Committee for Risk Assessment

RAC

Annex 1

Background document

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

Triflumizole

EC number: -

CAS number: 946578-00-3

CLH-O-0000001412-86-40/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

04 December 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

Triflumizole (ISO); (1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1H-imidazol-1-yl)-2-propoxyethanimine

EC Number: not available

CAS Number: 68694-11-1

Index Number: not available

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Version number: 3 **Date:** August 2013

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Triflumizole
EC number:	Not available
CAS number:	68694-11-1
Annex VI Index number:	Not available
Degree of purity:	≥ 98%
Impurities:	Toluene ≤ 0.1%

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	None	None
Current proposal for consideration by RAC	Acute Tox. 4, H302 Skin Sens. 1, H317 STOT RE Cat. 2 (H373) Repr. 1B; H360D Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Acute M-factor of 1 Chronic M-factor of 1	Repr. Cat. 2; R61 Xn; R22, R43 N; R50/53 SCL: N; R50-53: C ≥ 25 % N; R51-53: 25 % > C ≥ 2.5% R52-53: 2,5 % > C ≥ 0.25%
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4, H302 Skin Sens. 1, H317 STOT RE Cat. 2 (H373) Repr. 1B; H360D Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Acute M-factor of 1 Chronic M-factor of 1	Repr. Cat. 2; R61 Xn; R22, R43 N; R50/53 SCL: N; R50-53: C ≥ 25 % N; R51-53: 25 % > C ≥ 2.5% R52-53: 2,5 % > C ≥ 0.25%

As triflumizole is a plant protection product that is proposed for harmonized classification for the first time, RAC is requested also to assess the correctness of this proposal that no classification is needed for all other hazard classes.

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

According to the data presented in the DAR, the lowest LD_{50} value found was 1057 mg/kg bw (via oral route in the rat) and the substance was found to be a skin sensitiser. In accordance with the criteria of the CLP regulation, triflumizole should be classified as Acute Tox 4 (H302) and Skin Sens 1 (H317). Classification with STOT RE Cat. 2 (H373) is based on liver toxicity at oral dose levels below the relevant guidance values. Classification with Repr 1B; H360D and Repr. Cat2; R61 is based on the increase in post implantation loss in the developmental study in rats.

Triflumizole is classified as Aquatic Acute 1 and Aquatic Chronic 1. A harmonized M-factor (both acute and chronic in accordance with the 2nd ATP criteria) and SCLs for triflumizole are proposed.

RAC general comment
Triflumizole is an active substance in pesticidal products, and has not previously been assessed for harmonised classification by the Risk Assessment Committee (RAC). The conclusions on the peer review of pesticide risk assessment of triflumizole were published in the EFSA journal (7(12):1415, 2009).

Table 3: Proposed classification according to the CLP Regulation

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	None	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	None	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	None	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	None	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	None	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	None	Not classified	conclusive but not sufficient for

					classification
2.15.	Organic peroxides	Not classified	None	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	None	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 4, (H302)	None	Not classified	
	Acute toxicity - dermal	Not classified	None	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	None	Not classified	data lacking
3.4.	Skin sensitisation	Skin Sens. 1, H317	None	Not classified	
3.5.	Germ cell mutagenicity	Not classified	None	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	None	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Repr. 1B; H360D	None	Not classified	
3.8.	Specific target organ toxicity –single exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE Cat. 2 (H373)	None	Not classified	
3.10.	Aspiration hazard	Not classified	None	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Acute M-factor 1 Chronic M-factor 1	Not classified	

5.1.	Hazardous to the ozone layer	Not classified	None	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:

Signal word:	Danger
Pictogram:	GHS07, GHS08, GHS09
Hazard statements:	H302, Harmful if swallowed H317, May cause an allergic skin reaction H360D, May damage the unborn child H373, May cause damage to the liver through prolonged or repeated exposure H410, Very toxic to aquatic life with long lasting effects
Precautionary statements:	No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

A note is not proposed.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	None	Not classified	Conclusive but not sufficient for classification
Oxidising properties	Not classified	None	Not classified	Conclusive but not sufficient for classification
Flammability	Not classified	None	Not classified	Conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	Not classified	None	Not classified	Conclusive but not sufficient for classification
Thermal stability	Not classified	None	Not classified	Conclusive but not sufficient for classification
Acute toxicity	Xn; R22	None	Not classified	
Acute toxicity – irreversible damage after single exposure	Not classified	None	Not classified	Conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	None	Not classified	Conclusive but not sufficient for classification
Sensitisation	R43	None	Not classified	
Carcinogenicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	None	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – development	R61	None	Not classified	
Toxicity to reproduction – breastfed babies. Effects on or via	Not classified	None	Not classified	Conclusive but not sufficient for classification

lactation				
Environment	N; R50/53	SCL: N; R50-53: C ≥ 25 % N; R51-53: 25 % > C ≥ 2.5% R52-53: 2,5 % > C ≥ 0.25%	Not classified	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger: T; N : Toxic; Dangerous for the environment

R-phrases:
- R22 : Harmful if swallowed
- R43 : My cause sensitising by skin contact
- R61 : May cause harm to the unborn child
- R50/53 : Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

S-phrases:
- S45 : In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
- S53 : Avoid exposure – Obtain special instructions before use.
- 60 : This material and its container must be disposed of as hazardous waste
- 61 : Avoid release to the environment. refer to special instructions/safety data sheets

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

According to the data presented in the DAR, the classification of triflumizole is: Xn; R22, 43, N; R50/53.

In a PRAPeR Expert Meeting TC 14 (26 June 2009) it was concluded that classification of Triflumizole (regarding human toxicity), as proposed in the DAR, did not change (Xn, R22, R43).

The conclusions on the peer review of pesticide risk assessment of triflumizole was published in the EFSA journal (7(12):1415, 2009). The classification was unchanged. The DAR can be requested via: http://dar.efsa.europa.eu/dar-web/provision. The final addendum is available via the EFSA website.

Triflumizole was added to Annex I of Directive 91/414/EEC (Council Directive 2010/27/EU of 23 April 2010) from 1 July 2010.

Triflumizole has not previously been assessed for harmonised classification by RAC or TC C&L.

The proposal for harmonised classification differs from the conclusions drawn by EFSA during their assessment.

2.2 Short summary of the scientific justification for the CLH proposal

In accordance with the criteria of the CLP regulation, triflumizole should be classified as Acute Tox 4 (H302) based on an oral LD50 of 1780 mg/kg bw and Skin Sensitiser 1B (H317) as more than 30% of the animals reacted positive in a guinea pig maximization test following 10% w/v intradermal induction. Classification with STOT RE Cat. 2 (H373) is based on liver toxicity at oral dose levels below or just above the relevant guidance values. Classification with Repr 1B; H360D and Repro Cat2; R61 is based on the increase in post implantation loss in the developmental study in rats in the presence of maternal toxicity.

Triflumizole is classified as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). In this dossier, a CLH proposal including harmonized M-factor (both acute and chronic according to the criteria of the 2nd ATP) and SCLs for triflumizole are proposed.

2.3 Current harmonised classification and labelling

Not applicable

2.3.1 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Not applicable

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)				
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng			23	
Aquatic Chronic 2	H411	H411						
		H400		GHS07 GHS09 Wng			4	
Acute Tox. 4	H302	H302						
Skin Sens. 1	H317	H317						
Aquatic Chronic 1	H410	H410						
Acute Tox. 4	H302	H302		GHS07 Wng			3	

2.4.2 (Date 5 July 2013)Current self-classification and labelling based on DSD criteria

Not applicable

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Triflumizole is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (CLP, article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

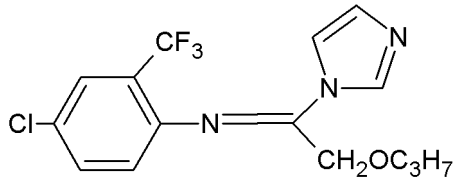
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	Not available
EC name:	Not available
CAS number (EC inventory):	68694-11-1
CAS number:	68694-11-1
CAS name:	1-[(1E)-1-[[4-chloro-2-(trifluoromethyl)phenyl]imino]-2-propoxyethyl]-1H-imidazole
IUPAC name:	(1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1H-imidazol-1-yl)-2-propoxyethanimine
ISO name	triflumizole
CLP Annex VI Index number:	Not applicable
Molecular formula:	$C_{15}H_{15}ClF_3N_3O$
Molecular weight range:	345.75 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Triflumizole	Minimum 980 g/kg	-	-

Current Annex VI entry: Not applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
toluene		≤ 0.1%	Based on the DAR there are no (eco)toxicological relevant impurities present.

Current Annex VI entry: Flam. Liquid 2, H225; Skin Irrit. 2, H315; Asp. Tox. 1, H304; STOT Single Exp. 3, H336; STOT RE. 2*, H373; Repr. 2, H361.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

1.3 Physico-chemical properties

The information provided in table 9 is based on triflumizole. The physico-chemical properties of triflumizole were assessed in the Draft Assessment Report prepared in the context of the possible inclusion of triflumizole in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005, volume 3, B2 and subsequent addendum February 2009, RMS the Netherlands)

Table 9: 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	White granulate material with a scentless odour (99.9%)	DAR	
Melting/freezing point	63ºC (99.3%)	DAR	Measured
Boiling point	Not applicable, decomposition before boiling	DAR	
Relative density	1.35 g/cm3 at 20 °C	DAR	Measured
Vapour pressure	1.91x10-4 Pa at 25ºC (99%)	DAR	Measured
Surface tension	49.4 mN/m at 20 °C (at 90% of the saturation concentration). The formulation is surface active.	DAR	Measured
Water solubility	In water and buffer solutions (pH 7 and 8): 10.5, 10.2 and 9.6 mg/L, respectively (20ºC) In water at 30ºC: 10.2 mg/L (99.9%) In buffer (pH 4): 21 mg/L (20ºC) (99.7%)	DAR	Measured
Partition coefficient n-octanol/water	Calculated from the measured solubilities: $LogP_{ow}$ pH 4= 4.46 $LogP_{ow}$ pH 7= 4.77 $LogP_{ow}$ pH 8= 4.80 The measured value of LogPow = 5.1 (pH 7) is in good agreement with the calculated value of logPow = 4.77.	DAR	Calculation: solubility in n-octanol / solubility water. Using the measured solubility of triflumizole in n-octanol and water at different pH's, the logPow is calculated as log(Soctanol/SwaterpH): $S_{water\text{-}pH\ 4}$ = 21 mg/L $S_{water\text{-}pH\ 7}$ = 10.2 mg/L $S_{water\text{-}pH\ 8}$ = 9.6 mg/L $S_{n\text{-}octanol}$ = 605 g/L
Flash point	Not required	DAR	
Flammability	Not highly flammable (99.2%)	DAR	Measured
Explosive properties	Not explosive (statement)	DAR	
Self-ignition temperature	Not self-ignitable	DAR	
Oxidising properties	Not oxidising (statement)	DAR	
Granulometry	No data	DAR	

Stability in organic solvents and identity of relevant degradation products	No data	DAR	
Dissociation constant	pKa= 3.7 at 25ºC	DAR	Measured
Viscosity	No data	DAR	
Henry's law constant	H = 6.29 x 10^{-3} Pa.m^3/mol at 25˚C Calculated using: Vapour pressure: 1.91 x10^{-4} Pa (25˚C) Water solubility: 0.0105 g/L (20 ˚C) Molar weight: 345.75 g/mol	DAR	Calculated by RMS No to GLP. It is considered acceptable to use the value for the water solubility at 20˚C to support a calculation of the Henry's constant at 25˚C (water solubility at 30˚C was 0.0102 g/L, which does not significantly differ from the solubility at 20˚C considering the error in the test method).

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier

2.2 Identified uses

Triflumizole is a fungicide with protective and curative action. It inhibits the biosynthesis of ergosterol by inhibiting the C14-demethylation in sterol. Ergosterol is considered to function as a stabilizer of the cell wall membranes of fungi.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of triflumizole were assessed in the Draft Assessment Report prepared in the context of the possible inclusion of triflumizole in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005, volume 3, B2 and subsequent addendum February 2009, RMS the Netherlands) concerning the placing of plant protection products on the market.

Triflumizole is not flammable and not self-ignitable and does not evolve flammable gasses in contact with water. The substance has no oxidizing or explosive properties. Therefore, no classification for physico-chemical properties is proposed.

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of triflumizole were assessed in the Draft Assessment Report and the Addendum to the Draft Assessment Report prepared in the context of the possible inclusion of triflumizole in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005, volume 3, B6 and subsequent addendum February 2009, RMS The Netherlands) concerning the placing of plant protection products on the market.

The summaries included in this proposal are copied from the DAR (and its addenda and assessment reports when these contain updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarized in a table. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda which are available at the EFSA website.

For several toxicological studies, the synonym NF-114 is used for triflumizole.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

See Section 4.1.3.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

ABSORPTION

In the rat, based on radiolabel recovered from urine, tissues and carcass 48 h after administration, the oral absorption of triflumizole ^{14}C-equivalents after single and repeated low dose (10 mg/kg bw) was at least 72%. The oral absorption after single high dose (300 mg/kg bw) was at least 79% after 96 h. The oral absorption after high dose was considerably slower than after low dose, as evidenced by the ca. 2x lower excretion 24 h after administration and the much higher t_{max}.

The percutaneous absorption of [^{14}C]-triflumizole was studied *in vitro*, using flow-through diffusion cells. Dermatomed skin from rats and humans were exposed to either 1.3 mg/cm^2 or 1.4 µg/cm^2. The test substance remained in contact with the skin for 8 hours. Samples of receptor fluid were taken at 0-1 h and 1-2 h, followed by 2-h intervals until 24 h after application. In human skin, the mean flux constants for the absorption of triflumizole were 0.186 µg/cm^2/h (high dose) and 0.002 µg/cm^2/h (low dose). In rat skin, the mean flux constants for the absorption of triflumizole formulated as Rocket EC were 2.343 µg/cm^2/h (high dose) and 0.005 µg/cm^2/h (low dose).

Total mean recovery of the high dose and low dose was 92.2 and 94.1% for human skin and 89.1 and 91.7% for rat skin. At the high dose 0.34% AR had penetrated through the human skin at the 24 hour time point. Rat skin exposed under the same conditions was more permeable, as 5.91% AR penetrated within 24 hours. At the low dose 3.37% and 9.01% of the applied dose penetrated within 24 hours through human and rat skin, respectively. At 24 hours still increasing levels of radiolabel were recovered from the receptor fluid. Therefore, radiolabel from the skin membranes should be included into the potentially absorbed amount. Furthermore, it is not clear whether the amount in the tape strips could be absorbed. During EPCO 23 in May 2005 it was decided to regard the amount of AR in tape strips 1 and 2 as exfoliated and to regard the amount of AR in the following tape strips as potentially absorbed. This results in a mean total absorption of 5 and 20% through human skin after exposure to 1.3 mg/cm^2 or 1.4 µg/cm^2, respectively. The mean total absorption through rat skin is 25 and 27% after exposure to 1.3 mg/cm^2 or 1.4 µg/cm^2, respectively.

EXCRETION

In the rat, excretion of triflumizole ^{14}C-equivalents after oral low dose (10 mg/kg bw), both single and repeated, was relatively fast: ca. 90% of the administered radiolabel was excreted in the first 24 h. This is also reflected in the plasma terminal half-life of ca. 14 h after single oral low dose. Two days after administration of the oral low dose, ca. 95% of the radiolabel had been excreted. Excretion after single oral high dose was considerably slower: only 45-35% (m-f) of the administered dose had been excreted after the first 24 h. Four days after administration of single high dose 99-92% was excreted. Irrespective of dose regimen or sex, most radiolabel was excreted via urine: after sacrifice ca. 75% of the administered dose was recovered from urine, while ca. 20% was found in faeces.

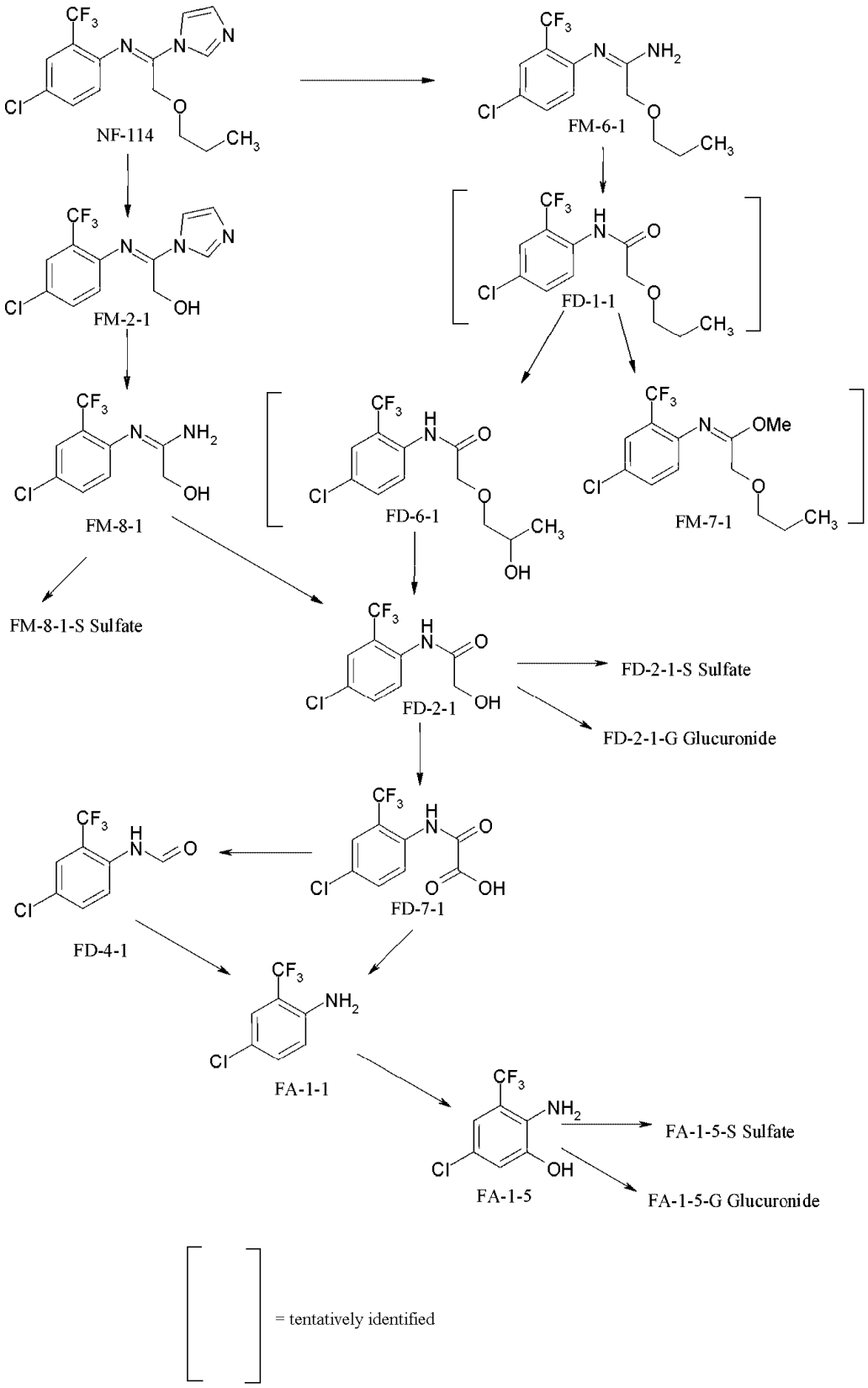
DISTRIBUTION

In the rat, irrespective of dose regimen or sex, ca. 2% of the triflumizole radiolabel was retained in tissues and carcass 48 (low dose) or 96 h (high dose) after administration. In all instances, the liver retained the highest concentration of radiolabel (ca. 1 mg eq./kg after low dose, 14.5-8.5 mg eq./kg after high dose (m-f)). All other tissues and organs retained approximately half this concentration or less, well perfused tissues tending to have higher concentrations. Fat was among the tissues retaining the lowest concentration of radiolabel: approximately one tenth of the liver concentration. It should be noted that the brain retained relatively high concentrations (one third to half the liver concentration). This was also the case with the thyroid, however this organ was only measured in the single low dose group.

METABOLISM

In the rat, irrespective of dose regimen or sex, triflumizole is extensively metabolised: less than 2% of the radiolabel recovered from urine or faeces was identified as parent compound. Repeated oral (low) dosing may induce some enzymes involved in triflumizole metabolism. There are indications of saturation of (some) metabolic pathways after oral high dose. However, still less than 1% of the radiolabel recovered represented parent compound. A few differences in metabolite pattern were observed between males and females after repeated low and single high dose, but not after single low dose. The major urinary metabolites are the sulphate conjugates of N-(4-chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamidine and 2-amino-5-chloro-3-trifluoromethylphenol. In faeces, N-(4-chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide is a major metabolite in all dose regimens (ca. 6-10% of the recovered radiolabel). Considerable quantitative differences between dose regimens exist with respect to other major faecal metabolites (2-(4-chloro-2-trifluoromethylphenylimino)-2-imidazol-1-ylethanol, 4-chloro-2-trifluoromethylphenylamine and N-(4-chloro-2-trifluoromethylphenyl)-2-propoxyacetamide). In total 16 metabolites were identified in urine and faeces, representing 60-75% of the administered radiolabel.

Proposed metabolic pathway



RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

The physico-chemical properties of triflumizole were assessed in the Draft Assessment Report (DAR) prepared in the context of the possible inclusion of triflumizole in Annex I of Council Directive 91/414/EEC (December 2005, volume 3, B2 and subsequent addendum February 2009, RMS the Netherlands). The information on physico-chemical properties of triflumizole were provided by the dossier submitter (DS) in the CLH report.

Triflumizole is not flammable and not self-ignitable and does not evolve flammable gases in contact with water. The substance has no oxidising or explosive properties. Therefore, no classification for physical hazards was proposed by the DS.

Comments received during public consultation

No comments were received for any of the physical hazard classes during public consultation.

Assessment and comparison with the classification criteria

Triflumizole does not meet the classification criteria for physical hazards according to CLP. There were no comments during public consultation. RAC supports the proposal of the DS not to classify triflumizole for physical hazards.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Study 1

reference	:	Nishibe *et al.*, 1983a	exposure	:	once by gavage
type of study	:	acute oral toxicity study	doses	:	Male : 395, 593, 889, 1333 and 2000 mg/kg bw Female : 592, 888, 1333, 2000, 3000 and 4500 mg/kg bw
year of execution	:	1979	vehicle	:	1% CMC (carboxymethylcellulose)
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 401
species	:	rat, Wistar -SLC	acceptability	:	acceptable
group size	:	10/sex/dose	**LD_{50}**	:	1057 mg/kg bw

The test substance in 1% carboxymethylcellulose was administered to groups of 10 male and 10 female Wistar rats at dose levels 395, 593, 889, 1333 and 2000 mg/kg bw (males) and 592, 888, 1333, 2000, 3000 and 4500 mg/kg bw (females) by gavage. The study was performed in accordance with OECD Guideline 401. The mortality was 4/10, 8/10, 9/10 in males and 1/10, 2/10, 8/10, 8/10, 10/10 in females in the highest dose groups Symptoms of toxicity included ataxia, hypotonia, ventral position, lacrimation, incontinence of urine, decreased body temperature, decreased heart rate and respiration rate and ptosis. Body weight decreased in the high dose groups on the first day, though they had recovered by the second day after dosing. haemorrhages of intestine mucosa,

thymus and stomach mucosa, and dark reddish lung were observed in dead rats. However, no gross pathological change was observed in the rats surviving for 14 days. The acute oral LD50 of the test substance was calculated to be 1057 mg/kg bw for male rats, and 1780 mg/kg bw for female rats.

Study 2

reference	:	Nishibe *et al.*, 1983b	exposure	:	once by gavage
type of study	:	acute oral toxicity study	doses	:	Male : 104, 156, 234, 351, 527, 790, 1185, 1778, 2667, 4000 and 6000 mg/kg bw Female : 156, 234, 351, 527, 790, 1185, 1778, 2667, 4000 and 6000 mg/kg bw
year of execution	:	1979	vehicle	:	1% CMC (carboxymethylcellulose)
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 401
species	:	mice, ICR-CRJ	acceptability	:	acceptable
group size	:	10/sex/dose	**LD_{50}**	:	2000 mg/kg bw

In the study with ICR-CRJ mice according to OECD Guideline 4011, the test substance in 1% carboxymethylcellulose was administered at dose levels of 104, 156, 234, 351, 527, 790, 1185, 1778, 2667, 4000 and 6000 mg/kg bw (males) and 156, 234, 351, 527, 790, 1185, 1778, 2667, 4000 and 6000 mg/kg bw (females) to groups of 10 animals/sex/dose. The mortality was 1/10, 5/10, 9/10, 9/10, 10/10 in males and 1/10, 3/10, 8/10, 10/10 in females in the highest dose groups. Symptoms of toxicity included ataxia, hypotonia, lacrimation, decreased body temperature, decreased heart rate and respiration rates. Body weight decreased in the high dose groups on the first day, though they had recovered by the second day after dosing. Haemorrhages of intestine mucosa, and stomach mucosa, and dark reddish lung and gas in digestive tracts were observed in dead mice. However, no gross pathological change was observed in the mice surviving for 14 days. The acute oral LD50 of the test substance was calculated to be 2000 mg/kg bw for male mice and 2800 mg/kg bw for female mice.

4.2.1.2 Acute toxicity: inhalation

Study 1

reference	:	Nishibe *et al.*, 1983d	exposure	:	4 hours (whole body)
type of study	:	acute inhalation toxicity study	concentration (actual)	:	0 and 3.2 mg/L, GMD[1] (±gsd): 5.8 μm (± 2.7)
year of execution	:	1981	vehicle	:	air
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	inhalation	guideline	:	not in accordance with OECD 403
species	:	rat, SLC:SD	acceptability	:	not acceptable
group size	:	10/sex/concentration	**LC_{50}**	:	n/a

[1] THE AUTHORS OF THE REPORT CALCULATED THE GEOMETRIC MEAN DIAMETER (GMD) IN STEAD OF THE MMAD.

The study was performed in accordance with OECD guideline 403. One group (5/sex) of Wistar Crl:WI rats was exposed to an atmosphere containing the test material for 4 h at a mean actual concentration of 3.6 mg/L (maximum attainable exposure concentration) in a dynamic flow inhalation apparatus (nose only). The nominal exposure concentration was 80.4 mg/L. The test substance was administered to the animals in an exposure chamber as a dust generated from triflumizole. The mean mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined twice. The MMAD was 3.6 and 3.8 μm respectively and the GSD was 1.9 and 2.0 respectively.
The post-exposure observation period was 14 days. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice.

No mortalities occurred. Hunched posture, lethargy, chromodacryorrhoea (head and/or snout) were noted among the majority of the animals between day 1 and day 4. Rales in one animal at day 1 and periorbital alopecia in an other animal were observed between days 4 and 13. The body weight gain shown by the animals over the study period was considered to be normal. Macroscopic post mortem examination of the animals did not reveal abnormalities.
The acute 4-hour inhalatory LC50 in rats is >3.6 mg/L (maximum attainable exposure concentration).

4.2.1.3 Acute toxicity: dermal

Study 1

reference	:	Nishibe *et al.*, 1983c	exposure	:	24 hours (occlusive)
type of study	:	acute dermal toxicity study	doses	:	2000 and 5000mg/kg bw
year of execution	:	1982	vehicle	:	Physiological saline (moistened)
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	dermal	guideline	:	in accordance with OECD 402
species	:	rat, SLC:SD	acceptability	:	acceptable
group size	:	10/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw

In the OECD Guideline 402 acute dermal toxicity study with SLC:SD rats, the test substance moistened with physiological saline was administered to groups of 10 animals/sex/dose at dose levels of 2000 and 5000 mg/kg bw/day under occlusive dressing for 24 hours. There were no mortalities. No toxic signs were observed in male rats. Incontinence of urine was observed only in female rats on the 2nd and 3rd days. Body weights decreased in all groups at day 1, but recovered thereafter. No treatment-related findings were noted at pathology. The acute dermal LD50 of the test substance was found to be > 5000 mg/kg bw for male and females rats.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

The results of the acute toxicity studies relevant for the classification update are summarized in Table.10. Only reliable and validated acute toxicity tests accepted for risk assessment from Draft Assessment Reports are shown in this table.

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral toxicity			

OECD 401 OECD 401	LD_{50}: 1057 mg/kg bw (males) LD_{50}: 1780 mg/kg bw (females) LD_{50}: 2000 mg/kg bw	Rat, Wistar SLC Mice, ICR-CRJ	Nishibe et al., 1983a[a] Nishibe et al., 1983b [a]
Dermal toxicity			
OECD 402	LD_{50}: > 5000 mg/kg bw	Rat, SLC:SD	Nishibe et al., 1983c [a]
Inhalation toxicity			
OECD 403	LC_{50}: >3.6 mg/L Maximal achievable exposure concentration (aerosol)	Rat, Wistar Crl:WI	Nishibe et al., 1983D [a]

[a]As summarized in the DAR, updated addendum of February 2009

4.2.4 Comparison with criteria

CLP

According to the CLP triflumizole should be classified as Acute Tox. 4 because the LD_{50} of 1057 mg/kg bw in male rats is within the limits, 300 < ATE ≤ 2000 (oral, mg/kg bw).

Based on LD50 > 5000 mg/kg bw/day triflumizole does not need to be classified for acute dermal toxicity.

The acute 4-hour inhalatory LC50 in rats is >3.6 mg/L (maximum attainable exposure concentration). Triflumizole does not need to be classified for acute inhalation toxicity, as no mortalities occurred at the highest achievable concentration.

67/548/EEC

According to 67/548/EEC triflumizole should be classified as Xn;R22, because the LD_{50} of 1057 mg/kg bw in male rats is within the limits, 200 < LD_{50} ≤ 2000 mg/kg.

Based on LD50 > 5000 mg/kg bw/day triflumizole does not need to be classified for acute dermal toxicity.

The acute 4-hour inhalatory LC50 in rats is >3.6 mg/L (maximum attainable exposure concentration). Triflumizole does not need to be classified for acute inhalation toxicity, as no mortalities occurred at the highest achievable concentration.

4.2.5 Conclusions on classification and labelling

Table 11 Conclusion on classification for acute toxicity

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised	Acute Tox. 4 (H302)	Xn; R22

classification (future entry in Annex VI, CLP Regulation)		

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS gave an overview on toxicokinetic data and summarised the results from available acute toxicity studies.

According to the CLP criteria triflumizole should be classified for acute oral toxicity category 4, because the LD_{50} was 1057 mg/kg bw in male rats (Nishibe *et al.*, 1983a), which was within the limits of 300 < ATE ≤ 2000 (oral, mg/kg bw).

Based on a dermal LD_{50} > 5000 mg/kg bw in rats (Nishibe *et al.*, 1983c), triflumizole does not meet the classification criteria for acute dermal toxicity.

The acute 4-hour inhalatory LC_{50} in rats was >3.6 mg/L (maximum attainable exposure concentration; Nishibe *et al.*, 1983d). Triflumizole does not meet the classification criteria for acute inhalation toxicity, as no mortalities occurred at the highest achievable concentration.

Comments received during public consultation

Four member states (MS) supported the proposed classification for acute oral toxicity of triflumizole.

Assessment and comparison with the classification criteria

Comparison with the criteria

Oral

The acute oral toxicity of triflumizole was tested in both rats and mice, according to OECD TG 401. The acute oral LD_{50} of triflumizole for male rats was 1057 mg/kg bw and for female rats 1780 mg/kg bw (Nishibe *et al.*, 1983a). The acute oral LD_{50} of triflumizole for male mice was 2000 mg/kg bw and for female mice 2800 mg/kg (Nishibe *et al.*, 1983b).

Taking into account that the oral LD_{50} in male and female rats were within the limits of 300 < ATE ≤ 2000 mg/kg, triflumizole should be classified as Acute Tox. 4; H302 according to the CLP criteria.

Dermal

Triflumizole was tested for acute dermal toxicity in rats, in a study performed according to OECD TG 402, at doses of 2000 and 5000 mg/kg bw (Nishibe *et al.*, 1983c). No mortalities were observed.

Based on an LD_{50} >5000 mg/kg bw triflumizole should not be classified for acute dermal toxicity since the dermal LD_{50} is above 2000 mg/kg bw, the upper limit for classification by the dermal route.

Inhalation

In an Addendum to the DAR, it is stated that an acute inhalation toxicity study with

triflumizole was presented, but this study was not acceptable. Instead a new study was conducted (Janssen, 2005; in rats, OECD TG 403). In this new, reliable inhalation study in rats, the highest attainable exposure concentration was 3.6 mg/L/4h (the same as in Nishibe *et al.*, 1983d) and this concentration did not result in lethality. Based on this triflumizole does not need to be classified for acute toxicity via the inhalation route.

Overall

Triflumizole does not meet the CLP classification criteria for acute dermal or acute inhalation toxicity. It does, however, meet the classification criteria for acute oral toxicity and RAC agreed that triflumizole should be classified as Acute Tox. 4: H302 (Harmful if swallowed).

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

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

In the available acute toxicity studies according to OECD Guidelines 401, 402 and 403 no evidence of specific target organ toxicity was noted (see Section 4.2.1). In the acute neurotoxicity study with rats, described in Section 4.12.1, no specific neurotoxic effects of the test substance were observed.

4.3.2 Comparison with criteria

As no evidence of specific target organ toxicity (including neurotoxicity and respiratory tract irritation) was observed in the available acute toxicity studies, no classification of the substance for STOT-SE is warranted in accordance with CLP criteria.

4.3.3 Conclusions on classification and labelling

Table 12 Conclusion on classification for Specific target organ toxicity – single exposure

	CLP Regulation
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Not classified

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

Summary of the Dossier submitter's proposal

In the available acute toxicity studies conducted according to OECD TG 401, 402 and 403, no evidence of specific target organ toxicity was noted. In the acute neurotoxicity study with rats, no specific neurotoxic effects of the test substance were observed.

Comments received during public consultation

No comments were received for this hazard class during public consultation.

Assessment and comparison with the classification criteria

As no evidence of specific target organ toxicity after single exposure was observed (including neurotoxicity and respiratory tract irritation) in the available acute toxicity studies, no classification for STOT SE is warranted in accordance with CLP criteria.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404, The study was considered acceptable.	Not irritating to skin		Nishibe et al., 1983e[a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.4.1.1 Non-human information

Study 1

reference	:	Nishibe *et al.*, 1983e	exposure	:	24 hours (occlusive)
type of study	:	skin irritation study	doses	:	0.5 g
year of execution	:	1979	vehicle	:	moistened with water
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	dermal	guideline	:	in accordance with OECD 404
species	:	rabbit, Angola	acceptability	:	acceptable
group size	:	6 males	**Effect**	:	not irritating to skin

The skin irritation study with 6 male Angola rabbits was performed in accordance with OECD 404, except for the fact that the surface area was 9 cm^2 instead of 6 cm^2. In addition to application on intact skin the test substance was also applied to abraded skin. 0.5 g of the test substance moistened with water was applied for 24 hours under occlusive conditions. The erythema and edema scores were zero in all animals at all observation time points. The substance was considered not irritating to skin.

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

Triflumizole is not irritating to skin in a study in animals. No data on skin irritation due to exposure of humans to triflumizole were available.

4.4.1.4 Comparison with criteria

No skin irritation occurs following exposure to triflumizole at the limit values for classification set by 67/548/EEC or EC 1272/2008. No data on skin irritation due to exposure of humans to triflumizole were available.

4.4.1.5 Conclusions on classification and labelling

Triflumizole is considered not irritating to skin according to the criteria mentioned in 67/548/EEC or EC 1272/2008. Triflumizole does not need to be classified for skin irritation.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Triflumizole was not corrosive or irritating to skin in a study conducted according to OECD TG 404 in Angola rabbits (6 males, dose of 0.5 g, 24 h occlusive exposure, non-GLP; Nishibe *et al.*, 1983e). No data on skin corrosion/irritation after exposure of humans to triflumizole were available.

Comments received during public consultation

No comments were received for this hazard class during public consultation.

Assessment and comparison with the classification criteria

No skin oedema or erythema were seen in any of 6 rabbits following exposure to triflumizole in an OECD TG 404 study (Nishibe *et al.*, 1983e), and therefore, triflumizole is not considered irritating nor corrosive to skin and therefore does not meet the classification criteria for skin corrosion/irritation.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405, The study was considered acceptable.	Not irritating to eyes		Nishibe et al., 1983f[a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.4.2.1 Non-human information

Study 1

reference	:	Nishibe *et al.*, 1983f	exposure	:	single instillation in conjunctival sac
type of study	:	eye irritation study	doses	:	0.1 g (ground into fine powder)
year of execution	:	1979	vehicle	:	none
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	ocular	guideline	:	in accordance with OECD 405 (1987)
species	:	rabbit, Japanese white	acceptability	:	acceptable
group size	:	9 males	**Effect**	:	not irritating to eyes

The study was performed partly in accordance with OECD 405 (1987). 0.1 g of the test substance grounded to a fine powder were instilled into a conjunctival sac of 9 male Japanese White rabbits. The treated eyes of 6 rabbits remained unwashed and the treated eyes of the three other animals were flushed for one minute with lukewarm water starting 20-30 seconds after application. No irritation scores 1 hour after application were reported. The mean cornea, iris and chemosis scores in the unwashed eyes were 0 at 24, 48 and 72 hours. Redness of conjunctiva (average score 1.33) was observed after 24 hours in 5 out of 6 animals (unwashed eyes). After 48 hours mild redness of conjunctiva (score 1) was observed in only 2 animals (unwashed eyes); all scores were 0 at 72 hours. Based on the results of the study, the substance was considered to be not irritating to rabbit eyes.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

Triflumizole is not an eye irritant in a study in animals. No data on eye irritation due to exposure of humans to triflumizole were available.

4.4.2.4 Comparison with criteria

No eye irritation occurs following exposure to triflumizole at the limit values for classification set by 67/548/EEC or EC 1272/2008. No data on eye irritation due to exposure of humans to triflumizole were available.

4.4.2.5 Conclusions on classification and labelling

Triflumizole does not need to be classified for eye irritation according to 67/548/EEC or EC 1272/2008.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Triflumizole was not irritating to eyes in a study conducted according to OECD TG 405 in Japanese white rabbits (9 males, dose of 0.1 g, single instillation in conjunctival sac, non-GLP; Nishibe *et al.*, 1983f). No human data on eye damage/irritation after triflumizole exposure were available.

Comments received during public consultation

No comments were received for this hazard class during public consultation.

Assessment and comparison with the classification criteria

No human data on eye damage/irritation after triflumizole exposure were available.

In a rabbit study (Nishibe *et al.*, 1983f), the mean cornea, iris and chemosis scores in the unwashed eyes of 6 rabbits in a study performed in accordance with OECD TG 405 were 0 at 24, 48 and 72 hours. Redness of the conjunctiva (average score 1.33) was observed

after 24 h in 5 out of 6 animals (unwashed eyes). After 48 hours, mild redness of the conjunctiva (score 1) was observed in only 2 animals (unwashed eyes); all scores were 0 at 72 hours.

Since scores for inflammatory changes in the eyes of rabbits were below the scores defined in the CLP classification criteria, the substance is considered to be not irritating to rabbit eyes and does not meet the classification criteria for serious eye damage/eye irritation.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In the available acute inhalation toxicity study, no signs of respiratory tract irritation were noted (see Section 4.2.1)

4.4.3.2 Human information

The results of its yearly health examination of the personnel involved in the production of triflumizole at the Takaoka plant (Japan) in the period May 1996 to May 2002, performed as a consequence of the Japanese “Occupational Safety and Health Law” (Takami, 2002) , revealed no adverse health effects attributable to chemical exposure. Commercial production of triflumizole started in 1985 at this plant. The health examination consisted of physical examination, haematology, urinalysis and blood chemistry. In addition Takami (2002) reported that in the period covered no events of acute poisoning by exposure, nor skin and/or eye irritation were observed.

4.4.3.3 Summary and discussion of respiratory tract irritation

No signs of respiratory tract irritation were noted either in experimental animals in the acute inhalation toxicity studies, or in humans employed at the plant manufacturing triflumizole.

4.4.3.4 Comparison with criteria

Based on the lack of respiratory tract irritation signs in the available studies, classification of the substance is not warranted according to 67/548/EEC.

4.4.3.5 Conclusions on classification and labelling

Table 15 Conclusion on classification for Specific target organ toxicity – single exposure

	Directive 67/548/EEC (DSD)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Not classified

RAC evaluation of respiratory tract irritation
Summary of the Dossier submitter's proposal There were no signs of acute inhalation toxicity seen in the OECD TG 403 study in rats (Nishibe *et al.*, 1983d). There were no complaints of respiratory tract irritation from humans employed at a plant manufacturing triflumizole (Takami, 2002). **Comments received during public consultation** No comments were received for this hazard class during public consultation. **Assessment and comparison with the classification criteria** Based on the lack of respiratory tract irritation signs in the new, reliable study by Janssen (2005; OECD TG 403; Addendum to DAR), classification for respiratory track irritation of triflumizole is not warranted.

4.5 Corrosivity

Table 16: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
OECD 404, The study was considered acceptable.	Negative		Nishibe et al., 1983e[a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.5.1 Non-human information

In studies in animals no skin corrosion was observed after exposure to triflumizole. See 4.4.1.1 for the study summary.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

There are no indications that triflumizole has corrosive properties.

4.5.4 Comparison with criteria

There are no indications that triflumizole has corrosive properties.

4.5.5 Conclusions on classification and labelling

It is not necessary to classify triflumizole for corrosive effects according to 67/548/EEC or EC 1272/2008.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406, The study is considered acceptable.	Sensitising to skin	Maximisation test	Nishibe et al., 1983g[a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.6.1.1 Non-human information

Study 1

reference	:	Nishibe *et al.*, 1983g	exposure	:	intradermal and topical induction, topical challenge (occlusive, 48h)
type of study	:	skin sensitisation study (Maximisation test)	doses	:	10% w/v intradermal induction; 25% (w/w)% in vaseline topical induction and 25% w/w challenge
year of execution	:	1980	vehicle	:	olive oil (intradermal) and vaseline (topical induction)
test substance	:	NF-114, lot no. YS-0155, purity 98.2%, white crystal	GLP statement	:	no
route	:	dermal	guideline	:	in accordance with OECD 406
species	:	guinea pig, Hartley	acceptability	:	acceptable
group size	:	12 controls, 12 test animals (females only)	**Effect**	:	sensitising to skin

A skin sensitization study was performed in accordance with OECD 406 (maximization test). The following doses were used: 10% w/v in olive oil for intradermal induction; 25% (w/w)% in vaseline for topical induction and 25% w/w challenge. Groups of 12 female Hartley guinea pigs (one test and one control group) were used. The challenge exposure was 48 hours under occlusive conditions. No information was provided with regard to the study on which the dose selection was based. One week after the injections the skin was pretreated with 10% sodium lauryl sulphate (SLS) in white vaseline 24 hours before the topical application.
Triflumizole is sensitising to the skin of guinea pigs in a Maximisation study. Following challenge with 25% w/w, dermal responses were observed in 8 of the 12 test animals. Control animals showed no skin reactions.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

Triflumizole was sensitising in the Guinea pig maximisation test. Eight out of 12 animals showed a dermal response after challenged versus none in the controls.

4.6.1.4 Comparison with criteria

CLP

In the CLP Regulation, a substance should be classified as a skin sensitiser (category 1B, H317) when a positive response in a GPMT test (in ≥30% of the animals at >1% intradermal induction dose or ≥30% to <60% of the animals at >0.1% to ≤1% intradermal induction dose) is observed. This criterion is fulfilled (66% positive at 10% induction dose). Subcategory 1A is required when ≥ 30% of the animals react positive at <0.1% intradermal induction dose, or ≥ 60% of the animals react positive at <0.1% to ≤1% intradermal induction dose. However, as no information is available after intradermal induction at ≤1%, it cannot be fully excluded that triflumizole will not require subclassification in 1A. This could be considered as data not sufficient for sub-categorisation as in paragraph 3.4.2.2.1.1 of CLP and therefore category 1 without subclassification is proposed for triflumizole.

67/548/EEC

According to 67/548/EEC triflumizole should be classified as Xi;R43 (May cause sensitisation by skin contact), because of a positive Maximisation test as more than 30% of the animals reacted. No SCL is required.

4.6.1.5 Conclusions on classification and labelling

Table 18 Conclusion on classification for sensitisation

	CLP Regulation	Directive 67/548/EEC (DSD)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin sens. 1 (H317)	Xi; R43

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Based on a positive Guinea Pig Maximisation Test (GPMT), the DS proposed to classify triflumizole as Skin Sens. 1: H317.

Comments received during public consultation

Four MS supported the proposed classification of triflumizole for skin sensitisation.

Assessment and comparison with the classification criteria

In the skin sensitization study (Nishibe *et al.,* 1983g) performed in accordance with OECD TG 406 (GPMT), dermal responses were observed in 8 out of the 12 test animals (66.6%) after a challenge with 25% (w/w) triflumizole. Control animals showed no skin reactions. The intradermal induction concentration was 10%, and the topical induction concentration was 25%.

According to the CLP Regulation, a substance should be classified as a skin sensitizer in category 1B (H317) when in a GPMT test ≥30% of the animals respond at >1% intradermal induction dose. This criterion is fulfilled for triflumizole (66% positive at 10% induction dose).

To fulfil the category 1A criteria, ≥ 30% of the animals should have a positive reaction at a <0.1% intradermal induction dose, or ≥ 60% of the animals at a <0.1% to ≤1% intradermal induction dose. Taking into account that no information is available on frequency of dermal responses after intradermal induction by triflumizole at ≤1%, it cannot be excluded that triflumizole would require sub-categorisation in category 1A. As proposed by the DS it could be considered that the available data is not sufficient for sub-categorisation (as stipulated in Annex I, 3.4.2.2.1.1, CLP) and therefore category 1 without subcatergorisation is proposed for triflumizole.

Taking into account the above data and considerations, RAC is of the opinion that triflumizole should be classified as Skin Sens. 1; H317 (May cause an allergic skin reaction).

4.6.2 Respiratory sensitisation

Table 19: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data			DAR

4.6.2.1 Non-human information

No data that indicate that triflumizole cause respiratory sensitization in animals were found.

4.6.2.2 Human information

No data that indicate that triflumizole cause respiratory sensitization in humans were found.

4.6.2.3 Summary and discussion of respiratory sensitisation

There is no indication that triflumizole cause respiratory sensitization.

4.6.2.4 Comparison with criteria

There is no indication that triflumizole cause respiratory sensitization.

4.6.2.5 Conclusions on classification and labelling

It is not necessary to classify triflumizole for respiratory sensitization according to EC 1272/2008 and 67/548 based on insufficient data.

RAC evaluation of respiratory sensitisation
Summary of the Dossier submitter's proposal There is no indication that triflumizole causes respiratory sensitization. **Comments received during public consultation** No comments were received for this hazard class during public consultation. **Assessment and comparison with the classification criteria** Based on the lack of respiratory sensitisation data, classification of the substance is not warranted.

4.7 Repeated dose toxicity

Table 20: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
28-days oral study in rat	NOAEL is 2.3 mg/kg bw/day	increased ovary weights	Nishibe et al., 1980a[a]
28-days oral study in mouse	NOAEL is 40 mg/kg bw/day	reduced spleen weight, increased liver and heart weights, reduction in body weight gain	Nishibe et al., 1980b [a]
21-days dermal study in rat	NOAEL is 100 mg/kg bw/day	liver and blood effects	Goldenthal, 1980 [a]
90-days oral study in rat	NOAEL is 15 mg/kg bw/day	decreased body weight combined with increased food consumption, liver effects, increased kidney weights, decreased plasma cholinesterase activity	Nishibe et al., 1980c [a]
90-days oral study in mouse	NOAEL is 33 mg/kg bw/day	decreased body weight combined with increased food consumption, liver effects	Nishibe et al., 1980d [a]
1-year oral study in dog	NOAEL is 9 mg/kg bw/day	decreased PCV, Hb and RBC and increase in liver weight	Chesterman, 1984 [a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Study 1

reference	:	Nishibe *et al.*, 1980a	exposure	:	28 days, diet
type of study	:	28-day oral toxicity study	dose	:	0, 20, 200, 2000 ppm[1]
year of execution	:	1979	vehicle	:	acetone
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 407
species	:	rat, SD	acceptability	:	acceptable
group size	:	10/sex/dose	**NOAEL**	:	20 ppm (2.3 mg/kg bw/d)

[1] Equal to 0, 2.3, 22, and 265 mg/kg bw/d for males and 0, 2.3, 22, and 309 mg/kg bw/d for females.

A 28-day oral toxicity study with Sprague-Dawley rats was performed partly in accordance with OECD 407. Groups of 10 animals/sex/dose received the test substance in diet at dose levels of 0, 20, 200 and 2000 ppm daily for 28 days, equal to 0, 2.3, 22, and 265 mg/kg bw/day for males and 0, 2.3, 22, and 309 mg/kg bw/day for females. Acetone was used as a vehicle. Deviations from the guideline were that haematological parameters were only determined in five instead of 10 animals/sex/dose and that blood clotting potential and creatinine were not determined. Further, sensory stimuli tests and functional observations were not conducted. However, these deviations are not considered to have influenced the conclusions of the study. Blood for the blood chemistry tests was sampled after overnight fasting. Plasma cholinesterase activity was measured using the DTNB method with S-butyrylthiocholine iodide as substrate.
Oral exposure of rats at concentrations of 2000 ppm for 28 days resulted in significantly lower body weight gain, increased food consumption and, consequently, decreased food efficiency. Compared to control animals, the reduction in body weight gain was 9 and 21% for males and females, respectively. Increased absolute and relative liver weight was observed in both sexes, which correlated with the macroscopic and microscopic findings of macula and fatty metamorphosis in the liver. Changes in several proteins and enzymes associated with liver function were also observed in this dose group. The increased food consumption and decreased body weight gain may indicate an increased catabolism, which may have caused the increased levels of cholesterol, total protein, and albumin. Relative weights of the adrenals and gonads were significantly increased in females, while plasma cholinesterase activity was decreased with 33%. The significance of effects on adrenal weight was concluded not to be considered toxicologically relevant. The increase in relative weight of the ovaries was dose-dependent (10 and 21% of the control values at 200 and 2000 ppm, respectively), and could not solely be explained by the reduction in body weight.
At the lower dose levels, changes in clinical chemistry (at 200 and 2000 ppm) and weights of the liver (at 200 ppm) were observed as well. However, as differences from the controls were either nonsignificant or smaller than 10%, these changes were not considered to be toxicologically relevant.
In conclusion, based on the increased relative ovary weight, the NOAEL was set at 20 ppm, which was equal to 2.3 mg/kg bw/day.

Results of the 28-day oral study in rats

Dose (ppm)	**0**		**20**		**200**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	no treatment-related findings								
Body weight							d (5%)	d (9%)	
Body weight gain							dc (9%)	dc (21%)	

Dose (ppm)	0		20		200		2000		dr
	m	f	m	f	m	f	m	f	
Food consumption							ic	ic	
Water consumption	no treatment-related findings								
Haematology	no treatment-related findings								
Clinical chemistry									
- cholesterol					ic (7%)		ic (12%)	ic (12%)	m
- total protein			ic (4%)		ic (5%)		ic (7%)	ic (9%)	m
- albumin					ic (6%)		ic (10%)	ic (8%)	m
- cholinesterase								dc (33%)	
Urinalysis	no treatment-related findings								
Organ weights									
- liver						ic^r (8%)	ic^{ar} (26, 33%)	ic^{ar} (16, 27%)	f
- adrenals								ic^r (17%)	
- ovaries						ic^r (10%)		ic^r (21%)	f
Pathology									
macroscopy									
Liver									
- greyish macula							+		
microscopy									
Liver									
- fatty metamorphosis		+		+		+	++	++	

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++ present in most/all animals

Microscopic observations in the liver (Nishibe, 1980a)

	0	20	200	2000
males				
Focal necrosis	0 (0.0; 0.0)	0 (0.0; 0.0)	1 (1.0; 10.0)	1 (1.0; 10.0)
Fatty metamorphosis	0 (0.0; 0.0)	0 (0.0; 0.0)	0 (0.0; 0.0)	10 (1.7; 100.0)
females				
Fatty metamorphosis	1 (1.0; 10.0)	1 (2.0; 10.0)	1 (1.0; 10.0)	10 (1.9; 100.0)

( ): mean grade and percent

Study 2

reference	:	Nishibe *et al.*, 1980b	exposure	:	28 days, diet
type of study	:	28-day oral toxicity study	dose	:	0, 20, 200, 2000 ppm[1]
year of execution	:	1979	vehicle	:	acetone
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 407
species	:	mouse, ICR	acceptability	:	acceptable
group size	:	10/sex/dose	**NOAEL**	:	200 ppm (40 mg/kg bw/d)

[1] Equal to 0, 3.8, 40, and 397 mg/kg bw/d for males and 0, 4.8, 52, and 552 mg/kg bw/d for females.

In a 28-day study with ICR mice, groups of 10 mice/sex/dose received the test substance at dose levels 0, 20, 200 and 2000 ppm in diet daily for 28 days. Acetone was used as a vehicle. The average daily consumed doses were equal to 0, 3.8, 40, and 397 mg/kg bw/d for males and 0, 4.8, 52, and 552 mg/kg bw/d for females. The study was performed mostly in accordance with OECD 407. Deviations from the guideline were that haematological parameters were only determined in five instead of ten animals/sex/dose. Further, blood clotting potential and creatinine were not determined. No plasma cholinesterase activity was measured.
Exposure to 20, 200, and 2000 ppm resulted in a dose-related decrease in body weight gain in both sexes, which was only statistically significant in females at the highest dose. At the highest dose level, absolute and relative weights were decreased for the spleen (males), increased for the liver (both sexes), and heart (males). The change in liver weight was associated with the microscopic finding of the swelling of the livers of all males in the highest dose group. At 200 and 2000 ppm, the absolute and relative weights of the adrenals of females were decreased by 15% or more of the control values. The significance of effects on adrenal weight was concluded to be not toxicologically relevant.
Based on the reduced spleen weight, the increased weights of liver and heart and the statistically significant reduction in body weight gain at the next higher dose, the NOAEL for NF-114 to mice is set at 200 ppm, which was equal to 40 mg/kg bw/day.

Results of the 28-day oral study in mice

Dose (ppm)	**0**		**20**		**200**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	no treatment-related findings								
Body weight gain			d (5%)	d (17%)	d (10%)	d (22%)	d (16%)	dc (25%)	m+f
Food consumption	no treatment-related findings								
Haematology	no treatment-related findings								
Clinical chemistry	no treatment-related findings								
Organ weights									
- spleen							dc[ar] (22, 18%)		
- liver							ic[ar] (28, 35%)	ic[ar] (24, 26%)	
- heart							ic[ar] (20, 26%)		
- adrenals[1]						dc[ar] (18 15%)		dc[ar] (17, 16%)	
Pathology									
Macroscopy	no treatment-related findings								
microscopy									
Liver									
- swelling							10/10		

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

[1] mean of relative increase of right and left adrenal, only increase of left adrenal reached statistical significance

Study 3

reference	:	Nishibe *et al.*, 1980c	exposure	:	90 days, in diet
type of study	:	90-day oral toxicity study	doses	:	0, 20, 200, 2000 ppm[1]
year of execution	:	1979	vehicle	:	acetone
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	partly in accordance with OECD 408
species	:	rat, Charles River SD	acceptability	:	acceptable
group size	:	20/sex/dose	**NOAEL**	:	200 ppm (15 mg/kg bw/d)

[1] Equal to 1.4, 15, and 177 mg/kg bw/d for males and 1.8, 17, and 218 mg/kg bw/d for females.

In the 90-day oral toxicity study with rats, performed partly in accordance with OECD Guideline 408, groups of Sprague-Dawley rats received the test substance at dose levels 0, 20, 200 and 2000 ppm (equal to 1.4, 15, and 177 mg/kg bw/d for males and 1.8, 17, and 218 mg/kg bw/d for females) in diet for 90 days. Acetone was used as a vehicle. Deviations from the guideline were that the animals were checked for morbidity and mortality once instead of twice a day and that no sensory stimuli tests and ophthalmological and functional observations were included. Further, blood clotting potential, urea, and creatinine were not determined and histopathological examinations of the spinal cord, aorta, female mammary gland, and peripheral nerve were not performed.
An additional group of 10 animals/sex was used for haematology and blood chemistry tests at the start of the dosing period. At the end of the study, all animals in the test were subjected to haematology and blood chemistry tests. Blood for the blood chemistry tests was sampled after overnight fasting. Plasma cholinesterase activity was measured using the DTNB method with S-utyrylthiocholine iodide as substrate.
Oral exposure at a concentration of 2000 ppm for 13 weeks resulted in a significantly lower body weight gain of females and an increased food consumption in both sexes, mainly in the first weeks of the study. This may indicate a rise in catabolism, which may explain the increased concentrations of BUN, cholesterol, total protein, and albumin observed at the highest dose level. Further, female red blood cell parameters and plasma cholinesterase activity were affected. Kidney weights were increased in both sexes and a dose dependency was found for absolute and relative kidney weight in males. Decreased adrenal weights were found in males, and decreased thymus and increased spleen weights for females. The significance of effects on adrenal weight was concluded not to be toxicologically relevant. Absolute and relative weights of the liver were increased in both sexes, which correlated with the microscopic finding of fatty metamorphosis in the livers of all animals in this dose group. Increased liver weights were also seen in males of the 20 and 200 ppm groups, as well as increased kidney weights at 200 ppm. The absolute increases may to a considerable extent be explained by the higher body weights (ca. 5%) in these dose groups as compared to controls. The relative increases were low (<10%) and no changes in relating parameters were present. Therefore, these deviations are not considered to be toxicologically relevant.
Based on the decreased body weight gain combined with increased food consumption, the liver and kidney enlargement, and fatty metamorphosis and decreased cholinesterase activity at 2000 ppm, the NOAEL was set at 200 ppm, which was equal to 15 mg/kg bw/day.

Results of the 90-day oral study in rats

Dose (ppm)	**0**		**20**		**200**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								

Dose (ppm)	0		20		200		2000		dr
	m	f	m	f	m	f	m	f	
Clinical signs	no treatment-related findings								
Body weight gain									
- week 1 – 3							dc	dc	
- week 4 - 13								dc	
Food consumption									
- week 1 - 4							ic	ic	
- week 7 - 10							ic		
Water consumption	no treatment-related findings								
Haematology									
- RBC								dc	
- Hb								dc	
- MCHC								dc	
- MCV								ic	
Clinical chemistry									
- BUN								ic	
- cholesterol								ic	
- total protein								ic	
- albumin								ic	
- ChE								dc	
Urinalysis	no treatment-related findings								
Organ weights									
- liver			ic[a] (12%)		ic[ar] (11, 7%)		ic[ar] (27, 31%)	ic[ar] (18, 29%)	
- kidney					ic[a] (9%), i[r] (4%)		ic[ar] (14, 17%)	ic[ar] (8, 18%)	m
- adrenals							dc[ar] (15, 12%)		
- thymus								dc[ar]	
- spleen								ic[r]	
Pathology									
macroscopy	no treatment-related findings								
microscopy									
Liver									
- fatty metamorphosis	+						++	++	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals

Study 4

reference	:	Nishibe *et al.*, 1980d	exposure	:	90 days, in diet
type of study	:	90-day oral toxicity study	doses	:	0, 20, 200, 2000 ppm[1]
year of execution	:	1979	vehicle	:	acetone
test substance	:	NF-114, lot no. YS-200, purity 98.7%, white crystal	GLP statement	:	no
route	:	oral	guideline	:	partly in accordance with OECD 408
species	:	mouse, Charles River ICR	acceptability	:	acceptable
group size	:	20/sex/dose	**NOAEL**	:	200 ppm (33 mg/kg bw/d)

[1] Equal to 0, 3.2, 33, and 381 mg/kg bw/d for males and 0, 4.2, 43, and 466 mg/kg bw/d for females.

In the 90-day study with mice, groups of ICR mice (20/sex/dose) received the test substance in diet at dose levels of 0, 20, 200 and 2000 ppm (equal to 0, 3.2, 33, and 381 mg/kg bw/d for males and 0, 4.2, 43, and 466 mg/kg bw/d for females) for 90 days. Acetone was used as a vehicle. The study was performed partly in accordance with OECD 408. Deviations from the guideline were that the animals were checked for morbidity and mortality once instead of twice a day and that no sensory stimuli tests and ophthalmologic and functional observations were included. Further, blood clotting potential, urea, and creatinine were not determined and histopathological examinations of the spinal cord, aorta, female mammary gland, and peripheral nerve were not performed.
An additional group of 10 animals/sex was used for haematology and blood chemistry tests at the start of the dosing period. No plasma cholinesterase activity was measured.
A reduction of body weight gain and a slight increase of food consumption was found in both sexes of the highest dose group. Hb (males) and MCHC levels (both sexes) were significantly decreased as well. Increased levels of potassium were measured in both sexes. In combination with the increase in relative kidney weight and ketone bodies in urine of females, this may be indicative of kidney failure. Relative adrenal weights were also increased in females. Absolute and relative liver weights were increased in both sexes, which corresponded with the microscopic finding of swelling of cytoplasm in the central zone of all male livers at 2000 ppm.
At 200 ppm, liver weights of males (absolute and relative weight) and liver, adrenal, and kidney weights of females (relative weight) were increased and Hb levels of males were significantly decreased. Relative liver and adrenal weight was also decreased in females at 20 ppm.
The changes in liver weight at mid and low dose are not considered toxicologically relevant as the increases in relation to controls were less than 10%. The changes in blood parameters at mid and high dose are also considered to be toxicologically irrelevant, as the relative changes are small, and the decrease in HB level is not more severe at high dose. The significance of effects on adrenal weight was concluded to be not toxicologically relevant.
In conclusion, based on the combination of decreased body weight gain and increased food consumption, and effects on the liver at 2000 mg/kg bw/day, the NOAEL was set at 200 ppm, which was equal to 33 mg/kg bw/day.

Results of the oral 90-day study in mice

Dose (ppm)	**0**		**20**		**200**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	no treatment-related findings								
Body weight gain							dc (27%)	d (15%)	
Food consumption									
- week 1-4							ic (10%)	i (<10%)	
- week 5-13							i (<10%)	i (<10%)	
Haematology									
- Hb					dc (6%)		dc (5%)		
- MCHC							dc (4%)	dc (6%)	
Clinical chemistry									
- potassium							ic (12%)	ic (17%)	
Urinalysis									
- presence of ketone									

Dose (ppm)	0		20		200		2000		dr
	m	f	m	f	m	f	m	f	
bodies, score 2/3+								4/10	
Organ weights									
- liver				ic[r] (7%)	ic[ar] (8, 9%)	ic[r] (9%)	ic[ar] (19, 29%)	ic[ar] (20, 32%)	
- kidney						ic[r] (11%)		ic[r] (8%)	
- adrenal				ic[r] (19%)		ic[r] (20%)		ic[r] (21%)	
Pathology									
macroscopy	no treatment-related findings								
microscopy									
Liver									
- swelling of cytoplasm in central zone							20/20		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a absolute organ weight, relative organ weights were not indicated

Study 5

reference	:	Virgo *et al*, 1984	exposure	:	104 weeks, in diet
type of study	:	combined toxicity/carcinogenicity study	doses	:	0, 100, 400, 1600 ppm[1]
year of execution	:	1981-1983	vehicle	:	None
test substance	:	NF-114, lot no. TK-1116, purity 98.6%, brown, crystalline powder	GLP statement	:	No (study performed before GLP existed)
route	:	oral	guideline	:	Mainly in accordance with OECD 453
species	:	Rat, CD	acceptability	:	acceptable
group size	:	70/sex/dose (and 10/sex/dose for interim kills)	**LOAEL**	:	100 ppm (3.5 mg/kg bw/d)

[1] Equal to 0, 3.5, 14, 59 mg/kg bw/d for males and 0, 4.5, 18, 77 mg/kg bw/d for females

A combined chronic toxicity/carcinogenicity study in rats exposed through the diet to 100, 400 or 1600 ppm triflumizole was further evaluated in the addendum to the DAR (February 2009) with the following conclusions:
The survival of the animals was about 80% up to 18 months. At 24 months, the mortality of the male rats of the carcinogenicity study was in all cases within the background range (52 – 75% mortality) of the historical controls for the strain of rats in the laboratory where the study was performed, except for the male high dose group, where mortality was lower. Mortality of females, however, tended to be at or below the lower end of the historical-control range (38.6 – 61.7), with the exception of the 100 ppm group, where mortality was unexpectedly high.
The main target organ was the liver. The relative liver weight was increased in both the highest dose groups and males administered 400 ppm. Females administered 400 or 1600 ppm had more macroscopic liver lesions than females in the control group. Microscopically, the number of observed effects increased with increasing doses and were more prominent in females than in males (see below). The incidence of diffuse hepatocytic fatty vacuolation indicated that the liver damage increases with time. At 104 weeks, the severity also increased by dose.
Centriacinar, periacinar and midzonal vacuolation was also observed, but without a clear dose related pattern. The effects were reflected in the liver enzyme levels, notably an increase in ALAT in males in the highest dose group. In the absence of additional adverse effects, the periacinar hepatocytic hypertrophy observed in females given 100 ppm would not be considered to be toxicologically relevant. However, the incidence of focal inflammation and necrosis was increased

at doses ≥ 100 ppm in females (both at 54 and 104 weeks) and doses ≥ 400 ppm in males (after 104 weeks) (no statistical analysis performed). This is an effect that warrants classification.

Incidence of microscopic liver effects in the toxicity part of the study, rats killed after 54 weeks (n=10/sex/dose)

Dose (ppm)	0		100		400		1600	
	m	f	m	f	m	f	m	f
Centriacinar fatty vacuolation hepatocytes	0	0	0	4	4	1	6	7
Diffuse fatty vacuolation hepatocytes	0	0	1	0	3	2	4	3
Periacinar hepatocytic hypertrophy	0	0	0	1	0	2	1	9
Focal inflammation and necrosis	8	4	6	10	9	10	10	9
Basophyllic foci	2	1	2	4	2	5	0	1
Eosinophyllic foci	6	1	4	2	4	0	3	0
Hyaline degeneration/fibrosis bile duct	2	0	0	0	2	0	1	2

Incidence of microscopic liver effects in the carcinogenicity part of the study, rats killed after 104 weeks plus rats killed or dying during treatment (n=70/sex/dose, except for male control group (n=69))

Dose (ppm)	0		100		400		1600	
	m	f	m	f	m	f	m	f
Centriacinar fatty vacuolation hepatocytes	14 (4)	16 (6)	2 (1)	33 (16)	11 (2)	17 (16)	16 (8)	11 (8)
Diffuse fatty vacuolation hepatocytes	10 (0)	13 (0)	15 (0)	15 (1)	26 (0)	43 (12)	35 (3)	55 (33)
Periacinar hepatocytic hypertrophy	0	0	5	18	10	13	17	28
Focal inflammation and necrosis	13	19	12	29	24	31	24	35
Basophyllic foci	4	9	1	12	3	23	9	32
Eosinophyllic foci	6	4	13	3	16	8	15	26
Hyaline degeneration/fibrosis bile duct	39	19	31	24	28	32	32	33

( ): moderate to marked

Chronic oral administration of NF-114 to rats also provoked neurotoxic effects. A dose of 1600 ppm caused an increased number of convulsive episodes (violent jerking movements, ataxia, tremors) in comparison to the control groups. For females in the highest dose group the increased incidence reached statistical significance. The convulsions tended to start earlier with increasing dose, in males as well as in females, indicating they are probably treatment-related.
Remarkable is the high incidence of convulsions in the male control group. The notifier submitted historical control data on convulsive episodes. The historical control data are from the laboratory where this study has been conducted. The background data are from a relevant period and are

acceptable. It is concluded that the incidence of convulsive episodes among controls and animals receiving 100 or 400 ppm was consistent with that reported in a range of similar studies and only at the highest dietary concentration (1600 ppm) the incidence was above the background range, particularly in females.
Further indications of neurotoxic effects were the reduced levels of brain butyrylcholinesterase at week 54 in all the female dose groups and the two highest male dose groups. However, the decreases were not dose-related and were not evident at 104 weeks. After one year of exposure, brain butyrylcholinesterase values in most groups (control and exposed) were ca. 2 times higher than the values after two years of exposure. Apparently the temporal variation is larger than any treatment-related effect. No consistent decrease in plasma or erythrocyte cholinesterase activity was observed, nor a decrease in brain acetylcholinesterase. Based on the current knowledge on brain butyrylcholinesterase activity (see above) and the absence of a dose-response, the decrease in brain butyrylcholinesterase activity is considered not toxicologically relevant.
In conclusion, the NOAEL is set at 100 ppm (3.5 mg/kg bw/d) based on effects on the liver.

Study 6

reference	:	Yamagata *et al.*, 1984	exposure	:	104 weeks, in diet
type of study	:	combined toxicity/carcinogenicity study	doses	:	0, 100, 400, 1600 ppm[1]
year of execution	:	1981-1983	vehicle	:	None
test substance	:	NF-114, lot no. TK-116, purity 98.6%, light yellow solid	GLP statement	:	Yes
route	:	Oral	guideline	:	Mainly in accordance with OECD 453
species	:	Mouse, SPF, $B6C3F_1$	acceptability	:	acceptable
group size	:	50/sex/dose (and 10/sex/dose for 3 interim kills)	**NOAEL**	:	100 ppm (16 mg/kg bw/d)

[1] Equal to 16, 67, 296 mg/kg bw/d for males and 22, 88, 362 mg/kg bw/d for females.

Mice were exposed through the diet to 100, 400 or 1600 ppm triflumizole. Chronic oral administration of NF-114 to mice at doses of 400 ppm and above caused primarily liver effects. The absolute and/or relative liver weight was increased in animals in the mid-and highest dose groups. An increased number of animals in the highest dose group, compared to the control group, had macroscopic liver effects. These effects were also seen to a lesser degree in males and/or females in the 400 ppm group. Effects on liver enzymes were observed as increased levels of GOT and GPT in males administered 1600 ppm. The non-neoplastic lesions were also found primarily in the liver, where several effects increased dose-related in the mid-and high dose groups. A decrease in body weight gain was noted in the highest dose group in males (significant) and females (not significant). The increase in liver fatty metamorphosis observed in males administered 100 ppm is not considered to be a toxicologically relevant effect, in absence of additional liver effects. The decreases in the number of WBC in males of all dose groups do not show a consistent pattern across time or dose groups. In absence of effects on lymphoid organs, its toxicological significance is not clear. As neither its relation to treatment nor its toxicological significance clear, the reduction in number of WBC observed in this study is not considered relevant. In conclusion, the NOAEL was set at 100 ppm (16 mg/kg bw/day).

Results combined toxicity/carcinogenicity study in mice

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
Mortality	8/50	4/50	4/50	5/50	11/50	7/50	2/50	8/50	
Clinical signs	No treatment-related effects								
Body weight gain							dc (44%)	d (10%)	

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
Food consumption	No treatment-related effects								
Ophthalmoscopy	not performed								
Haematology									
-WBC (wk 26)					dc (44%)		dc (44%)		
-WBC (wk 52)			dc (33%)		dc (57%)		dc (47%)		
-WBC (wk 78)			d (44%)		dc (63%)		d (44%)		
-WBC (wk 104)			dc (60%)		dc (44%)		d (40%)		
Clinical chemistry									
-inorganic phosphate							ic (25%)[1]	ic (26%)[2]	
-GOT							ic (116%)[3]		
-GPT							ic (400%)[2]		
Urinalysis	no treatment-related effects								
Organ weights									
-liver (wk 26)					ic[r] (14%)	ic[a] (13%)	ic[a,r] (38, 59%)	ic[a,r] (40, 40%)	
-liver (wk 52)					ic[r] (11%)	ic[a] (9%)	ic[a,r] (41, 61%)	ic[a,r] (33, 39%)	
-liver (wk 78)							ic[a,r] (33, 46%)	ic[a,r] (32, 46%)	
-liver (wk 104)							ic[a,r] (39, 69%)		
-kidneys (all)							ic[r] (19%)[2]		
Pathology									
Macroscopy									
-liver enlargement					+	+	+	+	
-liver, white zone						+	+	+	
-liver nodule							+	+	
microscopy *neoplastic lesions*	no treatment-related effects								
microscopy *non-neoplastic lesions*									
Liver									
-hepatic nodule	8/60	5/60	15/60	7/60	12/60	9/60	20/60	17/60	f
-fatty metamorphosis	11/50		17/50		20/50	17/50	30/50	24/50	m,f
-granulomatous inflammation					+		+		m
-cytological alterations					+		++		
-pigmentation					+		++		f
-necrosis					+		++	+	m
Kidneys									
-regenerating epithelium					+		+	+	
Spleen									
-pigmentation							++		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls

d/i	decreased/increased, but not statistically significantly compared to the controls
a,r	absolute organ weight, relative organ weight
+	a few more affected than in control group
++	many more affected than in control group
[1]	significant in week 26 and 78; % increase is averaged
[2]	% increase of 4 weeks is averaged
[3]	significant in week 26, 78, and 104; % increase is averaged

Study 7

reference	:	Chesterman, 1984	exposure	:	52 weeks, in diet
type of study	:	1-year oral toxicity study	doses	:	0, 100, 300, and 1000 ppm[1]
year of execution	:	1982-1983	vehicle	:	none
test substance	:	NF-114, batch no. TK-1114, purity 98.7%, brown crystalline powder	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 409
species	:	dog, Beagle	acceptability	:	acceptable
group size	:	6/sex/dose	**NOAEL**	:	300 ppm (9 mg/kg bw/d)

[1] Equal to 3, 9, 32 mg/kg bw/d for males and for females.

In the 1-year study with Beagle dogs, groups of 6 animals/sex/dose received the test substance at dose levels of 0, 100, 300, and 1000 ppm (equal to 3, 9, 32 mg/kg bw/d for males and for females) for 52 weeks in diet. The study was performed in accordance with OECD guideline 409. There was an interim kill of 2 males and 2 females from each group after 13 weeks. For the remaining animals, treatment continued for 52 weeks. No plasma cholinesterase activity was measured. Oral exposure of dogs to the test substance at concentrations of 1000 ppm for 1 year resulted in decreased PCV, Hb and RBC and increased MCV, ALP and relative liver weight. At a dose level of 300 ppm, no adverse effects were observed. Therefore, the NOAEL was set at this level (equal to 9 mg/kg bw/day).

Results of the 1-year oral study in dogs

Dose (ppm)	**0**		**100**		**300**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	no treatment-related findings								
Body weight gain	no treatment-related findings								
Food consumption	no treatment-related findings								
Ophthalmoscopy	no treatment-related findings								
Haematology									
PCV							dc (8%)		
Hb							dc (11%)		
RBC							dc (19%)		
MCV							ic (12%)		
Clinical chemistry									
ALP							ic (79%)**	ic* (63%)	
Urinalysis	no treatment-related findings								
Organ weights									
liver							ic[r][ic][r] (18, 16%)	ic[r] [ic][r] (25, 16%)	
Pathology									

Dose (ppm)	0		100		300		1000		dr
	m	f	m	f	m	f	m	f	
macroscopy - lobular pattern and granular texture of liver							¼[1/2]	¼[1/2]	
microscopy	no treatment-related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
[..] the results of the 13-week kills, males and females were grouped
***** significant in week 12 and 26, % increase is averaged
****** % increase of 4 weeks is averaged

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

Study 1

reference	:	Goldenthal, 1990	exposure	:	21 days, 6 h/d, semi-occlusive (ca. 10% of the total body surface area)
type of study	:	21-day dermal toxicity study	dose	:	0, 10, 100, 1000 mg/kg bw/d
year of execution	:	1990	vehicle	:	distilled water
test substance	:	Triflumizole Technical, lot no. 2112, purity 97%, tan powder	GLP statement	:	yes
route	:	dermal	guideline	:	in accordance with OECD 410
species	:	rat, Charles River CD	acceptability	:	acceptable
group size	:	6/sex/dose	**NOAEL**	:	100 mg/kg bw/d

The 21-day dermal toxicity study with CD rats was performed in accordance with OECD 410, except that only the treated skin, liver, and kidney were histopathologically examined. Since the liver is the target organ in the subacute oral toxicity studies, this deviation probably did not affect the derivation of a NOAEL. Groups of 6 animals/sex/dose received the test substance in distilled water at dose levels 0, 10, 100 and 1000 mg/kg bw/day 6 hours/day under semi-occlusive dressing (ca. 10% of the total body area).
Dermal exposure of rats to triflumizole at a concentration of 1000 mg/kg bw/day for 21 days resulted in a significant increase in relative liver weight of males. A slight increase in the incidence of vacuolar fatty change in the livers of females of the high dose group was seen, as well as an increase of the severity of the effect. It cannot be excluded that this effect is test substance related. The number of animals with skin inflammation was slightly higher in the high dose groups compared to the control groups. This is thought to be due to the application procedure and is not considered to be related to the test substance.
Based on the significantly increased relative liver weights in males and the histopathological liver changes in females of the highest dose group, the NOAEL for Triflumizole Technical to rats is set at 100 mg/kg bw/day.

Results of the 21-day dermal study in rats (Goldenthal, 1990)

Dose (mg/kg bw/d)	0		10		100		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	none								

Dose (mg/kg bw/d)	0		10		100		1000		dr
	m	f	m	f	m	f	m	f	
Clinical signs	no treatment-related findings								
Body weight gain	no treatment-related findings								
Food consumption	no treatment-related findings								
Haematology	no treatment-related findings								
Clinical chemistry	no treatment-related findings								
Urinalysis	no treatment-related findings								
Organ weights - liver							ic[r] (12%)		
Pathology									
macroscopy	no treatment-related findings								
microscopy[1] *Liver* - vacuolar fatty change		1/6						3/6	
Skin - inflammation (trace)	2/6	1/6					3/6	3/6	

dr dose related

ic statistically significantly increased compared to the controls

r relative organ weight

[1] only determined in control and high dosage group

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

In all short-term studies increased liver weights were observed, although this was not always among the critical effects. In all oral short-term studies, decreased body weight gain combined with increased food consumption was observed, often among the critical effects.
In Table 21 the compilation of effects observed at dose levels approximately equal to the limits of classification (100 mg/kg bw/day and 50 mg/kg bw/day according to EC 1272/2008 and 67/548, respectively, in 90-day rodent studies and in 28-day rodent studies of 300 mg/kg bw/day and 150 mg/kg bw/day) are presented.
The repeated dose neurotoxicity study in rats confirms the liver effects observed in the previous rat studies and does not indicate a specific neurotoxic effect. (see chapter 4.12).

Table 21: Summary of the effects observed at dose levels approximately equal to the limits of classification

Method	Effect level	Observed effect	Reference
28-days oral study in rat	22 mg/kg bw/day 265 mg/kg bw/day (males), 309 mg/kg bw/day (females)	Males: cholesterol ↑, total protein ↑, albumin ↑, Body weight gain ↓ Females: liver weight ↑, ovaries weight ↑, Body weight gain ↓ Food consumption ↑, Body weight gain ↓, liver weight ↑, ovaries weight ↑, fatty metamorphosis of liver, greyish macula in liver (males), cholesterol ↑, total protein ↑, albumin ↑, cholinesterase ↓ (females)	Nishibe et al., 1980a[a]
28-days oral study in mouse	40 mg/kg bw/day (males); 52 mg/kg bw/day (females) 397 mg/kg bw/day (males), 552 mg/kg bw/day (females)	Body weight gain ↓ Body weight gain ↓, spleen weight ↓ (males), liver weight ↑, heart weight ↑, ovaries weight ↑, liver swelling (males)	Nishibe et al., 1980b[a]

90-days oral study in rat	15 mg/kg bw/day (males), 17 mg/kg bw/day (females) 179 mg/kg bw/day (males), 218 mg/kg bw/day (females)	Males: liver weight ↑, kidney weight ↑ Body weight gain ↓, food consumption ↑, liver weight ↑, kidney weight ↑, spleen weight ↑ (females), thymus weight ↓ (females), fatty metamorphosis of the liver , BUN↑ (females), cholesterol ↑ (females), total protein ↑ (females), albumin ↑ (females), cholinesterase ↓ (females), RBC ↓ (females), HB ↓ (females), MCHC ↓ (females), MCV ↑ (females)	Nishibe et al., 1980c[a]
90-days oral study in mouse	33 mg/kg bw/day (males), 43 mg/kg bw/day (females) 381 mg/kg bw/day (males), 466 mg/kg bw/day (females)	Hb ↓ (males), liver weight ↑, kidney weight ↑ (females) Body weight gain ↓, food consumption ↑, Hb ↓ (males), MCHC ↓, potassium ↑, liver weight ↑, kidney weight ↑ (females), swelling of cytoplasm in the central zone of the liver (males)	Nishibe et al., 1980d[a]
1-year study in dogs	32 mg/kg bw/day	PCV ↓ (male), Hb ↓ (male), RBC ↓ (male), MCV ↑ (male), ALP ↑ relative liver weight ↑	Chesterman, 1984[a]
Chronic toxicity/carcinogenicity study in rats	< 4.5 mg/kg bw (females), 3.5 mg/kg bw/day (males) 14 mg/kw bw/day (males), 3.5 mg/kg bw/day (females)	NOAEL Brain butyrylcholinesterase ↓, liver weight ↑ (males), swollen liver (females), dark depresse area in the liver (females), fatty vacuolation of liver ↑, peracinar hepatocytic hypertrophy ↑, basophilic foci/hepatocellular alteration ↑ (females), focal inflammation/necrosis ↑ (females), fibrosis of bile ducts ↑ (females)	Virgo et al., 1984[a]
Chronic toxicity/carcinogenicity study in mice	16 mg/kg bw/day (males), 22 mg/kg bw/day (females)	NOAEL	Yamagata et al., 1984[a]

[a]As summarized in the DAR, updated addendum of February 2009

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4.7.1.8 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

In accordance with EC 67/548, substances have to be classified for repeated dose toxicity if the significant adverse effects, which indicate irreversible functional impairment, occur at dose levels ≤ 50 (EEC 67/548) mg/kg bw/day in the 90-day rodent studies. Such effects may include, but are not limited, to mortality, significant functional changes in various organ systems, significant adverse changes in clinical biochemistry, haematology, or urinalysis parameters, significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; wide-spread or severe necrosis, fibrosis or granuloma formation in vital organs; severe morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction and/or evidence of appreciable cell death in vital organs incapable of regeneration. For a 28-day study the guidance values are increased by a factor of three and are thus 150 mg/kg bw/day according to EC 67/548. For long-term studies, EC 67/548 recommends evaluation on a case by case basis. Assuming extrapolation using Habers rule for a 2 year study would result in 6.25 mg/kg bw/day.

In the available 28-day toxicity studies with mice and rats, the effects in the liver (weight increase and fatty metamorphosis) occurred at the dose level of 265 mg/kg bw/day in male rats, which is above the classification limit of 150 mg/kg bw/day for 28-day studies according to EC 67/548. Fatty metamorphosis occurred in all animals at the top dose, but the severity was only slight to moderate (mean grade 1.7 and 1.9 in males and females) and the effects are considered not severe enough for classification. Since also the effects on body weight were slight at this dose (< 10%), classification is not warranted based on this study.

In the 90-day study with rats the observed LOAELs were 177 and 218 mg/kg bw/day for males and females, respectively, and are thus above the cut-off for classification according to 67/548. The observed effects included liver weight increase and fatty metamorphosis of the liver, as well as accompanying changes in clinical chemistry parameters in female rats (cholesterol increase, total protein increase, albumin increase and cholinesterase decrease). Again, interpolation of the effects to a dose level of 50 mg/kg bw/day is difficult. Due to the uncertainty of the severity of the effects at the relevant dose level it is not shown that classification is needed based on this study according to EC 67/548.

For mice, the respective LOAELs were 381 and 466 mg/kg bw/day for males and females, respectively. This is based on an increased liver weight (20% absolute, 30% relative) in males and females, and cytoplasmic swelling of the liver in all males at this dose. Seen the limited effects at 33 and 43 mg/kg bw/day in males and females, respectively (increase in liver weight <10%), which is close to the guidance value of 50 mg/kg bw/day, no classification is required.

In the available chronic toxicity/carcinogenicity study with rats, severe liver effects, including focal inflammation/necrosis and bile ducts fibrosis were observed. The incidence of focal inflammation and necrosis was increased at doses ≥ 4.5 mg/kg bw/day in females (both at 54 and 104 weeks) and doses ≥ 18 mg/kg bw/day in males (after 104 weeks) (no statistical analysis performed). This is below the guidance value of 6.25 mg/kg bw/day when extrapolated to a 2 year study, or the guidance value of 12.5 mg/kg bw/day when extrapolated to a 1 year study (effects were also

observed in the satellite group). However, since the observed necrosis was not widespread or severe, the criteria for classification as R22/48 are not fulfilled. In mice, the NOAEL was above the extrapolated guidance value of 6.25 mg/kg bw/day.

Therefore, no classification for oral repeated dose toxicity is required according to EG 67/548.
For repeated dose toxicity after dermal exposure only a 21-day study is available. Effects were only observed at the limit dose of 1000 mg/kg bw/day. This is clearly above the extrapolatated guidance value of approximately 433 mg/kg bw/day. Further, the effects at this dose were limited to increased liver weight in males and liver vacuolar fatty change in some female rats. Therefore, no classification is required.

4.7.1.9 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

It is not necessary to classify triflumizole for repeated dose toxicity according to EC 67/548.

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

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See section 4.7.1.7.

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

In accordance with EC 1272/2008, substances have to be classified for repeated dose toxicity if the significant adverse effects, which indicate functional impairment, occur at dose levels ≤100 (EC 1272/2008) mg/kg bw/day in the 90-day rodent studies. Such effects may include, but are not limited, to mortality, significant functional changes in various organ systems, significant adverse changes in clinical biochemistry, haematology, or urinalysis parameters, significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs; morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction and/or evidence of appreciable cell death in vital organs incapable of regeneration. For a 28-day study the guidance values are increased by a factor of three and are thus 300 mg/kg bw/day according to EC 1272/2008. For long-term studies, EC 1272/2008 recommends the use of extrapolation similar to Haber's law which states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure. This results in a guidance value of 12.5 mg/kg bw/day for a 2-year study.

In the available 28-day toxicity studies with mice and rats, the effects in the liver (weight increase and slight fatty metamorphosis) occurred at the dose level of 265 mg/kg bw/day in male rats, which is below the classification limit of 300 mg/kg bw/day for 28-day studies according to EC 1272/2008. Body weight was decreased <10%. With respect to the liver effects, fatty metamorphosis was observed in all animals at this dose, where 100% of the liver was affected, with a mean grade of 1.7 in males and 1.9 in females. In addition, necrosis was observed in 1 male of the mid dose and 1 male of the high dose group. The effects are considered not severe enough for classification.

In the 90-day study with rats the observed LOAELs were 177 and 218 mg/kg bw/day for males and females, respectively. The observed effects included liver weight increase and fatty metamorphosis of the liver, as well as accompanying changes in clinical chemistry parameters in female rats (cholesterol increase, total protein increase, albumin increase and cholinesterase decrease). As no information is available whether the observed fatty metamorphosis could be considered as severe fatty change it is also unclear whether this effect warrants classification. Although, the combination of effects (decreased weight gain, increased food consumption and changes of the liver) probably is sufficient, the effects occur at a dose level twice above the cut-off for classification according to EC 1272/2008 (100 mg/kg bw/day).

For mice, the respective LOAELs were 381 and 466 mg/kg bw/day for males and females, respectively. Interpolation of the effects to the guidance level of 150 mg/kg bw/day, makes it unlikely that the effects observed in this mouse study are severe enough for classification.

In the available chronic toxicity/carcinogenicity study with rats, severe liver effects, including focal inflammation/necrosis and bile ducts fibrosis were observed. Most of these effects were observed at a dose level of 18 mg/kg bw/day in females. This is just above the guidance value of 12.5 mg/kg bw/day when extrapolated to a 2 year study.
However, the incidence of focal inflammation and necrosis was already increased at doses ≥ 4.5 mg/kg bw/day in females (both at 54 and 104 weeks) (and in males after 104 weeks at doses ≥ 18 mg/kg bw/day in) (no statistical analysis performed). This is below the guidance value of 12.5 mg/kg bw/day when extrapolated to a 2 year study, or the guidance value of 25 mg/kg bw/day when extrapolated to a 1 year study (effects were also observed in the satellite group). Considering the severity of the observed effects (including necrosis), classification as STOT RE Category 2 is justified.

In mice, the NOAEL was above the extrapolated guidance value 12.5 mg/kg bw/day.

Based on the fact that histopathological changes in the liver occurred already following 28 days exposure at dose levels below the limit for classification according to EC 1272/2008, and at a more severe level (including necrosis) at dose levels the guidance values in longer oral rat studies it is proposed to classify triflumizole as STOT RE Category 2, H373 according to EC 1272/2008.

The difference in the proposed classification between CLP and DSD is due to the higher guidance values and the more limited effects required for classification under CLP.

For repeated dose toxicity after dermal exposure only a 21-day study is available. Effects were only observed at the limit dose of 1000 mg/kg bw/day. This is just above the extrapolated guidance value of approximately 866 mg/kg bw/day. The effects at this dose were limited to increased liver weight in males and liver vacuolar fatty change in some female rats. The effects are similar (although not as severe) to the effects on which classification for STOT-RE is based. Since only a 21 day dermal toxicity study is available, it is possible that animals may not have fully adapted to the exposure and that a longer exposure would have resulted in more severe effects, which would warrant classification.

In addition, no information is available on the effects after repeated inhalation exposure.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Conclusion on classification for repeated dose toxicity

	CLP Regulation	Directive 67/548/EEC (DSD)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	STOT RE Cat. 2 (H373) Target organ liver No limitation of the routes	Not classified

Due to a lack of data on long term exposure via the dermal and inhalation route, it is not possible to conclude that the effects are route-specific. Therefore, no route is included in the classification for STOT-RE.

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

Summary of the Dossier submitter's proposal

The DS proposed to classify triflumizole as STOT RE 2; H373 based on the results of the studies summarised in Table 1 below.

Table 1. Summary of relevant repeated dose toxicity studies

Method	NOAELs	Critical effects	Reference
28-day oral study in rat Partly in accordance with OECD TG 407 Non-GLP	NOAEL: 2.3 mg/kg bw/d	Dose-dependent increase in relative ovary weights	Nishibe *et al.*, 1980a
28-day oral study in mouse Mainly in accordance with OECD TG 407 Non-GLP	NOAEL: 40 mg/kg bw/d	Reduced spleen weight, increased liver and heart weights, reduction in body weight gain	Nishibe *et al.*, 1980b
21-day dermal study in rat OECD TG 410 GLP	NOAEL: 100 mg/kg bw/d	Relative liver weight increase in males, histopathological liver changes in females	Goldenthal, 1980
90-day oral study in rat Partly in accordance with OECD TG 408 Non-GLP	NOAEL: 15 mg/kg bw/d	Decreased body weight gain combined with increased food consumption, liver and kidney enlargement, fatty metamorphosis, decreased cholinesterase activity	Nishibe *et al.*, 1980c
90-day oral study in mouse Party in accordance with OECD TG 408 Non-GLP	NOAEL: 33 mg/kg bw/d	Decreased body weight gain combined with increased food consumption, liver effects	Nishibe *et al.*, 1980d

2-year oral study in rat; combined toxicity/carcinogenicity study; Mainly in accordance with OECD TG 453 Non-GLP	NOAEL: 3.5 mg/kg bw/d	Liver effects: relative weight increased, macroscopic and microscopic lesions, hepatocytic fatty vacuolation, concurrent changes in liver enzyme levels, focal inflammation and necrosis	Virgo *et al.*, 1984
2-year oral study in mouse; combined toxicity/carcinogenicity study; Mainly in accordance with OECD TG 453 GLP	NOAEL: 16 mg/kg bw/d	Liver effects: increased absolute/relative weight, macroscopic effects, liver enzyme changes	Yamagata *et al.*, 1984
1-year oral study in dog OECD TG 409 GLP	NOAEL: 9 mg/kg bw/d	Decreased PCV, Hb and RBC and increase in relative liver weight, MCV and ALP	Chesterman, 1984

M=in males
F=in female
PCV=packed cell volume
Hb=hemoblobin
RBC=red blood cell count
MCV=mean cell volume
ALP=alkaline phosphatase

In the available chronic toxicity/carcinogenicity study with rats, severe liver effects, including focal inflammation/necrosis and bile duct fibrosis were observed. Most of these effects were observed at a dose level of 18 mg/kg bw/d in females. This is just above the guidance value of 12.5 mg/kg bw/d when extrapolated to a 2 year study. However, the incidence of focal inflammation and necrosis was already increased at doses $\geqslant$ 4.5 mg/kg bw/d in females (both at 54 and 104 weeks) (and in males after 104 weeks at doses $\geqslant$ 18
mg/kg bw/d in). This is below the guidance value of 12.5 mg/kg bw/d when extrapolated to a 2 year study, or the guidance value of 25 mg/kg bw/d when extrapolated to a 1 year study (effects were also observed in the satellite group). Considering the severity of the observed effects (including necrosis), the DS considered that classification as STOT RE Category 2 is justified.

Comments received during public consultation

Three MS supported the proposed classification for STOT RE 2, H373 for triflumizole.

One MS expressed doubts about the need to classify for STOT RE 2. They reasoned that although there are significant effects on the liver (increased weight and changes in clinical chemistry), liver specific enzymes were not modified. Moreover, they argued that the inflammation and necrosis seen in the liver was seen at a high rate also in the control group.

Assessment and comparison with the classification criteria

According to CLP, substances have to be classified for repeated dose toxicity if the significant adverse effects, which indicate functional impairment, occur at dose levels ≤100 mg/kg bw/d in a 90-day rodent study.

Such effects may include, but are not limited to, mortality, significant functional changes

in various organ systems, significant adverse changes in clinical biochemistry, haematology, or urinalysis parameters, significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs; morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction and/or evidence of appreciable cell death in vital organs incapable of regeneration.

In the 28-day oral toxicity study in rats (Nishibe *et al.*, 1980a), groups of Sprague-Dawley rats received triflumizole in the diet at concentrations 20, 200 and 2000 ppm (equal to doses of 0, 2.3, 22, and 265 mg/kg bw/d for males and 0, 2.3, 22 and 309 mg/kg bw/d for females). Exposure to 20, 200 and 2000 ppm resulted in a dose-related decrease in body weight gain in both sexes, which was only statistically significant in females at the highest dose. At the highest dose level, absolute and relative weights were decreased for the spleen (males), and increased for the liver (both males and females), and heart (males). The change in liver weight was associated in microscopic investigations with fatty metamorphosis in all males and females in the highest dose group (i.e. 265 mg/kg bw/d for males and 309 mg/kg bw/d for females). Based on the increased relative ovary weight, the NOAEL was set at 20 ppm (equal to 2.3 mg/kg bw/d). Thus, the severity of the effects in liver (increased weight and the associated fatty changes in hepatocytes) meet the classification criteria for STOT RE 2, and they were observed at levels (265 mg/kg bw/d for males and 309 mg/kg bw/d for females) below or very close to the guidance value of ≤ 300 mg/kg bw/d for a 28-day study.

In the 28-day oral toxicity study in mice (Nishibe *et al.*, 1980b), groups of 10 ICR mice received triflumizole in the diet at concentrations 20, 200 and 2000 ppm (equal to doses of 0, 3.8, 40 and 397 mg/kg bw/d for males and 0, 4.8, 52 and 552 mg/kg bw/d for females). Exposure to 20, 200 and 2000 ppm resulted in a dose-related decrease in body weight gain in both sexes, which was statistically significant only in females at the highest dose (552 mg/kg bw/d). At the highest dose level (397 and 552 mg/kg bw/d in males and females, respectively), absolute and relative weights were decreased for the spleen (males) and increased for the liver (both sexes) and heart (males). The change in liver weight was associated with the microscopic finding 'swelling of the liver' in all males in the highest dose group (397 mg/kg bw/d). Based on the reduced spleen weight, the increased weights of liver and heart and the statistically significant reduction in body weight gain at the next higher dose, the NOAEL for to mice was set at 200 ppm, which was equal to 40 mg/kg bw/d. These mice data do not support classification of triflumizole as STOT RE 2 because no significant adverse effects were observed at the dose level equal to or below the CLP guidance value of ≤ 300mg/kg bw/d for a 28-day study.

In the *90-day oral toxicity study in rats* (Nishibe *et al.*, 1980c), groups of Sprague-Dawley rats received triflumizole in the diet for 90 days, at dose levels equal to 1.4, 15, and 177 mg/kg bw/d for males and 1.8, 17, and 218 mg/kg bw/d for females. Oral exposure at 177 and 218 mg/kg for males and females, respectively, for 3 weeks resulted in a significantly lower body weight gain of females and increased food consumption in both sexes, mainly during the first weeks of the study, associated with increased concentrations of blood urea nitrogen, cholesterol, total protein, and albumin. Absolute and relative liver weights were increased in both sexes, which correlated with the microscopic finding of fatty metamorphosis in the livers of all animals in the highest dose group. No fatty metamorphosis was observed in liver of animals exposed at the lower dose levels, although it was observed in the liver of a few control males. Hence, although the severity of effects in the liver (increased absolute and relative weight which correlated with fatty changes in hepatocytes) meet the classification criteria for STOT RE 2, they were observed at levels (177 and 218 mg/kg bw/d) higher than the guidance value of ≤ 100mg/kg bw/d. Thus, these data do not justify classification as STOT RE 2, although it is noted that there is a large span between the mid-dose (15 and 17 mg/kg bw/d for males and females, respectively) and high-dose (177 and 218 mg/kg bw/d,

respectively) group.

In the *90-day study in mice* (Nishibe *et al.*, 1980d), groups of ICR mice (20/sex/dose) received triflumizole in the diet for 90 days, at dose levels equal to 0, 3.2, 33 and 381 mg/kg bw/d for males and 0, 4.2, 43 and 466 mg/kg bw/d for females.
A reduction of body weight gain and a slight increase in food consumption was found in both sexes of the highest dose group. Absolute and relative liver weights were increased in both sexes, which corresponded with the microscopic finding of swelling of cytoplasm in the central zone of all male livers at 381 mg/kg bw/d. The changes in liver weight at the low and mid dose are not considered toxicologically relevant as the increases in relation to controls were less than 10% and they were not associated with microscopic changes in the liver. It is concluded that the observed effects do not warrant classification as STOT RE 2, because the significant adverse changes in the liver occurred at dose levels (381 and 466 mg/kg bw/d for males and females, respectively) above the guidance value of ≤ 100mg/kg bw/d. However, it is noted that there is a large dose span between the mid-dose (33 and 43 mg/kg bw/d for males and females, respectively) and high-dose (381 and 466 mg/kg bw/d, respectively) groups

In the *2-year combined chronic toxicity/carcinogenicity study in rats* (Virgo *et al.*, 1984) the animals received triflumizole in the diet at dose levels equal to 0, 3.5, 14 and 59 mg/kg bw/d for males and 0, 4.5, 18 and 77 mg/kg bw/d for females. The mortality of the male and female rats is considered unaffected by the treatment. The relative liver weight was increased in both the highest dose groups (59 mg/kg bw/d and 77 mg/kg bw/d for males and females, respectively) and in males administered triflumizole at 14 mg/kg bw/d. Females administered triflumizole at doses of 18 and 77 mg/kg bw/d had more microscopic liver lesions, such as diffuse fatty vacuolation of hepatocytes and focal inflammation and necrosis, than females in the control group. The incidence of these changes in the control group and rats exposed at 3.5 and 4.5 mg/kg bw/d (males and females, respectively), seems comparable, although no statistical analysis was included in the CLH report.

Focal inflammation and necrosis were observed in the control group in 13 males and 19 females (out of 69 males and 70 females examined) and in 12 males exposed at 3.5 mg/kg bw/d and 29 females at 4.5 mg/kg bw/d (out of 70 tested animals/sex). The differences between these incidences were reported as not statistically significant in the Addendum to the DAR (February 2009). Therefore, it is concluded that significant adverse effects in liver such as diffuse fatty vacuolation of hepatocytes and focal inflammation and necrosis were observed at doses of 14 and 59 mg/kg bw/d in males and at 18 and 77 mg/kg bw/d in females, respectively. This is above the guidance value of 12.5 mg/kg bw/d (extrapolation from the guidance value of 100 mg/kg bw/d for 90-day studies to a 2-year study, using a factor of 8 according to Haber's law). Therefore these results do not justify classification as STOT RE 2.

However, it is noted that adverse effects were observed at doses of 14 and 18 mg/kg bw/d (males and females, respectively), which is not much higher than the extrapolated guidance value of 12.5 mg/kg bw/d. On the other hand, the chronic oral exposure to triflumizole of male rats at 3.5 mg/kg bw/d and female rats at 4.5 mg/kg bw/d did not significantly increase the frequency of adverse effects in the liver, which seem to be the most sensitive organ after triflumizole exposure.

In the *2-year combined chronic toxicity/carcinogenicity study in mice* (Yamagata *et al.*, 1984) the animals received triflumizole in the diet at dose levels equal to 0, 16, 67 and 296 mg/kg bw/d for males and 0, 22, 88 and 362 mg/kg bw/d for females. The study was performed mainly in accordance with OECD TG 453. The absolute and/or relative liver weight was increased in animals in the mid and highest dose groups. An increased number of animals in the highest dose group, compared to the control group, had macroscopic liver effects. Effects on liver enzymes were observed as increased levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) in males administered

296 mg/kg bw/d. In microscopic evaluations, adverse effects such as inflammation, fatty metamorphosis and necrosis were found in males exposed at 67 and 296 mg/kg bw/d, while in females liver necrosis and alterations in kidney were observed only at a dose of 362 mg/kg bw/d. Thus, the effects were seen at doses much higher than the guidance value of 12.5 mg/kg bw/d. Therefore, these results do not justify classification as STOT RE 2.

In the *1-year study with Beagle dogs* (Chesterman, 1984), groups of 6 animals/sex/dose received triflumizole at dose levels of 0, 100, 300 and 1000 ppm (equal to 3, 9 and 32 mg/kg bw/d for males and female) for 52 weeks in the diet. The study was performed in accordance with OECD TG 409. Oral exposure of dogs to triflumizole at concentrations of 1000 ppm (32 mg/kg bw/d) for 1 year resulted in decreased packed cell volume (PCV), haemoglobin (Hb) and red blood cell counts (RBCs) (in a range of 8-12%) and increased mean corpuscular volume of red cells (MCV) by 12% in males, and in increased alkaline phosphatase level (ALP) in both males and females (79% and 63%), as well as increased relative liver weight (16%). In microscopic examinations, no treatment related findings were noted in any of the exposed dogs. At a dose level of 300 ppm (9 mg/kg bw/d), no adverse effects were observed. Therefore, the NOAEL was set at this level (9 mg/kg bw/d). It is concluded that no significant, adverse effects meeting the classification criteria were seen. The effects observed at the dose level of 32 mg/kg bw/d corresponded rather to effects which do not justify classification that may be seen in humans and/or animals (see point 3.9.2.8.1, Annex 1, CLP). Thus, it is concluded that the small changes in haematology, clinical biochemistry and the changes in liver weight, with no evidence of organ dysfunction, which were observed at dose of 32 mg/kg bw/d (slightly higher than the extrapolated guidance value of 24 mg/kg bw/d for a 1-year study) do not provide sufficient evidence for classification of triflumizole as STOT RE 2.

There are no data for assessment of specific target organ toxicity - repeated exposure for the inhalation route.

In the *21-day dermal toxicity study in rats* (Goldenthal, 1990; in accordance with OECD TG 410 except that only the treated skin, liver, and kidney were histopathologically examined), groups of 6 CD rats/sex/dose received the test substance in distilled water at dose levels 0, 10, 100 and 1000 mg/kg bw/d, 6 hours/day, under semi-occlusive dressing (ca 10% of the total body area). Dermal exposure of rats to triflumizole at a concentration of 1000 mg/kg bw/d for 21 days resulted in a significant increase in relative liver weight of males. A slight increase in the incidence of vacuolar fatty change in the livers of females of the high-dose group (1000 mg/kg bw/d) was seen, as well as an increase in the severity of the effect. It cannot be excluded that this effect is test substance related. The number of animals with skin inflammation was slightly higher in the high-dose groups compared to the control groups. These data do no justify classification as STOT RE 2, since adverse effects were seen at exposure level higher than the extrapolated guidance value of ca 800 mg/kg bw/d.

<u>Neurotoxicity</u>

In the re-evaluation of the 13-week neurotoxicity study (Goldenthal, 2004; Addendum to DAR, February 2009), the effects on motor activity were considered not adverse because there was no dose-response relationship, there were no effects in females, and the changes in locomotor activity were within the normal range of behaviour. In the chronic toxicity/carcinogenicity study in rats, the incidence of convulsive episodes was above the background range at the highest dose of 1600 ppm in females (77 mg/kg bw/d), far above the guidance value for STOT RE 2. This dose level also induced severe general toxicity, with liver being the main target organ. The suggested NOAEL of 400 ppm (18 mg/kg bw/d) for convulsions is higher than the NOAEL for general toxicity (100 ppm; 4.5 mg/kg bw/d). The observed decrease in brain butyrylcholinesterase activity at 54 weeks in the same study was considered not toxicologically relevant (Addendum to DAR,

February 2009). Therefore, the neurotoxicity data from the long-term studies with triflumizole does not fulfil the classification criteria for STOT RE 2. Based on the lack of the specific neurotoxic effects in the acute, 28-day and 90-day repeated toxicity studies, it is not justified to classify triflumizole as STOT RE based on neurotoxic effects.

In summary, RAC is of the opinion that the effects of triflumizole observed in a 28-day repeated dose toxicity study meets the CLP criteria for classification as STOT RE 2, taking into account the significance and severity of the adverse effects occurring after oral exposure at the level below, or very close to, the respective guidance values. RAC also takes into account the adverse effects in the liver of rats in a 2-year study which were seen at doses of 14 and 18 mg/kg bw/d (males and females, respectively) i.e. very close to the extrapolated guidance value of 12.5 mg/kg bw/d. RAC has also considered that a different selection of doses in the 90-day repeated toxicity studies in rats and mice, respectively, instead choosing exposure doses just below the respective guidance values, could have revealed adverse effects of triflumizole meeting the classification criteria for STOT RE 2.

Hence, the liver effects seen in the 28-day repeated dose toxicity study in rats, the 2-year study in rats and the 90-day repeated dose toxicity studies in rats and mice, and also taking into account the consistency of the effects seen in these studies, are considered to support classification as STOT RE 2; H373 (May cause damage to organs (liver) through prolonged or repeated exposure).

4.9 Germ cell mutagenicity (Mutagenicity)

Table 22: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
In vitro			
OECD 471	The test substance did not induce point mutations in *S. typhimurium.*	5, 15.8, 50, 158, 500, 1575 and 5000 µg/plate (with and without S9-mix) solvent: DMSO	Nishibi, 1987[a]
OECD 471	The test substance did not induce point mutations in S. typhimurium or in *E. coli*.	8, 24, 80, 240, 800, 2400 and 8000 µg/plate (with and without S9-mix) solvent: DMSO	Inoue *et al.*, 1983 [a]
OECD 476	The test substance did not induce gene mutations in Chinese hamster V79 cells.	1.22, 2.44, 4.88, 9.75 and 19.5 µg/mL (- S9-mix); 9.75, 19.5, 39.0, 78.0 and 156 µg/mL (+S9-mix) solvent: DMSO	Seeberg and Forster, 1989 [a]
OECD 473	The test substance did not induce chromosome aberrations in Chinese hamster lung cells.	5, 10, 20 and 40 µg/mL (with and without S9-mix) solvent: DMSO	Nishibe, 1988 [a]
OECD 482	The test substance did not induce unscheduled DNA synthesis in mammalian cells.	12.5, 15.0, 20.0, 25.0, 30.0 and 40.0 µg/mL solvent: DMSO	Cifone, 1984 [a]
In vivo			
OECD 474	The test substance did not induce micronuclei in mouse bone marrow cells.	160, 533.3 and 1600 mg/kg bw, administered by single oral gavage, sacrifice 24 and 48 h after dosing vehicle: : DMSO	Ivett, 1984 [a]
OECD 474	The test substance did not induce micronuclei in bone marrow cells of Chinese hamsters.	1000, 2000 and 4000 mg/kg bw, by single oral application; sacrifice at 12, 24 and 48 h after dosing vehicle: 0.5% CMC	Mosesso, 1989 [a]

[a]As summarized in the DAR, updated addendum of February 2009

4.9.1 Non-human information

4.9.1.1 In vitro data

Study 1

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.* TA 98	point mut.	-	-	rat liver	Pheno-barbital	5, 15.8, 50, 158, 500, 1575 and 5000 µg/plate (with and without	Nishibi, 1987

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
TA 100 TA 1535 TA 1537	point mut. point mut. point mut.	- - -	- - -		and 5,6-benzo-flavone	S9-mix) solvent: Dimethylsulfoxide (DMSO)	
Test substance: Triflumizole, lot/batch no. NF-114, purity 98.2 %, pale yellow crystal Cytotoxicity observed at dose level: ≥50 µg/plate Precipitation observed at dose level: ≥1575 µg/plate GLP statement: yes According to OECD 471 (1983): yes							

The substances was tested in *Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537 strains at concentration levels 5, 15.8, 50, 158, 500, 1575 and 5000 µg/plate (with and without S9-mix), using DMSO as a solvent. Cytotoxicity observed at dose level: ≥50 µg/plate. Precipitation was observed at dose level ≥1575 µg/plate. The results were negative in all strains, both with and without metabolic activation.

Study 2

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.* TA 98 TA 100 TA 1535 TA 1537 TA 1538 B : *E. coli* WP2uvrA	 point mut. point mut. point mut. point mut. point mut. point mut.	 - - - - - -	 - - - - - -	rat liver	Phenobar bital and 5,6-Benzoflav one	8, 24, 80, 240, 800, 2400 and 8000 µg/plate (with and without S9-mix) solvent: Dimethylsulfoxide (DMSO)	Inoue *et al.*, 1983.
Test substance: NF-114, lot no. TK-1116, purity 98.6%, light yellow solid Cytotoxicity observed at dose level: ≥ 80 µg/plate Precipitation observed at dose level: not reported GLP statement: no According to OECD 471: yes							

The substance was tested in Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and *Escherichia coli* strain WP2uvrA at concentration ranges 8, 24, 80, 240, 800, 2400 and 8000 µg/plate (with and without S9-mix) in DMSO. The test substance did not induce point mutations in *S. typhimurium* or in *E. coli*.

Study 3

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	gene mutations (HGPRT)	-	-	rat liver	Phenobar-bital and betanaftho-flavone	1.22, 2.44, 4.88, 9.75 and 19.5 µg/mL (- S9-mix); 9.75, 19.5, 39.0, 78.0 and 156 µg/mL (+S9-mix) solvent: Dimethylsulfoxide (DMSO)	Seeberg and Forster, 1989.
Test substance: NF-114, batch no. TK-4121, purity 98.3%, light brown powder. Cytotoxicity observed at dose level: ≥ 39.1 µg/mL (-S9-mix) and ≥ 313 µg/mL (+S9-mix), observed in a separate toxicity test using dose levels of 1, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250 and 2500 µg/mL. Precipitation observed at dose level: ≥ 2500 µg/mL (the lowest concentration at which some of the substance formed a visible precipitation in DMSO) GLP statement: yes							

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
According to OECD 476: yes							

A gene mutation HGPRT test was performed in Chinese hamster V79 cells at concentration levels 1.22, 2.44, 4.88, 9.75 and 19.5 μg/mL without S9-mix, and 9.75, 19.5, 39.0, 78.0 and 156 μg/mL with S9-mix in DMSO. Cytotoxicity was observed at dose level: > 39.1 μg/mL (-S9-mix) and > 313 μg/mL (+S9-mix), observed in a separate toxicity test using dose levels of 1, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250 and 2500 μg/mL. Precipitation was observed at dose level > 2500 μg/mL. The test substance did not induce gene mutations in Chinese hamster V79 cells.

Study 4

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster lung (CHL) cells	chromosome aberration	-	-	rat liver	Phenobarbital and 5,6-Benzoflav one	5, 10, 20 and 40 μg/mL (with and without S9-mix) solvent: Dimethylsulfoxide (DMSO)	Nishibe, 1988.
Test substance: NF-114, lot no. TFB-020, purity 98.2%, pale yellow crystal Cytotoxicity observed at dose level: ≥ 40 μg/mL (-S9-mix and +S9-mix) Precipitation observed at dose level: not reported GLP statement: yes According to OECD 473: yes							

Triflumizole was tested in a chromosome aberration assay according to OECD Guideline 473 with Chinese hamster lung (CHL) cells at concentration levels 5, 10, 20 and 40 μg/mL(with and without S9-mix), using DMSO as a solvent. In the first range finding test, performed with doses of 51.2, 128, 320, 800, 2000 and 5000 μg/mL, complete cell lethality was observed at all doses (with and without S9-mix). In the second range finding test, doses tested were 3.2, 8, 20 and 50 μg/mL (with and without S9-mix). Reduction in cell growth was only seen in the 20 and 50 μg/mL dose groups. The test substance did not induce chromosome aberrations in Chinese hamster lung cells.

Study 5

Indicator cells	Endpoint	Result	Dose range	Reference
primary rat hepatocytes	DNA repair (unscheduled DNA synthesis)	-	12.5, 15.0, 20.0, 25.0, 30.0 and 40.0 μg/mL solvent: Dimethylsulfoxide (DMSO)	Cifone, 1984
Test substance: NF-114, lot no. TK 3081, purity 98.7%, off-white powder Cytotoxicity observed at dose level: ≥ 40 μg/mL Precipitation observed at dose level: not observed GLP statement: yes According to OECD 482: yes				

The test substance did not induce unscheduled DNA synthesis in mammalian cells when tested in primary rat hepatocytes at concentration levels 12.5, 15.0, 20.0, 25.0, 30.0 and 40.0 μg/mL in DMSO.

4.9.1.2 In vivo data

Study 1

Species	Endpoint	Result	Dose range	Reference
mouse, CD-1 5/sex/dose	micronuclei (bone marrow)	-	160, 533.3 and 1600 mg/kg bw, administered by single oral gavage sacrifice 24 and 48 h after dosing vehicle: : Dimethylsulfoxide (DMSO)	Ivett, 1984
Test substance: NF-114, lot no. TK 3081, purity 98.7%, off-white powder GLP statement: no According to OECD 474: yes				

The substance was tested in a mouse bone marrow micronucleus assay according to OECD guideline 474. Triflumizole was administered at dose levels of 160, 533.3 and 1600 mg/kg bw, by single oral gavage to 5 CD-1 mice/sex/dose, followed by sacrifice 24 and 48 h after dosing, using DMSO as a vehicle. All the animals of the high (1600 mg/kg bw) and medium (533.3 mg/kg bw) dose group and several animals of the low (160 mg/kg bw) dose group had difficulty breathing. Approximately 24 hrs after dosing one female of the 24 hrs exposure group, administered with 1600 mg/kg bw, was observed to convulse. Before the second sacrifice, after 48 hrs, two high dose males were found dead. The remaining high dose males seemed barely alive. However, all animals from the medium and low dose groups appeared healthy. The test substance did not induce micronuclei in mouse bone marrow cells.

Study 2

Species	Endpoint	Result	Dose range	Reference
Chinese hamster, 5/sex/dose	Chromosome aberration (bone marrow)	-	1000, 2000 and 4000 mg/kg bw, by single oral application; sacrifice at 12, 24 and 48 h after dosing vehicle: 0.5% carboxymethylcellulose sodium salt (CMC)	Mosesso, 1989
Test substance: Triflumizole, batch no. TK-4121, purity 98.3%, pale yellow powder Toxicity observed at dose level: 5000 mg/kg bw (mitotic inhibition and poor quality of metaphases) GLP statement: no According to OECD 474: yes				

Triflumizole was also tested in a chromosome aberration assay in Chinese hamsters (5/sex/dose) at dose levels of 1000, 2000 and 4000 mg/kg bw, by single oral application in 0.5% carboxymethylcellulose sodium salt, followed by a sacrifice at 12, 24 and 48 h after dosing. A preliminary test showed no lethality at concentrations up to 5000 mg/kg bw. The test substance did not induce chromosome aberrations in bone marrow cells of Chinese hamsters.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

In vitro, triflumizole tested negative in point mutation tests with *S. typhimurium* strains TA 98, 100, 1535, 1537 and 1538 and *E. coli* strain WP2uvrA, in a gene mutation test with Chinese hamster V79 cells, in a chromosome aberration test with Chinese hamster lung cells and in an unscheduled DNA synthesis test with rat primary hepatocytes.
In vivo, triflumizole tested negative in a micronucleus test in mice and in a chromosome aberration test with Chinese hamsters, both with bone marrow as the observed target organ. Based on these tests, triflumizole does not possess genotoxic potential.

4.9.5 Comparison with criteria

The available data base indicates that triflumizole is not genotoxic.

4.9.6 Conclusions on classification and labelling

It is not necessary to classify triflumizole for mutagenicity according to 67/548/EEC or EC 1272/2008.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In vitro, triflumizole tested negative in point mutation tests with *S. typhimurium* strains TA 98, 100, 1535, 1537 and 1538 and *E. coli* strain WP2uvrA (Nishibi, 1987; Inoue *et al.*, 1983), in a gene mutation test with Chinese hamster V79 cells (Seeberg and Forster, 1989), in a chromosome aberration test with Chinese hamster lung cells (Nishibe, 1988) and in an unscheduled DNA-synthesis test with rat primary hepatocytes (Cifone, 1984).
In vivo, triflumizole tested negative in a micronucleus test in mice (Ivett, 1984) and in a chromosome aberration test with Chinese hamsters (Mosesso, 1989), both with bone marrow as the observed target organ. Based on these tests, according to the DS, triflumizole does not possess genotoxic potential.

Comments received during public consultation

No comments were received for this hazard class during public consultation.

Assessment and comparison with the classification criteria

The available data base indicates that triflumizole is not mutagenic in *in vitro* and *in vivo* assays. Triflumizole does not warrant classification for mutagenicity according to CLP criteria.

4.10 Carcinogenicity

Table 23: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
2-year combined toxicity/ carcinogenicity study in rats	$NOAEL_{carcinogenicity}$ 1600 ppm (59 mg/kg bw/day), no evidence of carcinogenicity was found. No increase in neoplastic lesions	Animals received doses of 0, 100, 400, 1600 ppm in food (equal to 0, 3.5, 14, 59 mg/kg bw/d for males and 0, 4.5, 18, 77 mg/kg bw/d for females)	Virgo et al., 1984[a]
2-year combined toxicity/ carcinogenicity study in mice	NOAEL 1600 ppm (296 mg/kg bw/day), no evidence of carcinogenicity was found. No increase in neoplastic lesions	Animals received doses of 0, 100, 400, 1600 ppm in food (equal to 0, 16, 67, 296 mg/kg bw/d for males and 0, 22, 88, 362 mg/kg bw/d for females)	Yamagata *et al.*, 1984[a]

[a]**As summarized in the DAR, updated addendum of February 2009**

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Two combined chronic toxicity/carcinogenicity studies were performed, in which, respectively, rats and mice were exposed through the diet to 100, 400 or 1600 ppm triflumizole. The studies were conducted mostly in accordance with OECD guideline 453.

Study 1

reference	:	Virgo *et al*, 1984	exposure	:	104 weeks, in diet
type of study	:	combined toxicity/carcinogenicity study	doses	:	0, 100, 400, 1600 ppm[1]
year of execution	:	1981-1983	vehicle	:	None
test substance	:	NF-114, lot no. TK-1116, purity 98.6%, brown, crystalline powder	GLP statement	:	No (study performed before GLP existed)
route	:	oral	guideline	:	Mainly in accordance with OECD 453
species	:	Rat, CD	acceptability	:	acceptable
group size	:	70/sex/dose (and 10/sex/dose for interim kills)	**LOAEL**	:	100 ppm (3.5 mg/kg bw/d)

[1] Equal to 0, 3.5, 14, 59 mg/kg bw/d for males and 0, 4.5, 18, 77 mg/kg bw/d for females

In the rat study, after 54 weeks, 10 animals per sex per group were killed (satellite group). In these small groups, no treatment-related effect on neoplasms incidence was found. In addition, in animals killed or deceded during the treatment period, no treatment-related effects on tumour incidence occurred. There was a lower incidence of fibromas in the subcutis of males which had received the highest dosage of triflumizole ($P<0.01$) than in controls. In animals killed after 104 weeks of treatment the incidence of neoplasms did not suggest an effect of the administration of triflumizole. There were, however, lower than control incidences of both benign and malignant mammary gland fibroepithelial tumours and pituitary adenomas in females which had received the highest dosage of triflumizole (statistically significant in all cases). It is concluded that triflumizole when fed to CD rats for 104 weeks, did not increase the incidence of tumours, nor shorten the induction period of tumours, nor alter the type of tumours found; indeed there were generally fewer tumours in rats treated at 1600 ppm than controls. This is probably a result of the lower bodyweight gain and food consumption observed in rats which had received the highest dosage of triflumizole.

Results of the chronic study in rats.

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
Mortality	52/70	31/70	50/70	47/70	46/70	25/70	32/70	22/70	
Clinical signs									
-convulsive episodes[1]	3/80 (2)	0/80 (0)	4/80 (4)	2/80 (2)	2/80 (2)	2/80 (0)	6/80 (1)	15/80 [tc] (2)	
Body weight gain							dc (24%)[tc]	dc (35%)[tc]	
Food consumption							dc (12%)[tc]	dc (10%)[tc]	
Ophthalmoscopy	No treatment-related effects								
Haematology	No treatment-related effects								
Clinical chemistry									
-ALAT							ic		
-brain butyryl-cholinesterase (wk 54)				dc (24%)	dc (29%)	dc (22%)	dc (22%)	dc (16%)	
-brain butyryl-cholinesterase (wk 104)								ic (20%)	
Urinalysis	No treatment-related effects								
Organ weights									
-liver					ic[r] (19%)[c]		ic[r] (34%)[tc]	ic[r] (41%)[tc]	
-ovaries[2]								ic[a, r] (88[c], 185%)[tc]	
-kidneys							ic[r] (36%)[c]	ic[r] (37%)[tc]	
Pathology									
macroscopy									
-liver, swollen						ic		ic	
-liver, dark, depressed area						ic		ic	
-liver, pale								ic	
-cystic ovary								ic	
microscopy *neoplastic lesions*	No treatment-related effects								
microscopy *non-neoplastic lesions*									
Liver									
-fatty vacuolation: -periacinar hepatocytic					ic	ic	ic	ic	m,f
hypertrophy: -basophilic foci/ hepatocellular alteration				ic	ic	ic	ic	ic	
						ic		ic	
-eosinophilic foci/ hepatocellular alteration								ic	
-focal inflammation/ necrosis						ic		ic	
-hyaline degeneration/ fibrosis of bile ducts						ic		ic	
Pancreas									
-lobular acinar atrophy							ic		

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
Ovary -follicular cysts								ic	

Study 2

reference	:	Yamagata *et al.*, 1984	exposure	:	104 weeks, in diet
type of study	:	combined toxicity/carcinogenicity study	doses	:	0, 100, 400, 1600 ppm[1]
year of execution	:	1981-1983	vehicle	:	None
test substance	:	NF-114, lot no. TK-116, purity 98.6%, light yellow solid	GLP statement	:	Yes
route	:	Oral	guideline	:	Mainly in accordance with OECD 453
species	:	Mouse, SPF, $B6C3F_1$	acceptability	:	acceptable
group size	:	50/sex/dose (and 10/sex/dose for 3 interim kills)	**NOAEL**	:	100 ppm (16 mg/kg bw/d)

[1] Equal to 16, 67, 296 mg/kg bw/d for males and 22, 88, 362 mg/kg bw/d for females.

In the mouse study (B6C3F1), interim kills were performed in weeks 26, 52 and 78 after initiation of exposure. An increase in the number of hepatic nodules was observed in all male dose groups and the mid-and high female dose groups, in comparison to the control group. The term ‘nodule’ has been used in (early) scientific publications to denote an adenoma. However, the terms "hepatic nodule" and "hepatocellular adenoma" are listed individually in the study report, and are, therefore, apparently no synonyms. Furthermore, the term "hepatic nodule" is not commonly used, while the term "hyperplastic nodule" is synonym with hepatocellular adenoma. In this summary it has therefore been assumed that these two terms "hepatic nodule" and “hepatocellular adenoma" indicate different lesions. Whatever the definition of "hepatic nodule", the authors of the study report consider it a non-neoplastic lesion. In view of the absence of a clear dose-effect relationship in males at the two lower doses, and the not significant increase in incidence observed in females of these dose groups, only the highest dose is considered to have resulted in a treatment-related increase of hepatic nodules.

Results of the chronic study in mice.

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
Mortality	8/50	4/50	4/50	5/50	11/50	7/50	2/50	8/50	
Clinical signs	No treatment-related effects								
Body weight gain							dc (44%)	d (10%)	
Food consumption	No treatment-related effects								
Ophthalmoscopy	not performed								
Haematology -WBC (wk 26)					dc (44%)		dc (44%)		
-WBC (wk 52)			dc (33%)		dc (57%)		dc (47%)		
-WBC (wk 78)			d (44%)		dc (63%)		d (44%)		
-WBC (wk 104)			dc (60%)		dc (44%)		d (40%)		
Clinical chemistry -inorganic phosphate							ic (25%)[1]	ic (26%)[2]	

Dose (ppm)	0		100		400		1600		dr
	m	f	m	f	m	f	m	f	
-GOT							ic (116%)[3]		
-GPT							ic (400%)[2]		
Urinalysis	no treatment-related effects								
Organ weights									
-liver (wk 26)					ic[r] (14%)	ic[a] (13%)	ic[a,r] (38, 59%)	ic[a,r] (40, 40%)	
-liver (wk 52)					ic[r] (11%)	ic[a] (9%)	ic[a,r] (41, 61%)	ic[a,r] (33, 39%)	
-liver (wk 78)							ic[a,r] (33, 46%)	ic[a,r] (32, 46%)	
-liver (wk 104)							ic[a,r] (39, 69%)		
-kidneys (all)							ic[r] (19%)[2]		
Pathology									
Macroscopy									
-liver enlargement					+	+	+	+	
-liver, white zone						+	+	+	
-liver nodule							+	+	
microscopy *neoplastic lesions*	no treatment-related effects								
microscopy *non-neoplastic lesions*									
Liver									
-hepatic nodule	8/60	5/60	15/60	7/60	12/60	9/60	20/60	17/60	f
-fatty metamorphosis	11/50		17/50		20/50	17/50	30/50	24/50	m,f
-granulomatous inflammation					+		+		m
-cytological alterations					+		++		
-pigmentation					+		++		f
-necrosis					+		++	+	m
Kidneys									
-regenerating epithelium					+		+	+	
Spleen									
-pigmentation							++		

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

None

4.10.4 Summary and discussion of carcinogenicity

There are no indications that triflumizole is carcinogenic.

4.10.5 Comparison with criteria

There are no indications that triflumizole is carcinogenic.

4.10.6 Conclusions on classification and labelling

Triflumizole does not have to be classified for carcinogenic effects.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS did not propose to classify triflumizole as a carcinogen, based on the data summarised in Table 2 below.

Table 2. Summary of carcinogenicity studies

Method	Results	Remarks	Reference
2-year combined toxicity/ carcinogenicity study in rats	$NOAEL_{carc}$ 1600 ppm (59 and 77 mg/kg bw/d in males and females, respectively), no evidence of carcinogenicity was found. No increase in neoplastic lesions.	Animals received doses of 0, 100, 400, 1600 ppm in food (equal to 0, 3.5, 14, 59 mg/kg bw/d for males and 0, 4.5, 18, 77 mg/kg bw/d for females)	DAR (Virgo *et al.*, 1984)
2-year combined toxicity/ carcinogenicity study in mice	NOAEL 1600 ppm (296 and 362 mg/kg bw/d, in males and females, respectively), no evidence of carcinogenicity was found. No increase in neoplastic lesions.	Animals received doses of 0, 100, 400, 1600 ppm in food (equal to 0, 16, 67 and 296 mg/kg bw/d for males and 0, 22, 88 and 362 mg/kg bw/d for females)	DAR (Yamagata *et al.*, 1984)

Comments received during public consultation

No comments were received for this hazard class during public consultation.

Assessment and comparison with the classification criteria

Taking into account the negative results in the carcinogenicity studies in rats and mice, RAC is of the opinion that triflumizole does not meet the classification criteria for carcinogenicity.

4.11 Toxicity for reproduction

Table 24: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
OECD 416 2-generation toxicity study in rats	Parental: NOAEL is 4.8 mg/kg bw/day (70 ppm), increased liver and kidney weights at LOAEL of 12 mg/kg bw/day Developmental: NOAEL is 4.8 mg/kg bw/day (70 ppm), reduced litter size at LOAEL of 12 mg/kg bw/day Reproduction: NOAEL is 4.8 mg/kg bw/day (70 ppm), matin/fertility parameters, macroscopy male reproductive organs at LOAEL of 12 mg/kg bw/day	doses: 0, 30, 70, 170 ppm (equivalent to 0, 2.1, 4.8, and 12 mg/kg bw/d for F0 males, 0, 2.5, 5.8 and 14 mg/kg bw/d for F0 females, 0, 2.6, 5.8, and 13 mg/kg bw/d for F1 males and 0, 2.8, 6.6, and 16 mg/kg bw/d for F1 females)	Tesh et al., 1984[a]
OECD 414 Teratogenicity study in rats	Maternal: NOAEL is 10 mg/kg bw/day, reduced body weight, food consumption, water intake, increased liver and spleen weight at LOAEL of 35 mg/kg bw/day Developmental: NOAEL is 10 mg/kg bw/day, reduced viability, body weight, increased resorptions, placental weight at LOAEL of 35 mg/kg bw/day No teratogenicity effects: NOAEL is >120 mg/kg bw/day	Doses: 0, 10, 35 and 120 mg/kg bw/day	Nishibe et al, 1983h[a]
OECD 414 Teratogenicity study in rabbits	Maternal: NOAEL is 100 mg/kg bw/day, reduced body weight, food consumption, ovary weight, increased liver, spleen weight at LOAEL of 200 mg/kg bw/day Developmental: NOAEL is 100 mg/kg bw/day, reduced survival rate, body weight, decreased placental weight at LOAEL of 200 mg/kg bw/day No teratogenicity effects: NOAEL is >200 mg/kg bw/day	Doses: 0, 50, 100 and 200 mg/kg bw/day	Hattori, 1985[a]

[a]As summarized in the DAR, updated addendum of February 2009

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Study 1

Reference	:	Tesh *et al.*, 1984	exposure	:	continuously through the study period
type of study	:	2-generation study	doses	:	0, 30, 70, 170 ppm[1]
year of execution	:	1982-1983	vehicle	:	diet
test substance	:	NF-114, lot no. TK 1116, purity 98.6%, fine fawn powder	GLP statement	:	no
Route	:	oral	guideline	:	partly in accordance with OECD 416 (1983)
Species	:	rat, Charles River CD	acceptability	:	acceptable
group size	:	15-30/sex/dose (see study design)	**NOAEL**par	:	70 ppm (4.8 mg/kg bw/d)
			NOAELdev	:	70 ppm (4.8 mg/kg bw/d)

reproductive effects : yes, at 170 ppm (12 mg/kg bw/d)

[1] Equivalent to 0, 2.1, 4.8, and 12 mg/kg bw/d for F0 males, 0, 2.5, 5.8 and 14 mg/kg bw/d for F0 females, 0, 2.6, 5.8, and 13 mg/kg bw/d for F1 males and 0, 2.8, 6.6, and 16 mg/kg bw/d for F1 females. **Note**: food consumption was only measured in the first 13 weeks before the first pairing.

A two-generation study was conducted with CD rats who received triflumizole continiously in diet at concentration levels 0, 30, 70 and 170 ppm (equal to 0, 2.1, 4.8, and 12 mg/kg bw/d for F0 males, 0, 2.5, 5.8 and 14 mg/kg bw/d for F0 females, 0, 2.6, 5.8, and 13 mg/kg bw/d for F1 males and 0, 2.8, 6.6, and 16 mg/kg bw/d for F1 females). Food consumption was only measured in the first 13 weeks before the first pairing. Thirty F0 animals were treated for 13 weeks before pairing twice in succession. The first pairing produced the F1A litters, which were discarded at weaning. After the second pairing, half of the females (n=15) were killed on Day 21 *post coitum* to permit teratological examination and the remainder were allowed to litter (F1B litters) from which the F1 generation was selected. This procedure was repeated for the F1 generation: thirty F1 animals were treated for 13 weeks before pairing twice in succession. The first pairing produced the F2A litters, which were discarded at weaning. After the second pairing, half of the females were killed on Day 21 *post coitum* to permit teratological examination and the remainder of females were allowed to litter (F2B litters). From the F2B litter, 10 animals/sex/dose were selected and treated for 13 weeks after weaning. Another group of 30 animals/sex/dose from the F2B litter underwent the abovementioned procedure, however, the results of this third generation were not described in the report. Physical development and auditory and visual function were examined in the F1B and F2B litters. Selected animals (10/sex/dose) from the F0 and F1 parents (after 29 weeks of treatment), and the F2 adults (13 weeks after weaning) were subjected to a detailed necropsy procedure and several organs were weighed. Microscopic examination was performed according to the following scheme:

F0 parental animals, high dose and control groups: 5/sex/dose full tissue list and 5/sex/dose reproductive organs only

F1 generation, high dose and control groups: 5/sex/dose full tissue list and 5/sex/dose reproductive organs only

F2 generation (13 weeks), all dose groups, 10/sex/dose full tissue list

All other animals were examined externally and internally for macroscopic abnormalities. In addition to the OECD (1983), organ weights of several organs were determined, and more tissues were examined than indicated in OECD (1983).
The study was performed partly in accordance with OECD 416 (1983); the main deviations were (1) the reproductive organs of not all parental animals were subjected to a full histopathological examination, and (2) food consumption was only measured until the animals were mated for the first pairing.
Dose levels were based on the results of preliminary studies.

The main effects were observed at the high dose of 170 ppm. Slight developmental toxicity was observed as the litter size of the F1A generation was decreased, hence the NOAEL for developmental effects is set at 70 ppm, corresponding to 4.8 mg/kg bw/day (LOAEL 12 mg/kg bw/day). Effects on reproduction consisted of increased gestation length in both generations, and in the second generation of decreased conception rate, fertility and percentage mating, and an increased incidence of changes in male reproductive organs. Minimal changes in male reproductive organs were also observed at the low and mid dose group, however, these changes did not correspond with concomitant decrease in mating/fertility parameters and is therefore considered not adverse at these 2 dose levels. Parental toxicity consisted of increased kidney weights at 70 and 170 ppm, and increased liver weights at 170 ppm. Moreover, one pregnant female in the high dose

group died of dystocia, and a relation to treatment cannot be excluded. The parental effects at 70 ppm were rather slight, significantly affecting only absolute kidney weights of females of the F1-generation. Therefore, effects at this dose level are not considered adverse. The NOAEL for parental effects is consequently set at 70 ppm, equal to 4.8 mg/kg bw/day.

Results of the 2-generation study.

Dose (ppm)	**0**		**30**		**70**		**170**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals[1]									
Mortality	none								
Clinical signs	no treatment-related effects[2]								
Body weight	no treatment-related effects								
Food consumption (pre-mating)	no treatment-related effects								
Mating/fertility/gestation									
first pairing (F1A)									
- gestation length								ic (2%)	
second pairing (F1B)	no treatment-related effects								
Organ weight (n=10)	no treatment-related effects								
Pathology									
macroscopy	no treatment-related effects								
microscopy (n=10)			-	-	-	-	no treatment-related effects		
F1 pups									
Litter size									
- F1A								dc (12%)	
- F1B	no treatment-related effects								
Survival index	no treatment-related effects								
Sex ratio	no treatment-related effects								
Body weight	no treatment-related effects								
Physical development	no treatment-related effects								
Auditory/visual function	no treatment-related effects								
Pathology									
macroscopy	no treatment-related effects								
F1 animals[1]									
Mortality	1	0	0	0	0	0	0	1[3]	
Clinical signs	no treatment-related effects[2]								
Body weight	no treatment-related effects								
Food consumption	no treatment-related effects								
Mating/fertility/gestation									
First pairing (F2A)									
- conception rate							d (14%)	d (13%)	

Dose (ppm)	0		30		70		170		dr
	m	f	m	f	m	f	m	f	
- fertility							d (16%)	d (16%)	
- % mating							d (4%)		
- gestation length								i (1.5%)	
Second pairing (F2B)	no treatment-related effects								
Organ weight (n=10)									
- liver							$i^{a,r}$ (11, 15%)	$ic^{a,r}$ (14, 10%)	
- kidneys						ic[a] (12%), i^{r} (7%)		$ic^{a,r}$ (13, 11%)	f
Pathology									
macroscopy									
- changed colour/size of reproductive organs	0/30		3/30		3/30		7/30		
microscopy (n=10)			-	-	-	-	no treatment-related effects		
F2 pups									
Litter size	no treatment-related effects								
Sex ratio	no treatment-related effects								
Body weight	no treatment-related effects								
Physical development	no treatment-related effects								
Auditory/visual function	no treatment-related effects								
Pathology									
macroscopy	no treatment-related effects								
F2 animals (13 weeks)									
Organ weight (n=10)									
- kidneys							i^{a}, ic^{r} (6, 9%)	$i^{a,r}$ (12, 8%)	
Pathology									
macro/microscopy (n=10)	no treatment-related effects								

4.11.1.2 Human information

No data available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Study 1

reference	:	Nishibe et al, 1983h	exposure	:	days 6-16 of gestation, gavage
type of study	:	teratogenicity study	doses	:	0, 10, 35, 120 mg/kg bw/d
year of execution	:	1982	vehicle	:	5% Arabic gum aqueous solution
test substance	:	Technical NF-114, lot no. YS-200, purity 98.7%, white crystals	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 414 (1981)
species	:	rat, Sprague-Dawley (Crj:CD)	acceptability	:	acceptable
group size	:	24 females/dose	**NOAEL**mat	:	10 mg/kg bw/d
			NOAELdev	:	10 mg/kg bw/d
			teratogenic effects	:	not observed

A teratogenicity study was performed with triflumizole in Sprague-Dawley rats (24 females/dose). The study was performed in accordance with OECD guideline 414 (1983). In addition the following organs of the dams were weighed: liver, kidney, spleen, ovaries, adrenals. The test substance was administered on days 6-16 of gestation by gavage in 5% Arabic gum aqueous solution at dose levels of 0, 10, 35 and 120 mg/kg bw/day. Maternal effects were observed at dose levels of 35 and 120 mg/kg bw/day, and consisted of significant reductions in body weight, food consumption, water intake, and significant increases of spleen and liver weight. At these dose levels a reduction in the number of viable foetuses and in foetal body weight, and an increase in the number of late resorptions and increased placental weight was also observed. The macroscopic findings in foetuses of all treated groups were considered either not treatment-related in the absence of a dose-response, or not considered adverse as the lesion is observed more often as a spontaneous finding. The NOAEL for maternal and developmental effects is therefore set at 10 mg/kg bw/d.
The late resorption in a high dose female might potentially have been caused by a teratological effect in the foetuses. However, since placental weight and the number of late resorptions increased as the dose level increased, it was considered in the DAR that the late resorption had occurred as an effect through the placenta damaged by the test substance, rather than a direct effect on foetuses. Therefore it is considered that triflumizole did not produce a teratogenic response in rats in this teratogenicity study.
The effects on maternal body weight (minus uterus and contents) are only 5% in the mid dose group and 8% in the high dose group. It is unlikely that such a small effect on body weight would increase post implantation loss or cause other foetal effects. In addition, the individual data (confidential) show that, in 8 animals of the mid dose group with body weights between 306 and 348 g and in 4 animals of the high dose group with body weights between 305 and 319 g, 3 or more late resorptions were found. In these animals, body weight is comparable of that of controls (body weight control animals range from 301.4 - 370.4). This indicates that it is unlikely that the late resorptions (at least in these animals) are caused by maternal toxicity.

Results of the developmental study in rats

Dose (mg/kg bw/d)	**0**	**10**	**35**	**120**	**dr**
Maternal effects					
Mortality	none				
Clinical signs	no treatment-related effects				
Pregnant animals	24	24	24	24	
Body weight gain			dc (16%)	dc (20%)	
Body weight minus uterus and contents			dc (5%)	dc (8%)	
Food consumption			dc (8%)	dc (13%)	
Water intake			d (2%)	dc (9%)	

Dose (mg/kg bw/d)	0	10	35	120	dr
Organ weight - liver - spleen **Pathology** macroscopy			i[r] (6%) i[a,r] (10, 17%) no treatment-related findings	i[r] (11%) i[a,r] (14, 24%)	
Litter response **Live foetuses/pregnant female** **Foetal weight** - males - females **Placental weight** **Post implantation loss** - late resorptions or deaths **Sex ratio**	(1)	(0)	dc (20%) dc (6%) ic (73%) ic (66) no treatment-related effects	dc (20%) dc (7%) dc (8%) ic (86%) ic (73)	
Examination of the foetuses **External observations** **Skeletal findings** - 14th rib **Visceral findings** - renal pelvic dilatation	(11) (13)	no treatment-related effects i (16) ic (29)	i (16) i (24)	ic (55) ic (25)	

Study 2

reference	:	Hattori, 1985	exposure	:	days 6-18 of gestation, gavage
type of study	:	teratogenicity study	doses	:	0, 50, 100, 200 mg/kg bw/d
year of execution	:	1983-1984	vehicle	:	1.5% Arabic gum aqueous suspension
test substance	:	NF-114, lot no. TK-3081, purity 98.7%, pale yellow powder	GLP statement	:	no
route	:	oral	guideline	:	in accordance with OECD 414 (1983)
species	:	rabbit, New Zealand White	acceptability	:	acceptable
group size	:	15 females/dose	**NOAEL**mat	:	100 mg/kg bw/d
			NOAELdev	:	100 mg/kg bw/d
			teratogenic effects	:	not observed

The second teratogenicity study according to OECD guideline 414 was performed with New Zealand White rabbits (15 females/dose). In addition the following organs of the dams were weighed: heart, lung, liver, kidney, spleen, thymus, ovaries, adrenals. The test substance was administered by gavage at dose levels 0, 50, 100 and 200 mg/kg bw/day as a suspension in 1.5% Arabic gum aqueous solution on days 6-18 of gestation. Maternal effects were observed at a dose level of 200 mg/kg bw/d and consisted of a reduction of food consumption and body weight, ovary weight and an increase in liver and spleen weight. At the foetal observations, lower survival rate and decreased body weights and placental weight were observed at 200 mg/kg bw/d. The

reduction in food consumption at 100 mg/kg bw/day was only slight and had no concurrent effect on body weight. It is therefore not considered to be adverse, and the NOAEL for maternal and developmental effects is set at 100 mg/kg bw/d. No teratogenic effects were observed in this teratogenicity study in rabbits.

Results of the developmental study in rabbits

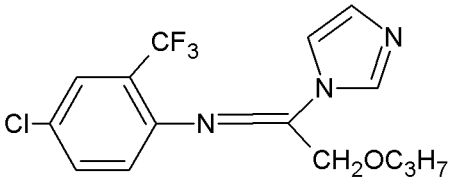
Dose (mg/kg bw/d)	**0**	**50**	**100**	**200**	**dr**
Maternal effects					
Mortality	none				
Clinical signs	no treatment-related effects				
Pregnant animals	13	14	13	14	
Abortions	0	0	0	0	
Body weight gain				dc (25%)	
Food consumption			dc (4%)	dc (11%)	
Organ weight					
- liver				ic[a,r] (15, 22%)	
- spleen				ic[r] (23%)	
- ovaries				dc[a] (22%)	
Pathology					
macroscopy	no treatment-related findings				
Litter response					
Live foetuses	no treatment-related findings				
Foetal weight				dc (13%)	
Placental weight				dc (16%)	
Post implantation loss	no treatment-related effects				
Sex ratio	no treatment-related effects				
24-h Survival rate				dc (21%)	
Examination of the foetuses					
External observations	no treatment-related effects				
Skeletal findings	no treatment-related effects				
Visceral findings	no treatment-related effects				

4.11.2.2 Human information

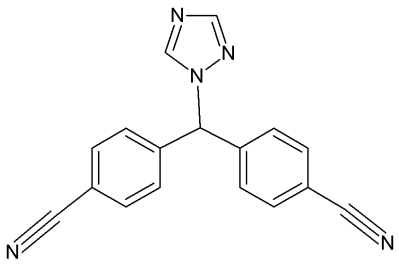
No data available

4.11.3 Other relevant information

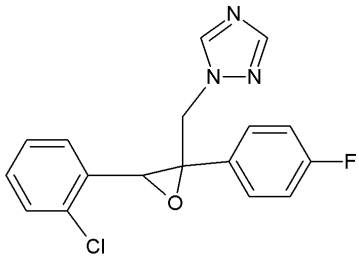
Late resorptions (and placental effects) are also observed after exposure to other azoles (epoxiconazole, letrozole).



triflumizole



letrozole



epoxiconazole

Epoxiconazole causes an increase in late resorptions at doses ≥ 45 mg/kg bw/day (i.e. similar dose level as triflumizole). Mechanistic studies in which epoxiconazole is administered together with estradiol cyclopentylproprionate (ECP) showed that in rats, depletion of estradiol (by administration of epoxiconazole) resulted in placental damage and late resorptions. Co-administration with ECP dose-relatedly increased (but not normalized) the estradiol serum levels and reduced the effect on placental damage and late resorptions. In guinea pigs, estradiol levels and placentas and the number of late resorptions or post-implantation loss were not affected. Clearly, there is a species difference with regard to the late resorptions (Additional information report for a Substance under Harmonised Classification and Labelling Process: Epoxiconazole (available at http://echa.europa.eu/documents/10162/1a1bc71c-1543-423b-849e-137a606205d4)). However, there are no adequate data that the mechanism (endocrine disruption) observed in rats is not relevant for humans. Therefore, it cannot be excluded that the effects observed in rats (and rabbits) can also occur in humans.

4.11.4 Summary and discussion of reproductive toxicity

In a 2-generation reproduction study rats were exposed through the diet to 30, 70 or 170 ppm triflumizole. Parental toxicity resulted in increased liver and kidney weights in the F1-animals at the highest dose level. Based on these effects the NOAEL for parental toxicity was set at the next lower dose level, equal to 4.8 mg/kg bw/day (LOAEL 12 mg/kg bw/day). Also at the highest dose level developmental (reduced litter size) and reproductive effects (reduced mating/fertility) were observed. Therefore the NOAELs for reproduction and development were likewise set at 4.8 mg/kg bw/day (LOAEL 12 mg/kg bw/day). However, the effects on fertility and male reproductive organs were not consistent between the different generations.

Two teratogenicity studies were executed with rats and rabbits, which were exposed by gavage to, respectively 10, 35 or 120 mg/kg bw/day and 50, 100 and 200 mg/kg bw/day.
Maternal toxic effects were virtually identical in both species and comprised of reduced body weight and food consumption and increased liver and spleen weights. Based on these effects, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day (LOAEL 35 mg/kg bw/day) for rats and 100 mg/kg bw/day (LOAEL 200 mg/kg bw/day) for rabbits. In the rat, the observed developmental effects were a reduction in the number of viable foetuses and in foetal body weight, and an increase in the number of late resorptions and increased placental weight, while in the rabbit a lower pup survival rate, decreased pup weights and decreased placental weight were observed. Based on these respective effects, the NOAEL for developmental toxicity was set at 10 mg/kg bw/day (LOAEL 35

mg/kg bw/day) for rats and 100 mg/kg bw/day (LOAEL 200 mg/kg bw/day) for rabbits. In neither study irreversible structural effects were observed (NOAEL >120 mg/kg bw/day for rats and >200 mg/kg bw/day for rabbits).

4.11.5 Comparison with criteria

The effects on fertility were small and only seen in one generation but not repeated in another mating or in another generation. Also the changed colour/size of the reproductive organs was only observed macroscopically in adult F1 animals but not confirmed microscopically and not observed in the 13-week F2 animals and not in the repeated dose toxicity studies. These effects are considered as incidental findings. Therefore, no classification for effects on fertility is required.

A clear increase in late resorptions and related decrease in live fetuses per pregnant female was observed at the two highest dose levels in the developmental study in rats. A reduced body weight gain, food consumption and water intake was also observed in the dams. However, it is unclear whether the foetal effects were secondary to the maternal toxicity or whether the maternal effects where due to the decrease in fetuses present in the womb. Nevertheless, the effects on maternal body weight minus uterus and contents was less than 10%. In addition, the individual data show that late resorptions in the mid and high dose group does also occur in dams with a normal body weight. It is therefore unlikely that the late resorptions are caused by maternal toxicity. The strong increase in placental weight may be an indication that the observed increase in post-implantation loss is not a direct effect of the substance on the fetus.
For another azole (epoxiconazole), which also induced late resorptions and increased placental weights, it is shown that depletion of estradiol results in placental damage and late resorptions. This is considered to be an effect on development and not to be a secondary non-specific consequence of maternal toxicity. As for triflumazole, a species difference was observed as late resorptions occurred in rats, but not in guinea pigs. Seen the resemblance in molecular structure and developmental effects between triflumizole and epoxiconazole, it is very likely that the increase in late resoptions with triflumizole are induced via the same mechanism and should also be considered as specific. However, there are no adequate data that the mechanism (endocrine disruption) observed in rats is not relevant for humans. It can therefore not be excluded that the effects observed in rats can also occur in humans.

In the developmental study in rabbits, there was decreases in foetal weigth and placental weight which are considered to be secondary to the observed maternal toxicity (decreased body weight gain, decreased food consumption and changes in certain organ weights) at the highest dose level. For the reduced 24-h survival rate it is unclear whether this could be secondary to the observed maternal toxicity. However, seen the reduced foetal weight this cannot be excluded.

4.11.6 Conclusions on classification and labelling

An increase in post implantation loss in the developmental study in rats (Nishibe et al., 1983h) was observed at the two highest dose levels. The effects are unlikely to be caused by maternal toxicity, since they are also observed in dams with normal body weights. The strong increase in placental weight might indicate that the observed embryo toxicity is not be a direct effect of triflumizole on the embryo. In addition, data from another azole (epoxiconazole) have shown that the mechanism for the late resorptions is endocrine disruption. Seen the resemblance in molecular structure and developmental effects between triflumizole and epoxiconazole, it is very likely that the increase in late resoptions with triflumizole are induced via the same mechanism and should also be considered as specific. There is no information showing that the mechanism (endocrine disruption) is not

relevant for humans. Therefore, it is proposed to classify triflumizole as Repr Cat 1B; H360D and DSD Cat 2; R61.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify triflumizole as Repr. 1B; H360D based on the results of the studies summarised in Table 3 below.

Table 3. Summary of relevant reproductive toxicity studies according to the DS.

Method	Results	Remarks	Reference
2-generation toxicity study in rats OECD TG 416	Parental NOAEL: 4.8 mg/kg bw/d (70 ppm); increased liver and kidney weights at LOAEL of 12 mg/kg bw/d. Developmental NOAEL: 4.8 mg/kg bw/d (70 ppm); reduced litter size at LOAEL of 12 mg/kg bw/d Reproduction NOAEL: 4.8 mg/kg bw/d (70 ppm); effects on mating/fertility parameters, and macroscopy of male reproductive organs at LOAEL of 12 mg/kg bw/d.	Doses: 0, 30, 70, 170 ppm (equivalent to 0, 2.1, 4.8 and 12 mg/kg bw/d for F0 males, 0, 2.5, 5.8 and 14 mg/kg bw/d for F0 females, 0, 2.6, 5.8, and 13 mg/kg bw/d for F1 males and 0, 2.8, 6.6 and 16 mg/kg bw/d for F1 females)	Tesh *et al.*, 1984
Teratogenicity study in rats OECD TG 414	Maternal NOAEL: 10 mg/kg bw/d; reduced body weight, food consumption, water intake, and increased liver and spleen weight at LOAEL of 35 mg/kg bw/d. Developmental NOAEL: 10 mg/kg bw/d; reduced viability, body weight, increased resorptions, and placental weight at LOAEL of 35 mg/kg bw/d. No teratogenicity effects, NOAEL: >120 mg/kg bw/d.	Doses: 0, 10, 35 and 120 mg/kg bw/d	Nishibe *et al.*, 1983h
Teratogenicity study in rabbits OECD TG 414	Maternal NOAEL: 100 mg/kg bw/d; reduced body weight, food consumption, ovary weight and increased liver and spleen weight at LOAEL of 200 mg/kg bw/d. Developmental NOAEL: 100 mg/kg bw/d; reduced survival rate and body weight, and decreased placental weight at LOAEL of 200 mg/kg bw/d. No teratogenicity effects, NOAEL: >200 mg/kg bw/d.	Doses: 0, 50, 100 and 200 mg/kg bw/d	Hattori, 1985

The DS's argument for classification was that an increase in post implantation loss in the developmental study in rats (Nishibe et al., 1983h) was observed at the two highest dose levels, and was unlikely to be caused by maternal toxicity since they are also observed in dams with normal body weights. The strong increase in placental weight might indicate that the observed embryo toxicity is not be a direct effect of triflumizole on the embryo.

In addition, data from another azole (epoxiconazole) have shown that the mechanism for the late resorptions is endocrine disruption. Considering the resemblance in molecular structure and developmental effects between triflumizole and epoxiconazole, it is very likely that the increase in late resoptions with triflumizole are induced via the same mechanism and should also be considered as specific. There is no information showing that the mechanism (endocrine disruption) is not relevant for humans. Therefore, it is proposed to classify triflumizole as Repr Cat 1B; H360D.

Comments received during public consultation

One MS agreed with the proposed classification as Repr 1B; H360D.

One MS proposed that Repr. 2 would be more appropriate taking into account that no teratogenic effects were seen, and that the increased post-implantation loss occurred only at dose levels where maternal toxicity was also seen.

One industry comment presented an extensive review of data and arguments that classification as a suspected human reproductive toxicant (Repr. 2) is a more appropriate than category 1B. In particular, they submitted 14 published papers during public consultation with the aim to compare the mode of action of different azoles and demonstrate that triflumizole does not induce teratogenic effects. However, the DS responded in the RCOM that there is no conclusive information available on the mode of action of triflumizole itself. The DS agreed that for triflumizole, endocrine disruption is a possible (hypothetical) mechanism for the developmental effects, but not a proven mechanism. The DS further argued that there were no adequate data to exclude the relevance for humans.

Assessment and comparison with the classification criteria

Fertility and sexual function

1) In a non-GLP range-finding reproductive toxicity study (Tesh & Willoughby, 1982), groups of 6 male and 6 female Sprague-Dawley rats were treated with triflumizole in the diet at doses of 0, 400 or 1200 ppm (equal to approximately 0, 20 and 60 mg/kg bw/d) for 2 weeks prior to mating, throughout the mating period, gestation and lactation, and up to termination after day 21 postpartum.

The mean litter size was slightly reduced at 400 ppm (10.5 versus 13.3 for controls; statistical significance not known), but the number of live births and viability were the same as for controls. Gestation length in the group receiving 400 ppm was increased by 1 day compared to controls, but the gestation index (number of live litters born/number of pregnant dams × 100) was not affected. No other differences were noted in this dose group compared to controls. Body weight gain in females was reduced compared to controls over gestation days (GD) 0–13 (approximately 25% less than control body weight gain; $P < 0.01$).

Of the 6 females in the 1200 group, only one gave birth to live foetuses and survived. Of the three female rats that gave birth, one gave birth to live foetuses but was killed in extremis on postpartum day 1; necropsy revealed retained dead foetuses *in utero*. The other female gave birth to dead foetuses. Of the 3 females that did not give birth, one female was found dead on day 23 postcoitum, and one female was killed in extremis on day 24 postcoitum. Both of these females had dead foetuses *in utero*. The remaining female did not deliver a litter, and necropsy revealed evidence of one early resorption. Necropsy of animals that died or were killed in extremis did not reveal any abnormalities other than the dead foetuses. In surviving foetuses in the high-dose group, foetal birth weight was reduced compared with controls, but it increased during lactation. The study revealed developmental toxicity seen as reduced number of live foetuses at birth and

increased number of dead foetuses at birth.

2) In a three-generation reproductive toxicity study (Tesh, Willoughby & Whitney, 1984, quoted from Triflumizole IMPR, 2013), groups of 30 male and 30 female CD rats were treated with doses of triflumizole (purity not stated) in the diet at doses of 0, 70, 170 or 420 ppm (equal to 0, 4.8, 11.7 and 29.0 mg/kg bw/d for F0 males and 0, 5.5, 13.5 and 33.3 mg/kg bw/d for F0 females). Continuous dietary administration of triflumizole at 420 ppm resulted in slight decreases in body weight gain, increased length of estrous cycles, reduced vaginal cornification and extended precoital interval. Eventual mating performance and conception rate were unaffected, but gestation length was extended, and severe parturition difficulties resulted in maternal death and high perinatal mortality of offspring. In surviving offspring, body weight gain to weaning was reduced. At a dose of 170 ppm, similar but less severe effects on gestation length, parturition and perinatal mortality were observed. At a dose of 70 ppm, only slight increases in gestation length were observed. There were no organ weight changes or pathological findings in males that were considered related to treatment at any dose. There was a marginal increase in relative liver weights in females in the 420 ppm dose group, but no microscopic findings. The only treatment-related macroscopic finding in offspring was a statistically significant increase in the incidence of hydronephrosis at 420 ppm. In conclusion, the NOAEL for parental and reproductive effects was 70 ppm (equal to 4.8 mg/kg bw/d). Based on the results observed at 170 and 420 ppm, the decision was made to terminate this study after weaning of the F1A litters and to conduct a second study at levels of 0, 30, 70 and 170 ppm in the diet (see below; Tesh *et al.*, 1984).

3) In a three-generation reproductive toxicity study (Tesh *et al.*, 1984), groups of 30 male and 30 female CD rats were treated with triflumizole in the diet at doses of 0, 30, 70 or 170 ppm (equal to 0, 2.1, 4.8 and 12 mg/kg bw/d for F0 males and 0, 2.5, 5.8 and 14 mg/kg bw/d for F0 females; 0, 2.6, 5.8 and 13 mg/kg bw/d for F1 males and 0, 2.8, 6.6, and 16 mg/kg bw/d for F1 females; and 0, 2.6, 6.0 and 15 mg/kg bw/d for F2 males and 0, 3.0, 6.9 and 16 mg/kg bw/d for F2 females).

Parental toxicity consisted of increased kidney weights at 70 and 170 ppm and increased liver weights at 170 ppm. Moreover, placental weights were increased at 170 ppm in all generations. The parental effects at 70 ppm were slight, significantly affecting only absolute kidney weights of females of the F1 generation. Therefore, effects at this dose level are not considered adverse. The percentage of mating, conception rates and fertility indexes were not affected by treatment in F0, F1 and F2 generations neither in the first (F1A-F3A) nor the second pairing (F1B-F3B).

At 170 ppm, an increased gestation length was observed in the first two generations. At 170 ppm, the litter size of the F1A generation was decreased; however, this was not seen in any generation thereafter. At 170 ppm in the F3A generation, statistically significant reductions were seen in live birth index, birth weight and viability index. However, these effects were seen only in this third generation and were not consistent or dose related in the other generations.

The NOAEL for parental toxicity was 70 ppm (equal to 4.8 mg/kg bw/d), based on increased placental weights and increased liver and kidney weights at the high dose (170 ppm, equal to 12 mg/kg bw/d).

The LOAEL for offspring toxicity was 170 ppm (equal to 12 mg/kg bw/d), the highest dose tested (Tesh, Willoughby & Secker, 1986) at which a decrease in litter size in the F1A generation and reduced live birth index, birth weight and viability index in the F1B generation were observed; however, these effects were most probably related to developmental toxicity of triflumizole because they were mostly seen at birth.

Taking into account the above data, RAC is of the opinion that triflumizole does not affect sexual function and fertility, since the effects observed in the studies were presumably

induced by the action of triflumizole on developing foetuses *in utero*, suggesting that triflumazole induces developmental toxicity.

Developmental toxicity

1) In a non-GLP developmental toxicity study in rats (Nishibe *et al.*, 1983), groups of 24 pregnant female Sprague-Dawley (Crj:CD) rats were treated with 0, 10, 35 or 120 mg/kg bw/d triflumizole by gavage on GD 6–16. The study was performed partly in accordance with OECD TG 414.

Maternal effects were observed at dose levels of 35 and 120 mg/kg bw/d and consisted of significant reductions in body weight gain (16 and 20%, respectively), feed consumption (8 and 13%) and water intake (2 and 9%) and of significant increases in spleen (17 and 24%) and liver (6 and 11%) weights. At the same dose levels, a reduction in the number of viable foetuses (by 20%), a reduction in foetal weight (6 and 7%), an increase in the number of late resorptions (18.1 and 19.8%) and increased placental weight (73% and 86%) were also observed. At the highest dose, there was an increase in foetuses with 14th rudimentary rib as compared to the low- and mid-dose groups, but the percentage of foetuses affected was comparable to that in controls. The incidences of renal pelvic dilatation showed no dose–response relationship in the percentage of foetuses affected. Therefore, these histopatological findings were not considered adverse. The study demonstrated moderate developmental toxicity of triflumizole, although without teratogenic effects, at doses showing moderate maternal toxicity.

2) In a developmental toxicity study in rats, groups of 24 pregnant female Sprague-Dawley (Crj:CD) rats were treated from GD 6 up to GD 16 with triflumizole by gavage at doses of 0, 3, 7 or 35 mg/kg bw/d (Gotoh, 1986). The study was performed partly in accordance with OECD Test Guideline 414.

Maternal toxicity was evident at 35 mg/kg bw/d. Body weight gain was reduced compared with controls over GDs 17–18 as well as over the whole dosing interval (-18% and -15%, respectively; $P < 0.05$). The reduction in mean body weight gain was accompanied by reductions in feed consumption on GD 7 and daily over GDs 12–19, ranging from -9% to -16% of control values. No statistically significant differences were noted in absolute body weight or water intake. Changes in organ weights could not definitively be considered adverse effects of treatment. The placental weight was significantly increased compared with controls (+45%). Gross necropsy did not reveal any adverse effects of treatment. No adverse effects of treatment were observed in females at 3 or 7 mg/kg bw/d.

Evidence of developmental toxicity was observed at 35 mg/kg bw/d. The incidence of late resorptions/dead foetuses was significantly increased (17% versus 0% for controls/4.8% versus 0% for controls, respectively). Although the number of viable foetuses was slightly reduced compared with controls (13.2 versus 14.1 for controls), this reduction did not attain statistical significance. Foetal weight was not affected. No statistically significant, treatment-related external, visceral or skeletal malformations or variations were noted.

Table 4. Foetal toxicity in a developmental toxicity study in rats (Gotoh, 1986)

Litter response	**Dose (mg/kg bw/d)**			
	0	**3**	**7**	**35**
Live foetuses/ pregnant female	14.1	14.9	14.3	13.2 (-6%)

Dead or resorbed foetuses	23	13 (-43%)	24	35 (+52%)
- Early deaths	23	12	21	18
- Late deaths	0	1	3	17*

*: $P < 0.05$;

Although the number of viable foetuses was slightly reduced compared to controls (13.2 vs. 14.1 for controls), this reduction did not attain statistical significance. Foetal weight was not affected. No statistically significant, treatment-related external, visceral or skeletal malformations or variations were noted. The increased number of late resorptions/dead foetuses at 35 mg/kg bw/d is concordant with the same effect observed in a developmental toxicity study in rats (Nishibe *et al.*, 1983)

3) In a non-GLP developmental toxicity study (Hattori, 1985) in rabbits, groups of 15 pregnant female New Zealand White rabbits were treated with triflumizole by gavage at doses of 0, 50, 100 or 200 mg/kg bw/d.

Treatment with triflumizole produced maternal toxicity, as shown by reduced body weight and feed consumption, at 200 mg/kg bw/d. A slight, temporary depression of feed consumption was also recorded at 100 mg/kg bw/d. Treatment with triflumizole did not result in any external, visceral or skeletal malformations. Pups from the high-dose group had a reduced 24-h survival rate (77% vs. 98% for controls) and statistically significantly reduced pups weight when compared with controls. Values were also low when compared with the laboratory historical control data. No other effects on reproduction or foetal development were noted.

4) In the other developmental toxicity study in rabbits (Keller, 1988), groups of 16 pregnant female New Zealand White rabbits were treated with triflumizole by gavage at doses of 0, 5, 25 or 50 mg/kg bw/d at GD 7–19. There was no clear evidence of maternal toxicity at any of the doses used. No effects on foetal development were noted.

Taking into account that oral exposure of female rats and rabbits to triflumizole, at doses causing moderate maternal toxicity, lead to reduced number of live foetuses at birth, increased number of dead foetuses at birth, increased number of late resorptions, reduction of foetal weight and increased placental weight, RAC is of the opinion that triflumizole warrants classification as Repr. 1B - H360D (May damage the unborn child).

It is considered unlikely that the late resorptions were caused by maternal toxicity because triflumizole exposure only slightly reduced maternal body weight gain and food consumption. The strong increase in placental weight may be an indication that the observed increase in late resorptions is not a direct effect of the substance on the foetus and might be due to placental dysfunction.

Late resorptions (and placental effects) were also observed after exposures to other azoles (epoxiconazole, letrozole). For epoxiconazole, it has been shown that depletion of estradiol resulted in placental damage and late resorptions. This was considered to be an effect on development and not to be a secondary non-specific consequence of maternal toxicity. For epoxiconazole, a species difference was observed as late resorptions occurred in rats, but not in Guinea pigs. Mechanistic studies, in which epoxiconazole was administered together with estradiol cyclopentylproprionate (ECP), showed that in rats, depletion of estradiol (by administration of epoxiconazole) resulted in placental damage and late resorptions. Co-administration with ECP led to a dose-dependent increase (but not normalisation) of the estradiol serum levels and reduced the effect on placental damage and late resorptions. In Guinea pigs, estradiol levels, placentas and the incidence of late resorptions or post-implantation loss were not affected. Clearly, there was a species difference with regard to the late resorptions. However, there were no adequate

data to show that the mechanism (endocrine disruption) observed in rats was not relevant for humans. Therefore, it could not be excluded that the effects observed in rats (and rabbits) could also occur in humans.

In summary, RAC concludes that classification of triflumizole as Repr. 1B; H360D is justified, based on clear adverse effects observed after triflumizole exposure in a rat developmental toxicity study (reduced number of live foetuses at birth, increased number of late resorptions and death, as well as reduced foetal weights) and also taking into account the decreased 24-h survival rate in a rabbit developmental toxicity study. RAC concludes that the observed effects across a number of studies, co-occurring with only slight to moderate maternal toxicity, justifies the classification as Repr. 1B:H360D, even without additional comparison with other azole substances (e.g. letrozole and epoxiconazole).

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Method	Results	Remarks	Reference
Acute oral neurotoxicity study in rats, single exposure	NOAEL of 25 mg/kg bw Critical effects: clinical findings and functional and motor activity effects No specific neurotoxic effects	Animals received doses of 0, 25, 100, 400 (males)/200 (females) mg/kg bw	Goldenthal, 2003[a]
13-week neurotoxicity study in rats	$NOAEL_{neurotoxicity}$ of 117 mg/kg bw/day No specific neurotoxic effects	Animals received doses of 0, 70, 700, 2000 ppm (equal to 0, 4.1, 41, 117 mg/kg bw/d for males and 4.9, 48, 133 mg/kg bw/d for females)	Goldenthal, 2004[a]

[a]As summarized in the DAR, updated addendum of February 2009

Study 1

reference	:	Goldenthal, 2003	exposure	:	once by gavage
type of study	:	acute oral neurotoxicity study	doses	:	0, 25, 100, 400 (males)/200 (females) mg/kg bw
year of execution	:	2003	vehicle	:	1% carboxymethylcellulose
test substance	:	triflumizole, lot no. TBC-343, purity 99.2%, off-white powder	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 424
species	:	rat, CD® [Crl: CD® (SD)IGS BR]	acceptability	:	acceptable
group size	:	11/sex/dose	**NOAEL**	:	25 mg/kg bw

Acute neurotoxicity study was performed in accordance with OECD guideline 424. Animals received a single dose of 0, 25, 100, 400 (males)/200 (females) mg/kg bw by gavage in 1% carboxymethylcellulose. Clinical observations and FOB evaluations were performed on days -4, 1 (before and 2 h after test substance administration), 8 and 15. Motor activity evaluations were conducted immediately following each FOB interval. Following neurobehavioral evaluations 6 animals per sex/dose were randomly selected for neuropathology.

No specific neurotoxic effects of the test substance were observed in this study. Based on the clinical findings, the functional and motor activity effects observed at the next higher dose, the NOAEL of this study was set at 25 mg/kg bw.

Study 2

reference	:	Goldenthal, 2004	exposure	:	13-weeks, in diet
type of study	:	13-weeks oral neurotoxicity study	doses	:	0, 70, 700 and 2000 ppm[1]
year of execution	:	2003	vehicle	:	none
test substance	:	triflumizole, lot no. TBC-343, purity 99.2%, off-white powder	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 424
species	:	rat, CD® [Crl: CD® (SD)IGS BR]	acceptability	:	acceptable
group size	:	16/sex/dose	**LOAEL**	:	70 ppm (4.1 mg/kg bw/d)

[1] Equal to 0, 4.1, 41, 117 mg/kg bw/d for males and 4.9, 48, 133 mg/kg bw/d for females.

The 90-day neurotoxicity study with CD rats was performed in accordance with OECD guideline 424. Dose levels were based on the results of unspecified previous studies. The test substance was administered for 13 weeks in diet at dose levels of 0, 70, 700 and 2000 ppm, equal to 0, 4.1, 41 and 117 mg/kg bw/day for males and 4.9, 48, 133 mg/kg bw/day for females. The clinical observations which may indicate neurotoxicity included autonomic effects such as salivation and nervous system effects including tremors, convulsions, reactivity to handling and bizarre behaviour. Functional observational battery (FOB) evaluations were conducted on designated animals without knowledge on the part of the testers of the treatment groups. Examinations were conducted on ten animals/sex/group prior to initiation of exposure to the test article, and during Weeks 4, 8, and 13 of test article administration. The same animals were tested at all time points. During open-field evaluations, each animal was observed for a minimum of three minutes in a black plexiglass, open-field observation box measuring 20 x 24 x 8 inches.
The parameters evaluated in the FOB included, but were not limited to, evaluation of activity and arousal, posture, rearing, bizarre behaviour, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration. The amount of defecation and urination was also recorded. Forelimb and hind limb grip strength and hind limb splay were measured. Pain perception was assessed by measuring the latency of response to a nociceptive (thermal) stimulus when each animal was placed on a hot plate apparatus set to 52 ± 1 °C. Body weight and temperature were also measured.
All ten animals/sex/group designated for behavioural testing were also tested for motor activity prior to initiation of exposure to the test article and during the Weeks 4, 8, and 13. Activity was assessed by placement in a Digiscan° Activity Monitor measuring 16 by 16 by 12 inches and equipped with a computer analyser. Animals were monitored (recorded) for three consecutive 10-minute intervals (ca. 10 seconds per interval) allowing for examination of both exploratory and acclimation activity levels. Movement was recorded by 16 photocells each in two horizontal and one vertical plane. Two of these planes were used to record horizontal activity and intersected at right angles to form a grid pattern. The third plane was located above the first two and recorded vertical activity. A range of different activities were recorded but only the following were used in comparisons between treated and control animals as the most representative activity parameters: horizontal activity, vertical activity, total distance (centimetres), and stereotypic behaviour.
Following the FOB and motor activity evaluations, six rats/sex/group were randomly selected for neuropathology evaluation from the ten rats/sex/group designated as behaviour test animals. Complete necropsies were performed on these animals. The brain (including cerebrum, cerebellum/pons and medulla oblongata), proximal sciatic nerve (2), sural nerve (2), tibial nerve (2), spinal cord including cervical swelling {C3-C6} and lumbar swelling (L1-L4), trigeminal ganglia (2), dorsal root ganglia (C3-C6, LI-L4), and dorsal and ventral root fibres (C3-C6, L1-L4) were collected. After fixation, both sciatic nerves with tibial, fibular and sural extensions were dissected

free from the carcass to a point below the hock from Groups 1 and 4. Those nerves were stapled onto cards, labelled left or right, proximal or distal. No neuropathology animals died on study or were euthanised in extremis. For the remaining animals (ten/sex/group), complete necropsy examinations were performed.
No cholinesterase activities were measured.

Within-session habituation
A clear habituation pattern was evident within a single session in all groups, *i.e.* male and female rats and at all time point measured (pretest, week 4, 8 and 13). No differences are observed between the dose groups. A significant decrease in horizontal activity was seen in control male rats in week 13 compared with the rats exposed to triflumizole. This decrease was observed for all motor activities.

Inter-session habituation
A unique inter-session habituation pattern was evident only in males of the control group (motor activity decreased constantly over the course of the study). This pattern was not seen in the treated males. A statistically significant test article-related change was observed in the magnitude of various calculated locomotor activity output measures (*i.e.* horizontal activity, vertical activity, total distance, and stereotypy) following 13 weeks of treatment in male rats. While male control animals demonstrated a decrease in the relative magnitude of locomotor activity parameters over the course of the study (*i.e.*, at successive evaluation intervals; pre-dose, Weeks 4, 8, 13), test article treated groups of male animals did not show this pattern of attenuated activity over time (*i.e.*, habituation). In the females, both in control and treatment groups, the inter-session habituation was not evident. While there did not appear to be any consistent dose-response pattern characterising this effect in male animals, the relatively increased levels of locomotor activity observed in the treated groups is considered test article-related. The locomotor findings in males are however not a clear adverse effects. The locomotor changes observed in this study were caused by the test article, but, according to the study author, the changes are within the normal range of behavior for rats.
Unfortunately there are no historical control data from the performing laboratory, but historical control data from other labs (same time period and same strain of rats) show that the decrease in motor activity over time in control male background data is less pronounced than control rats of the triflumizole study. Moreover, the motor activity data of treated rats fall within the range of the historical control data of control male rats. Therefore, the motor activity pattern of the rats exposed to triflumizole can be considered as not adverse.

Rearing
A statistically significant change in rearing was noted in males at 700 ppm and 2000 ppm at week 13 and 4, respectively. These effects were most likely related to the pretest difference in total number of rears between control rats and do not reflect test-article related changes in the functional behavior. There was no trend in the data, and there was no dose- or time-dependency. These effects, while statistically significant, are not considered to be physiologically relevant.
In conclusion, the effects on motor activity are considered not adverse because there is no dose-response relationship, there is no effects in females and the locomotor changes are within normal range of behavior.

Based on the liver effects, the NOAEL for non-neurotoxic effects is set at 70 ppm, equal to 4.1 mg/kg bw/d. The NOAEL for neurotoxicity is considered to be the highest dose tested, *i.e.* 2000 ppm (117 mg/kg bw/day).

In addition, in the 2-year chronic toxicity/carcinogenicity study with rats for triflumizole, convulsive episodes occurred at an increased incidence, compared to the controls, in animals receiving 1600 ppm. In animals receiving 0, 100, 400 or 1600 ppm, the incidence (in 80 animals) was 3, 4, 2 and 6 in males and 0, 2, 2 and 15 in females. When excluding the animals killed for interim examination at Week 54, the above incidences were the same (in this case, out of 70 animals), representing percentage incidences of 4.3, 5.7, 2.9 and 8.6% in males at 0, 100, 400 and 1600 ppm, respectively, and 0, 2.9, 2.9 and 21.4% in females at 0, 100, 400 and 1600 ppm, respectively. In males, the group incidence of convulsions was within the background study range (0-6.7%) for the controls and those receiving 100 or 400 ppm, but was slightly above the background range in those receiving 1600 ppm. In females, the incidence in controls was zero and that for females receiving 100 or 400 ppm was only minimally above the control range (0-2%). At 1600 ppm, the incidence of convulsions far exceeded the background range.
It is concluded that the incidence of convulsive episodes among controls and animals receiving 100 or 400 ppm was consistent with that reported in a range of similar studies and only at the highest dietary concentration (1600 ppm) was the incidence above the background range, particularly in females. Based on these results, 1600 ppm was considered an effect level for convulsions and 400 ppm as a NOAEL for convulsions.
In the same study, reduced levels of brain butyrylcholinesterase were observed at week 54 in all the female dose groups and the two highest male dose groups compared to control groups. However, the decreases were not dose-related and were not evident at 104 weeks. After one year of exposure, brain butyrylcholinesterase values in most groups (control and exposed) were ca. 2 times higher than the values after two years of exposure. Apparently the temporal variation is larger than any treatment-related effect. After two years, no consistent decrease in plasma or erythrocyte cholinesterase activity was observed, nor a decrease in brain acetylcholinesterase. Based on the current knowledge on brain butyrylcholinesterase activity (see above) and the absence of a dose-response, the decrease in brain butyrylcholinesterase activity is considered not toxicologically relevant.

4.12.1.2 Immunotoxicity

No immunotoxic studies were available for the DAR. There are no indications from acute and repeated dose studies that triflumizole has immunotoxic properties.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

4.12.2 Summary and discussion

There are no indications that triflumizole is neurotoxic or immunotoxic.

4.12.3 Comparison with criteria

There are no indications that triflumizole is neurotoxic or immunotoxic.

4.12.4 Conclusions on classification and labelling

In the acute neurotoxicity study, no specific neurotoxic effects of the test substance were observed.

After reevaluation of the semichronic neurotoxicity study (Addendum to DAR February 2009), the effects on motor activity were considered not adverse because there was no dose-response relationship, there was no effects in females and the locomotor changes were within normal range of behavior.

In the chronic toxicity/carcinogenicity study with rats, the incidence of convulsive episodes was above the background range at the highest dose of 1600 ppm in females. However, this dose level also induced severe general toxicity, with liver being the main target organ. The suggested NOAEL of 400 ppm for convulsions is higher than NOAEL for general toxicity (100 ppm). The observed decrease in brain butyrylcholinesterase activity at 54 weeks in the same study is considered not toxicologically relevant.

Based on the lack of the specific neurotoxic effects in the acute and 90-day neurotoxicity study, it is not necessary to classify triflumizole as STOT SE and STOT RE for neurotoxicity according to 67/548/EEC or EC 1272/2008.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of triflumizole were assessed in the Draft Assessment Report, the Addendum to the Draft Assessment Report and Assessment Report prepared in the context of the possible inclusion of triflumizole in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2005 and subsequent addendum February 2007, February 2009, May 2009, RMS The Netherlands) concerning the placing of plant protection products on the market.

Based on a review of the available data on aquatic toxicity, a change in the environmental classification is not needed. The summaries included in this proposal are partly copied from the DAR, its addenda and assessment reports. Details of some of the summaries were not included when not considered important for a decision on the classification and labelling of this substance. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda.

5.1 Degradation

Table 25: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis: guideline EPA proposed in Federal Register Vol. no 132, non-GLP	DT50[a] values at 20 ºC 0.5 mg/L pH 3: 18.5 hours (corresponding[b] to 0.8 days) pH 6: 519 hours (corresponding[b] to 21.6 days) pH 9: 111 hours (corresponding[b] to 4.6 days) 5 mg/L pH 3: 18.5 hours (corresponding[b] to 0.8 days) pH 6: 472 hours (corresponding[b] to 19.6 days) pH 9: 92.0 hours (corresponding[b] to 3.83 days)	Test substance: Triflumizole, ca. 99% purity	Anonymous, 1981

Hydrolysis: guideline EPA 161-1 not under GLP	DT50[a] values at 20ºC 5 mg/L, nominal concentration 10x diluted buffer concentration pH 5: 13 days pH 7: 101 days pH 9: 6 days	Test substance: Triflumizole, > 99% purity	Soeda Y & Shiotani H, 1987[c]
Photolysis SETAC ; OECD draft guideline	DT50 of 12.3 days under natural sunlight conditions	Test substance: Triflumizole, ca. 99.6% purity	Noorloos B, 2005[c]
Aerobic water sediment study: OECD 308 GLP	DT50 values = days Goorven system (sand) **[^{14}C-phenyl] [^{14}C-imidazole]** DT50,water 1.9 2.6 DT50,sediment 105 114 DT50,system 48.7 64 Schoonrewoerdse Wiel system (clay loam) **[^{14}C-phenyl] [^{14}C-imidazole]** DT50,water 3.1 3.5 DT50,sediment 209 138 DT50,system 117 123	Test substance: Triflumizole, ca. 99.6% purity	Willems H, 2005a[c]

[a]Converted from 25˚C data. RMS converted DT50 values to 20ºC using the Arrhenius equation.
[b]NL-CA converted DT50 values from hours to days.
[c]As summarized in the DAR, updated addendum of February 2009, Volume 3, Annex 8B

5.1.1 Stability

Hydrolysis

A non-GLP hydrolysis experiment tested according to a EPA guideline proposed in Federal Register Vol. no 132 was conducted in sterile buffers at temperatures of 25 ºC and 50 ºC at three pH levels (3, 6 and 9) and two concentrations (0.5 and 5 mg/L) (Anonymous, 1981).
More than 90% triflumizole equivalents were recovered. The study indicates that hydrolysis occurs and is pH dependent. DT50 values for Triflumizole are provided in Table 26. Triflumizole hydrolyses quickly under acidic conditions with a DT50 of less than 1 day. The substance was most stable at pH 6 with DT50 ranging from 472 to 519 hours between the two concentrations (DT50 ca. 20 days). Data on the percentages of metabolites were not provided, however the DAR reports FD-1-1 as the major degradation product.

Table 26: DT50 (hours) for hydrolysis of triflumizole at various temperatures and pH pH value	**DT50 value as calculated by RMS from the results**					
	20* °C		**25 °C**		**50 °C**	
	0.5 mg/L	**5 mg/L**	**0.5 mg/L**	**5 mg/L**	**0.5 mg/L**	**5 mg/L**
3	18.5	18.5	12.4	12.4	1.34	1.31
6	519	472	348	317	58.8	50.8
9	111	92	74.4	61.8	2.28	3.0

*Converted data from 25ºC. RMS converted DT50 values to 20ºC using the Arrhenius equation.

In a second study conducted under guideline EPA 161-1 not under GLP the hydrolysis rate was investigated at different buffer concentration at 25 ºC and pH 5, 7 and 9 over a period of 30 days (Soeda Y & Shiotani H, 1987). The test was carried out at a nominal concentration of 5 mg/L. The ^{14}C recoveries for all test tubes were between 89.6 and 106.9%. The pH was stable up to the end of the experiment.

The hydrolysis rate was affected by the concentration of buffer solute. . Table 27 shows the DT50 values (in days) for hydrolysis of triflumizole. The results are converted from 25˚C to 20˚C. DT50 values at different concentrations and pH values at 20 ºC were, pH 5: 5.2 to 17.3 days, pH 7: 20.7 to 171 days and pH 9: 4.9 to 22.8 days. Triflumizole is degraded almost completely into FD-1-1 (84-93.3%, 40.8% for the 50x dilution).

Table 27 DT50 (hours) for hydrolysis of triflumizole at different pH values and buffer concentrations.

pH value	**DT50 value [d] at different buffer concentrations at 25 °C**				**DT50 value [d] at different buffer concentrations at 20* °C**			
	1 x	**5 x**	**10 x**	**50 x**	**1 x**	**5 x**	**10 x**	**50 x**
5	3.5	7.7	8.7	11.6	5.2	11.5	13	17.3
7	13.9	46.2	68.2	115	20.7	68.8	101	171
9	3.3	3.8	4.0	15.3	4.9	5.7	6.0	22.8

*Converted data from 25 °C

Remarks from RMS: To minimise the influence of buffer solute the authors take into consideration the values from the 10x dilution only. At this dilution, pH values were still stable enough. RMS can agree on this. The DT50 values from the 10x dilution, at 20˚C were used for risk assessment.

Photolysis

In a non-GLP, non-guideline photodegradation study, the DT50 value of triflumizole in natural light was 32.6 hours respectively). One metabolite, FD-1-1, was formed at > 5% radioactivity (53% of initial applied radioactivity after 96 hours). In the experiment with artificial light, DT50 value of 56

minutes was established. One metabolite FM-3-1 was formed at > 5% (17.2% of initial radioactivity after 180 minutes). This metabolite was not formed in the experiment with natural light. The RMS considered the study with artificial light not acceptable.
A second study, a GLP-compliant SETAC; OECD draft guideline, was performed using Xenon lamp artificial light (Noorloos, B,2005). The DT50 under artificial light was 4.1 days and in the dark control, it was 13.7 days. The calculated photolytic half-life was 5.9 days. Three metabolites at concentrations >10% were detected.
Based on sunlight intensity of 25.87 W/m^2 at 40ºN and the average light intensity of the Xenon lamp of 53.86 W/m^2 the photolytic half-life of triflumizole in the test system is equivalent to a DT50 of 12.3 days under natural sunlight conditions.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available.

5.1.2.2 Screening tests

There is no ready biodegradability study available.

5.1.2.3 Simulation tests

Water/sediment

Two aerobic water/sediment studies with two sediment systems were conducted according the OECD 308 guideline using [Phenyl-^{14}C] triflumizole or [imidazole-14C] triflumizole at 20°C (Willems H, 2005a).

Freshly sampled water and sediment from two locations in the Netherlands, Goorven and Schoonrewoerdse Wiel were collected and filtered (water:125 µm, sediment: 2mm). The systems were prepared so that a wet sediment layer of 2 cm was covered by approximately 6 cm overlying water.
In both studies the water was spiked with 0.208 mg/L radio-labeled triflumizole (0.288 MBq) corresponding to a field application of 0.624 kg as/ha[1]. Volatiles were trapped by PUF, a liquid MeEtOH trap and two NaOH traps. Test systems were incubated for 101 and 95 days and two flasks were harvested at several time intervals. The water layer was decanted and passed through a Buchner filter. Aliquots were used for LSC and a subsample of 200 ml was extracted with 100 mL dichloromethane three times. The dichloromethane layers were combined and the total radioactivity in the extract and the residual water layer was determined by LSC. To the combined dichloromethane phase 10 ml acetonitril and 0.2 ml 1% glycerol in acetone was added and the extract was concentrated to an endvolume of 10 mL. This was stored in the freezer until analysis by HPLC and TLC.
The sediment layer including the filter contents was transferred to a centrifuge bottle using 100 mL MeOH. After extraction and centrifugation the supernatant was removed and the procedure was repeated trice. Radioactivity in the combined MeOH extracts was determined by LSC. A subsample of 200 mL was concentrated to an endvolume of 20-30 mL. The concentrate was extracted three

[1] as/ha = active substance/hectare

times with 25 mL dichloromethane. The dichloromethane layers were combined and the total radioactivity of the extract and the residual waterphase was determined by LSC. To the combined dichloromethane phase 10 mL acetontril and 0.2 ml 1% glycerol in acetone was added and the extract was concentrated to an endvolume of 10 mL. This was stored in the freezer until analysis (HPLC and TLC).
Later on in the experiment with [Phenyl-^{14}C] triflumizole concentration and dichloromethane extraction were not performed but the MeOH extracts were analysed directly. .
For selected samples in the study with [imidazole-14C] triflumizole the residual water from the DCM extraction was freeze dried and the residual dissolved in MeOH/water 1:1 v/v. Furthermore the residual water of the Goorven system at t=3 was extracted once again with DCM after acidification to pH 3 but this was not done for other samples as no additional radioactivity was extracted in this way. The sediment layer including the filter contents was transferred to a centrifuge bottle using 100 mL MeOH. After extraction and centrifugation the supernatant was removed and the procedure was repeated trice. Radioactivity in the combined MeOH extracts was determined by LSC.
In both studies the post-extraction sediment was allowed to air dry prior to combustion/LSC analysis. For samples with unextractable residues ≥ 10% Soxhlet extraction with MeOH was performed. The radioactivity in the extract was determined by LSC. Organic volatiles were determined by extracting the PUF plugs with acetonitril and determining the radioactivity in the extract by LSC. HPLC and TLC analysis was performed on a selection of the extracts. The total radioactivity in the NaOH traps was determined by LSC. $^{14}CO_2$ was confirmed by adding Ba salt.

Results of the study with [Phenyl-^{14}C] triflumizole
The mass balance of the systems ranged between 89 and 104% for the Goorven system and between 95 and 99 % for the Schoonrewoerd system. The results of microbial biomass analysis (expressed as % of organic carbon) indicate viable conditions at the end of the incubation period. Up to 33.2 and 15.3% of applied radioactivity was recovered from the polyurethane foam (PUF) plugs for the Goorven and Schoonrewoerdse Wiel, respectively, which indicates a high loss through volatilisation. In the liquid volatile traps negligible amounts of radioactivity were found. $^{14}CO_2$ accounted for 0.17 and 0.29% in the two systems respectively after 101 days. The decrease of the amount of radioactivity in the water is mainly caused by rapid partitioning to the sediment. The total radioactivity in the sediment reached a maximum of 81% after 7 days (Goorven) or 80% after 14 days (Schoonrewoerdse Wiel) and then decreased to 45.5% or 72% respectively. The majority of the sediment associated radioactivity was extractable with MeOH.
Non-extractable residues before Soxhlet extraction amounted to 10 and 19% of the applied radioactivity for the two systems respectively.
By HPLC analysis, the identity of five metabolites was confirmed. FA-1-1 can be regarded as relevant metabolite in the water and sediment phase (>10% a.r. and/or 2x>5% a.r.). None of the other identified metabolites (FD-1-1; FM-6-1; FM-5-1 and FD-2-1) are considered potentially relevant.

Results of the study obtained with [imidazole-14C] triflumizole
The mass balance of the systems ranged between 86 and 98% for the Goorven system and between 90 and 99 % for the Schoonrewoerd system. The results of microbial biomass analysis (expressed as % of organic carbon) indicate viable conditions at the end of the incubation period. Only 0.66 and 0.15% of applied radioactivity was recovered from the polyurethane foam plugs for the Goorven and Schoonrewoerdse Wiel system respectively and in the liquid volatile traps negligible amounts of radioactivity were found. $^{14}CO_2$ accounted for 39.5 and 19.8% in the two systems respectively after 95/94 days hence mineralization of the imidazole moiety plays an important role.

The decrease of the amount of radioactivity in the water is mainly caused by rapid partitioning to the sediment. The total radioactivity in the sediment reached a maximum of 71.5%of applied r.a. after 14 days (Goorven) or 80% after 14 days (Schoonrewoerdse Wiel) and then decreased to 39% or 65.572% respectively. The majority of the sediment associated radioactivity was extractable with MeOH. Nonextractable residues before Soxhlet extraction amounted to 16.2 and 18.5% of the applied radioactivity for the two systems respectively.
In HPLC analysis up to five metabolites were detected, the only major (>10% or >5% at two consecutive timepoints) metabolite in the Goorven system detected at approx. 5 minutes was identified as imidazole and appeared in the water and sediment phase. In the other system this metabolite was minor. None of the other metabolites is considered potentially relevant.

Summary of water/sediment studies
Degradation in water-sediment systems was tested in two studies (2 different radioactivity label positions) with water and sediment from two pond systems (See Table 28, DT50 values in days) For triflumizole, the $DT_{50,water}$ was 1.9 and 3.1 days for the phenyl label, and 2.6 and 3.5 days for the imidazole label (overall geomean 2.7 days). The $DT_{50,system}$ was 48.7 and 117 days (phenyl ^{14}C) and 64 and 123 days (imidazole 14C) (overall geomean 81.3 days). FA-1-1 was formed in the sand system (phenyl label), the maximum formation rate in water was 10% and in sediment 12.9%. No reliable DT50 could be calculated. Imidazole was formed in the sand system (imidazole label), the maximum formation rate in water was 14.6% and in sediment 10%. No reliable $DT_{50,water}$ could be calculated for the metabolite imidazole, the $DT_{50,system}$ was 13.2 days. Mineralisation (phenyl ^{14}C label) was maximally 0.17% of AR after 101 days in the Goorven system and in the Schoonrewoerdse Wiel system it was 0.3% of AR after 59 days. Bound residue was maximally 10 and 19% of AR after 59 and 101 days in the 2 systems respectively. For the imidazole ^{14}C label study the maximum mineralisation was 39.5% of AR after 95 days in the Goorven system and in the Schoonrewoerdse Wiel system it was 19.8% of AR. Bound residue was maximally 16.2 and 18.5% of AR after 28 and 94 days in the 2 systems respectively.

Table 28: DT50 values for [14C-phenyl] triflumizole and [14C-imidazole] triflumizole in water/ sediment systems.

Compartment	**Goorven system (sand)**		**SchoonrewoerdseWwiel (clay loam)**	
	[^{14}C-phenyl] DT50 [d]	**[^{14}C-imidazole] DT50 [d]**	**[^{14}C-phenyl] DT50 [d]**	**[^{14}C-imidazole] DT50 [d]**
Water	1.9	2.6	3.1	3.5
Sediment	105	114	209	138
Total System	48.7	64	117	123

Figure 1: Metabolic pathway of triflumizole in water

5.1.3 Summary and discussion of degradation

In the aquatic environment the hydrolysis of triflumizole is pH dependent. Data indicate that it is most stable at neutral pHs. In one study, triflumizole was most stable with a DT50 value of ca. 20 days at pH 6 and in a supplemental study a DT50 of 101 days at pH 7 was obtained. The photolytic DT50 of triflumizole was determined to be 12.3 days under natural sunlight conditions. Information on photochemical degradation is difficult to use for classification and labeling purposes (CLP guidance Annex II.2.3.9). It can be concluded that in none of the abiotic processes at least 70% of triflumizole degraded within 28 days, so this compound is not considered rapidly degradable in

abiotic processes in water. No read biodegradability study is available. Triflumizole is therefore regarded not readily biodegradable. The water sediment tests showed that triflumizole disappears rapidly from the water phase. Triflumizole is partly transported to the sediment (maximum concentration 71.9% after 28 days) where it is transformed. The average (overall geomean) DT50 (system) value was determined to be 81.3 days. This means that triflumizole does not meet the criterion of > 70% degradation within 28 days. Major metabolites in water phase of one system are FA-1-1 and imidazole (max. 10 and 14.6% respectively). For FA-1-1 volatilization is the main disappearance route (a DT50 could not be determined). Imidazole is rapidly degraded in the system, DT50 = 13.2 days.

The conclusion on the degradation of triflumizole is that this compound is neither readily biodegradable nor rapidly degradable in the environment.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

There is no experimental data available. The Koc value is calculated to be 2764 L/kg for triflumizole (DAR).

5.2.2 Volatilisation

There is no test available. The Henry's law contsant value is calculated to be 6.29E-03 $Pa.m^3$ /mol at 25 °C for triflumizole (DAR), using a vapour pressur eof 1.91E-04 Pa (at 25 °C), a water solubility of 0.0105 g/L (at 20 °C) and a molar weight of 345.75 g/mol.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Table 29 Summary of relevant information on aquatic bioaccumulation

Method	Remarks	Results	Reference
OECD 305, GLP 60d Flow-through *Cyprinus carpio*	Concentration: nominal 0.6 and 6.0 µg/L Purity: chemical 99.7% radiochemical 99.4%	BCF 1417 L/kg (at 0.6 µg/L) BCF 699 L/kg (at 6 µg/L) Lipid content is not reported	Bouwman, 2006[a]

[a]As summarised in the DAR, Revised addendum to B9, Annex B, July 2009.

5.3.1.1 Bioaccumulation estimation

There is measured Kow value available. Based on a calculated log Kow value of 4.8 triflumizole is expected to have a bioaccumulative potential. See Table 9, summary of physico–chemical properties, for information on the derivation of log Kow (Part B, section 1.3).

5.3.1.2 Measured bioaccumulation data

Study 1
In a GLP-compliance OECD 305 bioconcentration test carp (*Cyprinus carpio*) were exposed for 60 days to two target concentrations (0.60 and 6.0 µg/L) in a continuous flow-through system at 21-23 °C (Bouwman, 2006). The depuration time was 43 days. The weight and the size of the fish was 1.00 ± 0.1 g and 3.2 ± 0.1 cm, respectively. The loading was 0.15 g fish/L/d. The fish were fed with Nutra, 1.5-3% of bw per day. The photoperiod was 16 h dimmed light. Total hardness (as $CaCO_3$) of the (tap)water was 179-250 mg $CaCO_3$/L. There were no replicate test vessels The number of animals per test vessel was 22 in control, 44 in test groups. The water sampling regime was 3 replicate samples after -1, 0, 3, 6, 10, 20, 32 and 60 days. Three fish (control) or 4 fish (treatments) were sampled after 3, 6, 10, 20, 32, 60, 62, 68, 75, 89 and 103 days
Water analysis: ^{14}C by LSC and (after extraction) by radio-TLC. Fish analysis : tissue dissolves in Soluene 350 overnight, than 14C by LSC. Identification of metabolites was no part of the study

No clinical effects on the fish were observed during the test period. The measured concentrations in water were 0.58 to 0.68 µg/L (time weighed average 0.62 µg/L) and 5.8 to 6.5 µg/L (time weighed average 6.1 µg/L). 14C in the water was for > 96% present in triflumizole. The pH ranged from 7.4 to 8.1; D.O. ranged from 5.5 to 9.6 mg/L. After 60 days the steady-state level was not reached in the 0.60 µg/L target concentration and almost reached in the 6.0 µg/L target concentration. The depuration half-life (CT50) for the 0.60 µg/L target concentration was 5.8 d (fast phase) and 38 d (slow phase), and for the 6.0 µg/L target concentration 7.5 d. The CT90 for the 0.60 µg/L target concentration was 19.3 d (fast phase) and 126 d (slow phase), and for the 6.0 µg/L target concentration 24.9 d.
The BCF was calculatedrom the ratio: concentration in fish to concentration in water at the end of the uptake phase. The BCF calculated in this way was 955 L/kg for the 0.60 µg/L target concentration and 725 L/kg for the 6.0 µg/L target concentration.
The BCF of the experiment with 6.0 µg/L was also calculated from the mean uptake rate (64 L/kg.d) and the excretion rate (0.0922 d-1), using a one-compartment model. In this way the BCF was calculated to be 699 L/kg, which agrees well with the equilibrium BCF.

Since the depuration curve of the 0.60 µg/L indicated a biphasic elimination (a slow phase and a fast phase), a two-compartment model was used to calculate the BCF of this target concentration. This was based on two excretion rates: 0.1191 d-1 and 0.0183 d-1. Thus two uptake rates were obtained: 91 L.kg-1d-1 and 26 L.kg-1d-1. The corresponding BCF values were: 765 and 1417 L/kg.

Remarks
As the study was done with very young animals, growth dilution could have impacted the concentrations measured in the uptake and depuration phase. As the measured as based on total radioactivity, metabolism of the compound is not accounted for. Furthermore, the lipid content is not reported.

Study 2
In an older bioconcentration test, carp (*Cyprinus carpio*) were exposed for 8 weeks 60 days to two target concentrations (1 and 10 µg/L) in a continuous flow-through system at 25 °C (Anonymous, 1984). There was no depuration period and identification of metabolites was no part of the study. The weight, the size and lipid content of the fish was 32.4 g and 10.6 cm and 4.6%, respectively. The feeding regime, photoperiod, hardness and type of vessel were not reported.
The number of animals per test vessel was 20. The water and fish samples were taken after 2, 4, 6 and 8 weeks (two replicates). Chemical analysis was done with gaschromatography (no further details).

The measured concentrations in water were 0.85 and 8.29 µg/L . The bioconcentration factors as calculated from the water concentrations and the concentrations in fish are given in Table 30.

Table 30: BCF values in carp during 8 weeks of exposure to triflumizole

Exposure concentration	2 weeks	4 weeks	6 weeks	8 weeks
0.85	53, 65	97, 61	87, 87	81, 166
8.3	92, 127	147, 82	44, 75	83, 71

Remark
The study was considered not acceptable as the study was carried out before the introduction of GLP and therefore report contains no GLP. The method used was very briefly described. The raw data on the biological and the chemical analytical part were not given. The study was carried out without a control group and there was no depuration period

5.3.2 Summary and discussion of aquatic bioaccumulation

Both bioconcentration studies have shortcomings. It is unknown how the growth dilution and the metabolism in the most recent study have impacted the actual BCF values. In older study indicate the BCF value could be lower, but the method description is too limited, to validate the BCF values. In the absence of better data, the measured BCF of 1417 L/kg will be used as a worst case for the classification and labelling of triflumizole.

5.4 Aquatic toxicity

The results of the critical, reliable and validated aquatic toxicity data relevant for the classification are summarised in Table 31.

Table 31: Summary of relevant information on aquatic toxicity

Test Guideline	Purity	Species	Remarks	Endpoint	Toxicity values in mg/L
Short-term toxicity to fish					
EPA Vol. 43, no. 132, 1978	98.2%	Rainbow trout (*Oncorhynchus mykiss*)	Static, nominal	96-h LC50	0.57
OECD 203; 1992; JMAFF 2-7-1; OPPTS 850.1075	98.6%	Carp (*Cyprinus carpio*)	Semi-static, nominal	96-h LC50	0.96
Long-term toxicity to fish					
EPA 72-4	99.1%	Fathead minnow (*Pimephales promelas*)	Flow-through, nominal	35- d NOEC	0.044
Short-term toxicity to aquatic invertebrates					
OECD 202; JMAFF 2-7-2-1; OPPTS 850.1010	98.6%	*Daphnia magna*	Semi-static, nominal	48-h EC50	2.11
Long-term toxicity to aquatic invertebrates					
OECD 211, 1998	99.2%	*Daphnia magna*	Semi-static, nominal	21-d NOEC	0.18
Toxicity to Algae					
OECD 201; JMAFF 2-7-3; OPPTS 850.5400	98.6%	Green algae (*Pseudokirchneriella subcapitata*)	Static, mean measured filtered samples	72-h ErC50 72-h NOErC	1.9 0.40

The results of the reliable and validated aquatic toxicity test for the most relevant metabolites are summarised in Table 32. As this information is not critical for the classification and labelling of triflumizole no further detailed description is given.

Table 32: Summary of relevant information on aquatic toxicity of triflumizole metabolites

Test Guideline	Metabolite	Species	Remarks	Endpoint	Toxicity values in mg/L

Short-term toxicity to fish					
EC C.1, 1992; OECD 203, 1992	FD-1-1	Carp (*Cyprinus carpio*)	Static, mean measured	96-h LC50	2.8
-	FA-1-1	Rainbow trout (*Oncorhynchus mykiss)*	semistatic	96-h LC50	5.3
Short-term toxicity to aquatic invertebrates					
OECD 202, Part I (1984); ISO 6341	FA-1-1	*Daphnia magna*	Semi-static, initial measured	48-h EC50	1.64
Toxicity to Algae					
ISO 8692; OECD 201	FA-1-1	Green algae (*Pseudokirchneriella subcapitata*)	Static	72-h ErC50 72-h NOErC	24 2.6

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1: The critical study for short-term toxicity to fish was performed with rainbow trout (*Oncorhynchus mykiss*) in accordance with EPA Vol. 43, no. 132, 1978 but not under GLP, the study was considered acceptable. Triflumizole with a 98.2% purity was dosed using N,N-dimethylformamide and Tween 80 as solvents; the nominal concentrations tested were 0, 0 (solvent), 0.30, 0.36, 0.43, 0.52, 0.62, 0.75, and 0.90 mg a.s./L.

The dilution water had a dissolved oxygen concentration of 7.08 ml/L and a pH value of ca. 7. The test temperature varied between 10.3 and 10.7 °C. No mortality or adverse effects were observed in the control and solvent control groups. At test concentrations of 0.43 mg a.s./L and higher, adverse effects (loss of equilibrium) and mortality was observed. The 96-h LC50 was 0.57 (0.56 - 0.58) mg a.s./L.

The triflumizole concentration was not measured by chemical analysis. Therefore, the correct dosing and stability of triflumizole cannot be confirmed. The loading of fish exceeded the recommended maximum loading of 1.0 g fish/L given in OECD guideline 203. It is not clear whether the volume can have influenced the test substance concentration. However, the results of the test are in line with the other fish tests.

Study 2: Another short-term toxicity to fish study which was performed according to GLP and OECD guideline 203 with carp (*Cyprinus carpio*) was also considered acceptable. Triflumizole with a 98.6% purity was dosed from concentrated solutions in acetone; the nominal concentrations tested were 0, 0 (solvent), 0.156, 0.313, 0.625, 1.25, 2.5, 5.0, and 10 mg a.s./L. The test solutions of 5.0 and 10 mg a.s./L were non-homogenous with un dissolved material on the base of the test vessel. All other solutions were clear and colourless. At the three highest test substance concentrations (2.5, 5.0 and 10.0 mg a.s./L) all animals died; it can therefore be assumed that physical effects of un-dissolved material in the two highest concentrations have not influenced the calculated LC50 value. Chemical analysis showed that substantial losses occurred during the

filtration process. In the unfiltered solutions of 2.5 mg a.s./L and lower, the recovery was above 80% of nominal and it is allowed according to the guideline to express the results of the test in nominal concentrations.
The lowest measured dissolved oxygen concentration was 79% of the air saturation level; pH values varied between 7.0 and 7.4 and the test temperature between 21.1 and 22.3 °C.

In the control and solvent control groups, the percentage of affected and dead fish was ≤ 10%. At increasing test concentrations, the symptoms changed from mild toxic effects (increased cough frequency, swimming at different position in test vessel than control fish) to severe toxic effects (swimming abnormally, lying at bottom of tank) and finally death. The 96-h LC50 value (based on nominal concentrations) and found a value of 0.960 (0.808 - 1.14) mg a.s./L.

5.4.1.2 Long-term toxicity to fish

A GLP-compliant Early Life Stage test with fathead minnow (*Pimephales promelas*) conducted according to EPA 72-4 is available and considered acceptable. In this study fish eggs (60 at the start of the test) and hatched individuals (40 from day 5) were exposed to triflumizole (99.1% purity) at nominal test concentrations of 0, 0 (solvent), 22, 44, 88, 180, and 350 µg a.s./L for a total of 35 days under flow-through conditions.

The mean measured concentrations of triflumizole varied between 75 and 83% of the nominal values for all test levels. All concentrations measured on day 35 were much lower than the concentrations measured on previous days. Any disfunction of the diluter system or any irregularities in the analysis were not observed. It was assumed that the deviations from the expected concentrations were restricted to a small period of time which were considered not to influence the results of the test. When the analyses of day 35 were not taken into account, the mean recovery varied between 84 and 91%. The results of the test are therefore given in nominal concentrations.

The lowest measured dissolved oxygen concentration was 6.9 ± 0.9 mg/L; pH values varied between 6.6 and 7.4. The water temperature was 25 ± 0.7 °C.

The mean egg fertilisation rate in the pooled control and solvent control was 91.5%. The results of the test parameters with the statistical analyses are summarised in Table33. Fry survival after 35 days was significantly lower at 350 µg a.s./L , when compared with the control. Fry growth was significantly reduced at test concentrations of 88 µg a.s./L (wet weight) and 180 µg a.s./L (length) and higher. The overall 35-d NOEC to early life stages of fathead minnows is therefore 44 µg a.s./L (based on effects on fry growth).

Table 33 **Results for the various test parameters in the ELS test with PROCURE**

Test Parameter	Nominal Concentration PROCURE in µg triflumizole /L							
	0	0 (solvent)	22	44	88	180	350	NOEC
% embryo survival at hatch day 5	90	93	96	95	93	93	87	≥ 350
fry survival day 35	96	80	96	94	98	91	59[1]	180
fry growth day 35 (length in mm) ± SD	32 ± 2.8	33 ± 2.7	33 ± 3.0	33 ± 2.5	32 ± 2.3	29 ± 2.3[1]	23 ± 2.3[2]	88
fry growth day 35 (wet weigh in mg) ± SD	292 ± 81	327 ± 79	318 ± 86	293 ± 72	266 ± 61[1]	201 ± 53[1]	105 ± 33[2]	44

1 significant different from the pooled control and solvent control (α = 0.05).
2 data not analysed for significance, due to significant effects on larval survival.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The critical study for short-term toxicity to aquatic invertebrates was performed with *Daphnia magna* in accordance with OECD guideline 202 and GLP and was considered acceptable. Triflumizole with a 98.6% purity was dosed from concentrated solutions in acetone; the nominal concentrations tested were 0, 0 (solvent) 0.625, 1.25, 2.5, 5.0, and 10 mg a.s./L.

Samples for chemical analysis were taken at t = 0h, t = 24h (both new and old media) and at the end of the test (t = 48h; old medium). The test media were homogeneous liquid dispersions with white particles, the amounts increasing with increasing exposure concentrations. Samples were analysed both filtered and unfiltered. The mean measured concentrations in the unfiltered samples of nominal 0.625, 1.25, 2.5, 5.0, and 10 mg a.s. were 98, 84, 88, 91 and 54% of the nominal concentration, respectively. This indicates a correct dosing of the test substance (the highest concentration was dosed at the water solubility level). The filtered samples showed a recovery of 71, 63, 77, 84 and 51%, respectively (average = 69.2%), indicating losses by the filtration process. As the mean measured concentrations were above 80% of the nominal values it is allowed to express the results of the test in nominal concentrations. The lowest measured dissolved oxygen concentration was 89% of the air saturation level; pH values varied between 7.4 and 8.4 and the test temperature between 20.1 and 21.1 °C. In the control and solvent control groups, the number of immobilised animals was ≤ 10%.

The 48-h EC50 value was calculated with the Spearman-Kärber method (based on nominal concentrations) and was found to be 2.11 (1.69 - 2.63) mg a.s./L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A GLP-compliant reproduction study with *Daphnia magna* conducted according to OECD guideline 211 is available and considered acceptable. Triflumizole was dosed from concentrated stock solutions in acetone; the concentrations tested were 0, 0 (solvent), 0.056, 0.10, 0.18, 0.32, and 0.56 mg/L.

The mean measured concentrations of the test substance in the freshly prepared media and the spent media varied between 88 and 100% of the nominal values, which confirms the correct dosing and stability of the test substance. Therefore, it is allowed, according to the guidelines, to express the results of the test in nominal concentrations. The lowest measured dissolved oxygen concentration was 7.0 mg/L; pH values varied between 7.4 and 8.7 and test temperatures between 18.5 and 20.1 °C.

In the control groups the number of offspring per surviving female was > 60 and mortality was below the allowed 20%. Significant mortality was not observed at any of the test concentrations. Reproduction was significantly lower at 0.32 and 0.56 mg a.s./L, compared to control reproduction; aborted eggs and immobilised- and dead offspring were observed at these test concentrations. The body lengths at 0.32 and 0.56 mg a.s./L were significantly lower than the pooled data of the control and solvent control. The 21d-NOEC is considered 0.18 mg a.s./L based on reproduction.

5.4.3 Algae and aquatic plants

The critical study for toxicity to algae was a GLP-compliant OECD guideline 201 study performed with *Pseudokirchneriella subcapitata;* this study is considered acceptable. Triflumizole with a

98.6% purity was dosed from concentrated solutions in acetone; the nominal concentrations tested were 0, 0 (solvent), 0.625, 1.25, 2.5, 5, and 10 mg a.s./L.

The exposure medium of 10 mg/L appeared as a non-homogenous liquid dispersion with particulates at the bottom of the test vessels at the start of the test. All other media were clear (visually assessed). Comparison of the results for filtered and unfiltered samples showed substantial losses during the filtration process. The highest concentration (10 mg/L) most likely exceeded the solubility of the test medium in the growth medium. The results for the other (unfiltered) samples confirm the correct dosing; the mean recovery at 5.0 mg a.s./L and lower was > 80%.. pH values varied between 7.9 and 10.3 and temperatures between 21.8 and 24.1 °C.

The 72-h EC50 for growth rate based on mean measured is 1.9 mg a.s./L. The 72-h NOEC value for growth rate is 0.40 mg a.s./L.

5.4.4 Other aquatic organisms (including sediment)

No data available

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

Summary of the relevant toxicity and fate data for triflumizole:

Short-term toxicity		
Fish	*Oncorhynchus mykiss*	96-h LC50 = 0.57 mg/L
Invertebrates	*Daphnia magna*	48-h EC50 = 2.11 mg/L
Algae/aquatic plants	*Pseudokirchneriella subcapitata*	72-h ECr50 = 1.9 mg/L
Long-term toxicity		
Fish	*Pimephales promelas*	35-d NOEC= 0.044 mg/L
Invertebrates	*Daphnia magna*	21-d NOEC = 0.18 mg/L
Algae/aquatic plants	*Pseudokirchneriella subcapitata*	72-h NOErC = 0.40 mg/L

Degradation
In the aquatic environment, the hydrolysis of triflumizole is pH dependent. Data indicate that it is most stable at neutral pHs. The photolytic degradation of triflumizole was determined to be DT50 of 12.3 days under natural sunlight conditions. Information on photochemical degradation is difficult to use for classification and labeling purposes (CLP guidance Annex II.2.3.9). It can be concluded that in none of the abiotic processes at least 70% of triflumizole degraded within 28 days, so this compound is not considered rapidly degradable by abiotic processes in water. No read biodegradability study is available, triflumizole is therefore regarded as not readily biodegradable. An average (overall geomean) DT50 (system) value of 81.3 days was determined in a OECD308 simulation test. Therefore, triflumizole does not meet the criterion of > 70% degradation within 28 days. Major metabolites in water phase of one system are FA-1-1 and imidazole (max. 10 and 14.6% respectively). For FA-1-1 volatilization is the main disappearance route (DT50 could not be determined). Imidazole is rapidly degraded in the system, DT50 = 13.2 days.

The conclusion on the degradation of triflumizole is that this compound is neither readily nor rapidly degradable in the environment.

Bioaccumulation
The experimental BCF value of triflumizole is 1417 L/kg.

CLP Acute aquatic hazard

L(E)C50 values are available for all three trophic levels. The lowest L(E)C50 obtained for triflumizole is 0.57 mg/L in fish. Triflumizole therefore fulfils the criteria for classification as Aquatic Acute Cat. 1 (toxicity band: L(E)C50 ≤ 1 mg/l).

CLP Chronic aquatic hazard
NOEC values are available for all three trophic levels. The lowest NOEC value obtained for triflumizole is 0.044 mg/L in fish. Triflumizole therefore fulfils the criteria for classification as Aquatic Chronic Cat. 1 (toxicity band: L(Chronic NOEC or ECx ≤ 0.1 mg/l).

M-factor
Acute M-factor: A comparison of the L(E)C50 values obtained from short-term aquatic toxicity test indicates that the lowest value falls within the 0.1 < L(E)C50 ≤ 1 mg/L band. Based on this information the M-factor is 1.

Chronic M-factor: The substance is not rapidly degradable. A comparison of the NOEC values obtained from long-term aquatic toxicity test indicates that the lowest value falls within the 0.01 < NOEC ≤ 0.1 mg/L band. Based on this information M-factor is 1..

Directive 67/548/EEC
The lowest short-term aquatic toxicity value for triflumizole is 0.57 mg/L in fish. Triflumizole is not readily degradable and the experimental BCF value is 1,417 L/kg. Triflumizole therefore fulfils the criteria for classification with N;R50/53.

The specific concentration limits (SCL) as given in Directive 1999/45/EEC are:
$C_n \geq 25\%$ N; R50-53;
$2.5\% \leq C_n < 25\%$ N; R51-53;
$0.25\% \leq C_n < 2.5\%$; R52-53.

Where C_n is the concentration of triflumizole in a mixture are proposed.

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

Table 34 Conclusion on environnemental classification

	CLP Regulation	**Directive 67/548/EEC and 1999/45/EC**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410) M-factor Acute M-factor 1 Chronic M-factor 1	N; R50-53 SCL: $C_n \geq 25\%$: N; R50-53 $2,5\% \leq C_n < 25\%$: N; R51-53 $0,25\% \leq C_n < 2,5\%$: R52-53

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=1).

Degradation

A hydrolysis study according to an EPA guideline proposed in the Federal Register Vol. no 132 (non-GLP) was run at pH 3, 6 and 9, at temperatures of 25°C and 50°C and at two concentrations (0.5 and 5 mg/L). The study indicates that the hydrolysis was pH-dependent. Triflumizole hydrolysed quickly under acidic conditions with a DT_{50} of less than 1 day and it was more stable at pH 6 and 20°C (converted from 25°C data to 20°C using Arrhenius equation) with DT_{50}-values ranging from 472 to 519 h between the two concentrations (DT_{50} ca. 20 days). Data on the percentages of metabolites were not provided, but N-(4-chloro-2-trifluoromethylphenyl)-2-propoxyacetamide (FD-1-1) was stated to be the major degradation product.

In a second study, carried out according to EPA guideline 161-1 (non-GLP), the hydrolysis rate was investigated at different buffer concentrations at 25ºC and pH 5, 7 and 9 over a period of 30 days. The test was carried out at a nominal concentration of 5 mg/L. The hydrolysis rate was affected by the concentration of buffer solute. At different concentrations and at 20ºC the DT_{50}-values were 5.2 - 17.3 days at pH 5, 20.7 - 171 days at pH 7 and 4.9 - 22.8 days at pH 9. Triflumizole was degraded almost completely into FD-1-1.

The photodegradation of triflumizole in water was studied according to SETAC; OECD draft TG. The GLP-compliant study was carried out using a Xenon lamp at 25 ± 2°C for 15 days. The photolytic DT_{50} of triflumizole was determined to be 12.3 days under natural sunlight conditions.

No data on ready biodegradability were available.

Two water/sediment simulation studies carried out according to OECD TG 308 (in compliance with GLP), using two different radioactivity label positions, were run for 101 and 95 days at 20°C using two pond systems (sand and clay loam).
Triflumizole disappeared rapidly from the water phase. Triflumizole was partly transported to the sediment (maximum concentration 71.9% after 28 days) where it was transformed.
For triflumizole, the DT_{50}-values in water were 1.9 and 3.1 days for the phenyl label, and 2.6 and 3.5 days for the imidazole label (overall geometric mean 2.7 days). For the whole systems, DT_{50} were 48.7 and 117 days (phenyl label) and 64 and 123 days (imidazole label) (overall geomean 81.3 days). 4-chloro-2-trifluoromethylaniline (FA-1-1) was formed in the sand system (phenyl label), the maximum formation rate in water was 10% and in sediment 12.9%. For FA-1-1, volatilisation was the main disappearance route (no reliable DT_{50} could be calculated). Imidazole was formed in the sand system (imidazole label), the maximum formation rate in water was 14.6% and in sediment 10%. No reliable $DT_{50,water}$ could be calculated for the metabolite imidazole, the $DT_{50,system}$ was 13.2 days. Mineralisation (phenyl label) was maximally 0.17% of the applied radioactivity (AR) after 101 days in the sand system and in the clay loam system it was 0.3% of AR after 59 days. Bound residue was maximally 10% and 19% of AR after 59 and 101 days in the two systems, respectively. For the imidazole label study, the maximum mineralisation was 39.5% of AR after 95 days in the sand system and in the clay loam system it was 19.8% of AR. Bound residue was maximally 16.2% and 18.5% of AR after 28 and 94 days in the two systems, respectively.

Bioaccumulation

Triflumizole has a calculated log_{Kow} of 4.8 (calculated from the measured solubilities), and therefore triflumizole is expected to have bioaccumulation potential.

The DS provided two bioaccumulation studies on triflumizole.
In the first study (GLP-compliant, OECD TG 305, bioconcentration test), carp (*Cyprinus carpio*) were exposed for 60 days to two target concentrations (0.60 and 6.0 µg/L) in a continuos flow-through system. The depuration time was 43 days. After 60 days the steady-state level was not reached with the target concentration of 0.60 µg/L and almost reached with 6.0 µg/L.

BCF-values were calculated through the ratio concentration in fish to that in water: 955 L/kg (for 0,60 µg/L concentration), 725 L/kg (for 6.0 µg/L concentration).
BCF values were also calculated considering the ratio from uptake and excretion rate constants (Ku/Ke): 699 L/kg (for 6.0 µg/L concentration), 765 and 1417 L/kg (for 0.60 µg/L concentration, with a biphasic elimination: a slow phase and a fast phase).
For this study, the DS specified that the lipid content was not reported.

In the second study, carp were exposed for 60 days to two target concentrations (1 and 10 µg/L) in a continuous flow-through system. This study was considered not acceptable by the DS for several reasons including that the study was not GLP-compliant, there was no control group and there was no depuration period.

The DS concluded that the measured BCF of 1417 L/kg was used as a worst case for the classification and labelling of triflumizole. However, the measured bioaccumulation data showed that triflumizole was potentially bioaccumulative according to CLP (BCF $\geqslant$ 500).

Aquatic toxicity

The DS provided reliable and validated aquatic toxicity data for each throphic level on triflumizole and on the most relevant metabolites. However, the DS considered only information on triflumizole relevant for the classification because the toxicity values for metabolites were above 1 mg/L, and therefore not critical for classification and labelling.

Regarding short-term toxicity of Triflumizole, two tests on fish (*Oncorhynchus mykiss* and *Cyprinus carpio*), one on aquatic invertebrates *(Daphnia magna)* and one test on algae (*Pseudokirchneriella subcapitata*) were provided. Fish was shown as the most sensitive species and the study on *Oncorhynchus mykiss* (according to EPA Vol. 43, no. 132, 1978, non-GLP) was considered as the key study, with a 96-h LC_{50} = 0.57 mg/L (nominal concentration). The DS noted that the concentration was not measured by chemical analysis during the test. Therefore, the correct dosing and stability of triflumizole cannot be confirmed. However, the key study result was supported by a second study on *Cyprinus carpio*, perfomed according to GLP and according to OECD TG 203, with a 96-h LC_{50}-value (nominal concentration) of 0.960 mg/L.

The only result based on mean measured concentration of filtered solution was on green algae, resulting in a 72-h EC_{50} for growth rate of 1.9 mg/L.

Regarding long-term toxicity, there were two available tests, one on fish (*Pimephales promelas*) and one on aquatic invertebrates *(Daphnia magna)*.

Also for the chronic aquatic toxicity, fish was the most sensitive organism. An Early Life Stage test (EPA 72-4) was performed for 35 days under flow-through conditions, showing a NOEC of 0.044 mg/L, (nominal concentration) based on fry growth at day 35. The DS reported mean measured concentrations between 75% and 83% of the nominal values for all test levels, specifying that all concentrations measured on day 35 were much lower than the concentrations measured on previous days.

Table 5. Summary of the reported studies on aquatic toxicity

Test Guideline	Purity	Species	Remarks	Endpoint	Toxicity values (mg/L)
Short-term toxicity to fish					
EPA Vol. 43, no. 132, 1978	98.2%	Rainbow trout (*Oncorhynchus mykiss*)	Static, nominal	96-h LC_{50}	0.57
OECD 203; 1992; JMAFF 2-7-1; OPPTS 850.1075	98.6%	Carp (*Cyprinus carpio*)	Semi-static, nominal	96-h LC_{50}	0.96
Long-term toxicity to fish					
EPA 72-4	99.1%	Fathead minnow (*Pimephales promelas*)	Flow-through, nominal	35-d NOEC	0.044
Short-term toxicity to aquatic invertebrates					
OECD 202; JMAFF 2-7-2-1; OPPTS 850.1010	98.6%	*Daphnia magna*	Semi-static, nominal	48-h EC50	2.11
Long-term toxicity to aquatic invertebrates					
OECD 211, 1998	99.2%	*Daphnia magna*	Semi-static, nominal	21-d NOEC	0.18
Toxicity to Algae					
OECD 201; JMAFF 2-7-3; OPPTS 850.5400	98.6%	Green algae (*Pseudokirchneriella subcapitata*)	Static, mean measured filtered samples	72-h ErC50 72-h NOErC	1.9 0.40

Comments received during public consultation

Comments by four MS were submitted during public consultation supporting the proposed environmental classification.

One MS suggested only an addition, which however did not change the proposed classification. Another MS presented concern on the analyses of results and the potential impact on the M-factor. This MS provided 5 specific comments, to which the DS responded point by point. Among the most relevant comments was the observation that triflumizole is a surface active substance and therefore micelle formation and undissolved material in general could result in an overestimation of the bioavailability of the substance. As a solution the MS suggested to provide results based on filtered solutions and measured data.

The DS expressed general agreement with the MS considerations on the problems of testing unfiltered solutions. The DS also clarified that the information had been provided in the DAR documents, where the results were all considered acceptable by the

rapporteur member state (RMS). The DS specified that the reported data were the only information available on the aquatic toxicity and the classification had to be based on available information. However, the DS made a very rough estimation demonstrating that applying the commenting MS considerations would not change the conclusion on classification nor the M-factor.

In particular, the MS highlighted that in the Early life stage test (OECD TG 210) a test duration of 32 days was recommended for *Pimephales promelas* from start of test (or 28 days post-hatch). In the test provided in the CLH report, a 35-d NOEC was given, but the measured concentrations at day 35 were much lower than the concentrations measured on previous days. Therefore the commenting MS suggested determining the NOEC after 32 days for chronic toxicity on fish.

The DS agreed again with the MS but noted that it was not possible recalculate the NOEC on day 32 since the original study was not available.

RAC highlights that in the DAR, the RMS assumed that deviations from the expected concentrations did not influence the results of the test. Indeed the study duration was 35 days from the start of the test and 30 days post-hatch, while the OECD TG 210 foresaw a duration of 32 days or 28 days post-hatch. Considering the post-hatch duration, there was just a 2-day deviation, and therefore the RMS's assumption could be plausible.

Assessment and comparison with the classification criteria

Degradation
RAC agrees with the DS proposal to consider triflumizole as not rapidly degradable, based on the fact that less than 70% of triflumizole degraded within 28 days in the hydrolysis studies, no ready biodegradability study was available and less than 70% of the substance was ultimately degraded in the water/sediment studies.

Bioaccumulation
Triflumizole had a calculated $\log_{Kow}$ value of 4.8 (calculated from the measured solubilities).

The measured bioaccumulation data based on a GLP-compliant OECD TG 305 bioconcentration test showed that the substance is potentially bioaccumulative.
The measured BCF-values exceeded the CLP criteria (BCF ≥ 500).

Aquatic toxicity

Acute aquatic hazard:

Reliable and relevant acute toxicity data were available for all three trophic levels. The most sensitive taxonomic group is fish. The lowest reliable short-term aquatic toxicity result for *Oncorhynchus mykiss* was the 96-h LC_{50} of 0.57 mg/L (nominal concentration).

Chronic aquatic hazard:

Reliable and relevant long-term aquatic toxicity data were available for fish and aquatic invertebrates. The lowest value was for fish species *Pimephales promelas*, with a 35-d NOEC of 0.044 mg/L (nominal concentration).

Conclusion on classification
Triflumizole is considered not to be rapidly degradable and does fulfill the criteria for bioaccumulation.

The lowest available result obtained in an acute aquatic test for triflumizole is an LC_{50} of 0.57 mg/L in fish. Triflumizole therefore fulfils the criteria for classification as Aquatic

Acute 1 with an M-factor of 1, because the value is in the range of 0.1 mg/L ≤ L(E)C_{50} ≤ 1 mg/L.

The lowest available result obtained in a chronic aquatic test for triflumizole is a NOEC value of 0.044 mg/L in fish. Triflumizole therefore fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 1, because the value is in the range of 0.01 mg/L ≤ Chronic NOEC or ECx ≤ 0.1 mg/L and the substance is not rapidly degradable.

6 OTHER INFORMATION

7 REFERENCES

European Commission. Draft Assessment Report Triflumizole, prepared by The Netherlands, updated addendum of February 2009.

8 ANNEXES