Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling at EU level of

Triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate

EC number: N/A
CAS number: 126535-15-7

CLH-O-0000001709-67-02/F

Adopted

5 December 2013

5 December 2013

CLH-O-0000001709-67-02/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: Triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy) -1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate

EC number: N/A

CAS number: 126535-15-7

The proposal was submitted by **France** and received by the RAC on **26 March 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

France 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 **26 March 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 May 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Marianne van der Hagen**

Co-rapporteur, appointed by RAC: **Hans-Christian Stolzenberg**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **5 December 2013**; further scientific details are contained in the Background Document (BD; see Annex I) and the comments received from parties concerned are compiled in Annex 2.

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

OPINION OF THE RAC

The RAC adopted the opinion that **Triflusulfuron-methyl** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					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 Annex VI entry									
Dossier submitters proposal	607-714-00-7	triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M = 100 M = 10
RAC opinion		triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M = 100 M = 10
Resulting Annex VI entry if agreed by COM		triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M = 100 M = 10

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	No current Annex VI entry						
Dossier submitters proposal	607-714-00-7	triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. Cat. 3 R40 N; R50/53	Xn, N, R: 40-50-53 S: 36/37-60-61	N; R50-53: C ≥ 0.25% N; R51-53: 0.025% ≤ C < 0.25% R52-53: 0.0025% ≤ C <0.025%
RAC opinion		triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. Cat. 3 R40 N; R50-53	Xn, N, R: 40-50/53 S: 36/37-60-61	N; R50-53: C ≥ 0.25% N; R51-53: 0.025% ≤ C < 0.25% R52-53: 0.0025% ≤ C <0.025%
Resulting Annex VI entry if agreed by COM		triflusulfuron-methyl; methyl 2-({[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]carbamoyl}sulfamoyl)-3-methylbenzoate	-	126535-15-7	Carc. Cat. 3 R40 N; R50-53	Xn, N, R: 40-50/53 S: 36/37-60-61	N; R50-53: C ≥ 0.25% N; R51-53: 0.025% ≤ C < 0.25% R52-53: 0.0025% ≤ C <0.025%

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

The dossier submitter (DS) addressed explosive properties, flammability and oxidising potential. The chemical structure indicated that there is no cause for concern for any of these endpoints. Furthermore, tests have been performed for explosive properties and flammability with negative results. The DS concluded that classification for explosive properties, flammability or oxidising potential is not needed.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC agreed that the substance did not fulfil the criteria for classification as explosive, flammable or oxidising.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Six studies on acute toxicity were provided in the CLH report, three on oral, one on inhalation and two on the dermal route. No substance related mortalities were seen and only minor clinical signs observed. LD_{50} oral was determined to be >5000 mg/kg/d, dermal >2000 mg/kg/d and inhalation > 5.1 mg/L. No classification was proposed.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Acute toxicity: oral

The LD_{50} of triflusulfuron-methyl in rat and rabbit is >5000 mg/kg bw, and thus above the cut-off value of 2000 mg/kg bw for classification for acute toxicity by the oral route according to both CLP and DSD.

Acute toxicity: inhalation

No mortalities were observed in a study where rats were exposed to a concentration of 5.1 mg/L/4hr, i.e. a concentration above the cut-off value of 5.0 mg/L/hr for dusts and mists (CLP), and aerosols and particulates DSD.

Acute toxicity: dermal

The LD_{50} of triflusulfuron-methyl in rat and rabbit is>2000 mg/kg bw, and thus above the cut-off value of 2000 mg/kg bw for classification for acute toxicity by the dermal route according to both CLP and DSD.

RAC therefore supported no classification for acute toxicity as proposed by the DS.

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

Summary of the Dossier submitter's proposal

The DS stated that no effects relevant for this hazard class were observed in the acute toxicity studies and concluded that no classification was warranted.

Comments received during public consultation

No comments were received during public consultation

Assessment and comparison with the classification criteria

According to CLP, classification as STOT-SE should be considered when there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. As no specific target organ toxicity was observed after a single dose/exposure concentration of triflusulfuron-methyl, no classification was deemed necessary.

RAC supported no classification for STOT-SE as proposed by the DS.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Two studies performed in rabbits (conducted in accordance with EEC method B.4. and OECD test guideline (TG) 404, respectively) were presented in the report. Score 1 erythema was seen in one animal in one of the studies. The effect was reversed after 48 h. The DS concluded that the criteria for classification were not fulfilled.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In two skin irritation studies in rabbit, triflusulfuron-methyl was applied under a semi-occlusive dressing. Reversible erythema was observed in 1 of 3 animals in one study. In the other study no dermal irritation was observed. Since no irreversible damage to the skin was observed, classification as corrosive to skin is not warranted.

According to CLP, a reaction is normally needed in 2 out of 3 animals for classification as a skin irritant. However very definite positive reaction in only one animal may be sufficient for classification. In this case with triflusulfuron-methyl the reaction in one animal was so weak (erythema, score of 1) that no classification is considered justified.
No classification is required for skin irritation according to DSD, as the criteria for significant inflammation of the skin in two or more animals is not fulfilled.

RAC supported no classification for skin corrosion/irritation as proposed by the DS.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Two studies performed in rabbits (both according to EEC method B.5.) were presented in the CLH report, one with three and one with six animals. Conjunctival redness (scores 1 or 2) was seen in all animals. Conjunctival chemosis, discharge and corneal opacity (score 1 in one rabbit) were also reported. All effects were reversed after 48 h. The DS concluded that the results did not fulfil the criteria for classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In two eye irritation studies in rabbit, mean scores for corneal opacity, iritis, conjunctival redness and conjunctival oedema at 24 to 72 hours were below the criteria for classification and labelling in CLP and DSD.

According to CLP, Irritating to eyes (Category 2) is applied to substances that produce, a positive response at least in 2 of 3 tested animals, of:
– corneal opacity ≥ 1 and/or
– iritis ≥ 1, and/or
– conjunctival redness ≥ 2 and/or
– conjunctival oedema (chemosis) ≥ 2
– calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.
Irreversible eye effects should be assigned to Category 1.

No signs of irritation were present at 48 hours in either test. Signs observed after 24 hours were below the cut-off values for classification, both when compared to criteria in CLP and DSD.

RAC supported no classification for eye corrosion/irritation as proposed by the DS.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Two Magnusson and Kligman maximisation tests (OECD 406) were described in the report. Both were considered by the DS not to justify classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Two Magnusson and Kligman Guinea Pig Maximisation Tests (GPMT) are available, i.e. adjuvant tests in which sensitisation was potentiated by the injection of Freunds Complete Adjuvant (FCA). In one test, intradermal induction consisted of 0.1 ml of 1% solution of the test substance. In the other test the corresponding intradermal dose was 0.1 ml of 1.5 %. For cutaneous induction, 500 mg of the test substance (purity 98.7%) was used in the first test, and 0.2 ml of the test preparation (25% w/v) was applied in the second. No cutaneous reactions were observed following the exposure in either test. The DS considered the first study to be only indicative, as no positive control was used. In the other test 2,4-Dinitrochlorobenzene was used as a positive control.

RAC agreed with the DS that the first test provided only indicative results, and supported no classification according to CLP (including its 2nd ATP) or DSD for skin sensitisation, as no cutaneous reactions from exposure to triflusulfuron-methyl were observed.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Three 90 day OECD 408 studies (two in rats, one in mice), one 90 day OECD 409 study in dog and one 1 year OECD 452 study in dog conducted by the oral route, were included in the CLH report. The main target organs were liver and blood in rat and dog and liver in mice. In the 1 year dog study, mortalities were also seen. All effects occurred above the guidance value of 100 mg/kg/d or, in case of the 1 year study effects (mortality) were seen at 90 mg/kg/d. The DS conclude that classification was not warranted.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the 90 days oral studies in rats, mice and dogs the NOAELs were > 100 mg/kg bw for changes in the liver and red blood cells in rats and dogs and in the liver in mice. Thus the NOAELs were

above the cut-off values of 100 mg/kg bw for classification in STOT RE 2 and above the cut-off value of 50 mg/kg bw for repeated dose toxicity (R48) in DSD. Findings in a 1 year oral dog study at 94 mg/kg bw in females and 112 mg/kg bw in males were not sufficient for classification after the use of Haber´s rule adjusting the guidance values to 100/4 for the guidance values in CLP, and 50/4 according to Directive 67/548.

RAC supported no classification for repeated dose toxicity, as proposed by the DS.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The CLH report included seven *in vitro* (bacteria and mammalian cells) and three *in vivo* genetic toxicity tests (all in mice). All the tests were negative. The DS concluded that classification was not warranted.

Comments received during public consultation

A comment was received from one MSCA, agreeing with the proposal not to classify the substance for this hazard class.

Assessment and comparison with the classification criteria

The mutagenicity data base for triflusulfuron-methyl is extensive and consists of Ames tests in bacteria as well as tests *in vitro* and *in vivo* both in human and non-human mammalian cells. All tests had a negative outcome both with and without metabolic activation, except two *in vitro* chromosome aberration tests of human lymphocytes. As these were conducted with high (cytotoxic) concentrations (≥ 1700 µg/ml) and the three *in vivo* chromosome aberration tests were all negative, analysis of the weight of evidence indicates that triflusulfuron-methyl has no genotoxic potential, and should not be classified according to CLP. According to the DSD, substances showing positive results only in one or more in vitro mutagenicity assays should normally not be classified.

RAC therefore supported no classification for germ cell mutagenicity (CLP) / mutagenicity (DSD) as proposed by the DS.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

One two-year cancer bioassay in Sprague Dawley rats and one 18-month cancer bioassay in CD-1 mouse were described in the CLH report, as well as mechanistic studies related to hormone levels and aromatase effects. In mice a slight increase in hepatocellular adenomas was seen at the high dose. The DS concluded that this effect was not sufficient for classification.

In rats, Leydig cell hyperplasia and adenomas were seen at the two highest doses. Although arguments questioning the relevance for humans were discussed in the CLH report, the DS concluded that this effect was considered to be relevant to humans. Summary tables of the main results in the carcinogenicity studies presented by the DS are given below.

Table 1 - Two-year feeding study in rats: Triflusulfuron-methyl-induced microscopic effects in testes

Triflusulfuron-methyl (ppm):	**0**	**10**	**100**	**750**	**1500**
Number examined	*51*	*46*	*47*	*50*	*51*
Adenoma, interstitial cell	*0*	*2*	*1*	*7**	*7**
Hyperplasia, interstitial cell	*10*	*7*	*11*	*18**	*27**

*Statistically significant ($p \leq 0.05$).

Table 2– 18-month feeding study in mice: Triflusulfuron-methyl-induced microscopic effects

MALES

Triflusulfuron-methyl (ppm): Number of mice/group:	**0 81**	**10 80**	**150 81**	**2500 81**	**7000 80**
Liver					
Adenoma, hepatocellular[a]	*10*	*4*	*5*	*13**	*15**
Carcinoma, hepatocellular	*3*	*3*	*0*	*0*	*1*
Adenoma and/or Carcinoma[b]	*12*	*7*	*5*	*13*	*16**
Focus of hepatocellular alteration	*9*	*11*	*9*	*14*	*15*
Intracellular pigment accumulation, Kupffer cell/macrophage	*16*	*8*	*10*	*12*	*37#*
Intrahepatocellular erythrocytes	*0*	*0*	*0*	*5#*	*21#*
Necrosis, individual hepatocellular, increased	*0*	*1*	*2*	*2*	*14#*

FEMALES

Triflusulfuron-methyl (ppm): Number of mice/group:	**0 78**	**10 81**	**150 79**	**2500 83**	**7000 81**
Liver					
Adenoma, hepatocellular[a]	*0*	*0*	*0*	*4*	*1*
Carcinoma, hepatocellular	*0*	*0*	*0*	*1*	*0*
Adenoma and/or Carcinoma[b]	*0*	*0*	*0*	*5#*	*1*
Focus of cellular alteration	*2*	*1*	*3*	*6*	*7**
Intracellular pigment accumulation, Kupffer cell/macrophage	*24*	*34*	*27*	*22*	*26*
Intrahepatocellular erythrocytes	*0*	*0*	*0*	*0*	*9#*
Necrosis, individual hepatocellular, increased	*1*	*0*	*0*	*0*	*1*

a Includes single or multiple adenomas (there were no multiple adenomas in females)

b Total incidence of mice with hepatocellular tumours (adenoma, carcinoma, or both)

* Statistically significant by the Cochran-Armitage trend test ($p \leq 0.05$)

\# Statistically significant by Fisher's exact test ($p \leq 0.05$)

Several mechanistic studies were presented by the DS in relation to the increased incidences of Leydig cells hyperplasia and adenomas:

Hormone levels in serum of male rats fed with triflusulfuron-methyl for 1 year were analysed. Doses were 0, 10, 100, 750 and 1500 ppm. Testosterone, estradiol, Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) levels were affected at the higher doses (750 and 1500 ppm) while prolactin remained constant. The DS suggest that the reduction seen in estradiol (possibly caused by aromatase inhibition, see below) would lead to increased FSH and LH levels, in turn leading to the observed Leydig cell hyperplasia and increase in adenoma formation. At lower doses (10 and 100 ppm) where no effects on hormone levels were seen, no tumours were observed.

In a two-week oral study in the rat with high doses (0, 1000, 1500, 2000 mg/kg/day) of triflusulfuron-methyl, the relative and absolute weight of accessory sex glands was significantly reduced in comparison with pair-fed controls. Levels of estradiol were again decreased accompanied by a small increase in LH and FSH.

A number of *in vitro* studies were reported. It was shown that triflusulfuron-methyl acted as an aromatase inhibitor at all doses tested and that synthesis of estradiol was inhibited in cultured Leydig cells. However, in a 28 day oral Sprague-Dawley rat study dosed daily with low levels of triflusulfuron-methyl (up to 5 mg/kg bw/day), no decrease in estradiol or other hormone levels was seen.

The DS proposed classification as CLP Category 2 with H351.

Comments received during public consultation

Three Member States agree to the proposal. One industrial organisation disagreed with the classification and questioned the relevance of the animal data to humans.

During public consultation Industry stated that the tumours in male rats occurred in aged rats known to be especially susceptible to this tumour type. They further questioned the human relevance of the findings from a mechanistic point of view.

According to the Commission Working Group of Specialised Experts on carcinogenicity, mutagenicity and reprotoxicity (COM WG), studies on Fischer rats or other strains having a comparably high spontaneous Leydig cell tumour rate are normally not informative with regard to Leydig cell tumours (ECBI/08/04 Rev.2). However, the two-year study considered here was carried out with Sprague-Dawley (SD)-rats.

SD rats are reported not to be as susceptible as Fischer rats to the development of Leydig cell tumours in aged animals, even if such tumours in the case of triflusulfuron-methyl were not observed until late in the study. Thus RAC considers SD-rats to be a relevant species for findings on Leydig cell tumours leading to classification. Industry also questioned the human relevance of the mode of action of triflusulfuron-methyl on the Leydig cells. However RAC agreed with the DS and the COM WG that Leydig cell tumours in rats are relevant to humans if the mode of action is not through the perturbation of the hypothalamus-pituitary-testis (HPT) axis as a dopamine agonist or a gonadotropine releasing hormone (GnRH) agonist do. In the case of triflusulfuron-methyl the mode of action is probably mediated by aromatase inhibition, and considered relevant to humans.

<u>Comments received shortly before the RAC plenary</u>
Industry submitted a document entitled "Response to Opinion Development Document", with comments to the RAC draft opinion. In this document Industry expressed their disagreement with the draft opinion in relation to the conclusions contained in ECBI/08/04 Rev.2 regarding the possible human relevance of Leydig cell tumors (LCT). . In Industry's view, new information (published after 2004) on humans with mutations in the aromatase encoding gene (CYP-19) and new information from aromatase knock-out mice (CYP-19 knock out) supports an alternative mode of action. Industry summarised studies with 9 adult men aged 25-30 with aromatase deficiency where no instances of testicular neoplasia were reported from the clinical examination. Industry also stated that neither were any Leydig Cells Tomours seen in the aromatase knock-out mice, which in Industry's opinion is a better model than the rat for predicting LCT in humans.

Industry challenged the proposed mode of action from the COM WG and also described human cases as well as mice studies with the *opposite* MoA, where LCT is induced after increased aromatase expression, and not after inhibition.

These comments were received less than 10 calendar days prior to the RAC plenary meeting and the Committee was unable to take them fully into account. It was pointed out that considering the DS proposal for Repr. 2 under CLP which had been open for public consultation, this information could more appropriately have been submitted at that point in time. None of the supporting studies were new and the late introduction of these comments in the context of an opinion tabled for discussion/adoption was not explained to the Committee.

Assessment and comparison with the classification criteria

The carcinogenicity data for triflusulfuron-methyl consists of a 2-year carcinogenicity study in rats and an 18 month combined chronic toxicity/carcinogenicity study in mice. No information in humans was available. Thus classification in CLP Category 1A and Category 1 in DSD is not warranted.

In SD rats there was a statistically significant increase in incidences of interstitial cells hyperplasia (Leydig cells, testes) and adenomas at the two highest dose levels. No neoplastic lesions were observed in female rats. In mice, triflusulfuron-methyl slightly increased the incidence of hepatocellular adenomas in males at the two highest dose levels. There was no increase of tumours in female mice.

The slight increase of hepatocellular adenomas in male mice in the two highest dose groups and in female mice in the 2500 ppm dose group are not considered relevant for classification because they were within laboratory historical control ranges, only statistically significant in male mice, predominantly benign, and only observed in a single species. Also no effect was observed in mice on hepatic cell proliferation.

According to CLP a substance should be classified in Category 1B if a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. Substances may also be classified in Category 1B according to CLP if they produce an increased incidence of tumours in both sexes of a single species in a well-conducted study or if the substance leads to an unusual degree of malignant of neoplasms in one species and sex.

In this case the findings are not sufficient to justify classification in CLP Category 1B.

According to the DSD, classification in Category 2 requires either positive results in two animal species or clear positive evidence in one species, together with supporting evidence. For triflusulfuron-methyl classification in Category 2 according to DSD is not required.

CLP states that substances should be classified as Category 2 carcinogens when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. In the case of triflusulfuron-methyl, there is limited evidence of carcinogenicity in rats and mice manifested as benign tumours in different organs, i.e. testes (rats) and liver (male mice). The findings in mice are not considered relevant for classification for the reasons given above. There is no clear dose-response for the tumour findings in rats, as 7 testis adenomas were found in each of the two highest dose groups and none in the other dose groups. For interstitial cell hyperplasia a dose-response was present in male rats. Studies of a possible mode of action (MoA) in rats indicate that the carcinogenic effect from triflusulfuron-methyl on Leydig cells stems from a perturbation of the hypothalamus-pituitary-testis (HPT) axis mediated by aromatase inhibition. This MoA is considered to be relevant to humans.

RAC agreed with the DS that classification in CLP Category 2 with H351 was justified. In parallel, the DS proposal for classification in Category 3 with R40 according to DSD was also justified, as there was some evidence from appropriate animal studies.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

One two-generation study in rats (OECD 416) and two prenatal development studies (OECD 414) in rat and rabbit where included in the report. No treatment related adverse effects were seen in the two generation study in rats or the prenatal development study in rabbits. In the rat prenatal development study four pups with malformation were seen in four litters and there was an increase in variants. The DS concludes that the number of malformations were low and within the

historical control and where probably caused by maternal toxicity; they considered that classification was not warranted.

Comments received during public consultation

Two comments from MSCAs agree with not classifying the substance for this hazard class.

Assessment and comparison with the classification criteria

Triflusulfuron-methyl was tested in a two-generation toxicity study in SD-rats and in prenatal development toxicity studies in SD-rats and (NZW) SPF-rabbits. No fertility or developmental effects were seen in the two-generation study. No malformations were observed in the prenatal development toxicity study in rabbits. In rats some malformations were seen at the top dose. This was not statistically significant. In rats receiving 350 mg/kg bw/d some variations due to retarded development were seen in the prenatal development toxicity study, in four fetuses from four different litters in the top dose group. No consistent pattern of malformations was seen, as the malformations were found in various anatomical sites.

Some effects were seen on testes in rats and dogs in subchronic studies but the effects were not consistently found across these studies or in the chronic study in dogs. Testes seemed to be the target organ in rats and dogs in studies of subchronic toxicity, manifested as testicular atrophy and reduced testicular weights in the top dose group in one of the two 90-days rat studies (accompanied with high general toxicity and poor nutritional status), and as testicular atrophy and reduced testicular weights in dogs in the medium and high dose group in a 90-day study. This effect was however not seen in the 1 year dog study where the doses were lower. In the carcinogenicity study in rats testis was the target organ, see section on carcinogenicity.

RAC supported no classification for reproductive toxicity as proposed by the DS.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed classification as Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=10) according to CLP, and R 50/53 according to DSD with the following specific concentration limits:
N; R50-53: Cn ≥ 0.25%
N; R51-53: 0.025% ≤ Cn < 0.25%
R52-53: 0.0025% ≤ Cn < 0.025%

The proposal is based on the acute toxicity from test results for all three trophic levels, three fish studies (for *Oncorhynchus mykiss*, *Lepomis macrochirus* and *Cyprinus carpio*), one study of crustaceans (*Daphnia magna*), three studies of algae (two *Pseudokirchneriella subcapitata* and one *Anabaena flos-aquae*) and two tests with the duckweed *Lemna gibba*. In addition, chronic test results are available for *Oncorhynchus mykiss (two studies)*, *Daphnia magna*, *Pseudokirchneriella subcapitata* (two studies), *Anabaena flos-aquae* and *Lemna gibba* (two studies), which allows setting a separate M-factor.
The proposed classification is based on short- and long-term toxicity results on *Lemna gibba* 14-d ErC_{50} of 0.0035 mg/l and 14-d NOEC of 0.00127 mg/l, respectively, together with the fact that the substance is not rapidly degradable (or readily biodegradable).

Comments received during public consultation

Six Member States (MS) submitted comments on environmental classification during public consultation, all in agreement with the proposed classification.
Four MS had specific comments, mainly on the evaluation and selection of the key studies (e.g. the following suggestions were made: calculation and use of 7-day values for acute classification (2 MS), correction of the mean measured concentrations of one key study to initial measured concentrations (1 MS) and the use of another key study for classification (2 MS)).

The re-evaluation of the relevant studies and values revealed the results to be in the same concentration range with no effect on the final classification or setting of the M-factors. One MS asked for specification of the metabolites formed by hydrolysis and clarifications concerning the water sediment simulation tests and soil photolysis study. The DS provided this information in the RCOM document.

Assessment and comparison with the classification criteria

Degradability:
In one hydrolysis study on triflusulfuron-methyl two hydrolysis products and DT_{50} values of 3.7, 32 and 36 days were determined at 25°C and pH 5, 7 and 9, respectively.

According to an OECD 301 D screening test based on oxygen consumption only 25% of triflusulfuron-methyl was biodegraded within 28 days. Therefore the substance is considered as not readily biodegradable.

A water-sediment study with two aerobic water-sediment systems (pH water: 7.5) resulted in a half-life of 22-40 days for the whole system (measured with applied radioactivity) so that triflusulfuron-methyl also failed to achieve ultimate degradation within 16 days.

The aerobic route of degradation and degradation rate of triflusulfuron-methyl were analyzed in four soil types "Triflusulfuron-methyl exhibits low to moderate persistence in soil under dark aerobic conditions at 20°C (DT_{50} lab aerobic = 5.3 – 15 d) or 25°C (DT_{50} lab aerobic = 5.7 d [geometrical mean of the two labels]). Under dark anaerobic conditions at 25°C, degradation of triflusulfuron-methyl is slower than under aerobic conditions (DT_{50} anaerobic = 21 d)." Although not directly relevant for classification purposes, these results provide some further evidence for the lack of rapid degradability.

On this basis, triflusulfuron-methyl does not meet the criteria for being rapidly degradable or readily biodegradable in the environment.

Bioaccumulation:
No measured data on bioaccumulation are available for triflusulfuron-methyl. However, with log Kow values of 2.3 (pH 5, 25°C), 0.96 (pH 7, 25°C) and -0.066 (pH 9, 25°C), the substance does not show a potential for bioaccumulation.

Ecotoxicity:
Valid ecotoxicolgical data is available for all three trophic levels. The purity of triflusulfuron-methyl used in the key studies ranges from 95.6% – 98.7% and complies with the specified composition in Section 1 of the CLH report. The lowest results from all valid studies were as follows (the key study results proposed by the DS are highlighted in **bold**, RAC prefers however using for classification purposes the recalculated values highlighted in ***bold italics***):

Trophic level	**Species**	**Short-term result**	**Long-term result**
Fish	*Oncorhynchus myciss*	96-h LC_{50} = 730 mg a.s./L (m.m.)	21-day NOEC = 210 mg a.s./L (m.m.) 97-day NOEC = 57.7 a.s./L. (m.m.)
Aquatic invertebrates	*Daphnia magna*	48-h EC_{50} > 960 mg a.s./L. (m.m.)	21-day NOEC = 11 mg a.s./L. (m.m.)
Aquatic algae and	*Pseudokirchneriella subcapitata*	120-h EbC_{50} = 0.046 mg/L (nom.) ---------- <u>*Recalculated:*</u> 96-h ErC_{50} = 0.137 mg/L (nom.)	120-h NOEC = 0.036 mg/L (nom.) ---------- <u>*Recalculated:*</u> 96-h NOEC = 0.018 mg/L (nom.)

aquatic plants	*Lemna gibba*	**14-day ErC_{50} = 0.0035 mg/L (nom.)** --- <u>*Recalculated:*</u> 14-day ErC_{50} = 0.0025 mg/L (initial measured concentration) ***7-day ErC_{50} = 0.002 mg/L (initial measured concentration)***	14-day NOErC = 0.0015 mg/L (nom.) --- <u>*Recalculated:*</u> *14-day NOErC = 0.0011 mg/L (initial measured concentration)* ***7-day NOErC = 0.0011 mg/L (initial measured concentration)***
	Lemna gibba	14-day ErC_{50} = 0.00282 mg/L (nom.) --- <u>*Recalculated:*</u> 7-day ErC_{50} = 0.00269 mg/L (nom.)	**14-day NOErC = 0.00127 mg/L (nom.)** --- <u>*Recalculated:*</u> 7-day NOErC = 0.00127 mg/L (nom.)

m.m. = mean measured concentrations, nom = nominal concentrations

The toxicity values for fish and aquatic invertebrates are based on mean measured concentrations (m.m.), whereas the studies for aquatic algae and plants provide only nominal concentrations (nom.). The available ecotoxicological data for fish and aquatic invertebrates lie outside the classifiable range (for acute toxicity LC_{50} > 100 mg/L and for chronic toxicity NOEC > 1 mg/L); the test results for aquatic algae and plants show that *Lemna gibba* is the most sensitive aquatic species.

For one *Lemna gibba* study the initial (0 hours) measured test concentrations were only 73-78% of the nominal concentrations. One MS corrected the test results from nominal to initial measured concentrations. When the measured initial concentration falls below 80%, OECD test guideline 221 recommends the use of a semi-static test regime. As the present study was conducted under static conditions, RAC supports the approach to use initial measured instead of nominal concentrations. These corrections do however not affect the resulting classification. The **corrected 14-day ErC_{50}** (initial measured concentration) is **0.0025 mg/L** instead of the originally presented 14-day ErC_{50} of 0.0035 mg/L (nominal concentration) **for acute toxicity** and the **14-day NOErC = 0.0011 mg/L** instead of 14-day NOErC = 0.0015 mg/L **for chronic classification**. All test results range within the same order of magnitude.

The *Lemna gibba* studies were carried out according to EPA - or ASTM-guidelines respectively and were run over a period of 14 days. The OECD test guideline 221 recommends a test duration of 7 days and during public consultation one MS recalculated the 7-day ErC_{50} values for the *Lemna* studies. As for classification of the acute effects based on aquatic plant toxicity tests, the use of the EC_{50} values on day 7 (if available) is generally preferred instead of the data at day 14, RAC supports the approach to recalculate the 7-day ErC_{50} values for the *Lemna* studies. These are 7-day ErC_{50} = 0.002 and 7-day ErC_{50} = 0.00269, respectively and in the same concentration range as the key study proposed by the DS, so that this does not affect the CLH classification or the M-factors. For consistency, the 14-day NOErCs presented by the DS were also corrected to 7-day NOErCs and included in the table. The values are the same and consequently neither influences the classification nor the M-factors.

However, based on the correction of the results from nominal to initial measured concentrations in combination with the generally preferred use of test results of day 7 for classification purposes, RAC accepted this as the key study also for classification of the chronic toxicity.

Regardless of any re-calculations, the available data reveals an acute aquatic toxicity in the range of 0.001 < $L(E)C_{50}$ ≤ 0.01 and a chronic aquatic toxicity of 0.001 < NOEC ≤ 0.01, warranting a classification as Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=10).

Classification according to CLP:

***Acute aquatic hazard**:*
Acute toxicity data is available for all three trophic levels. With toxicity values of ≥ 700 mg/L, fish and aquatic invertebrates are outside the classifiable range.
The most sensitive aquatic species is *Lemna gibba*. The lowest reliable short-term aquatic toxicity result is 7-day ErC_{50} = 0.002 mg/L (initial measured concentration) for *Lemna gibba*. While the result for the aquatic algae is one magnitude higher, it is still in the classifiable range. Triflusulfuron-methyl is therefore classifiable as Aquatic Acute 1 (H400), with an M-factor of 100 ($0.001 < L(E)C_{50} \leq 0.01$).

***Chronic aquatic hazard**:*
Triflusulfuron-methyl is considered to be not rapidly degradable. Data is presented for all three trophic levels. The lowest long-term aquatic toxicity result is the 7-day NOErC = 0.0011 for *Lemna gibba*, leading to classification as Aquatic Chronic 1 (H410) with an M-factor of 10 ($0.001 < L(E)C_{50} \leq 0.01$ when not rapid degradable).

Classification according to DSD:

The lack of ready biodegradation together with an EC_{50} = 0.002 mg/L (initial measured concentration) for *Lemna gibba* mean that triflusulfuron-methyl fulfils the criteria for classification with N; R50-53. The following specific concentration limits are applicable:

Concentration of triflusulfuron-methyl in the mixture; C (w/w)	**Classification of the mixture**
Cn ≥ 0.25%	N; R50-53
0.025% ≤ Cn < 0.25%	N; R51-53
0.0025% ≤ Cn < 0.025%	R52-53

In summary, RAC agrees with the original proposal of the DS to classify triflusulfuron-methyl as **Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=10)**.

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 DS and rapporteurs' comments (excl. confidential information).