Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

EC number: -
CAS number: 68694-11-1

CLH-O-0000001412-86-40/F

Adopted

04 December 2014

4 December 2014

CLH-O-0000001412-86-40/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: Triflumizole (ISO); (1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1Himidazol-1-yl)-2-propoxyethanimine

EC number: -

CAS number: 946578-00-3

The proposal was submitted by **The Netherlands** and received by the RAC on **16 August 2013.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **18 March 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 May 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-rapporteur, appointed by RAC: **Pietro Paris**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2014.**

The RAC opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **Triflumizole (ISO)** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current entry										
Dossier submitters proposal	612-289-00-6	triflumizole (ISO); (1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1H-imidazol-1-yl)-2-propoxyethanimine	-	68694-11-1	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H302 H373 (liver) H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360D H302 H373 (liver) H317 H410		 M=1 M=1	-
RAC opinion	612-289-00-6	triflumizole (ISO); (1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1H-imidazol-1-yl)-2-propoxyethanimine	-	68694-11-1	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H302 H373 (liver) H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360D H302 H373 (liver) H317 H410		 M=1 M=1	-

Resulting Annex VI entry if agreed by COM	612-289-00-6	triflumizole (ISO); (1E)-N-[4-chloro-2-(trifluoromethyl)phenyl]-1-(1H-imidazol-1-yl)-2-propoxyethanimine	-	68694-11-1	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H302 H373 (liver) H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360D H302 H373 (liver) H317 H410		M=1 M=1	

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

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).

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.

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).

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.

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.

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.

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.

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).

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.

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 ⩾ 4.5 mg/kg bw/d in females (both at 54 and 104 weeks) (and in males after 104 weeks at doses ⩾ 18 mg/kg bw/d). 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 ≤ 300 mg/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.

Neurotoxicity. 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).

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.

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.

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).

ENVIRONMENTAL HAZARD ASSESSMENT

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, bioconcentrtion 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.

Additional references

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ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).