not aware of data/publications on consequences of absent subclavian arteries, however an aberrant subclavian artery in humans can lead to dysphagia (through disturbance in swallowing by compression of the oesophagus) or aneurysma of the adjacent descending thoracic aorta that can cause serious complications in patients and needs surgical intervention (Kouchoukos *et al.*, 2007). Aberrant subclavian arteries are reported as rarely occurring in humans (0.5-1.0% in the population).

RAC considers the effect 'absent subclavian artery' as a malformation and does not agree with the Industry comment interpreting the effect as a variation. The classification as a malformation has also been agreed by experts contributing to the IPCS Harmonisation Project on Terminology in Developmental Toxicology (Solecki *et al.*, 2003).

RAC follows the argumentation of the DS that the incidence was outside the HCD and that the finding occurred at a dose that produced maternal toxicity in rabbits. However, whether the incidence should be considered as marginally increased compared to the HCD where one study out of 34 (see Table 21 in the CLH dossier) showed an incidence of 2.05% and two other studies showed incidences of 0.58% and 0.64%, leading to a mean incidence of 0.09%, seems debatable.

RAC considered that for a lipophilic substance, the choice of a lipid vehicle would have been more appropriate than the use of aqueous CMC solutions and questioned whether the actual doses were possibly lower than the nominal ones administered. RAC members emphasised that also the single cases of absent subclavian arteries in the low and mid dose groups contribute to the evidence. Other members considered that the increased number of stillborn

pups and reduced pup viability during PND 0-4 seen in the two-generation study in rats are rather developmental effects than fertility effects, and suggested to take this evidence as supporting for the classification for developmental toxicity. RAC noted the remaining uncertainties on the long-term consequences from the absent artery.

RAC agrees with the DS's proposal that classification with **category 2 for developmental toxicity, Repr. 2; H361d,** is warranted.

Overall, RAC agrees to classify metaflumizone as **Repr. 2; H361fd and Lact., H362**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

The potential neurotoxicity was investigated in an acute oral study in rats (DAR: B.6.7.1) [39] and a 90-day study in rats (DAR: B.6.7.2) [40]. Both studies were carried out to guidelines and GLP standards.

In the acute neurotoxicity study, metaflumizone was administered to Wistar rats (10/sex/group) by oral gavage at doses of 0, 125, 500 and 2000 mg/kg bw. There were no signs of general toxicity or neurotoxicity observed.

In the sub-chronic neurotoxicity study, metaflumizone was administered to Wistar rats (10/sex/group) by oral gavage at doses of 0, 1, 12, 36 or 150 mg/kg bw/day and to males (n = 10) at 300 mg/kg bw/day for 90 days. Clinical signs of toxicity and reductions in bodyweight, bodyweight gain and food consumption were noted in males at 300 mg/kg bw/day and males and females at 150 mg/kg bw/day. No clinical or neuropathological signs of neurotoxicity were noted.

4.12.1.2 Immunotoxicity

In an immunotoxicity study carried out to GLP, Wistar rats (10 females/dose) were administered metaflumizone at dose levels of 0, 15, 40 or 75 mg/kg bw/day for 28 days (DAR: B.6.8.2) [41]. Clinical signs of reduced body weight were observed at doses of 40 mg/kg and above. Metaflumizone was not immunotoxic in female Wistar rats.

4.12.1.3 Specific investigations: other studies

No other information

5 ENVIRONMENTAL HAZARD ASSESSMENT

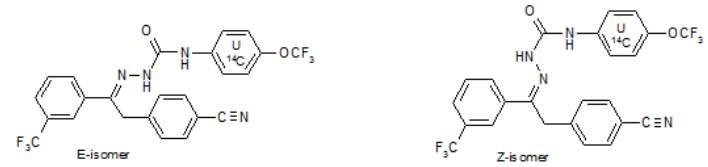
Metaflumizone (referred to in test reports as BAS 320I) is an insecticide intended for use against lepidopteran, coleopteran and other species which acts primarily via ingestion but also contact. Metaflumizone is a semicarbazone insecticide with a molecular mode of action which involves blocking the sodium channels in the nerve cells of the target insects - causing 'relaxed' paralysis. No metabolism of the insecticide is required for toxicity to occur in target insects. Available environmental fate and hazard studies have been considered under EU Regulation 1107/2009 and summarised in the Draft Assessment Report, 2012. The agreed endpoints from the peer review of metaflumizone (conducted according to the procedures and conditions of the previous Directive 91/414/EEC) are also included in the EFSA Conclusion (EFSA Journal 2013;11(10):3373).

The key information pertinent to determining a classification is presented below.

Metaflumizone is a mixture of E and Z isomers in a ratio of 90:10 in the proposed product. Technical metaflumizone used in fate and ecotoxicity studies generally reflects this ratio unless noted. Where data on individual isomer ratios are available, results are based on the sum of isomers as metaflumizone.

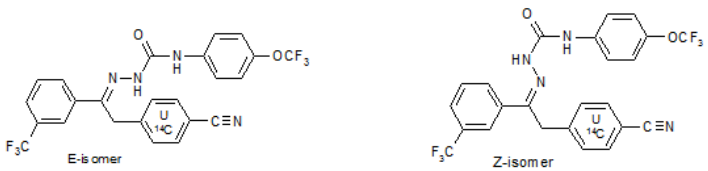
All radiolabelled studies used ^{14}C-metaflumizone with a purity of ≥95% and a combination of the labels shown in Figure 1.

Figure 1: Structure of metaflumizone indicating positions of the ^{14}C labels.

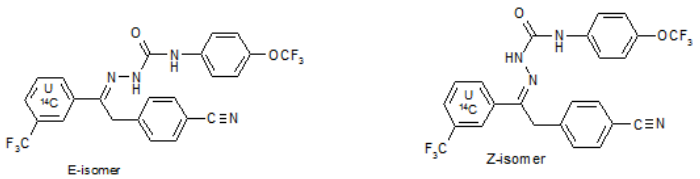


[trifluoromethoxyphenyl-U-^{14}C]-label

/



[benzonitrile-U-^{14}C]-label



[trifluoromethylphenyl-U-^{14}C]-label

The measured water solubility of metaflumizone in distilled water at 20 ºC is 1.81 µg/l (0.00181 mg/l) at pH 7 based on the sum of both isomers (Yan, 2001)[42]. In the same study a solubility of 1.35 µg/l was reported at pH 5 and 1.73 µg/l at pH 9 indicating pH does not significantly influence solubility. Additionally, the solubility of each isomer was determined as 1.43 µg/l for the E isomer and 2.03µg/l for the Z isomer (both 20 ºC and pH 8.1-8.7).

Metaflumizone does not have any dissociation constants in the range of 1 < pKa <12 (Petry, 2001)[9].

Where available, information on degradation products is included - details of degradant names and structures are presented in Annex I.

5.1 Degradation

A summary of available valid information on the fate of metaflumizone is presented in Table 22 below.

Table 22: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis US EPA Subdivision N, 161-1, GLP	~10% hydrolysis at pH 7 and 9 at 25 ºC by day 30 DT_{50} at pH 4, 12 ºC = 16.1-18.4 days DT_{50} at pH 5, 12 ºC = 77.2-88.8 days	Valid	Fang (2004a) [43]
Aquatic photolysis US EPA Subdivision N, 161-2, GLP	DT_{50} 2.4 – 6.3 days in spring sunlight at 40º North 1.7% mineralisation after 15 days	Valid	Ta (2004d), Ta (2004e) [45][46] Gericke, 2011c[47]
OECD Test Guideline 301 (CO_2 Evolution)	1.1-1.8% degradation	Valid	Heim *et al*, 2002[48]
Water/sediment simulation US EPA Subdivision N, 161-4, GLP	DT_{50} 394 – 715 days (total system) 3.7 – 4.5 % mineralisation after 100 days DT_{50} 322 – 581 days (total system) water/sediment kinetic evaluation according to FOCUS Guidance	Study run in the dark Valid	Rosenwald, 2003 [49] and Schriever, 2010b [50]
Water/sediment simulation US EPA Subdivision N, 161-4, GLP	DT_{50} 3.6 – 23.2 days (total system) 16.3 – 23.9 % mineralisation after 100 days DT_{50} 6.32 days (total system) water/sediment kinetic evaluation according to FOCUS Guidance	Irradiated study Valid	Ta, 2004f [51] and Schriever, 2010c [52]

5.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Fang, 2004a)[43] is available following GLP and US EPA Subdivision N Guideline, Series 161-1. The study used benzonitrile-U-^{14}C and trifluoromethoxyphenyl-U-^{14}C radio labelled metaflumizone (~1.6 µg a.s./l). Test solutions were incubated at 25± 1 ºC in at pH 4, 5, 7 and 9 the dark for 30 days. At pH 7 and 9 at study termination, mass balance based on E and Z isomers were ~90% indicating limited hydrolysis had occurred. At lower pHs, hydrolysis was observed with DT_{50} values calculated by single first order (SFO) linear regression based on the sum of isomers. The Evaluating Member State (eMS) has calculated DT_{50} values at 12 ºC which are presented below with study DT_{50} values.

Table 23: Hydrolysis DT_{50} values for metaflumizone based on SFO linear regression

Label	pH	DT_{50} (days) at study temperature 25 ºC	DT_{50} (days) at 12 ºC
benzonitrile	4	5.7	16.1
trifluoromethoxyphenyl	4	6.5	18.4
benzonitrile	5	31.4	88.8
trifluoromethoxyphenyl	5	27.3	77.2

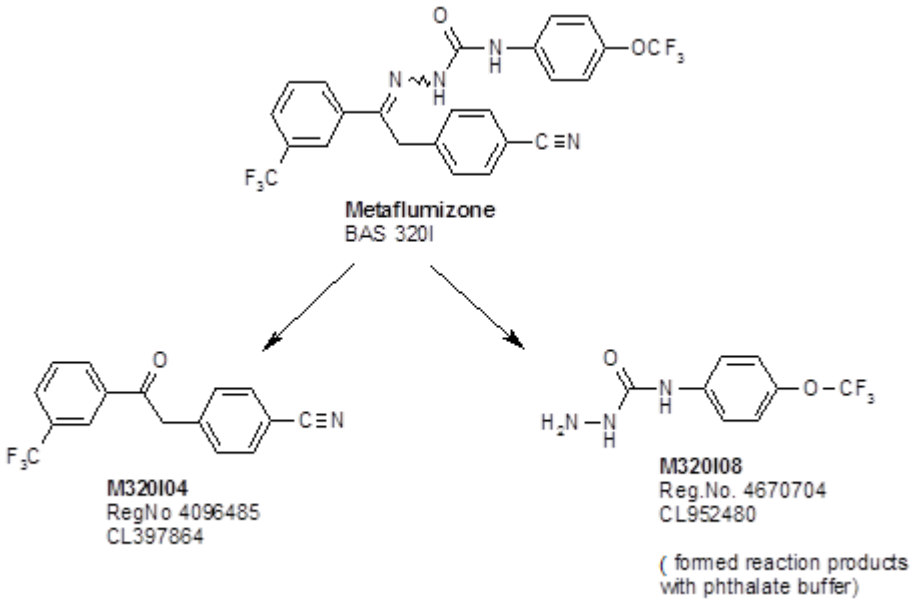
During the study the main hydrolysis products were identified as:

- M320I04 (max. 88.5% AR[2] at termination day 30, pH 4, benzonitrile label); and
- M320I08 (max. 70% AR at day 15, pH 4, trifluoromethoxyphenyl label).

The proposed hydrolytic pathway is presented in Figure 2. There was insufficient data to provide reliable DT_{50} values for hydrolysis products although they were considered stable under study conditions.

[2] Applied Radioactivity (AR)

Figure 2: Proposed hydrolytic pathway of metaflumizone at pH ≤5



Aqueous photolysis

Study 1

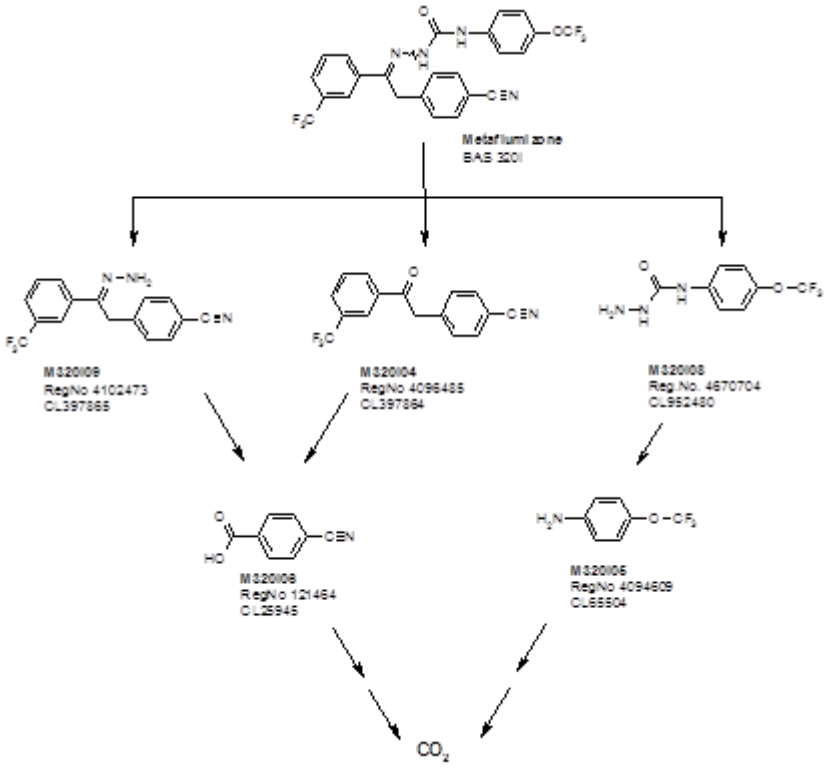
Aqueous photodegradation of metaflumizone was investigated (Ta, 2004d and Ta, 2004e)[45][46] following US EPA Subdivision N Guideline, Series161-2 and GLP, US EPA Guideline Subdivision N, Series 161-2, The study used benzonitrile-U-^{14}C and trifluoromethoxyphenyl-U-^{14}C radio labelled metaflumizone (~0.9 µg a.s./l). Test solutions were incubated at pH 9 for up to 15 days at 22 ^{o}C ± 1 ^{o}C under constant irradiation (wavelengths below 300 nm filtered out). This is considered representative of natural sunlight in spring at 40^{o} North latitude. Radiochemical balances were 96.4-101% AR. Review under EU Directive 91/414/EEC noted the study excluded light <300nm rather than <290nm and that the exclusion may have marginally underestimated photodegradation.

A number of degradants were identified:

- M320I04 (max. 9.7% AR on day 1, declining to 4.3% AR by termination at day 15);
- M320I06 (max. 27.4% AR);
- M320I09 (max. 6.1% AR);
- M320I05 (max. 1.7% AR, observed to be volatile with ~21% AR in trap); and
- M320I08 (max. 2.2% AR).

The proposed aqueous photolysis pathway is presented in Figure 3

Figure 3: Proposed aqueous photolysis pathway of metaflumizone at pH 9



Mineralisation was low accounting for 1.7% AR at study termination.

Metaflumizone DT_{50} values were determined based on the sum of isomers. Two methods were used: first order multi-compartment (FOMC) kinetics following non-linear regression of log transformed data, and single first order (SFO) kinetics.

Table 24: Aqueous photolysis DT_{50} values for metaflumizone and degradants (Ta, 2004)[45][46]

Label	First order multi-compartment DT_{50} (days)	Single First Order DT_{50} (days)
Metaflumizone parent (benzonitrile label)	3	6.3
Metaflumizone parent (trifluoromethoxyphenyl label)	2.4	3.6
M320I04	2.8	7.8
M320I08	12.7	12.3

Conversion of the E isomer to the Z isomer was noted in irradiated samples with concentrations of the Z isomer exceeding concentrations of the E isomer after 0.33 days irradiation.

Study 2
Gericke, 2011c[47] reanalysed the data from Ta, 2004 performing additional kinetic revaluation according to FOCUS kinetics guidance, 2006. The study generated the following best fit DT_{50} values using double first order in parallel (DFOP), first order multi-compartment (FOMC) kinetics and single first order (SFO) kinetics.

Table 25: Aqueous photolysis DT_{50} values for metaflumizone and degradants (Gericke, 2011c)[47]

Item	DT_{50} (days)
Metaflumizone parent (benzonitrile label)	4.12 - 4.19
Metaflumizone parent (trifluoromethoxyphenyl label)	2.4 - 2.44
M320I04	1.13
M320I09	28.3
M320I06	49.1
M320I05	$1.5x10^{-15}$
M320I08	0.008

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

A ready biodegradation study (Heim *et al*, 2002) is available following OECD Test Guideline 301 (CO_2 Evolution) and GLP. The study was run at ~36.5 mg/l corresponding to 20 mg C/l which is above the test item water solubility of 1.81 µg/l. Between 1.1 and 1.8% degradation was observed over 29 days. Validation criteria were met.

5.1.2.3 Simulation tests

Two GLP water sediment studies are available using radiolabelled metaflumizone (3 labels) and US EPA Guideline Subdivision N 162-4. Both studies employed concentrations of the test item in the water phase above the experimental water solubility.

Rosenwald, 2003[49] and Schriever, 2010b[50]
Two UK aerobic systems were employed: 'Emperor's Son Lake' and 'Millstream Pond'. The water and sediment test conditions with a ratio of 3:1 are included in Table 26 below. The systems were treated via the water surface with concentrations above the test item water solubility and conducted with separate samples for each of the three radio labels in Figure 1.

Table 26: Water-sediment system test conditions

Criteria	Emperor's Son Lake, UK	Millstream Pond, UK
Water properties	pH: 6.43 Dissolved organic carbon: 35.9 mg/l Oxygen: ≥20% saturation Redox potential: ≥300 mV	pH: 7.62 Dissolved organic carbon: 9.6 mg/l Oxygen: ≥20% saturation Redox potential: ≥300 mV
Sediment properties	74% sand; 9% silt; 17% clay Organic carbon: 1.2% pH: 6.8 Redox potential: ≤210 mV	25% sand; 42% silt; 33% clay Organic carbon: 8.1% pH: 7.7 Redox potential: ≤210 mV

The study was conducted at 20 ± 1 °C, in the dark under aerobic conditions for up to 100 days.

Radioactivity was determined by High Performance Liquid Chromatography (HPLC) and subsequent confirmatory analysis by Liquid Scintillation Counting (LSC). Total mean recoveries for both systems were generally >90% AR (87.2 to 98.9% AR) for all three labels at each sampling point.

Metaflumizone initially dissipated rapidly from the water phase to the sediment phase in both systems. Sediment residues continued to increase over the study period reaching 69.1 – 83.4% AR in the Emperor's Son Lake and 74.4 – 91.7% AR in the Millstream Pond at study termination.

Two degradants (M320I23 and M320I04) were detected throughout the study reaching a maximum of 7.5% AR in sediment and 6% AR in water. The degradant M320I04 was only detected on day in each study - 6% AR in Emperor's Son Lake, and 4.6% AR in Millstream Pond.

Metaflumizone dissipation DT_{50} values for water were determined based on the sum of isomers and Double First Order in Parallel (DFOP).

Water dissipation DT_{50} values combined for all labels were as follows:
$DT_{50\ water}$: 0.01 days for Emperor's Son lake system following DFOP kinetics
$DT_{50\ water}$: 0.01 days for Millstream pond system following DFOP kinetics

The rapid dissipation in water may be influenced by precipitation of non-dissolved residues.

Metaflumizone DT_{50} values for the total system were determined based on the sum of isomers and simple first order kinetics (SFO) non-linear regression. Whole system study DT_{50} values combined for all labels were as follows:
$DT_{50\ total\ system}$: 394 days for Emperor's Son lake system following SFO kinetics
$DT_{50\ total\ system}$: 715 days for Millstream pond system following SFO kinetics

Minimal mineralisation was observed with a maximum of 3.7% AR and 4.5% AR observed in each system after 100 days.

Schriever, 2010b[50] undertook a kinetic re-evaluation in accordance with FOCUS (2006) Guidance using the Rosenwald (2003)[49] data. This included visual and statistical fit using various models. The evaluation determined that metaflumizone dissipated rapidly from the water phase with the best model fit using Hockey Stick (HS) kinetics resulting in dissipation $DT_{50\ water}$ values between 0.556 and 0.733 days. The study extrapolated beyond the study endpoint of 100 days to calculate degradation $DT_{50\ total\ system}$ values between 322 and 581 days based on a Single First Order (SFO) model.

Ta, 2004f[51] and Schriever, 2010c[52]

One system from the USA 'White Lake' was employed in two experiments - one ran in the dark and the other in artificial light using a 12 hour light-dark cycle excluding light <300nm. Water and sediment test conditions with a ratio of 2:1 are included in table 27 below. The systems were treated via the water surface with concentrations above the test item water solubility and conducted with separate samples for each of the three radio labels in figure 1.

Table 27: Water-sediment system test conditions

Criteria	White Lake, USA
Water properties	pH: 8.3 Dissolved organic carbon: 15 mg/l Oxygen: 4.7 – 6.8 mg/l Redox potential: ~324 mV
Sediment properties	95% sand; 2% silt; 3% clay Organic carbon 0.12% pH: 8.3 Redox potential: -41 - -257 mV

The study was conducted at 25 ± 1 ^{o}C, under aerobic conditions for up to 120 days.

Radioactivity was determined by Liquid Scintillation Counting (LSC) and subsequent analysis by High Performance Liquid Chromatography (HPLC) was undertaken. Total mean recoveries for both systems were >92.27% AR for both labels at each sampling point.

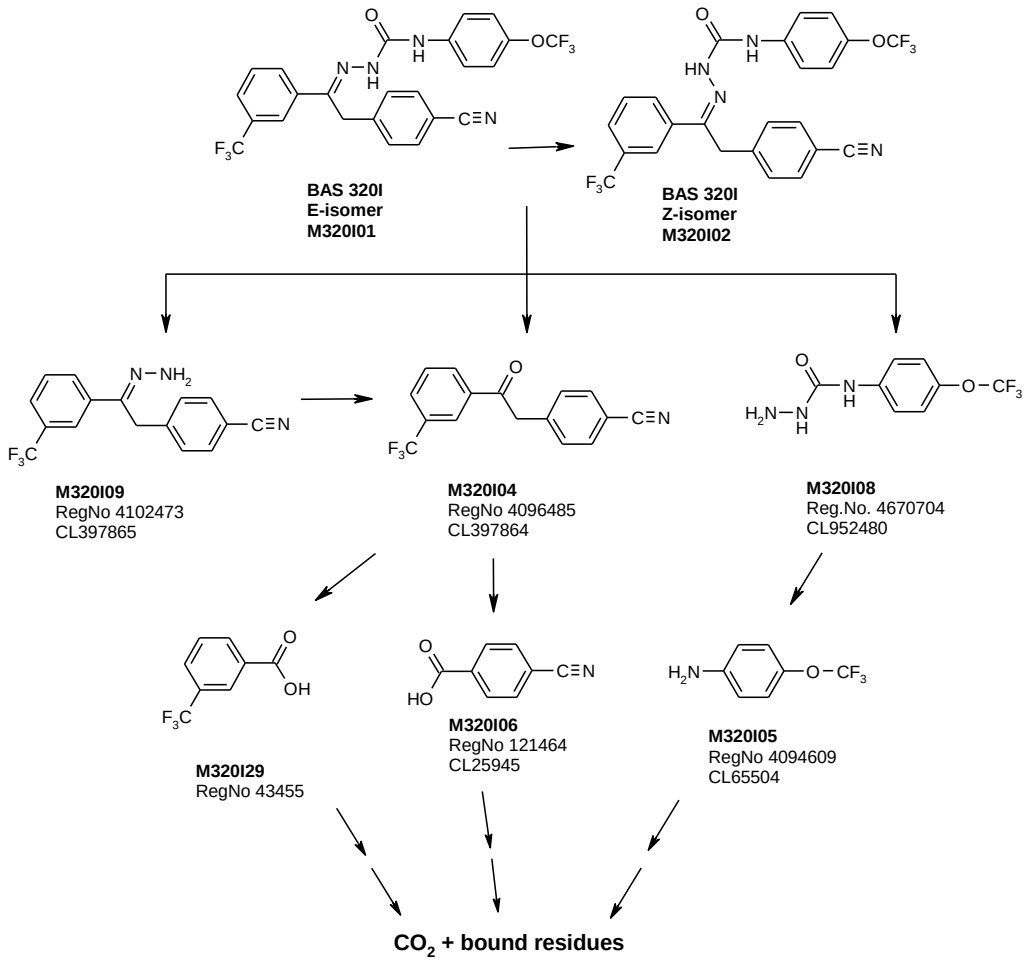
Under dark conditions, metaflumizone dissipated from the water phase to the sediment phase as per the previous study (Rosenwald, 2003)[49] and DT_{50} values were not calculated for this dark test system.

Under irradiation, metaflumizone dissipated from the water phase - day 0 95.3% AR to 27.5% AR by day 1. Concentrations of metaflumizone in sediment peaked on day 1 and steadily declined – 55.3% AR on day 1, 20% AR on day 30 and 11% AR on day 100. A number of degradants were formed with M320I04, M320I05, M320I08, M320I09 generally less than 10% AR.

The degradant M320I04 was observed in the aqueous and sediment phases. The maximum concentration in the water phase was 3.3% AR on day 2 from when it decline to <1% from day 3 to study termination on day 100. It was observed in sediment throughout the study at ≤2% AR.

Two further degradants, M320I06 and M320I29, were observed up to a maximum of 35 and 21% AR respectively in water. In addition, a change in the ratio of E and Z isomers was observed from 90:10 to approximately 35:65 after 1 day. The proposed degradation pathway in water-sediment systems under irradiation is presented in Figure 4.

Figure 4: Proposed degradation pathway in water-sediment systems under irradiation



NB: M320I05 was a volatile degradant detected in the ethylene glycol trap.

Metaflumizone dissipation $DT_{50\ water}$ values were determined for each radio label based on the sum of isomers and First Order Multi-compartment (FOMC): $DT_{50\ water}$: 0.5 to 1.5 days. The rapid dissipation in water may be influenced by precipitation of non-dissolved residues.

Calculated $DT_{50\ total\ system}$ values for metaflumizone and significant degradants following FOMC kinetics using non-linear regression of log transformed data are presented in Table 28.

Table 28: Water –sediment $DT_{50\ total\ system}$ values for metaflumizone and degradants under irradiated conditions

Item	DT_{50} (days)
Metaflumizone parent (benzonitrile label) Metaflumizone parent (trifluoromethoxyphenyl label) Metaflumizone parent (trifluoromethylphenyl label)	3.6 8.4 23.2
M320I04 (benzonitrile label) M320I04 (trifluoromethylphenyl label) [max. formation 6.7% AR in total system]	4.4 29.6
M320I06 (benzonitrile label) [max. formation 37.3% AR in total system]	1.6
M320I29 (trifluoromethylphenyl label) [max. formation 24.9% AR in total system]	7.7

Increased mineralisation was observed under irradiation with maximums of 16.3, 35.5 and 23.9% AR observed with each radio label after 100 days.

Schriever, 2010c[52] undertook a kinetic re-evaluation in accordance with FOCUS (2006) Guidance using the Ta (2004f)[51] data for the dark and irradiated systems. This included visual and statistical fit using various models. Tables 29 and 30 present DT_{50} values for each system.

Table 29: Water –sediment DT_{50} values for metaflumizone under dark conditions

	DT_{50} (days)
Metaflumizone $DT_{50\ water}$ (HS)	0.298
Metaflumizone $DT_{50\ sediment}$ (SFO)	674
Metaflumizone $DT_{50\ total\ system}$ (SFO)	376

Table 30: Water –sediment DT_{50} values for metaflumizone and degradants under irradiated conditions

	DT_{50} (days)
Metaflumizone $DT_{50\ water}$ (FOMC)	0.218
Metaflumizone $DT_{50\ sediment}$ (HS / SFO)	3 - 38
Metaflumizone $DT_{50\ total\ system}$ (DFOP)	6.32
M320I04 $DT_{50\ total\ system}$ (SFO)	31.9
M320I06 $DT_{50\ total\ system}$ (SFO)	6.27
M320I029 $DT_{50\ total\ system}$ (SFO)	40.2

5.1.3 Summary and discussion of degradation

Metaflumizone is considered hydrolytically stable at pH 7 and 9. Under acidic conditions metaflumizone undergoes hydrolysis. At 25 ^{o}C and pH 5, DT_{50} values were 27.3 to 31.4 days. Adjusting these to 12 ^{o}C results in hydrolysis DT_{50} values at pH 5 between 77.2 and 88.8 days.

Metaflumizone is susceptible to photodegradation under suitable conditions. The experimental DT_{50} in sterile pure water was 2.4 to 6.3 days at in spring sunlight 40^{o}N (approximating to Spain, southern Italy and Greece). After 15 days, mineralisation accounted for 1.7% AR. The degradant M320I05 was volatile and accounted for 21% AR in the trap. Various photodegradants were observed with only one >10% AR. The principal degradant M320I06 (max. 27.4% AR day 15) had a DT_{50} of 49.1 days. The actual degree of photodegradation in the aquatic environment depends on local conditions and seasons. Therefore, in reality the potential for aquatic photolysis is likely to be limited.

In a ready biodegradation study 1.1-1.8% degradation was observed.

In an aerobic water-sediment study performed in the dark, metaflumizone was observed to dissipate from the water column to sediment in two systems. Estimated study whole system degradation DT_{50} values for metaflumizone were between 394 and 715 days. Minimal mineralisation was observed (3.7 to 4.5% AR).

In a subsequent aerobic water-sediment study, conducted under artificial irradiation, increased degradation was observed. Mineralisation was significantly higher with 16.3 to 35.5% AR after 100 days. Total system degradation DT_{50} values of 3.6, 8.4 and 23.2 days (different radio labels) reflect the increased photodegradation. In addition, major degradants were observed. As noted above, the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Due to light attenuation in the overlying water phase in local environments, it is unclear if the available data is representative of European water bodies.

Subsequent kinetic assessment derived degradation $DT_{50\ total\ system}$ values of 376 days under dark conditions and 6.32 days for irritated conditions. Degradation $DT_{50\ total\ system}$ values were also derived for significant degradants (M320I06 and M320I029) with values ranging between 6.27 and 40.2 days.

The degradant M320I04 was observed in laboratory hydrolysis (at low pH) and photolysis studies. It was also observed in a 100-day irradiated fate simulation test where it was detected in both the aqueous and sediment phases. The maximum concentration of M320I04 in the water phase was 3.3% AR on day 2 - from this point it declined to <1% AR by day 3 and remained below 1% AR until study termination on day 100. M320I04 was observed in sediment throughout the study at ≤2% AR

Overall, the degradation information does not provide sufficient data to show metaflumizone is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, metaflumizone is considered not rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Two GLP adsorption/desorption studies are available using radiolabelled [benzonitrile-U-^{14}C] metaflumizone although the ratio of E and Z isomers is unknown. Both studies followed OECD Test Guideline 106. A clear relationship between sorption and soil properties was not evident in either study.

The first study (Zirnstein, 2004a)[53] employed a single test concentration of 0.02 mg a.s./l and five European soils. Following HPLC analysis recoveries were between 68.7 and 85.7% AR. This was considered due to sorption to glassware and K_{oc} values were corrected to reflect losses. Corrected K_{oc} values ranged from 16,500 ml/g for the silty sand to 43,800 ml/g for the loamy sand.

The second study (Zirnstein, 2004c)[54] employed a single test concentration of 0.0169 mg a.s./l and two North American soils. Recoveries were 45 to 65% AR which were also considered due to sorption to glassware and again K_{oc} values were corrected to reflect losses. Corrected K_{oc} values were 28,000 ml/g for the sandy silt loam and 51,000 ml/g for the loamy sand.

The DAR includes an arithmetic average of the seven K_{oc} values which is 30,714 ml/g indicating limited soil mobility. This is confirmed by the water-sediment simulation studies whereby rapid distribution of metaflumizone to the sediment compartment was observed.

Additional adsorption studies are available in the DAR and Addendum for several degradants. These are not presented further as they are not considered relevant for classification of metaflumizone.

5.2.2 Volatilisation

Experimental data (Yacoub, 2004a)[3] indicate the vapour pressure for metaflumizone is low at 1.24 x 10^{-8} Pa at 20 °C (sum of isomers) following OECD Test Guideline 104. A difference in vapour pressure of the isomers was observed with the E-isomer value as 7.94 x 10^{-10} Pa at 20 °C and the Z-isomer value as 2.42 x 10^{-7} Pa at 20 °C.

The Henry's Law Constant (Paulik, 2003)[55] was calculated for the E and Z isomers at 25 °C (using water solubility data generated at 20 °C) as follows:

E isomer 7.8 x 10^{-4} Pa m^3 mol^{-1}

Z isomer 0.11 x 10^{-4} Pa m^3 mol^{-1}.

This data indicates the Z isomer with its higher Henry's Law Constant is more volatile. However, this is expected to be minimal given the low Henry's Law Constant value which is supported by no significant volatile losses of metaflumizone in water-sediment simulation studies (see section 5.1.2.3).

5.2.3 Distribution modelling

Not relevant for classification and labelling.

5.3 Aquatic Bioaccumulation

Table 31: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water (HPLC method) using metaflumizone with purity 96.3% (E/Z isomer ratio of 90% min E: 10% max Z)	Z isomer: Log K_{ow} 4.4 at pH 5, 30°C E isomer: Log K_{ow} 5.1 at pH 5, 30°C	Valid	Holman and Petry, 2001[5]
Partition coefficient *n*-octanol/water (HPLC method) using E isomer (98.7%) and Z isomer (99.7%)	Z isomer: Log K_{ow} 4.2 at pH 7, 20°C Log K_{ow} 3.8 at pH 3, 20°C E isomer: Log K_{ow} 4.9 at pH 7, 20°C Log K_{ow} 4.4 at pH 3, 20°C	Valid	Class, 2006a and 2006b[6][7]
Experimental aquatic BCF test in fish to OECD Guideline 305, GLP, purity 97.8%, E:Z 92:8	Kinetic whole fish BCF_k: 7,800 to 8,100 l/kg based on Total Radioactive Residues Kinetic whole fish BCF_k: 5,769 and 4,099 l/kg based on Total Radioactive Residues (normalised for 5% lipid content) Depuration half-life DT_{50} whole fish: 14 - 17 days	Flow through, 42 days exposure, 56 days depuration Analysis of Total Radioactive Residues Valid	2004a[56]

Method	Results	Remarks	Reference
Experimental aquatic BCF test in fish to OECD Guideline 305, GLP, purity 97.2%, E:Z 93.5:6.5	Metaflumizone steady state whole fish BCF: 1,210 to 1,238 l/kg wet weight Metaflumizone steady state whole fish BCF: 1,667 to 1,705 l/kg wet weight (normalised for 5% lipid content) Kinetic whole fish BCF: 1,986 to 2,117 l/kg Kinetic whole fish BCF: 2,736 to 2,916 l/kg (normalised for 5% lipid content) Depuration half-life DT_{50} whole fish: 14.7 to 16 days	Flow through, 28 days exposure, 56 days depuration Analysis of parent metaflumizone Valid	2008a[57]

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No information submitted.

5.3.1.2 Measured bioaccumulation data

Three fish bioaccumulation studies using metaflumizone are available – two using aquatic exposure and one using dietary exposure. A further microcosm study using a metaflumizone preparation reflecting an intermittent pesticide application exposure pattern is available. The intermittent exposure method is not applicable to hazard classification so further details have not been included.

Study 1 (2004a)[56]

The study followed GLP and OECD Guideline 305. It used ^{14}C-metaflumizone (benzonitrile ring – U-^{14}C), a flow-through system with Bluegill Sunfish (*Lepomis macrochirus*) and two exposure concentrations; 0.04 and 0.45 µg/l with the aid of solvent dimethylformamide (DMF). The exposure period ran for 42 days followed by a 56-day depuration period. Analysis of Total Radioactive Residues (TRR) was by LSC with analysis of parent by HPLC.

A steady-state fish residue concentration was not obtained during the exposure phase so steady state Bioconcentration Factors (BCF) could not be determined. BCFs were calculated based on comparison of TRR in fish and water and kinetic rate constants for update and depuration. For the lower exposure concentration this resulted in BCF_k of 7,800 ± 1,200 l/kg. For the higher exposure concentration the BCF_k was 8,100 ± 390 l/kg. The lipid content at the end of the exposure phase was 6.76% for the lower exposure concentration and 9.88% for the higher exposure concentration. The 5% lipid normalised BCF_k values are 5,769 and 4,099 l/kg (2009) [58].

Selected samples of fillet and viscera were analysed by HPLC to determine the fraction of TRR as metaflumizone. For the lower exposure concentration this was ≥80% of the TRR. For the higher

exposure concentration this was ≥78% of the TRR. This indicates the parent metaflumizone remained mostly unchanged.

Radio-analysis of TRR on day 56 of the depuration phase indicated 86 to 91% depuration for whole fish with depuration half lives calculated as 14 to 17 days.

Study 2 (2008a)[57]

The study followed GLP and OECD Guideline 305. It used non-radio-labelled metaflumizone, a flow-through system with Common Carp (*Cyprinus carpio*) and two exposure concentrations; 0.15 and 1.5 µg/l with the aid of DMF. The exposure period ran for 28 days followed by a 56 day depuration period. Analysis of metaflumizone was by HPLC-MS. Lipid content was measured on day 0 of the uptake phase and days 0 and 56 of the depuration phase.

The concentration of metaflumizone in fish reached a plateau by day 14 indicating steady state had been achieved. Whole fish steady state bioconcentration factors (BCF_{ss}) for metaflumizone were 1,238 l/kg for the low exposure concentration and 1,210 l/kg for the higher exposure concentration. Lipid content can have a significant impact on BCF results and it is recommended BCFs are corrected to 5% lipid content where lipid data are available. Considering a conservative measured lipid content of 3.63% at the end of the uptake phase, 5% lipid normalised BCF_{ss} values are 1,667 to 1,705 l/kg.

BCF_k values were determined based on kinetic rate constants for update and depuration estimated from linear regression. Whole fish kinetic BCF_k values for metaflumizone were 1,986 l/kg for the low exposure concentration and 2,117 l/kg for the higher exposure concentration. Considering a conservative measured lipid content of 3.63% at the end of the uptake phase, 5% lipid normalised BCF_ks are 2,736 to 2916 l/kg (2009)[58].

The eMS notes that BCFs are based on analytical measurement of parent metaflumizone. Given that limited transformation or metabolism were observed in the Afzal, 2004a study, the eMS considers that the major component of bioaccumulated residues in fish is the parent and the approach to BCF calculation is acceptable.

Depuration half-lives of 14.7 and 16 days were calculated for the low and high exposure concentrations.

Additional information:

2009a[59]

This study is included for information as reliable criteria for classification are not available.

The study followed GLP and OECD Guideline 305 fish feeding (diet) method. It used non radio labelled metaflumizone, a flow-through system and Rainbow Trout (*Oncorhynchus mykiss*). The exposure period ran for 28 days followed by a 36 day depuration period. The fish were fed daily with a diet containing nominally 100 mg metaflumizone/kg.

Concentrations of metaflumizone in fish food determined by HPLC-MS were 59 to 66% of nominal with a mean concentration of 62.8 mg/kg. Analysis of the test item in treatment water confirmed no leaching of the test item from diet into the media initially. From the second half of the uptake phase, metaflumizone was detected at a maximum of 0.48 µg/l on day 28. This is considered due to uptake and excretion by fish through the gastrointestinal tract.

A steady state was not achieved.

The kinetic biomagnification factor (BMF) was 0.326. Accounting for the fish growth rate the growth corrected BMF was 0.554. Accounting for the lipid content in fish through the depuration phase and the lipid content in food, the lipid and growth corrected BMF was 1.2.

The depuration half-life was 12 days.

5.3.2 Summary and discussion of aquatic bioaccumulation

Experimental $logK_{ow}$ data are available for the E and Z isomers of metaflumizone:

Z : $logK_{ow}$ 4.4 at pH 5 and 4.2 at pH 7
E: $logK_{ow}$ 5.1 pH 5 and 4.9 at pH 7

Experimental $logK_{ow}$ data are also available for various degradants in the DAR. The highest values $logK_{ow}$ values were 3.1 at pH 7 for M320I04 and 3.8 at pH 7 for M320I23.

Based on two experimental BCF studies using radio-labelled metaflumizone, both steady state and kinetic BCF values exceed 1,000 l/kg following normalisation to a 5% lipid content.

Overall, the $logK_{ow}$ is considered to be above the CLP $logK_{ow}$ trigger value of ≥ 4 and representative fish BCFs are above the trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of metaflumizone is presented in Table 32. Studies were reviewed under EU Regulation 1107/2009. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the respective test guideline. They are considered reliable and suitable for use in hazard classification. Further details are presented for studies conducted on the active substance metaflumizone.

Metaflumizone is a mixture of E and Z isomers with a minimum ratio of 9:1 (w/w). The DAR and EFSA review considered that both isomers have comparable biological activity and toxicity. On this basis the conversion of the E isomer to the Z isomer under light conditions is not considered relevant for the purpose of classification and labelling.

The experimental water solubility for metaflumizone (as mixture E/Z 92.2:7.8) was 1.81 µg/l (0.00181 mg/l) at pH 7 and 20^{o}C. In deionised water it was determined to be 1.79 µg/l (0.00179 mg/l) also at 20^{o}C. Data indicate the Z isomer is slightly more soluble than the E isomer (see Table 8).

Exposure concentrations for ecotoxicity testing were based on maximum visual solubility of the test item in the test media at test temperature using a solvent. While solvents generally aid dissolution they do not significantly increase the limit of solubility. In addition, test media salts, temperature and pH can influence solubility to a degree. It is noted that exposure concentrations in acute ecotoxicity tests record test item concentrations significantly above the water solubility. This apparent increase in solubility may have been influenced by the presents of cations such as $Ca\ 2^{+}$. Analysis of measured solutions resulted in little variability and analysis of centrifuged and non-centrifuged samples (acute *Daphnia* study) were comparable. This supports the test item being dissolved.

Exposure concentrations for chronic ecotoxicity testing were prepared with nominal exposure ranges up to or above 0.002 mg/l (nominal) to reflect the quoted water solubility. Mean measured concentrations were slightly below the quoted water solubility but considered to reflect the maximum

achievable dissolved concentration in test media. Where no effects were observed, the NOEC or EC_{10}, is considered equal to or greater than the highest tested concentration. This is interpreted as no chronic effects up to the limit of water solubility for the purpose of classification.

The DAR includes ecotoxicity studies using the preparation 'BAS 320 00 I' which is an aqueous suspension concentrate containing 240 g metaflumizone/l. However, studies using metaflumizone technical are available for the same species and endpoints and take precedence. Therefore studies conducted with the preparation are not considered further in this report.

A summary of valid information for degradants is included in Annex II, Table 1. Further details are not included as degradants are either less toxic or not formed in significant quantities (refer to section 5.1.3) so are not considered further for classification of metaflumizone.

One degradant (M320I04) exhibits a 48-hour EC_{50} of 0.95 mg/l for *Daphnia* based on verified nominal concentrations. This is in the range for Aquatic Acute classification. However, as mentioned, M320I04 is not formed in significant amounts in water over the acute test period. Therefore the endpoint is not considered for acute classification of metaflumizone.

Table 32: Summary of relevant information on aquatic toxicity for metaflumizone (BAS 320I)

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Acute toxicity to fish US EPA 72-1, OECD 203, GLP, purity: 96.3%, E:Z 92:8	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Flow-through	96 hours	LC_{50}	>0.0378 (mm)	2001e[60]
Acute toxicity to fish US EPA 72-1, OECD 203, GLP, purity: 96.3%, E:Z 92:8	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Flow-through	96 hours	LC_{50}	>0.349 (mm)	2001c[61]
Acute toxicity to fish US EPA 72-3, OECD 203, GLP, purity: 96.3%, E:Z 92:8	Fathead Minnow (*Pimephales promelas*)	Mortality	Flow-through	96 hours	LC_{50}	>0.257 (mm)	2001d[62]
Fish Early Life-Stage (FELS) toxicity OECD 210, GLP, purity: 96.3%, E:Z 92:8	Rainbow Trout (*Oncorhynchus mykiss*)	Hatching success, survival and growth	Flow-through	93 days	NOEC	≥ 0.00147 (mm) for all endpoints based on no effects at the highest exposure concentration	2002a[63]
Fish Early Life-Stage (FELS) toxicity OECD 210, GLP, purity: 96.3%, E:Z 92:8	Fathead Minnow (*Pimephales promelas*)	Hatching success, survival and growth	Flow-through	41 days	NOEC	≥ 0.001157 (mm) for all endpoints based on no effects at the highest exposure concentration	2002[64]
Daphnia sp Acute Immobilisation US EPA 72-2, GLP,	*Daphnia magna*	Acute immobilisation	Flow-through	48 hours	EC_{50}	>331 (mm)	Aufderheide *et al*, 2001f[65]

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
purity: 96.3%, E:Z 92:8							
Daphnia sp Acute Immobilisation OECD 202, GLP, purity: 99.7% Z isomer	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	2.86 (mm)	Weltje and Glaeser, 2006b[66]
Acute toxicity US EPA 72-3, GLP, purity: 100%, E:Z 92.2:7.8	Mysid Shrimp (*Americamysis bahia*)	Acute	Flow-through	96 hours	LC_{50}	>0.289(mm)	Aufderheide *et al*, 2001b[67]
Acute toxicity US EPA 72-3, GLP, purity: 96.3%, E:Z 92:8	Oyster (*Crassostrea virginica*)	Mortality Shell growth	Flow-through	96 hours	EC_{50}	>0.136 (mm)	Aufderheide *et al*, 2002[68]
Daphnia magna Reproduction OECD Guideline 211, GLP, purity: 96.3%, E:Z 92:8	*Daphnia magna*	Survival; reproduction; growth	Semi-static	21 days	NOEC	≥ 0.00147 (mm) for all endpoints based on no effects at the highest exposure concentration	Olivieri *et al*, 2001[69]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 96.3%, E:Z 92:8	*Pseudo-kirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} ErC_{10}	>0.0256 (mm) >0.0256 (mm)	Aufderheide *et al*, 2001a[70]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 96.9%, E:Z 94:6	*Anabaena flos-aquae*	Cell multiplication inhibition	Static	72 hours	ErC_{50} ErC_{10}	>0.225 (mm) >0.225 (mm)	Hicks and Holmes, 2004b[71]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 96.9%, E:Z 94:6	*Navicula pelliculosa*	Cell multiplication inhibition	Static	72 hours	ErC_{50} ErC_{10}	>0.0264 (mm) >0.0264 (mm)	Hicks and Holmes, 2004c[72]
Marine Algal Growth Inhibition OECD Guideline 201, GLP, purity: 96.3%, E:Z 92:8	*Skeletonema costatum*	Cell multiplication inhibition	Static	72 hours	ErC_{50} ErC_{10}	>0.011 (mm) >0.011 (mm)	Hicks and Holmes, 2004d[73]
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 96.9%, E:Z 94:6	*Lemna gibba*	Growth	Static	7 days	ErC_{50} NOErC	>0.314 (mm) ≥ 0.314 (mm) based on no effects at the highest exposure concentration	Hicks and Holmes, 2004a[74]

Notes:
mm refers to mean measured
*formerly *Selenastrum capricornutum*

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Three valid acute toxicity to fish studies using metaflumizone are available following GLP.

Study 1 (2001e)[60]

Following US EPA test guideline 72-1 (similar to OECD Test Guideline 203) and using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) at 0.1 mg/l and a solvent control was included. Flow-through study conditions were within the test guideline range and validation criteria were met. The study was conducted using a 16 hour light and 8 hour dark photoperiod. No effects were observed in the control or solvent control but sub-lethal effects were observed at all exposure concentrations. Analytical verification by High Performance Liquid Chromatography (HPLC) with UV detection was 70 to 88% of nominal, with measured concentrations determined as: 17.4, 37.8, 75, 163 and 343 µg a.s./l. From the study report, it is unclear if solutions were filtered / centrifuged before analysis.

As 40% mortality was observed at nominal concentration 400 µg/l (343 µg/l measured concentration), the study 96-h LC_{50} was considered >343 µg a.s./l. During the DAR review, the RMS noted all test solutions contained undissolved particulates at 24 hours and for the remainder of the test in all exposure solutions except the lowest two concentrations. The RMS considered the 96-h LC_{50} restricted to the highest dose at which the test items appeared to be fully dissolved which was nominally 50 µg a.s./l. This corresponds to 37.8 µg a.s./l (0.0378 mg a.s./l) based on measured concentrations.

For the purpose of classification, the evaluating Member State (eMS) considers the 96-h LC_{50} is greater than the quoted limit of water solubility

Study 2 (2001c)[61]

Using Bluegill Sunfish (*Leopmis macrochirus*) the nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. The flow-through study followed US EPA guideline 72-1 and OECD Test Guideline 203. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Test solutions were observed to be clear and colourless although test material was observed coating the glass of the test vessels. Study conditions were within the test guideline range and validation criteria were met. The study was conducted using a 16 hour light and 8 hour dark photoperiod. Analytical verification by HPLC-UV was 64 to 105% of nominal with measured concentrations determined as: 16, 37.9, 75.8, 209 and 349 µg a.s./l. It is unclear if solutions were filtered / centrifuged before analysis.

Sub-lethal effects were observed at the highest two exposure concentrations with 5% mortality observed at the highest exposure concentration. The study 96-h LC_{50} was >343 µg a.s./l (>0.349 mg a.s./l) based on mean measured concentrations.

For the purpose of classification, the eMS considers the 96-h LC_{50} is greater than the quoted limit of water solubility.

Study 3 (2001d)[62]

Using Sheepshead Minnow (*Cyprinodon virginica*) the nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF 0.1 ml/l) and a solvent control was included. The study followed US EPA guideline 72-3. Fish were fed during the study which is not recommended in study guidelines. Given additional validation criteria were met, this is not considered sufficient to invalidate the study. The study was conducted using a 16 hour light and 8 hour dark photoperiod.

Analytical verification by HPLC-UV was 51 to 64% of nominal at study initiation. It is unclear if solutions were filtered / centrifuged before analysis. Test solutions were observed to be clear and colourless with no particulates reported during the test. Mean measured concentrations over the study were 12.7, 28.1, 60.8, 125 and 257 µg a.s./l. Sub-lethal effects were observed from treatment 60.8 µg a.s./l. At the highest exposure concentration 25% mortality was observed. On this basis the study 96-h LC_{50} was considered >257 µg a.s./l (>0.257 mg a.s./l) based on mean measured concentrations.

For the purpose of classification, the eMS considers the 96-h LC_{50} is greater than the quoted limit of water solubility.

Additional Studies

Two additional non-standard acute fish toxicity studies using spiked water-sediment systems are available and were included in the risk assessment under EU Regulation 1107/2009. The eMS does not feel these are valid for the purpose of hazard classification due to the exposure route – further details and explanation are presented below for completeness only.

Study 4 (2004c)[75]

A 96-hour acute toxicity study with Channel Catfish (*Ictalurus punctatus*) is available using ^{14}C-metaflumizone (96.9% purity, E:Z 94:6) and a static spiked water-sediment system. The system was treated with spiked sediment at 1,000 µg a.s./kg (nominal) and spiked water at 300 µg a.s./l (nominal) prepared with the aid of DMF as a solvent and a solvent control (0.33ml/l DMF and 10 ml acetone/kg) was included. The study followed US EPA test guideline 850.1075 and elements of OECD Test Guideline 203.

Overall a decrease in overlying water test item concentrations was observed with an increase of test item sediment concentrations. The measured overlying water concentration at 0 hours was 248 µg a.s./l based on HPLC-MS analysis. At study termination, this was considered to be 26.8 µg/l ^{14}C-metaflumizone equivalents. Measured concentrations in the interstitial water were 10.1 to 12.8 µg/l ^{14}C-metaflumizone equivalents at study initiation and 13.6 to 17.7 µg/l ^{14}C-metaflumizone equivalents at study termination. Measured concentrations in sediment were 1,030 to 1,110 µg ^{14}C-metaflumizone equivalents/l at study initiation and 1,010 to 1,620 µg ^{14}C-metaflumizone equivalents/l at study termination. No statistical difference was observed between controls and treatment systems. On this basis, the report 96-h LC_{50} was considered to be >248 µg a.s./l based on initial measured concentrations of metaflumizone.

Given the decline in ^{14}C in the aqueous phase over the study duration, the eMS does not feel the study 96-hour LC_{50} based on initial measure concentrations of metaflumizone is appropriate for classification. In addition, given the spike sediment, and that Catfish are bottom feeders, it is unclear

what concentration the test organisms were exposed to over the study period. On this basis, the eMS considers the study does not provide a valid endpoint for hazard classification.

Study 5 (2004d)[76]

A 96-hour acute toxicity study with Common Carp (*Cyprinus carpio*) is available using ^{14}C-metaflumizone (96.9% purity, E:Z 94:6) and a static spiked water-sediment system. The system was treated with spiked sediment at 1,000 µg a.s./kg (nominal) and spiked water at 300 µg a.s./l (nominal) prepared with the aid of a solvent (0.33ml/l DMF and 10 ml acetone/kg) and a solvent control was included. The study followed US EPA test guideline 850.1075 and elements of OECD Test Guideline 203.

A decrease in overlying water test item concentrations was observed with an increase of test item sediment concentrations. The measured overlying water concentration at 0 hours was 289 µg a.s./l. At study termination, this was considered to be 39.5 µg/l ^{14}C-metaflumizone equivalents. Analytical measurement of the interstitial water was not included. Measured concentrations in sediment were 859 to 1,060 µg ^{14}C-metaflumizone equivalents/kg dry sediment at study initiation and 1,040 to 2,000 µg ^{14}C-metaflumizone equivalents/kg dry sediment at study termination. No statistical difference was observed between controls and treatment systems. On this basis, the report 96-h LC_{50} was considered to be >289 µg a.s./l based on initial measured concentrations of metaflumizone.

Given the decline in ^{14}C in the aqueous phase over the study duration, the eMS does not feel the study 96-hour LC_{50} based on initial measure concentrations of metaflumizone is appropriate for classification. In addition, given the spike sediment, and that Carp are bottom feeders, it is unclear what concentration the test organisms were exposed to over the study period. On this basis, the eMS considers the study does not provide a valid endpoint for hazard classification.

5.4.1.2 Long-term toxicity to fish

Two valid chronic fish toxicity studies using metaflumizone are available following GLP.

Study 1 (2002a)[63]

The study followed GLP and OECD Test Guideline 210 and ran for 93 days under flow-through conditions. The study used Rainbow Trout (*Oncorhynchus mykiss*) and the following endpoints: hatching success, survival and growth (length and wet weight). General observations were also recorded. Exposure solutions were prepared with the aid of the solvent DMF at 0.018 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. The nominal exposure range was 0.13, 0.25, 0.5, 1.0 and 2.0 µg a.s./l, reflecting up to the quoted water solubility.

Results were based on mean measured values by HPLC-MS: 0.08, 0.17, 0.29, 0.57 and 1.47 µg a.s./l. Significant effects were determined by ANOVA followed by the Dunnett's multiple means comparison test for weight and length and Fishers-exact test for mortality. There were no statistical differences between controls and any exposure treatment for any endpoint. Therefore, the 93-day

NOEC was considered to reflect the highest tested concentration and 1.47 µg a.s./l (0.00147 mg a.s./l) based on mean measured concentrations.

Study 2 (2002)[64]

The study followed GLP and OECD Test Guideline 210 and ran for 41 days under flow-through conditions. The study used the marine fish species Sheepshead Minnow (*Cyprinodon variegatus*) and the following endpoints: hatching success, survival and growth (length and wet weight). General observations were also recorded. Exposure solutions were prepared with the aid of the solvent DMF at 0.007 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. The nominal exposure range was 0.13, 0.25, 0.5, 1.0 and 2.0 µg a.s./l reflecting up to the quoted water solubility.

Results were based on mean measured values by HPLC-MS: 0.067, 0.145, 0.279, 0.565 and 1.15 µg a.s./l. Significant effects were determined by ANOVA followed by the Dunnett's multiple means comparison test for weight and length and Fishers-exact test for mortality. There were no statistical differences between controls and any exposure treatment for any endpoint. Therefore, the 41-day NOEC was considered to reflect the highest tested concentration and 1.15 µg a.s./l (0.00115 mg a.s./l) based on mean measured concentrations.

Additional Studies

A non-standard full life cycle study using a static water-sediment system and Zebra fish (*Danio rerio*) is available (2004a[77] and 2004b[78]). This was included in the risk assessment under EU Regulation 1107/2009. The study used a suspension concentrate containing 21.8% w/w ^{14}C metaflumizone and broadly followed OECD Test Guideline 210. The system was dosed via the aqueous phase with the addition of sediment phase considered a simulation for pesticide dissipation following spay exposure. To reflect this scenario, endpoints were based on initial measured concentrations.

The eMS does not feel the study is valid for the purpose of hazard classification given the method of exposure and uncertainty regarding actual test item concentrations over the study period.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1 (Aufderheide *et al*, 2001f)[65]

A flow-through acute toxicity study with *Daphnia magna* using metaflumizone is available following GLP and US EPA guideline 72-2. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 mg/l and a solvent control was included. Details of the study photoperiod conditions were not reported. However, results were based on mean measured values by HPLC-MS: 20.7, 41.4, 80.2, 170 and 331 µg a.s./l. Test solutions were observed to be clear and colourless with no visible particles throughout the test. It is unclear if solutions were filtered / centrifuged before analysis. Immobilisation was observed at all exposure concentrations up to a maximum of 35%. Sublethal

effects were observed from 41.4 µg a.s./l. The study 48-hour LC_{50} was >331 µg a.s./l (>0.331 mg a.s./l) based on mean measured concentrations. The eMS considers this is above the quoted water solubility. The study 48-hour NOEC was 20.7 µg a.s./l (0.207 mg a.s./l) based on mean measured concentrations.

During the DAR review, the RMS noted 10% mortality at the lowest test concentration so considered the 48-hour NOEC as <20.7 µg a.s./l (0.207 mg a.s./l) based on mean measured concentrations. For the purpose of hazard classification, the eMS notes 10% mortality was also observed in the solvent control and that the study NOEC is valid, i.e. 20.7 µg a.s./l (0.207 mg a.s./l) based on mean measured concentrations.

Study 2 (Weltje and Glaeser, 2006b)[66]

A static acute toxicity study with *Daphnia magna* using metaflumizone Z isomer (99.7% purity) is available following GLP and OECD Test Guideline 202. The study was conducted using a 16-hour light and 8-hour dark photoperiod. The nominal exposure range was 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/l. Exposure solutions were prepared with the aid of a DMF and a solvent control was included. It is unclear if solutions were filtered / centrifuged before analysis. Initial measured concentrations of the Z isomer by HPLC-MS were 65.7 to 88.7% of nominal with low levels of the E isomer also detected. Considering the similar biological activity of both isomers, the concentrations of Z and E isomers were summed to determine metaflumizone initial measured concentrations: 0.177, 0.384, 0.730, 1.542, 2.837 and 7.33 mg/l.

Fischers-exact test was used to determine statistically significant differences compared to controls. The study endpoints presented in the DAR were based on initial mean measured values: the study 48-hour LC_{50} was 4.34 mg/l (95% confidence limits 3.05 to 6.20 mg/l) and the 48-hour NOEC was 0.384 mg/l. While these values were appropriate for pesticide risk assessment, the eMS considered that endpoints based on mean measured concentrations for the study duration are appropriate for hazard classification (ECHA, 2015b). On this basis, the study 48-hour LC_{50} was 2.864 mg/l (95% confidence limits 2.03 to 4.01 mg/l) based on mean measured concentrations which is above the quoted water solubility. The 48-hour NOEC was 0.281 mg/l based on mean measured concentrations.

Study 3 (Aufderheide *et al*, 2001b)[67]

A flow-through 96-hour acute toxicity study with the marine Mysid Shrimp (*Americamysis bahia*) is available using metaflumizone. The study was run to GLP and followed US EPA OPPTS 72-3. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. The study was conducted using a 16-hour light and 8-hour dark photoperiod. Exposure solutions were prepared with the aid of a solvent (DMF at 0.1 ml/l) and a solvent control was included. The exposure range was based on visual assessment of solubility during the range finding study. Test vessel solutions were observed to be clear and colourless with no visible particulates throughout the study. However, the test item was observed coating the glass of the mixing box throughout the study indicating that the test item was present above the limit of solubility in the saline test media. It is unclear if solutions were filtered / centrifuged before analysis.

Results were based on mean measured values: 19, 43.5, 94.6, 113 and 289 µg a.s./l. The 96-h LC_{50} was >289 µg a.s./l based on mean measured concentrations (>0.289 mg a.s./l) which is above the quoted water solubility. The 96-h NOEC was 19 µg a.s./l based on mean measured concentrations reflecting sublethal effects at all but the lowest exposure concentration.

Study 4 (Aufderheide *et al*, 2002a)[63]

A flow-through 96-hour acute toxicity study with the marine Eastern Oyster (*Crassostrea virginica*) is available using metaflumizone. The study was run to GLP and followed US EPA OPPTS 72-3 with the following endpoints: new shell growth and mortality. The initial nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF (≤ 0.034 ml/l) and a solvent control was included. Across this range no mortality was observed but reduced shell growth was observed at each treatment.

Subsequently a second test was conducted with the nominal exposure range prepared with the aid the solvent DMF at 0.034ml/l: 2.6, 4.3, 7.2, 12 and 20 µg a.s./l. A solvent control was run in parallel. Analysis of exposure solutions was undertaken by HPLC-MS with mean measured concentrations determined as 1.28, 2.03, 3.52, 6.01 and 10.6 µg a.s./l. No mortality was observed.

Based on statistical analysis following ANOVA and Dunnett's-test, shell growth was reduced at the highest two exposure concentrations. The 96-h EC_{50} was >136 µg a.s./l (>0.136 mg a.s./l) based on mean measured concentrations reflecting the first exposure series. This is above the quoted water solubility. The 96-h NOEC was 3.52 µg a.s./l based on mean measured concentrations during the second test.

Additional Studies

A second acute toxicity study with *Daphnia magna* using metaflumizone is available (Aufderheide *et al*, 2006a)[79]. However, the study was not conducted to GLP and was not considered valid during review under pesticide regulations. On this basis, further details are not included.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1 (Olivieri *et al*, 2001)[69]

A flow-through chronic toxicity to *Daphnia magna* study using metaflumizone is available following GLP and OECD Test Guideline 211. The study was conducted using a 16-hour light and 8-hour dark photoperiod. The study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.13, 0.25, 0.5, 1.0 and 2.0 µg a.s./l reflecting up to the quoted water solubility. Exposure solutions were prepared with the aid of a solvent DMF (0.01 ml/l) and a solvent control was included. Results were based on mean measured values by HPLC-MS: 0.091, 0.204, 0.343, 0.709 and 1.470 µg a.s./l. There were no reported signs of the test substance exceeding solubility in test media and test solutions were observed to be clear and colourless throughout the study.

Based on statistical analysis using Fishers-exact test of mortality and Dunnett's-test for growth, no significant effects were observed for any parameter at any test concentration. The 21-d NOEC was 1.470 µg a.s./l (0.00147 mg a.s./l) based on mean measured reflecting the highest exposure concentration.

Additional Studies

A chronic toxicity study with *Daphnia magna* (Bergtold and Janson, 2006)[80] is available in the DAR. However, this study used non-standard (variable pulsed/peak) exposure scenarios. It is not considered valid for the purpose of hazard classification and not discussed further.

Study 2 (Aufderheide *et al*, 2002b)[81]

A flow-through 28-day toxicity study with the non-standard marine species Mysid Shrimp (*Americamysis bahia*) is available using metaflumizone. The study was run to GLP and followed US EPA OPPTS 72-4. The study assessed the following endpoints: mortality (first and second generation), weight (biomass for male/female) and number of offspring. The study was conducted using a 16-hour light and 8-hour dark photoperiod. The nominal exposure range was 0.065, 0.13, 0.25, 0.5 and 1.0 µg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF, 0.023 ml/l) and a solvent control was included. Results were based on mean measured values following analysis by HPLC-MS: 0.0389, 0.0777, 0.144, 0.271 and 0.654 µg a.s./l.

ANOVA analysis was employed with Dunnett's test for growth and reproduction and Fishers-exact test for mortality. The study NOEC for reproduction was 0.654 µg/l based on mean measured concentrations (i.e. no effects at the highest concentration tested) compared to pooled solvent and procedural controls. Whilst not concentration related, lower survival of 1st and 2nd generation mysids was observed in mid doses groups.

The most sensitive endpoint was female biomass. Although a clear dose-response was not observed, higher exposure concentrations were considered statistical different to solvent controls (due to significant difference between the solvent control and procedural control) from a measured exposure concentration of 0.144 µg a.s./l. On this basis, the study 28-day NOEC was 0.0777 µg a.s./l (0.0000777 mg a.s./l) based on mean measured concentrations.

The EFSA Peer Review Conclusion (EFSA Journal 2013; 11(10):3373) noted that the available data indicated the Mysid Shrimp (*Americamysis bahia*) were more sensitive than daphnids. However, they referenced the 'uncertainties in the derivation of the endpoints from this study on the Mysid shrimp (i.e. some effects without clear concentration dependence/independence, low number of replications (2 replicates of 10 animals per exposure concentration) hence low power of the statistics)'. This relates to significant differences between the study control and solvent control for various endpoints including dry weight and mean number of young per reproductive day. The confounding effects of the solvent mean the data cannot be considered reliable. EFSA therefore considered that there was a data gap and that further information to address the chronic risk to aquatic invertebrates was required. They recommended further information to address the chronic risk to aquatic invertebrates with consideration to the sensitivity of Mysid Shrimps. At the time of writing no further information is available.

Overall, the study does not provide a reliable endpoint for the purpose of classification. At present is it also unclear if Mysid Shrimp are more sensitive than the standard invertebrate test species *Daphnia Magna*.

5.4.3 Algae and aquatic plants

Algae:

Study 1 (Aufderheide *et al*, 2001a)[70]

An algal growth inhibition test using metaflumizone and the freshwater green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available following GLP and OECD Test Guideline 201. The study was run under static conditions and continuous illumination. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF (0.1 ml/l) and a solvent control was included. Measured concentrations by Liquid Chromatography-Mass Spectrometry (LC-MS) at study initiation were 217*, 34.2, 80.6, 168 and 313 µg a.s./l. However, measured concentrations were 1 to 3% of nominal at 72 hours which is likely to reflect photodegradation. Throughout the study, solutions were observed to be clear and colourless with no visible particulates.

ANOVA analysis using Dunnett's test determined no significant difference between treatments and pooled controls. On this basis the study 72-hour endpoint reflected the highest exposure concentration: the 72-hour E_rC_{50} was >313 µg a.s./l and the 72-hour E_rC_{10} >313 µg a.s./l based on initial mean measured concentrations.

For the purpose of hazard classification, the eMS notes that endpoints should preferentially be based on measured concentrations over the study period where significant losses are observed (ECHA, 2015b). The eMS has calculated the 0 to 72 hour mean measured concentration for the highest exposure treatment as 25.6 µg a.s./l (0.0256 mg a.s./l). However, in this instance, no significant effects were observed and the highest exposure concentration is considered to be above the limit of water solubility. Therefore, the eMS considers the 72-hour E_rC_{50} and 72-hour E_rC_{10} are above the quoted limit of water solubility.

*value considered due to sample contamination

Study 2 (Hicks and Holmes, 2004b)[72]

A 120-hour algal growth inhibition test using metaflumizone and the freshwater blue-green alga *Anabaena flos-aquae* is available following GLP and OECD Test Guideline 201. The study was run under static conditions and continuous illumination. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF 0.1 ml/l) and a solvent control was included. Measured concentrations at study initiation by HPLC-UV were 107 to 208% of nominal. At 72 hours, measured concentrations were 11 to 28% of nominal which is likely to reflect photodegradation. Throughout the study, solutions were observed to be clear and colourless with no visible particulates.

ANOVA analysis of 72-hour results using Dunnett's test determined no significant difference between treatments and pooled controls. On this basis the study 72-hour endpoint reflected the highest exposure concentration: 72-hour E_rC_{50} was >400 µg a.s./l and the 72-hour E_rC_{10} >400 µg a.s./l based on nominal concentrations.

For the purpose of hazard classification, the eMS notes that endpoints should preferentially be based on measured concentrations over the study period where significant losses are observed (ECHA, 2015b). The eMS has calculated the 0 to 72 hour mean measured concentration for the highest exposure treatment as 225 µg a.s./l (0.2256 mg a.s./l). However, in this instance, no significant effects were observed and the highest exposure concentration is considered to be above the limit of water solubility. Therefore, the eMS considers the 72-hour E_rC_{50} and 72-hour E_rC_{10} are above the quoted limit of water solubility.

Study 3 (Hicks and Holmes, 2004c)[72]
An algal growth inhibition test using metaflumizone and the freshwater diatom *Navicula pelliculosa* is available following GLP and OECD Test Guideline 201. The study was run under static conditions and continuous illumination. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF 0.1 ml/l) and a solvent control was included. Measured concentrations at study initiation by HPLC-UV were 116 to 155% of nominal. At 72 hours, measured concentrations were 0 and 9% nominal which is likely to reflect photodegradation. Throughout the study, solutions were observed to be clear and colourless with no visible particulates.

ANOVA analysis using Dunnett's test determined no significant difference between treatments and pooled controls. On this basis the study 72-hour endpoint reflected the highest exposure concentration: 72-hour E_rC_{50} was >400 µg a.s./l and the 72-hour E_rC_{10} >400 µg a.s./l based on nominal concentrations.

For the purpose of hazard classification, the eMS notes that endpoints should preferentially be based on measured concentrations over the study period where significant losses are observed (ECHA, 2015). The eMS has calculated the 0 to 72 hour mean measured concentration for the highest exposure treatment as 26.4 µg a.s./l (0.0264 mg a.s./l). However, in this instance, no significant effects were observed and the highest exposure concentration is considered to be above the limit of water solubility. Therefore, the eMS considers the 72-hour E_rC_{50} and 72-hour E_rC_{10} are above the quoted limit of water solubility.

Study 4 (Hicks and Holmes, 2004d)[73]
An algal growth inhibition test using metaflumizone and the marine diatom *Skeletonema costatum* is available following GLP and OECD Test Guideline 201. The study was run under static conditions with a 14 hour light, 10 hour dark photo period. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. Exposure solutions were prepared with the aid of a solvent (DMF 0.1 ml/l) and a solvent control was included. Measured concentrations at study initiation by HPLC-UV were 81 to 111% of nominal. At 72 hours, measured concentrations were 2 to 7% of nominal. Throughout the study, solutions were observed to be clear and colourless with no visible particulates.

ANOVA analysis using Dunnett's test determined no significant difference between treatments and pooled controls. On this basis the study 72-hour endpoint reflected the highest exposure concentration: 72-hour E_rC_{50} was >443 µg a.s./l and the 72-hour E_rC_{10} >443 µg a.s./l based on initial measured concentrations.

For the purpose of hazard classification, the eMS notes that endpoints should preferentially be based on measured concentrations over the study period where significant losses are observed (ECHA, 2015b). The eMS has calculated the 0 to 72 hour mean measured concentration for the highest exposure treatment as 110.4 µg a.s./l (0.11 mg a.s./l). However, in this instance, no significant effects were observed and the highest exposure concentration is considered to be above the limit of water solubility. Therefore, the eMS considers the 72-hour E_rC_{50} and 72-hour E_rC_{10} are above the quoted limit of water solubility.

Aquatic plants:

A 14-day toxicity to *Lemna gibba* study (Hicks and Homes, 2004a)[74] using metaflumizone is available following GLP and OECD Test Guideline 221. Exposure solutions were prepared with the aid of a solvent (DMF, 0.1 ml/l) and a solvent control was included. The nominal exposure range was 25, 50, 100, 200 and 400 µg a.s./l. The study included one media renewal on day 7. Analytical measurement by HPLC-UV at study initiation was 168 to 291% of nominal. After 7 days recoveries were 14 to 32% of nominal before renewal. Throughout the study, solutions were observed to be clear and colourless with no visible particulates.

The study endpoints were frond number, area under growth curve, biomass and growth rate and available for 7 and 14 days. For the purpose of hazard classification, 7-day endpoints are preferred.

No growth rate inhibition was observed at any concentration and the 7-day endpoints were quoted based on nominals: 7-d E_rC_{50} >400 µg a.s./l, 7-d NOE_rC 400 µg a.s./l. For the purpose of hazard classification, the eMS notes that endpoints should preferentially be based on measured concentrations over the study period where significant losses are observed (ECHA, 2015b). The eMS has calculated the 0 to 7-day mean measured concentration for the highest exposure treatment as 314 µg a.s./l (0.314 mg a.s./l). However, in this instance, no significant effects were observed and the highest exposure concentration is considered to be above the limit of water solubility. Therefore, the eMS considers the 72-hour E_rC_{50} and 72-hour E_rC_{10} are above the quoted limit of water solubility.

5.4.4 Other aquatic organisms (including sediment)

Data are available using spiked sediment exposure and presented in Table 33 for completeness given metaflumizone target species are insects.

Two *Chironomus* studies are available using aqueous exposure in a water-sediment system – further details of these are presented in the table below. However, it is not possible to use the quoted study endpoints for hazard classification for two reasons. Firstly, analytical data is not available for the full exposure series to validate aqueous exposure concentrations. Secondly, given the sediment compartment present in test vessels, it is unclear if a contribution of the toxicity was due to sediment contact/ingestion.

Table 33: Summary of relevant information on sediment dwelling organisms toxicity for metaflumizone (BAS 320I)

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity	
US EPA 850.1735, GLP, purity: 96.9%, E:Z 94:6	*Hyalella azteca*	Mortality	Static Spiked sediment	10 days	LC_{50} NOEC	>995 mg/kg 105 mg/kg (mm)	Aufderheide and Holmes, 2004b[82]
US EPA 850.1740, GLP, purity: 96.9%, E:Z 94:6	*Leptocheirus plumulosus*	Mortality	Static Spiked sediment	10 days	LC_{50} NOEC	>935 mg/kg 397 mg/kg (mm)	Aufderheide and Holmes, 2004a[83]
ASTM E1706-95b, GLP, purity: 96.9%, E:Z 94:6	*Chironomus tentans*	Mortality	Static Spiked sediment	10 days	LC_{50} NOEC	1.8 mg/kg 0.93 mg/kg (mm)	Aufderheide and Holmes, 2002c[84]
OECD 218, GLP, purity: 95.8%, E:Z 94:6	*Chironomus riparius*	Emergence and development	Static Spiked sediment	10 days	NOEC	1.616 mg/kg (im)	Backfish and Weltje, 2010a[85]
OECD 219, GLP, purity: 95.8%, E:Z 94:6	*Chironomus riparius*	Emergence and development	Static Spiked water	28 days	NOEC	0.00256 mg/l (im) significant losses observed	Weltje, 2005[86]
OECD 218, GLP, purity: 99.7%, Z isomer	*Chironomus riparius*	Emergence and development	Static Spiked water	28 days	NOEC	0.0025 mg/l (im) significant losses observed	Weltje and Glaeser, 2006a[87]

Notes:
mg/kg measurement are dry weight
mm refers to mean measured concentration
im refers to initial measured concentration

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Metaflumizone undergoes hydrolysis at low pH and is susceptible to photodegradation. It is not readily biodegradable. In water-sediment simulation studies, metaflumizone dissipated rapidly from the water column to sediment with dissipation $DT_{50water}$ as low as 0.01 days. In addition degradation products were observed in both the aqueous and sediment phases. While primary degradation was observed, mineralisation accounted for 16.3 to 35.5% AR after 100 days. Kinetic assessment derived metaflumizone degradation $DT_{50\ total\ system}$ values of 376 days under dark conditions and 6.32 days for irritated conditions. Degradation $DT_{50\ total\ system}$ values were also derived for significant degradants (M320I06 and M320I029) with values ranging between 6.27 and 40.2 days.

Overall, the degradation information does not provide sufficient data to show metaflumizone is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, metaflumizone is considered not rapidly degradable for the purpose of classification and labelling.

The experimental log K_{ow} is 4.2 to 4.4 for the Z isomer and 4.9 to 5.1 for the E isomer. This is above the CLP log K_{ow} trigger value of ≥ 4. Representative fish BCFs (e.g. $BCF_{k\ lipid\ normalised}$ 4,099 to 5,769 l/kg) are above the trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

Aquatic Acute Toxicity

Aquatic acute toxicity data on metaflumizone are available for fish, invertebrates, algae and aquatic plants. No acute/short-term $L(E)C_{50}$ endpoints were observed for fish, invertebrates or algae/aquatic plants up to the quoted limit of water solubility using metaflumizone (0.00181 mg/l at 20°C and pH 7).

Overall, metaflumizone should not be classified for Aquatic Acute classification.

Aquatic Chronic Toxicity

Chronic toxicity data on metaflumizone are available for fish, invertebrates, algae and aquatic plants using standard test species. In each case, exposure concentration ranges were prepared up to or above 0.002 mg/l (nominal) to reflect the quoted water solubility. Mean measured concentrations were slightly below the quoted water solubility but considered to reflect the maximum achievable dissolved concentration in test media. In each case, the NOEC or EC_{10} was equal to or greater than the highest tested concentration. This is interpreted as no chronic effects up to the limit of water solubility for the purpose of classification.

While the Aufderheide *et al*, 2002b[81] chronic study endpoints on Mysid shrimp were not considered valid, the EFSA Conclusion highlighted that shrimp species may be more sensitive than daphnids. At present, there are no data to clarify this although should additional data become available, the environmental classification should be reconsidered.

The eMS also notes that metaflumizone is an insecticide and that daphnia and mysids are crustaceans - so the available invertebrate test species may not be fully representative of the insect class. Chironomid studies are available - but the reported endpoints are not considered relevant for hazard classification due to difficulties interpreting data from water-sediment test systems and a lack of analytical verification.

Metaflumizone meets the following criteria for classification as Aquatic Chronic 4:

- no acute toxicity recorded at levels up to the water solubility (poorly soluble substance with water solubility < 1 mg/l)

- not rapidly degradable
- experimentally determined BCF ≥ 500

However, valid chronic NOECs > 1 mg/l or > the quoted limit of water solubility are considered available for standard hazard classification surrogate test species. On this basis the eMS feels Aquatic Chronic 4 is not applicable although notes that should additional data become available, the environmental classification should be reconsidered. This may include additional insect ecotoxicology studies.

Overall, it is proposed that metaflumizone should not be classified for Aquatic Chronic hazard.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Not classified – conclusive but not sufficient for classification

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's (DS) proposal

Water solubility of the mixture of E/Z (92.2:7.8) according to EEC method A6 1.4.1 (column elution method) (DAR B.2.1.11)

- pH 5: 1.35 µg/L
- pH 7: 1.81 µg/L
- pH 9: 1.73 µg/L
- In deionized water: 1.79 µg/L
- Isomers: E: 1.43 µg/L; Z: 2.03 µg/L

Partition coefficient – EEC method A8 (HPLC) (DAR B.2.1.13)

- LogPow at pH 5 and 30 °C: Z isomer: 4.4 E isomer: 5.1
- LogPow at pH 7 and 20 °C: Z isomer: 4.2 E isomer: 4.9
- LogPow at pH 3 and 20 °C: Z isomer: 3.8 E isomer: 4.4

Dissociation: no dissociation in water.

Degradation

- ***Aquatic hydrolysis*** (Fang, 2004a) GLP, US EPA N 161-1. Incubation in dark for 30 days with two different radiolabelled metaflumizones: benzonitrile-U-^{14}C (B) and trifluoromethoxyphenyl-U-^{14}C (T). The degradation pathway has been identified.
  - DT_{50} at pH 4 25 °C – B: 5.4 and T: 6.5 days
  - DT_{50} at pH 4 12 °C – B: 16.1 and T: 18.4 days
  - DT_{50} at pH 5 25 °C – B: 31.4 and T: 27.3 days
  - DT_{50} at pH 5 12 °C – B: 88.8 and T: 77.2 days

  The DS has, thus, concluded that metaflumizone is hydrolytically stable

- ***Aquatic photolysis*** (Ta, 2004d; Ta, 2004e) GLP, US EPA N 161-2, later re-assessed by Gericke, 2011c using FOCUS kinetics guidance (2006). Incubation at pH 9 for up to 15 days at 22 °C under constant irradiation. B and T radiolabelled metaflumizone was used. Aqueous photolysis pathway proposed. Mineralization 1.7%.
  - Results of Gericke study between 2.4–4.2 days.

  Susceptible for photodegradation when irradiated, but under aquatic environmental conditions photolyticpotential is limited.

- ***Biodegradation***
  - ***Ready biodegradation*** (Heim *et al*., 2002) GLP, OECD 301 (CO_2 evolution). 1.1–1.8% degradation was observed over 29 days.

    The DS has, thus, concluded that metaflumizone is not readily biodegradable.

  - ***Simulation testing***
    Water–sediment simulation tests (Rosenwald, 2003; Schriever, 2010)
    US EPA N 162-4, at 20 °C, in dark, under aerobic conditions, for 100 days.
    Dissipation $DT_{50\ water}$: 0.01 days for both study (two different lakes).
    Re-evaluated $DT_{50\ water}$ (Schriever, 2010): 0.556 and 0.733 days.
    Dissipation $DT_{50\ total\ system}$: 394 (lake 1) and 715 days (lake 2).
    Re-evaluated $DT_{50\ total\ system}$: 322 and 581 days
    Mineralization: 3.7 and 4.5%.
  - Simulation test using 12 hour light–dark cycle (Ta, 2004f; Schriever, 2010c)
    Dissipation $DT_{50\ water}$: 0.5–1.5 d,
    Re-evaluated $DT_{50\ water}$ (Schriever, 2010c): 0.298 d (dark); 0.218 d (irradiated);
    Dissipation $DT_{50\ total\ system}$: 3.6, 8.4 and 23.2 days (different radiolabels).
    Re-evaluated (Schriever, 2010c):376 days in dark (only sediment, dark: 674 d); 6.32 days irradiated (only sediment:3–38 days)
    Mineralization: 16.3, 35.5 and 23.9% (different radiolabels).
    Due to irradiation conditions of low realism metaflumizone should be considered not ultimately degraded or transformed to non-classifiable products within 28 days.

The overall conclusion of the DS was that the substance is **not rapidly degradable** for the purpose of classification and labelling.

Aquatic Bioaccumulation

- ***Kow*** (Holman & Petry, 2001)
  E isomer: Log K_{OW} 5.1 at pH 5, 30 °C
  Z isomer: Log K_{OW} 4.4 at pH 5, 30 °C
  Class, 2006a,b
  E isomer: Log K_{OW} 4.9 at pH 7, 20 °C and 4.4 at pH 3, 20 °C
  Z isomer: Log K_{OW} 4.2 at pH 7, 20 °C and 3.8 at pH 3, 20 °C
- ***Experimental aquatic BCF*** – Unpublished study performed according to GLP, OECD 305, radioactive metaflumizone
  42 days exposure period, flow through, *Lepomis macrochirus, non-steady state*
  $BCF_{whole\ fish}$: 7,800 to 8,100 L/kg
  Lipid normalized $BCF_{whole\ fish}$: 5,769 to 4,099 L/kg

2008 – 28 days flow through, *Cyprinus carpio, steady state*
BCF: 1,986 to 2,117 l/kg
Lipid normalized BCF: 2,736 to 2,916 l/kg
Depuration half-life $DT_{50\ whole\ fish}$: 14–17 days and 14.7–16 days.

Metaflumizone has the potential to bioaccumulate.

Aquatic toxicity

- ***Fish***
  - ***Acute aquatic toxicity:*** 3 fish studies are considered relevant by the DS: 96 h, flow-through test, endpoint: mortality. The highest (dissolved) conc. (mm) did not reached 50%, but only 40.5% and 25% mortality.

US EPA 72-1, OECD 203, GLP, purity: 96.3%, E/Z 92:8	Rainbow Trout (*Oncorhynchus mykiss*)	LC_{50} >0.0378 mg/L (mm)	2001e
US EPA 72-1, OECD 203, GLP, purity: 96.3%, E/Z 92:8	Bluegill Sunfish (*Lepomis macrochirus*)	LC_{50} >0.349 mg/L (mm)	2001c
US EPA 72-3, OECD 203, GLP, purity: 96.3%, E/Z 92:8	Fathead Minnow (*Pimephales promelas*)	LC_{50} >0.257 mg/L (mm)	2001d

Two more studies were introduced in the CLH Report, which are not considered by the DS as appropriate for classification purposes. The DS's argumentation was: "*Given the decline in ^{14}C in the aqueous phase over the study duration, the eMS (evaluating member state) does not feel the study 96-hour LC_{50} based on initial measured concentrations of metaflumizone is appropriate for classification. In addition, given the spiked sediment, and that catfish are bottom feeders, it is unclear what concentration the test organisms were exposed to over the study period. On this basis, the eMS considers the study does not provide a valid endpoint for hazard classification*".

The DS considered that toxicity studies using spiked water-sediment systems were not valid for the purpose of hazard classification due to the exposure route (largely due to issues related to sediment ingestion and/or adsorption).

  - ***Chronic toxicity:*** early life-stage toxicity, measured in flow-through test system, the highest tested nominal concentration was 0.002 mg/L. No toxic effect was measured even in the highest concentration, so the NOEC is higher than or equal to the highest tested concentration.

OECD 210, GLP, purity: 96.3%, E:Z=92:8	Rainbow trout (*Oncorhynchus mykiss*)	Hatching success, survival, growth	93 days	NOEC ≥0.00147 (mm) based on no effects at the highest exposure concentration	2002a
OECD 210, GLP, purity: 96.3%, E:Z=92:8	Fathead minnow (*Pimephales promelas*)	Hatching success, survival, growth	41 days	NOEC ≥0.001157 (mm) based on no effects at the highest exposure concentration	2002

The DS stated that no statistical differences were observed between the controls and any treatments for any endpoint in both studies. Therefore, the NOEC was higher than the

highest tested concentration (≥0.00147 mg/L) in the 93 days and ≥0.00116 mg/L in the 41 days study based on mean measured concentrations).

- ***Invertebrates***
  - ***Aquatic acute:*** 2 relevant 48 h Daphnia tests: one static, one flow-through. Endpoint: immobilization. In the Aufderheide *et al*. (2001f) study, the highest (dissolved) concentration did not reach 50% immobilization, only a maximum of 35%. The EC_{50} for mean measured concentrations of the Weltje and Glaser (2006b) study was 2.86, much above water solubility of metaflumizone.

Daphnia sp US EPA 72-2, GLP, purity: 96.3%, E:Z=92:8	*Daphnia magna*	Flow-through	EC50 >0.331 mg/L (mm)	Aufderheide *et al.*, 2001f
Daphnia sp OECD 202, GLP, purity: 99.7%, Z isomer	*Daphnia magna*	Static	EC50 = 2.86 mg/L (mm)	Weltje & Glaeser, 2006b

  - ***Aquatic chronic:*** one 21 day, semi-static study is cited by the DS (Olivieri
  - *et al.*,2001) . The highest initial nominal concentration was 0.002 mg/L. The NOEC result – similar to fish – reflected that no toxicity was measured in the highest exposure concentrations.

OECD 211, GLP, purity: 96.3%, E:Z=92:8	*Daphnia magna*	Survival; reproduction, growth	NOEC ≥0.00147 (mm) based on no effects at the highest exposure concentration	Olivieri *et al*, 2001

- ***Aquatic plants/algae:*** freshwater and marine algae 72 h and *Lemna* 7 days static tests and their results are summarized in the table below.
  - ***Aquatic acute and chronic:*** 5 growth inhibition studies did not reveal any effects below the limit of water solubility.

OECD 201, GLP, purity: 96.3%, E:Z 92:8	*Pseudokirchneriella subcapitata*, freshwater algae	Cell multiplication inhibition	ErC_{50} >0.0256 (mm) ErC_{10} >0.0256 (mm)	Aufderheide *et al*, 2001a
OECD 201, GLP, purity: 96.9%, E:Z 94:6	*Anabaena flos-aquae* freshwater algae	Cell multiplication inhibition	ErC_{50} >0.225 (mm) ErC_{10} >0.225 (mm)	Hicks & Holmes, 2004b
OECD 201, GLP, purity: 96.9%, E:Z 94:6	*Navicula pelliculosa* freshwater algae	Cell multiplication inhibition	ErC_{50} >0.0264 (mm) ErC_{10} >0.0264 (mm)	Hicks & Holmes, 2004c
OECD 201, GLP, purity: 96.3%, E:Z 92:8	*Skeletonema costatum,* marine algae	Cell multiplication inhibition	ErC_{50} >0.011 (mm) ErC_{10} >0.011 (mm)	Hicks & Holmes, 2004d
OECD 221, GLP, purity: 96.9%, E:Z 94	*Lemna gibba duckweed*	Growth	ErC50 >0.314 (mm) NOErC ≥ 0.314 (mm)	Hicks & Holmes, 2004a

- ***Toxicity of metaflumizone to other aquatic and sediment dwelling organisms***

Two amphipod crustaceans and four *Chironomus* midge studies were introduced by the DS, all applying spiked sediments as the route of exposure.

Two Chironomus studies were available using aqueous exposure in a water-sediment system. However, it is not possible to use the quoted study endpoints for hazard classification for two reasons: (i) analytical data are not available for the full exposure series to validate aqueous exposure concentrations; (ii) the sediment compartment is present in the test vessels, so it is unclear if a contribution of the toxicity was due to sediment contact/ingestion.

The final conclusion of the DS is that no aquatic classification is proposed.

Comments received during public consultation

Two member states commented on the CLH report:

One agreed with the proposal of no classification for environmental hazards and added an editorial correction for the EC_{50} value of the Aufderheide et al. (2001f) *Daphnia magna* study. The DS stated that the latter was a typographical error and RAC subsequently used the correct value in the opinion.

The second MS disagreed with the DS proposal of no classification for environmental hazards and instead proposed a classification of Aquatic acute 1 (H400) and Aquatic chronic 1 (H410) (acute M-factor of 1 and chronic M-factor of 10). They also disagreed with the experimental value provided for water solubility. The DS responded that each study was considered individually and the presented results were based on analytical measurement and visual observations to reflect dissolved concentrations. Furthermore, in all acute and chronic studies considered, no effects up to the highest tested concentrations were observed, for the standard test species (fish, daphnia, algae and aquatic plants).

Additionally, this MS considered that Study 4 (2002) on short-term toxicity to fish as well performed and valid acute study with *Ictalarus punctatus,* of which the mean measured concentration (geometric mean) in the spiked water from the start (0 hours) to the end of the test (96 hours) is 0.154 mg/L. The DS did not accept this non-standard study because of the uncertainty in the exposure conditions, i.e. the concentration of metaflumizone in water and the rapid dissipation of metaflumizone from water, the spiked water and sediment and the potential ingestion of significant amounts of sediment by the fish.

Finally, the MS argued for the consideration of the chironomid study results to derive a Chronic Category 1 (M-factor=10) classification. The DS stated that these results reflected the highest tested exposure concentration and that at these exposure levels no significant effects were observed. Consequently, the substance should not be classified for environmental hazard based on these results.

The DS acknowledged that *C. riparius* data can be used in principle to derive a classification, but the study included in the CLH report (Weltje, 2005) has significant limitations, such as:

- The test system was static with no test item renewal
- Analytical data are not available to validate the aqueous exposure concentrations over the full study period
- The data support the rapid distribution of metaflumizone to the sediment phase, below detection limit in water by day 28

RAC agrees with the argumentation provided by the DS in all these points.

Assessment and comparison with the classification criteria

Degradation

Metaflumizone only undergoes hydrolysis at low pH, it is hydrolytically stable at pH 7 and 9. Hydrolysis DT_{50} values of the isomers at pH 5, 25°C are between 27.3–31.4 days, at 12°C between 77.2–88.8 days >16 days. Data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4–9 is shorter than 16 days. Accordingly, metaflumizone is hydrolytically stable.

Metaflumizone is susceptible to photodegradation, but in the aquatic environmental compartments the potential for aquatic photolysis is usually limited by local conditions such as turbidity. The applicability of metaflumizone photolysis results for classification purposes is therefore limited.

In a ready biodegradation study (OECD 301, 29 days), 1.1–1.8% degradation was observed.

Metaflumizone is therefore not considered to be readily biodegradable.

In an aerobic water-sediment study performed in the dark, DT_{50} values for metaflumizone were between 394 and 715 days >28 days. Metaflumizone is not ultimately degraded within 28 days or transformed to non-classifiable products.

RAC's overall conclusion on degradation is that metaflumizone is not rapidly degradable for the purpose of classification and labelling.

Aquatic bioaccumulation

The measured Log K_{OW} of metaflumizone at aquatic environmental pH is above the CLP trigger value of 4 and the experimental fish BCFs are above the trigger of 500 L/kg (up to 8,000 L/kg).

RAC's conclusion on bioaccumulation is that metaflumizone is a substance with a potential to bioaccumulate.

Aquatic toxicity

Aquatic acute toxicity data on metaflumizone are available for fish, invertebrates, algae and aquatic plants. No acute $L(E)C_{50}$ effects were observed up to the quoted limit of water solubility. Metaflumizone does not warrant classification for Aquatic Acute toxicity.

Aquatic chronic toxicity data on metaflumizone are available for standard species of fish, invertebrates, algae and aquatic plants. Exposure concentrations in the chronic studies range up to or above 0.002 mg/L (nominal) to reflect the quoted water solubility. In each case, the NOEC was equal to or greater than the highest tested concentration. Metaflumizone showed no chronic effects up to the limit of water solubility for the purpose of classification, so it does not warrant classification for Aquatic Chronic toxicity.

As Metaflumizone has no acute toxicity up to the measured water solubility, is not rapidly degradable and has a potential to bioaccumulate, classification as Aquatic Chronic 4 requires consideration. However, as there are reliable NOECs from long-term toxicity tests that show no toxicity up to the water solubility limit, Metaflumizone does not warrant classification for Aquatic Chronic toxicity.

6 OTHER INFORMATION

No other relevant information.

7. REFERENCES

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Metaflumizone - Volume 3, Annex B.2 : Physical and chemical properties – May 2012

Metaflumizone - Volume 3, Annex B.6 : Toxicology and Metabolism – May 2012

Metaflumizone – Volume 3, Annex B.8. Environmental Fate and Behaviour - May 20102

Metaflumizone – Volume 3, Annex B.9: Ecotoxicology – May 2012

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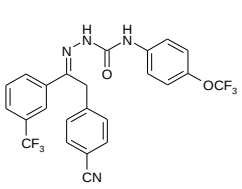
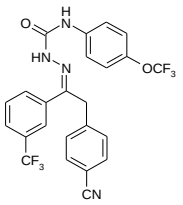
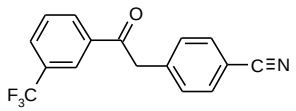
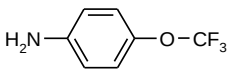
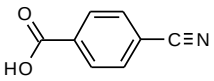
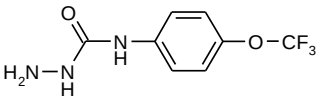
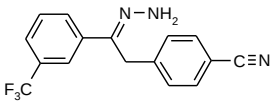
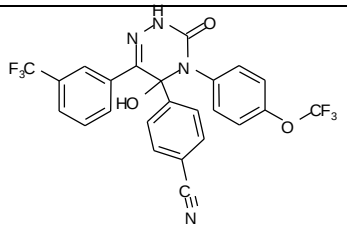
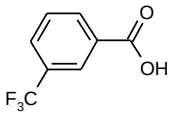
8 ANNEXES

ANNEX I: Parent and environmental degradant information: code, chemical name and structure.

ANNEX II: Aquatic toxicity data for metaflumizone degradants.

ANNEX I – Parent and environmental degradant information: code, chemical name and structure.

	Report name, Structure IUPAC name CAS name	Molecular formula molar mass
a.s.	**Metaflumizone (parent substance)**  BAS 320 I (*E*-isomer)  BAS 320 I (*Z*-isomer) A mixture of 85-100% E-2'-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-m-tolyl)ethylidene]-4-(trifluoromethoxy)carbanilohydrazide and 10-0% Z-2'-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-m-tolyl)ethylidene]-4-(trifluoromethoxy)carbanilohydrazide (IUPAC) CAS: 139968-49-3	$C_{24}H_{16}F_6N_4O_2$ 506.4 g/mole
M320I04 Soil and aquatic degradant	 4-(2-oxo-2-[3-(trifluoromethyl)phenyl]ethyl)-benzonitrile CAS: 146653-56-7	289.26 g/mole
M320I05 Soil and aquatic degradant	 Trifluroro-methoxy aniline CAS: 461-82-5	177.12 g/mole
M320I06 Soil and aquatic degradant	 4-cyano benzoic acid CAS: 619-65-8	147.13 g/mole
M320I08 Soil and aquatic degradant	 (N-[4-(trifluoromethoxy)phenyl]hydrazinecarboxamide) CAS: not available	235 g/mole

	Report name, Structure IUPAC name CAS name	**Molecular formula molar mass**
M320I09 Soil and aquatic degradant	 (4-(2-hydrazono-2-[3-(trifluromethyl)phenyl]ethyl)benzonitrile) CAS: 139972-23-9	303.29 g/mole
M320I23 Soil and aquatic degradant	 4-(5-hydroxy-3-oxo-4 [4-(trifluoromethoxy) phenyl] -6-[3-(trifluoromethyl) phenyl]-2,3,4,5- tetrahydro-1,2,4-triazin-5-yl)-benzonitrile) CAS: not available	520.39 g/mole
M320I29 Soil and aquatic degradant	 m-trifluoromethyl benzoic acid CAS: 454-92-2	190.12 g/mole

ANNEX II – Aquatic toxicity data for metaflumizone degradants.

Table 1: Summary of relevant information on aquatic toxicity for metaflumizone degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
M320I06							
Acute toxicity to fish OECD Guideline 203, GLP,	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>100 (n) Supported by analytical verification	2004a[1]
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>100 (n) Supported by analytical verification	Funk, 2004d[2]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	E_rC_{50} NOErC	>100 (n) ≥ 100 (n) Supported by analytical verification	Hoffmann, 2004b[3]
M320I04							
Acute toxicity to fish OECD Guideline 203, GLP, purity unknown	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>2.2 (n) Supported by analytical verification	2005a[4]
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	0.95 (n) Supported by analytical verification	Funk, 2004a[5]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	E_rC_{50} E_rC_{10}	2.92 (mm) 0.56 (mm)	Hoffmann, 2004a[6]
M320I023							
Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>0.07 (n) Saturated solution. Supported by analytical verification	2004c[7]
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>51.3 (n) Supported by analytical verification	Funk, 2004c[8]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} E_rC_{10}	>100(n) >100 (n) Supported by analytical verification	Hoffmann, 2004c[9]
M320I029							

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute toxicity to fish OECD Guideline 203, GLP	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>100 (n) Supported by analytical verification	2004b[10]
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>100(n) Supported by analytical verification	Funk, 2004e[11]
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} E_rC_{10}	>100(n) 63 (n) Supported by analytical verification	Hoffmann, 2004d[12]

Notes:
mm refers to mean measured concentrations
n refers to nominal concentrations
*formerly *Selenastrum capricornutum*

References to Annex II

[1] (2004a) Reg. No. 121 464 (metabolite of BAS 320 I) - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a static system over 96 hours. BASF AG, Germany. Unpublished.

[2] Funk, M. (2004d) Effect of Reg. No. 121 464 (metabolite of BAS 320 I) on the immobility of *Daphnia magna* STRAUSS in a 48 hours static, acute toxicity test. BASF AG, Germany. Unpublished.

[3] Hoffmann, F. (2004b) Effect of Reg. No. 121 464 (M320I06) on the growth of the green alga *Pseudokirchneriella subcapitata*. BASF AG, Germany. Unpublished.

[4] (2005) Reg. No. 4096485 (metabolite of BAS 320 I) - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a semi-static system over 96 hours. BASF AG, Germany. Unpublished.

[5] Funk, M. (2004a) Effect of Reg. No. 4096485 (metabolite of BAS 320 I) on the immobility of *Daphnia magna* STRAUS in a 48 hours static, acute toxicity test. BASF AG, Germany, Unpublished.

[6] Hoffmann, F. (2004a) Effect of Reg. No. 409 6485 (M320I04, metabolite of BAS 320 I) on the growth of the green alga *Pseudokirchneriella subcapitata*. BASF AG, Germany. Unpublished.

[7] (2004c) Reg. No. 4984051 (metabolite of BAS 320 I) - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a static system over 96 hours. BASF AG, Germany. Unpublished.

[8] Funk, M. (2004c) Effect of Reg. No. 4984051 (metabolite of BAS 320 I) on the immobility of *Daphnia magna* STRAUSS in a 48 hours static, acute toxicity test. BASF AG, Germany. Unpublished.

[9] Hoffmann, F. (2004c) Effect of Reg. No. 498 4051 (M320I123) on the growth of the green alga *Pseudokirchneriella subcapitata*. BASF AG, Germany. Unpublished.

[10] (2004b) Reg. No. 43455 (metabolite of BAS 320 I) - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a static system over 96 hours BASF AG, Germany. Unpublished.

[11] Funk, M. (2004e) Effect of Reg. No. 43455 (metabolite of BAS 320 I) on the immobility of *Daphnia magna* STRAUSS in a 48 hours static, acute toxicity test. BASF AG, Germany, Unpublished.

[12] Hoffmann, F. (2004d) Effect of Reg. No. 43 455 (M320I29) on the growth of the green alga *Pseudokirchneriella subcapitata*. BASF AG, Germany. Unpublished.