Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl- 1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate

EC Number: -
CAS Number: 79277-27-3

CLH-O-0000001412-86-136/F

Adopted

9 December 2016

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9 December 2016

CLH-O-0000001412-86-136/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 Regulation (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:

Chemical name: **thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate**

EC Number: **not available**

CAS Number: **79277-27-3**

The proposal was submitted by the **United Kingdom** and received by RAC on **4 February 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom 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 **7 March 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 April 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

Co-Rapporteur, appointed by RAC: **Paola Di Prospero Fanghella**

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 adopted on **9 December 2016** by **consensus**.

Existing Annex VI entry (CLP, Table 3.1)

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	016-096-00-2	thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) thiophene-2-carboxylate		79277-27-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	016-096-00-2	thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) thiophene-2-carboxylate		79277-27-3	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H400 H410	**Retain** GHS09 Wng	**Retain** H410		**Add** M=100 M=100	
RAC opinion	016-096-00-2	thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) thiophene-2-carboxylate		79277-27-3	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H400 H410	**Retain** GHS09 Wng	**Retain** H410		**Add** M=100 M=100	
Resulting Annex VI entry if agreed by COM	016-096-00-2	thifensulfuron-methyl (ISO); methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) thiophene-2-carboxylate		79277-27-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Due to discrepancies between the existing harmonised classification and the recommendations in the EFSA conclusion, the DS's CLH proposal is targeted at the hazard classes developmental toxicity and carcinogenicity. Additionally, the endpoints mutagenicity and repeated dose toxicity were assessed by RAC.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

The repeated dose toxicity of Thifensulfuron-methyl was investigated in 90-day oral feeding studies in rats and mice and in 90-day and 1-year dietary studies in dogs. These studies showed that there were no specific target organs showing repeated dose toxicity following exposure to THIFENSULFURON-METHYL in any of the three species investigated. Only body weight gain reduction and reduced food efficiency were observed at a relatively high dose of 2500 ppm (177 mg/kg bw/day) or greater in rats (bw gain reduction up to 18/29% in m/f at 7500 ppm) and 7500 ppm (200 mg/kg bw/day) in dogs (bw gain and food efficiency reduction of 60-70% and 60%, respectively in females in the 1 year study). There were no adverse effects in mice up to the highest dose of 7500 ppm (1427/2287 mg/kg bw/day in m/f) for 90 days, indicating a lower sensitivity to the substance in this species.

The DS concluded that classification of thifensulfuron-methyl for STOT-RE is not warranted.

Comments received during public consultation

No comments received on classification for this end-point during public consultation.

Assessment and comparison with the classification criteria

In the dietary repeated dose toxicity studies of thifensulfuron-methyl in rats, mice (90-day) and dogs (90-day and 1-year) no specific target organs were identified. The only effects observed in rats and dogs were reduced body weight gain and food efficiency starting at 177 mg/kg bw in rats (90 d exposure) and at 200 mg/kg bw in dogs (90 d and 1 year study). No adverse effects in mice up to the top dose were observed, indicating a lower sensitivity for this species. According to the CLH regulation, a substance meets the criteria for classification as STOT-RE category 2 if it can be presumed it has the potential to be harmful to human health following repeated exposure at concentrations below 100 mg/kg bw/day in rats after 90 days of exposure (300 mg/kg bw/day after correction with Haber's rule for 28 days of exposure). RAC notes that the effects reported after repeated oral exposures do not warrant classification because the severity of the findings was low at doses below the guidance values for classification. RAC agrees with the DS that **classification of thifensulfuron-methyl for STOT-RE is not warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS has assessed the available studies on mutagenicity, but also indicated that these have been presented in the CLH proposal because they could be relevant in the evaluation of the carcinogenic potential of the substance.

Thifensulfuron-methyl tested negative in several *in vitro* studies (three bacterial mutagenicity tests, one mammalian cell gene mutation assay, one chromosome aberration assay, one UDS test) and *in vivo* studies (one micronucleus and one chromosome aberration assay). No clear conclusions could be drawn on the potential of thifensulfuron-methyl to cause gene mutations in bacteria, since only low concentrations could be used due its bacteriostatic nature. However, based on the non-bacterial tests, the DS concluded that thifensulfuron-methyl is not genotoxic. The Ds proposes 'No classification' for mutagenicity.

Comments received during public consultation

Two comments from member state competent authorities (MSCA) and one from an individual have been received during public consultation (PC), all in favour of no classification for germ cell mutagenicity. Two related comments on the need for a higher level of detail in the reported studies in the CLH dossier were also received.

Assessment and comparison with the classification criteria

Gene mutation in bacteria was tested in three independent assays. The mutagenic potential of the substance has been further addressed in two *in vitro* mammalian cell test (UDS assay in isolated rat hepatocytes and hprt assay in CHO cells), all with negative results.

The ability of thifensulfuron-methyl to induce structural chromosome aberrations was tested in cultured human lymphocytes with a negative outcome. However, due to the lack of reproducibility between the two replicates of the positive control, findings from the study were considered to be equivocal.

The potential clastogenicity of thifensulfuron-methyl was also tested *in vivo*.

In the MN test in the bone marrow of mice, the tested dose of thifensulfuron-methyl was 5000 mg/kg bw (a dose exceeding the maximum recommended dose in accordance with current *in vivo* genotoxicity regulatory guidelines). This dose caused significant systemic toxicity (tremors, ptosis, body drop, decreased body tone and activity) and macroscopic findings (fluid-filled distended stomach, red lungs, discoloured intestine) in treated animals. The P/N ratio was affected in males sacrificed at 48 hr (0.78 vs 1.22 in the control group). thifensulfuron-methyl did not induce cytogenetic damage in the bone marrow MN test.

The potential clastogenicity of thifensulfuron-methyl *in vivo* was also tested in a rat chromosome aberration test. Animals were treated by gavage with 5000 mg/kg bw. Significant body weight loss was observed in the treated animals. No cytogenetic damage was observed in the bone marrow cells of treated rats. Although exposure of the bone marrow to thifensulfuron-methyl was not demonstrated in the study (no change in the mitotic index), it is noted that in the RAR an *in vivo* metabolism study is summarised where 0.001% of the administered dose (1774 -1900 mg/kg bw) was detected in the bone marrow.

RAC concludes that thifensulfuron-methyl is not genotoxic and agrees with the DS that **classification for germ cell mutagenicity is not warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The chronic toxicity and carcinogenicity of thifensulfuron-methyl in the diet were investigated in a 2-year carcinogenicity study in Sprague Dawley rats and in an 18-month carcinogenicity study in CD-1 mice.

In the mouse study, terminal body weights and mean body weight gain were statistically significantly decreased in females at the top dose of 1312 mg/kg bw/d. thifensulfuron-methyl did not show carcinogenic potential in female mice dosed up to the MTD (top dose) and in male animals dosed up to the limit dose (979 mg/kg bw/d).

In the rat, an increase in mammary gland adenocarcinoma was seen in females (29% and 32% at 500 and 2500 ppm respectively vs 21% in controls, not statistically significant). Although this increase was slightly above the laboratory HCD (range = 8.3-23.4%; mean = 17%), it was within contemporary published HCD (range = 7-31% ; mean = 18%). The increase stands against a very high background incidence of 21% in the concurrent controls; the incidence at the top-dose was only 1.5 x greater than that in the concurrent controls; the tumour incidences at the top two doses were not statistically significantly different from that in controls; the dose-response relationship was relatively flat over an approximate 100-fold exposure range (25 ppm to 2500 ppm); tumour latency was not shortened; and similar tumours were not seen in mice. No significant non-neoplastic lesions in the mammary gland were observed at 500 and 2500 ppm and the incidence of lobular hyperplasia was decreased compared to controls.

In addition, QSAR assessments show that thifensulfuron-methyl and its rat and groundwater metabolites (including triazine amine) do not bind to the oestrogen receptor or the dopamine receptors.

Furthermore, in an *in vitro* E-screen assay in MCF-7 human breast cancer-derived cells, thifensulfuron-methyl showed no oestrogenic activity. By taking into account that mammary gland tumours in rodents tend to arise as a consequence of oestrogenic activity or antagonism of dopamine receptors, the absence of such activity in thifensulfuron-methyl and its metabolites lends further support to the assertion that the mammary gland adenocarcinomas seen in the thifensulfuron-methyl rat cancer study are not treatment-related.

Overall, therefore, different strands of evidence lead to the conclusion that the slight increase in mammary gland adenocarcinoma observed at 500 and 2500 ppm thifensulfuron-methyl is not treatment-related but a chance finding in a strain of rats highly susceptible to mammary gland tumourigenesis. On this basis, the dossier submitter concluded that thifensulfuron-methyl is not carcinogenic to Sprague-Dawley rats.

Comments received during public consultation

Three MSCA commented on this endpoint. Two were in favour of no classification, indicating that the test was conducted with a rat strain known for high spontaneous incidences of mammary gland tumours. Also, new data showed that thifensulfuron-methyl does not have an endocrine tumorigenic mode of action in the rat. One MSCA raised some concern on the possible tumorigenic MoA of thifensulfuron-methyl in the rats similar to that seen with other triazinyl-sulfonylurea herbicides showing tumours in mammary glands and for which a possible endocrine mode of action has not been clarified. Two industry representatives and one individual argued that classification for carcinogenicity was not warranted.

A substantial amount of new information was also submitted by Industry, including studies on endocrine tumorgenic mode of action. The submitted information and argumentation is summarised in the section "additional key elements".

Assessment and comparison with the classification criteria

In the mouse carcinogenicity study, thifensulfuron-methyl did not induce tumour formation in males or females up to the highest tested dose.

In a two-year carcinogenicity oral study in SD rats, the incidence of various non-neoplastic changes among the treated groups were increased or decreased in some cases reaching statistical significance when compared to the control group. No significant non-neoplastic lesions in the mammary gland were found and the incidence of lobular hyperplasia was decreased in treated females compared to controls (97%, 72%, 69% and 82% at 0, 25, 500 and 2500 ppm, respectively). The incidence of tumour formation in male rats was very high in control and treatment group animals. No statistically significant difference between control and treated groups was observed, except a slight increase in the number of pituitary gland adenoma in the 500 ppm group, which was not observed at the highest dose and therefore was not considered treatment related (see table below).

Table: Incidence of tumours in male rats (data taken from the Renewal Assessment Report (RAR), 2014)

Male rats	**0 ppm**	**25 ppm**	**500 ppm**	**2500 ppm**
Animals with primary tumours	51/61 (84%)	55/61 (90%)	53/60 (88%)	52/60 (87%)
Animals with malignant tumours	7/61 (11%)	16/61 (26%)	12/60 (20%)	10/60 (17%)
Animals with benign tumours	48/61 (79%)	50/61 (82%)	53/61 (87%)	48/61 (79%)
Pituitary Gland Adenoma	35/61 (57%)	34/48 (71%)	37/46 (80%)*	40/60 (67%)

* Significantly different from controls at $P < 0.05$ (Fisher test)

Also, the incidence of tumour formation in female rats was generally very high in control and treatment group animals, with elevated total numbers of malignant tumours in the 500 and 2500 ppm groups and statistical significance at the top dose (see table below). However, the increased number of malignant tumours was not due to any particular statistically significantly increased type of tumour nor were any rare tumours observed. The formation of benign tumours in females was even lower in the treatment groups than in the control group.

RAC is of the opinion that the most relevant observation in this study was with 29% and 32% increases in mammary gland adenocarcinoma at 500 and 2500 ppm, respectively in females compared to 21% in control animals. However, the increase in adenocarcinoma was very shallow, without statistical significance and only slightly above the laboratory HCD (8.3-23.4%) and contemporary published HCD (range = 7-31%).

Table: Incidence of tumours in female rats

Female rats	**0 ppm**	**25 ppm**	**500 ppm**	**2500 ppm**
Animals with primary tumours	56/59 (95%)	56/59 (95%)	53/60 (88%)	61/62 (98%)
Animals with malignant tumours	16/59 (27%)	17/59 (29%)	25/60 (42%)	29/62 (47%)*
Animals with benign tumours	55/59 (93%)	53/59 (90%)	48/60 (80%)	53/62 (85%)
Mammary gland				
Fibroadenoma[a], multiple	7/58	6/54	5/52	5/62
Fibroadenoma[a], single	12/58	9/54	14/52	14/62
Adenocarcinoma, multiple	4/58	1/54	5/52	5/62
Adenocarcinoma, single	8/58	5/54	10/52	15/62
Adenocarcinoma, single+multiple	**(14%)** 12/58	**(9%)** 6/54	**(19%)** 15/52	**(24%)** 20/62
Adenosquamous cell carcinoma	**(21%)** -	**(11%)** -	**(29%)** 1	**(32%)** -

* significantly different from controls at P < 0.05 (Fisher test)

[a] Fibroadenoma most likely includes diagnosis of adenoma as adenoma was not reported separately

RAC pointed out that this rat strain is known to have a high spontaneous incidence of mammary gland tumours and that the weak dose-response relationship is insufficient to support the assumption of a treatment-related effect.

Overall, RAC agrees with the dossiers submitters proposal that **classification for carcinogenicity is not warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The potential effects of thifensulfuron-methyl on fertility and reproductive performance were investigated in a rat dietary 2-generation study. The animals were dosed with 0, 25, 500, 2500 ppm thifensulfuron-methyl in the diet (1.8, 34, 175 mg/kg bw/d in males; 2.4, 48, 244 mg/kg bw/d in females). In this study, there were no adverse effects on fertility, reproductive performance or offspring up to a dose (175 mg/kg bw/day), at which parental toxicity was seen.

Additionally, a preliminary one-generation study in rats dosed with 0, 100, 2500, 7500 ppm (0, 7, 177, 559 mg/kg bw/d in males; 0, 9, 216, 697 mg/kg bw/d in females) thifensulfuron-methyl was performed. Fertility was low in the control animals and therefore no meaningful comparison between reproductive parameters in the treated groups and in the control groups could be performed.

The DS concluded that classification of thifensulfuron-methyl for fertility is not warranted.

Development

The developmental toxicity potential of thifensulfuron-methyl was investigated in one standard rat and one rabbit study.

The rabbits were dosed with 0, 22, 158 or 511 mg/kg bw/d thifensulfuron-methyl via gavage. At the highest tested dose, maternal toxicity in the form of body weight loss during gestation day (GD) 7-9 and body weight gain reduction was observed. There were no adverse effects on development in rabbits up to the top dose of 511 mg/kg bw/day.

In the developmental toxicity study in Sprague-Dawley rats, thifensulfuron-methyl was administered in doses of 0, 30, 200, or 800 mg/kg bw/day from days 7-16 of gestation. The day of mating was designated as day 1 of gestation, and caesarean sections were performed on gestation day 21. The current guideline (OECD TG 414) designates the day of mating as day 0 of gestation, with caesarean sections performed on gestation day 21 (one day later that in the study with thifensulfuron-methyl). Limited maternal toxicity (small decrease in body weight gain) and observation of absent renal papilla (5 foetuses/5 litters vs 1/1 in controls) in fetuses, delayed ossification and reduced foetal weight was observed at the top dose level of 800 mg/kg bw/day. The renal papilla size was scored macroscopically and a microscopic evaluation to confirm the observation was not conducted. The dossier submitter argued that in this study the foetuses were examined one day prior to what would be the current standard, and as a result of missing the last 24 hours of in utero development, an increase in developmental effects in the fetuses was seen. This was especially applicable to the foetal kidney since renal development occurs late in gestation, and continues into the postnatal period. Although the increased incidence of absent renal papilla was above the laboratory historical control data for this finding, without histological confirmation, the DS stated that it is most likely that in some of the affected foetuses the renal papilla was not completely absent but only very small.

The DS argued further that the observed developmental toxicity appeared to be the consequence of retardation and was seen in the presence of some maternal toxicity.

The dossier submitter concluded that classification of thifensulfuron-methyl for developmental toxicity is not warranted.

Comments received during public consultation

Four MSCA, 2 IND representatives and one individual commented on this endpoint. One MSCA was in favour of no classification indicating that the effects seen in the fetuses were most likely the unspecific, secondary consequence of maternal toxicity. Two MSCA and one individual were in favour of classification with Repr. Cat 2; H361d, based on findings in renal papilla. One MSCA was indecisive and requested more information on the developmental toxicity rat study. Three industry representatives commented in favour of no classification.

New data were submitted by Industry including one new developmental toxicity study and one position paper. The new data is summarised in the section "additional key elements".

Assessment and comparison with the classification criteria

Fertility

In a guideline compliant 2-generation study, slightly decreased body weights or body weight gains (4 to 9%) were recorded in parental females of the F0 generation as well as parental males and females of the F1 generation. Reproductive parameters such as gestation index, percent pups born alive, 0-4 day viability index, 1-4 day viability index, lactation index, litter survival, number of pups born, number of pups alive, pup weight and number of pups weaned were not affected by treatment with thifensulfuron-methyl.

Overall, no adverse effects on fertility and reproductive performance were observed after continuous treatment of rats during two generations with thifensulfuron-methyl.

On this basis, RAC is of the opinion that there is no indication that thifensulfuron-methyl interferes with sexual function and fertility.

Developmental

Two standard developmental toxicity studies (one with rats and the other with rabbits) on thifensulfuron-methyl were evaluated in the CLH report. In addition, one new GLP study on rats submitted by Industry during public consultation was also taken into account in this assessment.

In a guideline-compliant developmental toxicity study in rabbits, body weight gains in dams of the high-dose group were reduced (58-69%, not statistically significant) from days 7 to 20 of gestation, indicating evidence of minimal maternal toxicity. At the high dose, during days 7-9 of gestation, mean maternal body weight loss was 36 g compared to a gain of 4 g in control animals. No significant differences between the control and experimental groups in pregnancy rate, number of nidations, abortions or total resorption of litters were observed, nor were any substance related effects in the foetuses detected.

The developmental toxicity study on rats (1984) was conducted according to the OECD guidelines available at the time and the DS stated that the day of mating was designated as day 1 of gestation, and caesarean sections were performed on GD 21 with dosing from GD 7 to 16, noting that the current guideline and practice is to designate the day of mating as day 0 of gestation. In the study from 1984, various findings were seen in the fetuses, which are reported in the two following tables.

Table: Incidence of malformations in rat fetuses (data from the published RAR, 2014)

	0 mg/kg bw	**30 mg/kg bw**	**200 mg/kg bw**	**800 mg/kg bw**
Visceral				
Number examined	180/25 (a)	156/22	159/23	168/24
Number affected	3/3	1/1	2/2	8/8 (*)
Kidneys : - Renal papilla - absent	1/1	1/1	-	5/5
Microphtalmia	-	-	1/1	2/2
Hydrocephaly	-	-	-	1/1
Skeletal				
Number examined	346/25	297/22	303/23	322/24
Number affected	1/1	-	-	-

Fetuses/litters. (*) significantly different from controls at P< 0.05 (Fischer test)

Table: Incidence of variations in rat fetuses (data from the published RAR, 2014)

	0 mg/kg bw	30 mg/kg bw	200 mg/kg bw	800 mg/kg bw
DEVELOPMENTAL VARIATIONS External Number examined	346/25(a)	297/22	303/23	322/24

Number affected	6/5	9/8	8/8	6/5
Visceral				
Number examined	180/25	156/22	159/23	168/24
Number affected	14/9	19/13	27/15	28/13
- Pulmonary arteries common trunk	6/5	7/7	13/9	5/4
- Renal papilla - small (§)	-	-	1/1	4/4(*)
- Renal pelvis - large	8/7	13/7	14/8	19/8
Skeletal				
Number examined	346/25	297/22	303/23	322/24
Number affected	24/15	23/14	30/18	27/17
Total with developmental variations	42/19	48/21	59/22	59/22
Mean % affected per litter	12.6%	16.0% (*)	19.8% (**)	18.2%
RETARDED OSSIFICATION				
Number examined	346/25	297/22	303/23	322/24
Number affected	106/23	77/22	103/20	119/22
- Sternebrae, partial or no ossification	54/18	49/17	62/16	70/15
- Skull bones partially ossified	1/1	-	1/1	10/5
TOTAL WITH VARIATIONS				
(including retarded ossification)	135/25	114/22	145/23	163/24
Mean % affected per litter	38.7%	39.0%	48.0%	49.1% (*)

foetuses / litters; (*) significantly different from controls at P < 0.05 (Fisher test);
(**) significantly different from controls at P < 0.01 (Fisher test)
(§) significant dose-related response (P < 0.01)

As can be seen in the tables, effects on renal papillae (incidence of absent and small papilla increased at 800 mg/kg bw) and microphtalmia were the most prominent findings in this study. Microphthalmia was dose dependently increased (1 foetus in 1 litter at mid dose, 2 foetuses in 2 litters at 800 mg/kg bw) with no statistical significance. The findings of microphathalia were not mentioned in the CLH report.

In a developmental reproductive toxicity study in rats submitted during public consultation, animals were dosed from GD 6 until GD 20 (therefore longer than in the preceding study and in compliance with modern guidelines) at 0 and 800 mg/kg bw/d, corresponding to the dose level at which the findings of absent/small renal papilla were seen in the original study from 1984. The kidneys, including renal papilla, of foetuses were examined at GD 21 (instead of GD 20, as in the preceding study). In addition, external examinations of the foetuses were performed. The findings reported in the previous study (small or absent renal papilla) were not reproduced.

In the 2 generation reproductive toxicity study in SD rats, no abnormalities of the kidneys in offspring were observed. According to comparative dosimetry assessment for female SD rats in the developmental and reproduction studies, the disparity in renal papillary findings cannot be

explained on the basis of lower doses used in the feeding study. This is probably because the maternal blood concentrations were predicted to be higher in the 2-generation study on GD19 than in the developmental toxicity study from 1984, in which dosing discontinued on GD 15. Additionally, in the published RAR (2014) it is reported that laboratory historical control data for microphthalmia in SD rats, indicate that these findings were not related to treatment with thifensulfuron-methyl (range of 0/0 – 2 foetuses/2 litters from 31 developmental toxicity studies conducted between 1982 and 1989). Historical control rat data have been also submitted during PC. Of the 17 studies conducted by gavage during the period 1981-1989, a mean (± SD) of 0.3 (± 0.7) foetuses with microphthalmia were calculated, with 14 studies with incidences of 0 foetuses; 1 study with an incidence of 1 foetus; and 2 studies with incidences of 2 foetuses.

RAC concluded that there is no evidence for developmental toxicity in rabbits. The malformation seen in the kidneys and eyes of rat foetuses in one development toxicity study could not be confirmed, either in the more recently conducted developmental reproductive toxicity study in rats with a longer exposure time, or in the 2 generation toxicity study. Both studies were conducted with the same (relevant) rat strain. Additionally, the incidences of microphthalmia observed were not statistically significant, and were within the historical control range.

Therefore, RAC concluded that the evidence for developmental toxicity was not sufficient for classification.

Overall, RAC agrees with the dossiers submitters proposal that **classification for reproductive toxicity is not warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

thifensulfuron-methyl is a pesticidal (herbicidal) active substance, currently listed in Annex VI to CLP. The existing harmonised entry includes a classification for the environment as Aquatic Acute 1; H400 - Very toxic to aquatic life and Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects. The DS proposed to retain this classification and to add separate acute and chronic M-factors of 100 and 100, respectively.

The DS indicated aquatic plants as the most sensitive trophic level. Based on available data, the DS proposed an environmental hazard classification as **Aquatic Acute 1** (H400) with an **M-factor of 100** based on acute aquatic toxicity for *Lemna gibba* (7d E_rC50 = 0.0011 mg/L), and **Aquatic Chronic 1** (H410) with an **M-factor of 100**, based on chronic aquatic toxicity for *Lemna gibba* (7d NOE_rC = 0.00037 mg/L) and being not rapidly degradable.

Degradation

Based on two reliable aqueous hydrolysis studies, the DS indicated that thifensulfuron-methyl is hydrolytically stable at certain pH and temperatures but to also degrade rapidly at others. At the more environmentally realistic temperature of 20°C, hydrolysis DT_{50}s were 6.3 days at pH 4, 199 days at a neutral pH 7 and 23.4 days at pH 9 (Wardrope, 2011). According to another aqueous hydrolysis study (Simmonds and Buntain, 2012) conducted at 25°C, DT_{50}s were 2.4, 137 and 7.1 days at pH 4, 7 and 9 respectively. The DS considers that hydrolysis half life is not consistently <16 days for all environmentally relevant pH, therefore thifensulfuron-methyl screens as stable to hydrolysis.

Aqueous photolysis is envisaged to contribute significantly to the degradation of thifensulfuron-methyl in certain natural water systems. Based on the results of reliable studies (Ryan, 1986; Lentz, 2001 and Oddy, 2012) photolysis half lives of <16 days could occur - even assuming that the maximum daylight and summer sunlight at relatively southern latitudes experienced in the tests did not occur across the EU. However, given the turbid nature of typical EU surface waters, lack of depth integration and lack of sunshine at northern latitudes and at other times of the year, the DS concluded that photolysis alone is not sufficiently consistent to determine thifensulfuron-methyl as stable to photolysis.

In a ready biodegradation study (Barnes, 2000) (OECD 301B) conducted at pH 7.3 to 7.6 and 19.8 to 22.9°C minimal biodegradation (1 %) of thifensulfuron-methyl was observed over 29 days. According to the criteria requiring ≥ 60% of the theoretical CO_2 production within 10 days of achieving 10 % biodegradation, the DS concluded that thifensulfuron-methyl can be considered as not readily biodegradable.

Two aerobic water/sediment studies are available. One of them was conducted in two systems at 20°C in the dark for 182 days (Spare, 2000). The further analysis of the results from this study derived whole system degradation DT_{50} values of 18.2 – 26.1 days depending upon the system studied and calculation method (van Beinum and Beulke, 2006). A second aerobic water/sediment study was conducted in two systems at 20°C in the dark for 104 days. Whole system degradation DT_{50}s for thifensulfuron-methyl were calculated to be 17.6 – 32.3 days in the system studied and with the calculation method used (Simmonds, 2012). The geomean DT_{50} across the 4 systems studied was 22.8 days. Mineralisation rates were low at <3 to <9%. Dissipation of thifensulfuron-methyl from the water column to the sediment was low in all the systems studied (max 1.08% found in sediment). A large number of mainly hydrolysis and photolysis degradants have been isolated from the water/sediment systems, some at >10% in the water phase. No major degradants (>10%) occurred in sediment. However, as the aquatic toxicity of the degradants is lower than the parent substance thifensulfuron-methyl, they are not considered further in relation to the hazard classification of the parent substance.

Overall, the DS concluded that despite evidence of rapid photolysis under certain aqueous conditions, the available degradation information does not indicate that thifensulfuron-methyl is ultimately degraded (>70%) within 28 days (equivalent to a degradation half-life of <16 days). Consequently, the DS considered thifensulfuron-methyl as not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

The log Kow of thifensulfuron-methyl at 25°C, pH 7 was -1.65 (Huntley and Edgar, 2000). This value is below the CLP log Kow trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate. For any major environmental degradants of thifensulfuron-methyl, log Kow values were modelled using up to four different methods based on the degradants' structure. All values of degradants was below the CLP trigger value of ≥ 4, indicating that the potential of bioaccumulation is very low for thifensulfuron-methyl and also for its degradants.

In addition to the log Kow, an experimental fish bioconcentration study was provided with Bluegill sunfish (*Lepomis macrochirus*) at a nominal concentration of 5 mg/L (Larkin, 1984). The flow-through test design consisted of a 28-days exposure phase followed by a 14-days depuration phase. Water temperature was maintained at 20-22°C and fish were sampled at regular intervals during the exposure and depuration periods. The calculated whole fish BCF was <0.8 L/kg on all sampling days during exposure.

Overall, the DS concluded not to consider thifensulfuron-methyl as having a low potential for bioaccumulation for the purpose of classification.

Aquatic Toxicity

The ecotoxicological tests results for thifensulfuron-methyl from available acute and chronic studies are summarised in the following table and sections. Only the valid acute and chronic studies on thifensulfuron-methyl which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below. Since thifensulfuron-methyl is considered as not rapidly degradable and degradants are not as toxic as the parent substance, degradants are not considered further in relation to the aquatic hazard classification of thifensulfuron-methyl.

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Reference
Fish			
Rainbow trout (*Oncorhynchus mykiss*) /	96-h LC_{50} = >100 mg/L (nominal)	-	Hall, C.L. (1983a)
Bluegill sunfish (*Lepomis macrochirus*) /	96-h LC_{50} = >100 mg/L (nominal)	-	Hall, C.L. (1983b)
Rainbow trout (*Oncorhynchus mykiss*) / OECD 204		21-d NOEC = 250 mg/L (mean measured)	Baer, K.N. (1991)
Aquatic invertebrates			
Water flea (*Daphnia magna*) /U.S. EPA 1600/4-85/012	48-h EC_{50} = 470 mg/L (mean measured)	-	Wetzel (1986)
Water flea (*Daphnia magna*) / OECD 202, U.S. EPA 72-2	48-h EC_{50} = >970 mg/L (mean measured)	-	Hutton (1989a)
Larvae (*Chironomous riparius*) / OECD 235	48-h EC_{50} = >100 mg/L (nominal)		Juckeland (2012)
Water flea (*Daphnia magna*) / OECD 202, U.S. EPA 72-4		21-d NOEC = 100 mg/L (mean measured)	Hutton (1989b)
Algae			
Pseudokirchneriella subspicata / OECD 201, U.S. EPA 122-2	24-48-h E_rC_{50} = 17 mg/L (nominal)	120-h NOE_rC = 5 mg/L (nominal)	M.T. Douglas and J.W. Handley (1987)
Pseudokirchneriella subspicata / U.S. EPA-FIFRA 122-2 & 123-2	120-h EC_{50} = >0.0157 mg/L (initial measured)	120-h NOEC = 0.0157 mg/L (initial measured)	Hicks, S.L. (1995)
Anabaena flos-aquae / U.S. EPA-FIFRA 122-2 & 123-2	120-h EC_{50} = >0.0263 mg/L (initial measured) 120-h E_rC_{50} = >0.0263 mg/L (initial measured)	120-h NOEC = 0.0263 mg/L (initial measured)	Hicks, S.L. (1995)

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Reference
Navicula pelliculosa / U.S. EPA-FIFRA 122-2 & 123-2	168-h E_bC_{50} = >0.0173 mg/L (initial measured) 24-48-h E_rC_{50} = 0.00162 mg/L (mean measured)	168-h NOEC = 0.00116 mg/L (mean measured)	Hicks, S.L. (1995)
Skeleonema costatum / U.S. EPA-FIFRA 122-2 & 123-2	120-h EC_{50} = >0.0175 mg/L (initial measured)	120-h NOEC = 0.0175 mg/L (initial measured)	Hicks, S.L. (1995)
Anabaena flos-aquae / U.S. EPA / OPPTS 850.5400	72-h EC_{50}=0.742 mg/L (mean measured) 96-h E_rC_{50} = 0.825 mg/L (mean measured)	72-96-h NOE_rC = <0.59 mg/L (mean measured)	Boeri, R.L., Magazu, J.P., Ward, T.J. (1999)
Aquatic macrophytes			
Duckweed (*Lemna minor*) / Draft OECD guideline for testing chemicals "Duckweed, static growth inhibition test", EPA pesticide assessment guidelines 122-2 and 123-2	14-d EC_{50} = 0.0013 mg/L (nominal) 14-d E_rC_{50} = 0.002 mg/L (nominal)	14-d NOE_rC = 0.0005 mg/L (nominal)	Douglas, M.T and Handley, J, W. (1988)
Duckweed (*Lemna gibba*) / U.S. EPA 123-2	14-d EC_{50} = 0.000866 mg/L (mean measured) 14-d E_rC_{50} = 0.00087 mg/L (mean measured)	14-d NOE_rC = 0.00023 mg/L (mean measured)	Kannuck, R.M., Samel, A., (1995)
Duckweed (*Lemna gibba*) / U.S. EPA OPPTS 850.4400, OECD 221	4-d E_rC_{50} = 0.0032 mg/L (nominal)	4-d NOEC = 0.00014 mg/L (nominal)	Porch, J.R., Kendall, T.Z., Krueger, H.O. (2011a)
Duckweed (*Lemna gibba*) / U.S. EPA 850, OCSPP Guideline 850.4400, OECD 221	7-d ErC50 = **0.0011** mg/L (mean measured)	7-d NOE_rC = **0.00037** mg/L (mean measured)	Arnie et al. (2015)
Ceratophyllum demersum / similar to OECD	14-d E_rC_{50} = 32.15 mg/L (mean measured)	14-d NOE_rC = <2.4 mg/L (mean measured)	Hoberg, J.R. (2011a)
Elodea canadensis / similar to OECD	14-d E_rC_{50} = 0.0217 mg/L (mean measured)	14-d NOE_rC = <0.058 mg/L (mean measured)	Hoberg, J.R. (2011b)
Myriophyllum aquaticum / similar to OECD	14-d E_rC_{50} = 0.1871 mg/L (mean measured)	14-d NOE_rC = <0.22 mg/L (mean measured)	Hoberg, J.R. (2011c)
Vallisneria americana / similar to OECD	14-d E_rC_{50} = **0.0011** mg/L (mean measured)	14-d NOE_rC = <**0.00025** mg/L (mean measured)	Hoberg, J.R. (2011d)
Myriophyllum spicatum / similar to OECD	14-d E_rC_{50} = 0.0516 mg/L (mean measured)	14-d NOE_rC = <0.20 mg/L (mean measured)	Hoberg, J.R. (2011e)

Based on available data, the DS concluded that thifensulfuron-methyl is most toxic to aquatic macrophytes. The DS stressed that although two acute fish toxicity studies (Hall, 1983a&b) were considered unreliable during the recent EFSA peer review, other evidence, including from a prolonged toxicity test and from thifensulfuron-methyl formulation studies on fish, suggests that the herbicide thifensulfuron-methyl is of low acute toxicity to fish. Also, the DS pointed out that no "true" chronic toxicity study on fish is available. However, the available prolonged 21-days study is considered sufficient to indicate a low chronic toxicity to fish. Despite this, the degree of difference between the available macrophyte toxicity endpoints and those for fish, invertebrates and even algae, indicate that acute and chronic classifications based only on macrophyte endpoints would be protective of other trophic groups and no consideration of surrogate approaches is required.

The DS identified that of the species tested, *Lemna gibba* appears, from the studies by Kannuck and Samel (1995) and Arnie *et al.* (2015), to be most sensitive with acute E_rC_{50}s of 0.00087 to 0.0011 mg/L and chronic NOE_rCs of 0.00023 to 0.00037 mg/L, both based on mean measured concentrations over 14 or 7 days. However, during the EFSA peer review of thifensulfuron-methyl, the Kannuck and Samel study was considered unreliable due to concerns that the ELISA and HPLC methods used to measure thifensulfuron-methyl were not sufficiently accurate or discriminatory regarding the parent substance and degradants. The original DAR (1996) *Lemna minor* study by Douglas and Handley (1988) was also considered unreliable in the EFSA peer review since it did not include analytical verification of test concentrations. The higher tier study on *Lemna gibba* by Porch *et al.* (2011) which made use of variable exposure and recovery durations is also considered unreliable for risk and hazard assessment in the EFSA peer review.

Due to the concerns expressed in the EFSA Conclusion (2015) relating to the earlier *Lemna* studies, it was proposed to use the next lowest aquatic macrophyte endpoints for *Vallisneria americana* (Hoberg, 2011d) for interim hazard assessment. These were an acute 14-days E_rC_{50} of 0.0011 mg/L and a chronic 14-day NOE_rC of <0.00025 mg/L, both based on mean measured concentrations. The 2015 EFSA Conclusion meanwhile stated that further data (especially a reliable *Lemna* sp. study on thifensulfuron-methyl) were still required to assess and conclude on the risk to aquatic organisms from the active substance. This was submitted in the form of the Arnie et al. (2015) study on *Lemna gibba*.

The DS does not consider that the *Vallisneria americana* endpoints should be used in isolation, particularly given there is not an accurate NOE_rC (<0.00025 mg/L). The *Lemna gibba* study by Kannuck and Samel (1995) may well also not be entirely accurate in its determination of measured concentrations (although it was otherwise performed and reported reliably). Therefore, the DS concluded that the recently submitted Arnie *et al.* (2015) study on *Lemna gibba* was considered fully reliable and relevant for aquatic hazard classification. The DS proposed to classify thifensulfuron-methyl as:

Aquatic Acute 1 (H400) based on the mean measured *Lemna gibba* 7-days E_rC_{50} of 0.0011 mg/L. As this value is in the range of 0.001 mg/L $<L(E)C_{50} \leq 0.01$ mg/L, the acute M-factor should be 100. The proposed value for acute classification is the same as the 14-days measured E_rC_{50} for *V. americana*. This value is slightly lower than, but still in the same range as the potentially unreliable 14-days nominal E_rC_{50} for *L. minor* (0.002 mg/L) and slightly higher than the potentially unreliable 14-days measured E_rC_{50} for *L. gibba* (0.00087 mg/L) from Kannuck and Samel (1995).

Aquatic Chronic 1 (H410) based on the substance being not rapidly degradable and the mean measured *Lemna gibba* 7-days NOE_rC of 0.00037 mg/L. As this value is in the range of 0.0001 mg/L <NOEC ≤0.001 mg/L, the proposed M-factor is 100. The value used for chronic classification is slightly higher than the imprecise 'less than' NOE_rC of <0.00025 mg/L proposed for *V. americana* and the potentially unreliable 14-days measured NOE_rC (0.00023 mg/L) for *L.*

gibba from Kannuck and Samel (1995). It is however slightly lower than the potentially unreliable 14-days nominal NOE_rC for *Lemna minor* (0.0005 mg/L). However, the values are still in the same range for determining the chronic M-factor.

Comments received during public consultation

Four MSCA and two individuals have submitted comments on the environmental part of the DS's proposal. All of them agree with the proposed classification of thifensulfuron-methyl as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100) without further justification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal that thifensulfuron-methyl does not meet the criteria for "rapid degradability" following the current CLP guidance degradation criteria. Based on available hydrolysis, photolytic degradation studies, results obtained in a biodegradation study and aerobic natural water/sediment systems studies,RAC agrees with the DS's conclusion that available degradation information does not indicate that thifensulfuron-methyl is ultimately degraded (>70%) within 28 days (equivalent to a degradation half-life of <16 days). Consequently, thifensulfuron-methyl is considered to be not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

thifensulfuron-methyl has a log Kow of -1.65 (at pH 7) which is below the CLP trigger of ≥ 4. Additionally, this was confirmed in an experimental study on *Bluegill sunfish* where the whole fish bioconcentration factor (BCF) was <0.8 L/kg and substantially less than the CLP BCF trigger of 500. Therefore, RAC agrees with the DS's conclusion that the substance has a low potential for bioaccumulation.

Aquatic Toxicity

RAC agrees that the herbicide thifensulfuron-methyl is most toxic to aquatic macrophytes. RAC notes that two acute fish toxicity studies (Hall, C.L., 1983a&b) were considered unreliable by the DS, however, it agrees with the DS that thifensulfuron-methyl has a low acute toxicity in fish. RAC also agrees with the DS that the available prolonged 21-day study is sufficient to indicate a low chronic toxicity in fish. Finally, RAC agrees with the DS's judgement that the most reliable and relevant study to assess and conclude on the risk and hazard to aquatic organisms from the active substance is the Arnie *et al.* (2015) study on *Lemna gibba*.

Acute toxicity

RAC agrees with the DS that the lowest, most reliable acute (short-term) result for aquatic acute classification of thifensulfuron-methyl is a 7-day E_rC_{50} of 0.0011 mg/L, based on mean measured concentration for aquatic plants (*Lemna gibba*).

Chronic toxicity

RAC agrees with the DS that the lowest most reliable chronic (long-term)result for aquatic chronic classification of thifensulfuron-methyl is a 7-day chronic NOE_rC=0.00037 mg/L, based on mean measured concentrations for aquatic plants (*Lemna gibba*).

Conclusion on classification

thifensulfuron-methyl is considered to be not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and most reliable information, RAC is of the opinion that thifensulfuron-methyl should be classified as:

Aquatic Acute 1 based on an E_rC_{50} = 0.0011 mg/L for *Lemna gibba*. As this acute toxicity value falls within the range of 0.001 < L(E)C_{50} ≤ 0.01 mg/L, the **acute M-factor is 100**.

The proposed classification is in line with another reliable acute toxicity study for *Vallisneria americana* (Hoberg, 2011d) with the same E_rC_{50}=0.0011 mg/L value.

Aquatic Chronic 1 based on being not rapidly degradable and a NOE_rC = 0.00037 mg/L for *Lemna gibba*. The is in line with another reliable chronic toxicity study for *Vallisneria americana* (Hoberg, 2011d) with a chronic NOE_rC < 0.00025 mg/L.

As this chronic toxicity value falls within the range of 0.0001 < NOEC ≤ 0.001 mg/L, the **chronic M-factor is 100.**

ANNEXES:

Annex 1 The 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 RAC (excluding confidential information).