ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Thifensulfuron-methyl

EC Number: **Not assigned**

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

Index Number: **016-096-00-2**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Thifensulfuron-methyl
EC number:	Not available
CAS number:	79277-27-3
Annex VI Index number:	016-096-00-2
Degree of purity:	≥ 97.9%
Impurities:	There are a number of impurities in the active substance. These have been taken into account and are not considered to impact on the proposed classification. Full information is provided in the technical dossier.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Aquatic Acute 1; H400 - Very toxic to aquatic life Aquatic Chronic 1: H410 - Very toxic to aquatic life with long lasting effects
Current proposal for consideration by RAC	Aquatic Acute 1; H400 - Very toxic to aquatic life M = 100 Aquatic Chronic 1: H410 - Very toxic to aquatic life with long lasting effects M = 100
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1; H400 - Very toxic to aquatic life M = 100 Aquatic Chronic 1: H410 - Very toxic to aquatic life with long lasting effects M = 100

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Not considered in this proposal
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Not considered in this proposal
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Not considered in this proposal
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Not considered in this proposal
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Not considered in this proposal
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Not considered in this proposal
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Not considered in this proposal
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	Not considered in this proposal
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Not considered in this proposal
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	Not considered in this proposal
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	Not considered in this proposal
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Not considered in this proposal

3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Not considered in this proposal
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	Not considered in this proposal
3.5.	Germ cell mutagenicity	**Not classified**	Not applicable	Not classified	**conclusive but not sufficient for classification**
3.6.	Carcinogenicity	**Not classified**	Not applicable	Not classified	**conclusive but not sufficient for classification**
3.7.	Reproductive toxicity	**Not classified**	Not applicable	Not classified	**conclusive but not sufficient for classification**
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	Not considered in this proposal
3.9.	Specific target organ toxicity – repeated exposure	**Not classified**	Not applicable	Not classified	**conclusive but not sufficient for classification**
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Not considered in this proposal
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1: H400 - Very toxic to aquatic life** **Aquatic Chronic 1: H410 - Very toxic to aquatic life with long lasting effects**	**M = 100** **M = 100**	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Not considered in this proposal

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

Labelling:

Pictogram(s): GHS09
Signal word: Warning
Hazard statements: H410 - Very toxic to aquatic life with long lasting effects
Precautionary statements: Not included in Annex VI

Proposed notes assigned to an entry: **None**

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The harmonised classification and labelling of Thifensulfuron-methyl (TSM) has been considered previously in the EU. In July 1998, it was agreed not to classify TSM for human health effects. The existing entry on Annex VI of CLP for TSM is as follows:

Aquatic Acute 1; H400 - Very toxic to aquatic life

Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects

At the time of submission, there are no REACH registration dossiers for this substance.

2.2 Short summary of the scientific justification for the CLH proposal

TSM is a pesticidal active substance. It was originally included in Annex I of the EU Council Directive 91/414/EEC on 1 July 2002. The active substance was subsequently approved under regulation (EC) 1107/2009 via Implementing Regulation (EU) 540/2011. In accordance with Commission Regulation (EU) 1141/2010 of 7 December 2010, DuPont and the EU TSM AIR 2 Task Force (representing Cheminova A/S and Rotam Agrochemical Europe Ltd) submitted separate dossiers to support the renewal of the approval of TSM. The UK, acting as the Rapporteur Member State (RMS), evaluated all aspects of the renewal dossiers via a Renewal Assessment Report (RAR). The RAR was the subject of a peer review by the Co-RMS Austria, MS and EFSA.

During the renewal peer-review process, EFSA concluded that classification with Repr Cat 2; H361d was appropriate (based on the same data available at the time of the first review) and that although classification for carcinogenicity was not warranted, it could not be excluded that the increase in mammary tumours seen in the rat carcinogenicity study was treatment-related.

Given the discrepancy between the harmonised classification and the recommendations in the EFSA Conclusion, a targeted CLH proposal for the endpoints of developmental toxicity and carcinogenicity has been presented in this document. In addition, as data on repeated dose toxicity, mutagenicity and fertility have been considered to aid interpretation of the developmental toxicity and carcinogenicity findings, a CLH proposal has also been included for these latter hazard classes/differentiations. **It is not proposed to classify for any human health hazard classes**.

The existing harmonised entry includes a classification for the environment of Aquatic Acute 1; H400 - Very toxic to aquatic life and Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects. This report proposes to retain this classification and to add acute and chronic **M-factors of 100 and 100** respectively.

2.3 Current harmonised classification and labelling

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

Aquatic Acute 1; H400 – Very toxic to aquatic life

Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

Aquatic Acute 1; H400 - Very toxic to aquatic life

Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects

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. Thifensulfuron-methyl is abbreviated to TSM throughout this opinion.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

TSM is an approved pesticidal active substance under Regulation 1107/2009. In accordance with Commission Regulation (EU) 1141/2010 of 7 December 2010, dossiers have been submitted to support the renewal of the approval.

During the renewal peer-review process, EFSA concluded that classification with Repr Cat 2; H361d was appropriate (based on the same data available at the time of the first review) and that although classification for carcinogenicity was not warranted, it could not be excluded that the increase in mammary tumours seen in the rat carcinogenicity study was treatment-related.

Given the discrepancy between the existing harmonised classification and the recommendations in the EFSA Conclusion, a targeted CLH proposal for the hazard classes of developmental toxicity and carcinogenicity has been presented in this document. In addition, as data on repeated dose toxicity, mutagenicity and fertility have been considered to aid interpretation of the developmental toxicity and carcinogenicity findings, a CLH proposal has also been included for these latter hazard classes/differentiations.

Finally, information is included in the report to propose the addition of M-factors to the existing entry.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

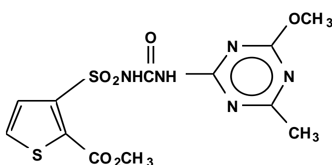
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	None available
EC name:	-
CAS number (EC inventory):	79277-27-3
CAS number:	79277-27-3
CAS name:	2-Thiophenecarboxylis acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-, methyl ester
IUPAC name:	Methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate
CLP Annex VI Index number:	016-096-00-2
Molecular formula:	$C_{12}H_{13}N_5O_6S_2$
Molecular weight range:	387.39 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Thifensulfuron-methyl	≥ 97.9%		

Current Annex VI entry: N/A

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in the substance. The impurities from each manufacturing source have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Current Annex VI entry: N/A

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: N/A

1.2.1 Composition of test material

The test material used for the physico-chemical, human health and environmental studies was considered to be equivalent to that outlined above for classification purposes.

1.3 Physico-chemical properties

Table 8: Summary of physico-chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	White/Off-white/Yellow Solid	Greenwood, 2002 (1) Denny, 2006a (2) Comb, 2012 (3) Pedersen, 2006 (4) RAR B.2.1.8	Observation GLP 96.5 – 99.2%
Melting/freezing point	171.1 ºC(*)	Huntley and Edgar, 1999 (5) RAR B.2.1.1	EEC A1 (capillary method) GLP 99.7%
Boiling point	Substance decomposes before boiling.	Comb, 2012 (3) RAR B.2.1.2 and B2.1.3 Huntley and Edgar 1999 (5) RAR B.2.1.3	EEC A2 (Siwoloboff method) GLP 99.2% EEC A1 (capillary method) GLP 99.7% *It is noted that the substance decomposes before boiling. In the Huntley and Edgar study the substance was observed to decompose after melting. However, the temperature of decomposition given in the Comb 2012 study was 162 ºC which is not consistent with the melting point reported in the earlier study.
Relative density	1.58 1.46	Greenwood, 2002 (6) RAR B.2.1.4 Comb, 2012 (3)	EEC A3 (gas comparison pyknometer) GLP 99.7% EEC method A 3 (gas comparison pyknometer) GLP 99.2%

Vapour pressure	5.6×10^{-11} mm Hg (20 °C) 1.3×10^{-10} mm Hg (25 °C) 5.19×10^{-9} Pa at 20 °C [Value extrapolated from 2.18×10^{-6} Pa (50 °C) 8.01×10^{-7} Pa (40°C)] 4×10^{-8} Pa at 25 °C	Barefoot, 1987 (7) RAR B.2.1.5 Ganesh, 2012 (8) RAR B2.1.5 Comb, 2012 (3) RAR B2.1.5	EEC A4 (Effusion method – Knudsen cell) Non-GLP 99.6% EEC A4 (gas saturation method) GLP 99.7% EEC A4 (method not stated) GLP 99.2%
Surface tension	63.8 mN/m at 19.5 °C (1% aq solution) 72.0 mN/m (90% saturated aq solution) 46.3 mN/m at 25 °C (saturated aq solution)	Huntley, 2000 (9) Comb, 2012 (3) Denny, 2006 (10) RAR B.2.1.24	EEC A5 (Ring method) GLP 98% EEC A5 (method not stated) GLP 99.2% NF ISO 304
Water solubility	54.1 mg/L at pH 4.09 and 20 °C 0.223 g/l at pH 5 and 25 °C 2.24 g/l at pH 7 and 25 °C 8.83 g/l at pH 9 and 25 °C	Greenwood, 2002 (11) RAR B.2.1.11 Barefoot and Cooke 1990 (12) RAR B.2.1.11	EEC A6 (shake flask) GLP 99.7% CIPAC Method 157 GLP (comparable to EEC A6 – shake flask) 98.3%
Partition coefficient n-octanol/water	log P_{ow} = 0.0253 at pH 5 log P_{ow} = -1.65 at pH 7 log P_{ow} = -2.10 at pH 9 at 25°C	Huntley and Edgar, 2000 (13) RAR B.2.1.13	EEC A8 (shake flask) GLP 99.7%
Flash point	Not applicable, substance is a solid.		
Flammability	Not flammable. Further, experience in handling and use indicates it is not pyrophoric and does not react with water to liberate flammable gases.	Denny, 2006 (14) RAR B.2.1.20	EEC A10 GLP 96.5%

Explosive properties	Not explosive (not sensitive to heat, impact or friction).	Gravell, 1995 (15) RAR B2.1.22	EEC A14 GLP 98.3%
Self-ignition temperature	Positive result in 100 mm cube at 140 ºC Negative result in 25 mm cube at 140 ºC No additional information available to derive classification	Gravell, 1995 (15) RAR B.2.1.20	UN RDTG Manual of tests and criteria N4 (modified Bowes-Cameron Cage test) GLP 98.3%
Oxidising properties	Not oxidising	Radhakrishnan, 2011 (16) and Denny, 2006 (17) RAR B.2.1.23	EEC A17 GLP 99% and 96.5%
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	pKa = 4	Huntley and Sarff, 1999 (18) RAR B.2.1.18	OECD 112 GLP 99.7%
Viscosity	Not applicable, substance is a solid.		

All references are taken from the RAR (2015) for Thifensulfuron-methyl

2 MANUFACTURE AND USES

2.1 Manufacture

The substance is manufactured outside of the EU.

2.2 Identified uses

The substance is used within the EU as a pesticidal (herbicide) active substance.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Physico-chemical Properties

Not addressed in this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

This information has been extracted from the renewal assessment report (RAR 2015) prepared by the UK under 1107/2009.

The toxicokinetics of thifensulfuron-methyl (TSM) were evaluated in rats using both a triazine- and a thiophene-labelled compound administered orally. Results were similar with both labelled compounds. The metabolism and disposition data indicated rapid absorption, metabolism, and elimination. Based on urinary excretion data, oral absorption of TSM was found to be extensive following single and repeated exposure to a low dose of 20 mg/kg bw (ranging from 73% to 89% of the administered dose). At the high dose of 2000 mg/kg bw, oral absorption appeared to be decreased, ranging from 52% to 77% of the administered dose.

Parent TSM represented the major fraction of radioactivity detected in urine and faeces in both male and female rats (70 to 95%). Five metabolites were identified in urine and faeces: Thifensulfuron acid (IN-L9225), O-demethyl Thifensulfuron-methyl (IN-L9226), triazine amine (IN-A4098), 2-acid-3-sulfonamide (IN-L9223) and 2-ester-3-sulfonamide (IN-A5546). Two thiophene-labelled metabolites (accounting for 5 to 20% of the radioactive compounds in urine) were not identified. The major pathway involved in the biotransformation of TSM in the rat was demethylation to O-demethyl Thifensulfuron-methyl (IN-L9226), which was subsequently hydrolysed to 2-ester-3-sulfonamide (IN-A5546) (further converted to 2-acid-3-sulfonamide – IN-L9223) and triazine amine (IN-A4098). Thifensulfuron-methyl was also deesterified by a non-specific esterase to yield Thifensulfuron acid (IN-L9225).

TSM and/or its metabolites were widely distributed around the body; however levels were low and no retention or accumulation in specific tissues was observed. No significant sex differences were detected in the kinetic behaviour of the substance. A 21 day pre-treatment of rats with TSM did not appear to alter the toxicokinetics of the compound.

In the rat, over 50% of the administered dose of TSM was excreted by 48 hours post-dosing and excretion was essentially complete by 72 hours post-dosing. The predominant excretory route was urine (60 to 80%), however, faecal excretion (10 to 30%) also contributed significantly to the elimination of the compound.

4.2 Acute toxicity

Not addressed in this proposal.

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

Not addressed in this proposal.

4.4 Irritation

Not addressed in this proposal.

4.5 Corrosivity

Not addressed in this proposal.

4.6 Sensitisation

Not addressed in this proposal.

4.7 Repeated dose toxicity

Although this proposal is targeted to carcinogenicity and developmental toxicity, this endpoint has been included as repeated dose toxicity information could be important in the interpretation of the carcinogenicity investigations. On this basis, a proposal for classification/non-classification has been presented.

The repeated dose toxicity of TSM was evaluated in rats, mice and dogs by the oral route.

Table 9: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
10-dose study SD **rats** (6 males/group) Oral gavage (in corn oil) Not guideline (range-finding study) Not GLP TSM 93.4%	0, 2200 mg/kg bw/d	**2200 mg/kg bw/d**: No adverse effects.	1984(26)
90-day study SD **rats** (10/sex/group) Dietary administration EU B26 method Conducted prior to the implementation of GLP, but QA statement confirming consistency with GLP requirements TSM 93.6%-95.6%	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) Dose levels relevant for classification (guidance value for subchronic rat study) ≤ 100 mg/kg bw/d	**7500 ppm (559/697 mg/kg bw/d in males/females):** ↓bw gain (18% males; 29% females); ↓food efficiency in males and females; Slight changes in some clinical-chemistry parameters (BUN, serum proteins and glucose) in males; Slight changes in some organ weights in males and females; **2500 ppm (177/216 mg/kg bw/d in males/females):** ↓bw gain (8% males; 17% females); ↓food efficiency in males and females; ↓glucose in males; Slight changes in some organ weights in males; **100 ppm (7/9 mg/kg bw/d in males/females):** No adverse effects NOAEL[$] = 100 ppm	1984a(35)
90-day study CD-1 **mice** (10/sex/group) Dietary administration EU B26 method Conducted prior to the implementation of GLP, but QA statement confirming consistency with GLP requirements TSM 93.6%	0, 500, 2500, 7500 ppm (0, 97, 528, 1427 mg/kg bw/d in males; 0, 123, 690, 2287 mg/kg bw/d in females) Dose levels relevant for classification (guidance value for subchronic rat study) ≤ 100 mg/kg bw/d	**7500 ppm (1427/2287 mg/kg bw/d in males/females):** No adverse effects **2500 ppm (528/690 mg/kg bw/d in males/females):** No adverse effects **500 ppm (97/123 mg/kg bw/d in males/females):** No adverse effects NOAEL[$] = 7500 ppm	1984b(36)

90-day study Beagle **dogs** (4/sex/group) Dietary administration EU B27 method GLP TSM 95-96.5%	0, 75, 1500, 7500 ppm (2.1, 41.3, 200 mg/kg bw/d in males; 2.1, 43.6, 207.5 mg/kg bw/d in females) Dose levels relevant for classification (guidance value for subchronic rat study) ≤ 100 mg/kg bw/d	**7500 ppm (200/207.5 mg/kg bw/d in males/females):** ↓bw gain in males and females; ↓abs adrenal wt (29%)* and rel adrenal wt (27%) in males; **1500 ppm (41.1/43.6 mg/kg bw/d in males/females):** No adverse effects **75 ppm (2.1 mg/kg bw/d in males and females):** No adverse effects NOAEL$ = 1500 ppm	1984 (25)
1-year study Beagle **dogs** (5/sex/group) Dietary administration EU B30 method GLP TSM 98.2-94.8%	0, 50, 750, 7500 ppm (1.3, 19.7, 195.3 mg/kg bw/d in males; 1.4, 22.5, 210.9 mg/kg bw/d in females) Dose levels relevant for classification (guidance based on application of Haber's Rule for long-term studies from CLP criteria Annex I 3.9.2.9.5, November 2012) ≤ 25 mg/kg bw/d	**7500 ppm (195.3/210.9 mg/kg bw/d in males/females)**: ↓bw gain (60-70%)* in females; ↓terminal bw (17%)* in females; ↓food efficiency (60%)* in females; ↑liver wt (abs 34%; rel 14%) in males; **750 ppm (19.7/22.5 mg/kg bw/d in males/females):** No adverse effects **50 ppm (1.3/1.4 mg/kg bw/d in males/females):** No adverse effects NOAEL$ = 750 ppm	(1984)(29)

$ As given in the RAR; *Statistically significant

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

<u>*Range-finding (10 dose) study*</u>

In a range-finding study, TSM was administered in corn oil by intragastric intubation to a group of 6 male Sprague Dawley rats (1984)(26). Rats were dosed 10 times over a 2-week period at a dose of 2200 mg/kg bw/day. A control group of 6 rats was dosed with corn oil only. Rats were weighed and

observed daily. At the end of treatment, 3 rats from each group were sacrificed for pathological examination. The remaining rats from each group were sacrificed and similarly examined after a 14-day recovery period.

No deaths occurred during the study. Clinical observations, body and organ weights, gross and histological examination revealed no compound-related toxic effects. A ten-dose exposure of male rats to TSM at 2200 mg/kg bw produced no signs of toxicity in rats.

90-day study

In a guideline 90-day study, TSM was administered to four groups of 10 male and female Sprague Dawley rats (1984a)(35). The substance was incorporated in the diet at 0, 100, 2500 and 7500 ppm. The calculated mean daily intake of TSM was 7, 177, and 559 mg/kg bw/day for males and 9, 216, and 697 mg/kg bw/day for females.

There were no compound-related effects on the incidence of clinical signs of toxicity and mortality. Decreases in mean body weight and overall body weight gain were observed in male and female rats at 2500 and 7500 ppm. Daily diet consumption was relatively uniform among test groups during the study. Food efficiency was slightly decreased in the 2500 and 7500 ppm male and female groups when compared to their respective controls. There was no effect of treatment at all dose levels on haematological and urologic parameters. Compound-related effects on clinical chemistry parameters occurred in the 7500 ppm males. These consisted of increased BUN at 1 month (40%), decreased serum proteins at 1, 2, and 3 months (-5 to -7%) and decreased serum globulin at 3 months (11%). Plasma glucose concentrations decreased in the 2500 and 7500 ppm males (16%). No changes in clinical chemistry parameters were observed in female rats.

Significant decreases in mean absolute spleen weights (13 to 17%) and increases in relative brain weights (+ 8 to 20%) occurred in the 2500 and 7500 ppm males. Significant decreases in mean absolute heart (13%) and liver (23%) weights and increases in relative kidney (20%) and testis (35%) weights were observed in the 7500 ppm males. Increased relative brain (13%) and heart (10%) weights were observed in the 7500 ppm female group. No gross or histopathologic changes attributed to dietary intake of TSM were observed. In the absence of any gross or microscopic observations, the organ weight effects may be considered secondary to body weight changes.

Based on the decreased body weight, reduced food efficiency, changes in clinical-chemistry parameters and organ weights, the NOAEL for TSM (90 day oral feeding) was 100 ppm for male (7 mg/kg bw/d) and female (9 mg/kg bw/d) rats.

Mouse

90-day study

In a guideline 90-day study, TSM was administered to four groups of 10 male and female CD-1 mice (1984b)(36). The substance was incorporated in the diet at 0, 500, 2500 and 7500 ppm. The calculated mean daily intake of TSM was 97, 528, and 1427 mg/kg bw/day for males and 123, 690, and 2287 mg/kg bw/day for females.

There were no compound-related effects on mortality or on the incidence of clinical signs of toxicity. There were no statistically significant or biologically important differences in overall mean body weights and in mean food consumption or food efficiency values for male or female mice.

There were no compound-related haematological effects. The only difference noted between test and control group mice was a statistically significant increase in relative kidney weights in male mice in the 2500 ppm group (13%) when compared to the male control group. Due to the absence of a dose-response or morphological changes, the organ weight changes were not attributed to the treatment. No compound-related gross or histopathologic changes were observed in mice sacrificed at the end of the study.

Following 90 days of dietary exposure to TSM, the NOAEL for male and female CD-1 mice was 7500 ppm, based on the lack of compound-related effects at this concentration. This concentration was equivalent to 1427 mg/kg bw/day and 2287 mg/kg bw/day in male and female mice, respectively.

Dog

<u>*90-day study*</u>

In a guideline 90-day study, TSM was administered to four groups of 4 male and 4 female beagle dogs for 13 weeks (1984)(25). The substance was incorporated in the diet at 0, 75, 1500 and 7500 ppm. The calculated mean daily intake of TSM was 2.1, 41.1, and 200 mg/kg bw/day for males and 2.1, 43.6, and 207.5 mg/kg bw/day for females.

There was no mortality. There was a small decrease in mean body weight and body weight gains relative to control in the 7500 ppm male group. Terminal body weight was slightly decreased in 7500 ppm males (- 8%). The mean body weight gain in the 7500 ppm females was slightly less than control over all intervals. Food consumption and food efficiency were comparable between treated and control groups. There were no treatment-related effects on clinical signs, clinical chemistry (except decreased lactate dehydrogenase (LDH) activities in all treated groups (30 to 50%) after 4 weeks of exposure in males and 13 weeks of exposure in females), haematology, and urinalysis. The decreases in LDH activities were considered to be of no toxicological significance as they occurred in isolation. Statistically significant decreases were noted in the mean absolute and relative adrenal weights in 7500 ppm males with a trend towards a dose-effect relationship. No gross or histopathological changes were noted in any of the organs or tissues which could be attributed to treatment.

Following 90 days dietary exposure to TSM, the NOAEL was 1500 ppm (41.1 and 43.6 mg/kg bw/day in male and female dogs respectively) based on body weight effects in both sexes, and on the decreased adrenal weights in male beagle dogs.

<u>One-year study</u>

In a guideline 1-year study, TSM was administered to four groups of 5 male and 5 female beagle dogs (1986)(29). The substance was incorporated in the diet at 0, 50, 750 and 7500 ppm. The calculated mean daily intake of TSM was 1.3, 19.7, and 195.3 mg/kg bw/day for males and 1.4, 22.5, and 210.9 mg/kg bw/day for females.

There were no mortalities or compound-related clinical signs. There was a compound-related decrease in the 7500 ppm female mean body weights. Female mean body weight gains relative to control were decreased in the 7500 ppm (60 to 70%, statistically significant between 0 and 39 weeks) and in the 750 ppm (overall 35% between 0 and 52 weeks, not statistically significant; but no reductions between 5-13 wks and 40-52 wks) groups. Terminal mean body weights in

females were reduced in the 750 (by 6%, not statistically significant) and 7500 (by 17%, statistically significant) ppm groups. For females there were no differences in mean total food consumption but mean food efficiency was reduced in the 750 (overall 40% between 1-52 wks; but no reductions between 5-13 wks and 40-52 wks) and 7500 (overall 60% between 1-52 wks) ppm groups. Individual body weights and body weight gains in the 750 ppm females were highly variable. This undermines the significance of the observed mean changes. Overall, the effects on body weight gain and food efficiency observed in females at 750 ppm were highly variable; were not statistically significant; were inconsistent over the duration of the study and resulted only in a 6% not statistically significant decrease in terminal body weight; therefore they were not considered to be adverse.

The most significant effect on clinical pathology parameters was a trend towards increased blood glucose concentrations, at the end of the exposure period (wk 52), in male (22 to 39%) and female (10 to 30%) dogs at 750 and 7500 ppm. In the absence of gross or microscopy findings in any organ, including the pancreas, this isolated change in blood glucose levels is considered to be unrelated to treatment. It is also noted that these increased glucose levels were similar to those observed in the control animals at weeks 26 and 39. This further undermines the relation to treatment of the reported finding. In addition, it is noted that there were no effects on glucose in the 90-day dog study up to a much higher dose of 7500 ppm. In the 7500 ppm groups, liver weights (absolute: +34%, $p \leq 0.05$ and relative: +14%, not statistically significant) were increased in males. There were no specific compound-related histopathologic lesions present in treated animals.

In conclusion, TSM had significant adverse effects at 7500 ppm in female dogs (decreased body weights, body weight gains, and food efficiency) and in male dogs (increased liver weights). These effects were not correlated with gross or microscopic findings. The NOAEL in male and female dogs was 750 ppm corresponding to 19.7 and 22.5 mg/kg bw/day respectively.

4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

4.7.1.3 Repeated dose toxicity: dermal

No data are available.

4.7.1.4 Repeated dose toxicity: other routes

No data are available.

4.7.1.5 Human information

No data are available.

4.7.1.6 Other relevant information

No data are available.

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

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

The repeated dose toxicity of TSM was investigated in 90-day oral feeding studies in rats and mice and in 90-day and 1-year dietary studies in dogs. All studies were considered to be acceptable. These studies showed that there were no specific target organs of repeated dose toxicity for TSM in all three species investigated, as only effects on body weight parameters and nutritional status were observed from a relatively high dose of 177 mg/kg bw/day in rats and from a dose of 200 mg/kg bw/day in dogs. There were no adverse effects in the mouse up to the top dose of 1427/2287 mg/kg bw/day (m/f) for 90 days, indicating a lower sensitivity for this species.

Classification of TSM for STOT-RE is not warranted.

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

The repeated dose toxicity of TSM was investigated in 90-day oral feeding studies in rats and mice and in 90-day and 1-year dietary studies in dogs. All studies were considered to be acceptable. These studies showed that there were no specific target organs of repeated dose toxicity for TSM in all three species investigated, as only effects on body weight parameters and nutritional status were observed from a relatively high dose of 177 mg/kg bw/day in rats and from a dose of 200 mg/kg bw/day in dogs. There were no adverse effects in the mouse up to the top dose of 1427/2287 mg/kg bw/day (m/f) for 90 days, indicating a lower sensitivity for this species.

Classification of TSM for STOT-RE is not warranted.

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

Not classified – conclusive but insufficient for classification

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 (TSM) 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 TSM 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 TSM 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 TSM 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 TSM for STOT-RE is not warranted.

4.9 Germ cell mutagenicity (Mutagenicity)

Although this proposal is targeted to carcinogenicity and developmental toxicity, this endpoint has been included as mutagenicity could be important in the interpretation of the carcinogenicity investigations. On this basis, a proposal for classification/non-classification has been presented.

The mutagenic potential of TSM has been investigated *in vitro* (3 bacterial mutagenicity tests, 1 mammalian cell gene mutation assay, 1 chromosome aberration study and 1 UDS test) and *in vivo* (mouse micronucleus test and rat chromosome aberration test).

Table 10: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bacterial mutagenicity OECD 471 Not GLP TSM 93.4% (Massado, 1983)(31)	*S. typhimurium* TA1535 TA97 TA98 TA100 Not TA102 or E coli WP2*uvr*A	0.1 – 20 µg/plate	**Negative ± S9** Cytotoxicity at ≥ 50 µg/plate; Study limited by the bacteriostatic activity of TSM and lack of 5th strain.
Bacterial mutagenicity OECD 471 GLP TSM 97.9% (Patel, 2007)(37)	*S. typhimurium* TA1535 TA1537 TA98 TA100 TA102	0.1 – 20 µg/plate	**Negative ± S9** Cytotoxicity at 20 µg/plate; Study limited by the bacteriostatic activity of TSM.
Bacterial mutagenicity OECD 471 GLP TSM 97.4% Bowles (2009) (21)	*S. typhimurium* TA1535 TA1537 TA98 TA100 *E coli* WP2*uvr*A	1.5 – 5000 µg/plate	**Negative ± S9** Cytotoxicity at ≥ 150 µg/plate for *S. typhimurium* strains; No evidence of cytotoxicity up to 5000 µg/plate in *E coli* strain. Study limited by the bacteriostatic activity of TSM in *S typhimurium*..
Mammalian cell gene mutation (HGPRT) OECD 476 GLP TSM 96.9% (McCooey & Richard, 1984 & 1987)(33)	CHO-K1 cell line	387 – 2712 µg/ml	**Negative ± S9** No excessive cytotoxicity observed, but the top concentration caused precipitation.
Chromosome aberration assay OECD 473 GLP TSM 96.9% (Vlachos, 1987)(45)	Human lymphocytes	250 – 2800 µg/ml	**Negative ± S9** Precipitation observed at top concentration; Study limited by weak response of positive controls and short harvest time (19 hr).

UDS OECD 482 GLP TSM 95.6% (McCooey, 1984)(32)	Primary culture of rat hepatocytes	0.39 – 2712 µg/ml	**Negative** Precipitation observed at the top 3 concentrations; cytotoxicity observed at the top 2 concentrations.
In vivo Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bone marrow micronucleus test Oral gavage Vehicle: corn oil OECD 474 GLP TSM 95.6% (1985;)(43)	Mice (15/sex in treated group; 5/sex in controls)	0, 5000 mg/kg bw (single dose) Mice sacrificed at 24 hr, 48 hr (including controls) and 72 hr post-treatment	**Negative** Mortality, clinical signs of toxicity (tremors, ptosis, body drop, decreased body tone and activity) and macroscopic findings (fluid-filled distended stomach, red lungs, discoloured intestine) were observed in the treated animals; P/N ratio affected only in males sacrificed at 48 hr.
Bone marrow chromosome aberration test Oral gavage Vehicle: corn oil OECD 475 GLP TSM 95.6% (1984)(44)	Rats, SD (15/sex/group)	0, 5000 mg/kg bw Animals sacrificed at 6, 24 and 48 hr	**Negative** Significant body weight loss observed in the treated animals; Mitotic index was not affected.

4.9.1 Non-human information

4.9.1.1 *In vitro* data

TSM was negative in a relatively old bacterial gene mutation assay. However, compared to current standards, this study lacked information from a bacterial strain (*E.coli* WP2*uvr*A or *S. typhimurium* TA102) able to detect oxidising mutagens and cross-linking agents. In addition, due to the bacteriostatic activity of TSM towards *S. typhimurium*, the highest concentration that could be tested in the assay was very low. This undermined the significance of the observed negative result. Since then, two additional bacterial mutagenicity tests have been conducted. These studies are modern investigations which have addressed the limitation of the lacking strain from the old test. However, similarly to the original assays, the highest concentration that could be tested in these assays was very low, undermining the significance of the observed negative results. Overall, no clear conclusions can be drawn about the potential of TSM to cause gene mutations in bacteria due its bacteriostatic nature. It is however noted that the gene mutation endpoint has been adequately

addressed in two *in vitro* mammalian cell test for gene mutation (UDS assay in isolated rat hepatocytes and *hprt* assay in CHO cells), with negative results returned.

TSM tested also negative *in vitro* for clastogenicity in human lymphocytes. Although the validity of the *in vitro* chromosome aberration study has been questioned (see table 10 for details), it is noted that there are two negative *in vivo* studies (mouse micronucleus and rat chromosome aberration), which have investigated adequately the potential clastogenicity of TSM.

4.9.1.2 *In vivo* data

In two *in vivo* studies (mouse micronucleus and rat chromosome aberration), TSM tested negative up to a dose (5000 mg/kg bw, a dose exceeding the maximum recommended dose in accordance with current *in vivo* genotoxicity regulatory guidelines) causing significant systemic toxicity.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

No data are available.

4.9.4 Summary and discussion of mutagenicity

TSM tested negative in several *in vitro* (bacterial mutagenicity, mammalian cell gene mutation, chromosome aberration, UDS) and *in vivo* studies (micronucleus and chromosome aberration). Overall, it can be concluded that TSM is not genotoxic. Classification for mutagenicity is not warranted.

4.9.5 Comparison with criteria

TSM tested negative in several *in vitro* (bacterial mutagenicity, mammalian cell gene mutation, chromosome aberration, UDS) and *in vivo* studies (micronucleus and chromosome aberration). Overall, it can be concluded that TSM is not genotoxic. Classification for mutagenicity is not warranted.

4.9.6 Conclusions on classification and labelling

Not classified – conclusive but insufficient for classification

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.

TSM 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 TSM 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 TSM 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.

Additional key elements

Due to the need for more study details, the following documents were also taken into account by RAC:

- The RAR 08 vol 3 B6 Tox

- EFSA opinion on TSM (EFSA Journal 2015;13(7):4201).

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 TSM 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 TSM was also tested *in vivo*.

In the MN test in the bone marrow of mice, the tested dose of TSM 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). TSM did not induce cytogenetic damage in the bone marrow MN test.

The potential clastogenicity of TSM *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 TSM 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 TSM is not genotoxic and agrees with the DS that **classification for germ cell mutagenicity is not warranted**.

4.10 Carcinogenicity

The carcinogenicity of TSM has been investigated in rats and mice by the oral route.

Table 11: Summary table of relevant carcinogenicity studies

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr>
<td>2-yr chronic toxicity/carcinogenicity study
EC B33
Not GLP but QA statement included in the report
Dietary administration
Rats, SD (72/sex/group)
TSM 95.6 – 98%
1986(23)</td>
<td>0, 25, 500, 2500 ppm (0.96, 20, 102 mg/kg bw/d in males; 1.3, 26 and 133 mg/kg bw/d in females)</td>
<td>2500 ppm (102/133 mg/kg bw/d in males/females):
↓ bw gain (up to 7%)# during 1st year in males;
↓ bw gain (6%) over 2 years in males;
↓ serum sodium (up to 8%)# in males and females at 9, 12, 18, 21 and 24 months;
500 ppm (20/26 mg/kg bw/d in males/females):
No adverse effects.

<u>Tumours</u>
<table>
<tr><th>Female rats</th><th>0 ppm</th><th>25 ppm</th><th>500 ppm</th><th>2500 ppm</th></tr>
<tr><td>Total tumours
Animals with primary tumours</td><td>56/59</td><td>56/59</td><td>53/60</td><td>61/62</td></tr>
<tr><td>Animals with malignant tumours</td><td>16/59 (27%)</td><td>17/59 (29%)</td><td>25/60 (42%)</td><td>29/62* (47%)</td></tr>
<tr><td>Animals with benign tumours</td><td>55/59</td><td>53/59</td><td>48/60</td><td>53/62</td></tr>
<tr><td>Animals with secondary tumours</td><td>2/59</td><td>6/59</td><td>4/60</td><td>7/62</td></tr>
<tr><td>Mammary gland
Animals with fibroadenoma[a],</td><td>19/58</td><td>15/54</td><td>19/52</td><td>19/62</td></tr>
<tr><td>Animals with adenocarcinoma,</td><td>12/58 (21%)</td><td>6/54 (11%)</td><td>15/52 (29%)</td><td>20/62 (32%)</td></tr>
<tr><td>Animals with adenosquamous cell carcinoma</td><td>-</td><td>-</td><td>1</td><td>-</td></tr>
</table>
* significantly different from controls at P < 0.05 (Fisher test)
[a] Fibroadenoma most likely includes diagnosis of adenoma as adenoma was not reported separately
<table>
<tr><td>Lab historical control data in female SD rats from 11 studies conducted between 1984 and 1990</td><td>Number of animals with mammary gland adenocarcinoma (%)
Range: 4 (8.3%) – 15 (23.4%); mean 17%</td></tr>
</table>
NOAEL$ (toxicity) = 500 ppm (20/26 mg/kg bw/d)
NOAEL$ (carcinogenicity) = 2500 ppm (102/133 mg/kg bw/d)</td>
</tr>
</table>

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
18-month carcinogenicity study EC B33 Not GLP but QA statement included in the report Dietary **Mouse**, CD-1 (80/sex/group) TSM 95.5-98% 1985, 1987 & 1990 (24)	0, 25, 750, 7500 ppm (3.2, 97 and 979 mg/kg bw/d in males; 4.3, 128 and 1312 mg/kg bw/d in females)	**7500 ppm (979/1312 mg/kg bw/d in males/females)**: ↓ terminal bw (4%) in females; ↓ bw gain (13%)* in females; **750 ppm (97/128 mg/kg bw/d in males/females)**: No adverse effects; **25 ppm (3.2/4.3 mg/kg bw/d in males/females)**: No adverse effects; NOAEL[$] (toxicity) = 750 ppm NOAEL[$] (carcinogenicity) = 7500 ppm

[$] As given in the RAR; #Statistically significant (level of significance not quantified in the RAR); *Statistically significant ($p \leq 0.05$)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat

In a guideline 2-year carcinogenicity study, TSM was administered to four groups of 72 male and 72 female Sprague Dawley rats (1986)(23). The substance was incorporated in the diet at 0, 25, 500 and 2500 ppm for 24 months. The calculated mean daily intake of TSM was 0.96, 20 and 102 mg/kg bw/day for males and 1.3, 26 and 133 mg/kg bw/day for females. Haematological, clinical chemistry and urine analysis parameters were measured 3, 6, 9, 12, 18, 21 and 24 months after study initiation on 10 rats from each sex and group. Ten rats/sex/dose were sacrificed at one year (interim sacrifice) and the remaining surviving rats were sacrificed at 24 months.

Male rats in the 2500 ppm group had mean body weights and body weight gains which were significantly lower (up to 7%) than control values over the first year of the study. These values remained below control levels for the remainder of the study, although the differences were not statistically significant. In females, no compound-related, significant body weight effects were observed during the study.

Serum sodium concentrations were slightly (up to 8%) but significantly lower than controls in male or female rats in the 2500 ppm groups at the 9, 12, 18, 21, and 24 month evaluations. Lower sodium concentrations were also observed in 500 ppm males at 24-months, 500 ppm females at 12 and 18-months, 25 ppm females at 12 and 18-months and 25 ppm males at 24 months. A statistically significant, compound-related decrease in serum sodium was determined to exist only for males and females at 2500 ppm, based on analysis of dose/time interactions. The changes observed at 25 and 500 ppm were minor, inconsistent with duration of exposure and within the ranges observed in control animals at other time points. Therefore, they were considered unrelated to treatment.

The total number of masses and total number of rats with masses were significantly elevated in females in the 500 and 2500 ppm groups (48 and 61 masses respectively *vs* 37 in controls). Part of these masses was identified as mammary tumours. There were no significant non-neoplastic lesions

in the mammary gland 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 number of female animals with malignant tumours was increased in the 500 and 2500 ppm groups (42% and 47% respectively *vs* 27% in controls) (Table 11). This increased number was not due to any one particular statistically significant increased type of tumour; they were not localised in any one particular organ or tissue; nor were there any unusual or rare tumours observed. However, there was a trend towards increased mammary gland adenocarcinoma in female rats (29% and 32% at 500 and 2500 ppm respectively *vs* 21% in controls, not statistically significant). There was no increase in the incidence of mammary gland adenomas (Table 11).

Relevant laboratory HCD for mammary adenocarcinoma show that in controls the incidence of such tumours can rise up to 23.4% (range = 8.3-23.4%; mean = 17%). Therefore the increased incidences of 29% and 32% observed at 500 and 2500 ppm respectively are slightly above the laboratory HCD.

A retrospective analysis of latency for these tumours showed that there was no trend (by Log-rank test) for decreased time-to-occurrence in TSM-treated females compared to controls, indicating that the mammary gland tumours seen in the treated females did not occur earlier in time compared to those occurring in controls, with the earliest malignant mammary tumour (on test day 119) observed among control animals

Overall, considering that the increase in mammary adenocarcinomas at the top two doses stands against a very high background incidence of 21% in the concurrent controls; the incidence at the top-dose was only 1.5-fold 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 was relatively flat over an approximate 100-fold exposure range (25 ppm to 2500 ppm); and tumour latency was not shortened, the slight increase in mammary adenocarcinoma observed at 500 and 2500 ppm TSM appears unlikely to be related to treatment.

It is well established that female Sprague-Dawley rats have a high spontaneous incidence of mammary gland tumours. Published HCD for Sprague-Dawley rats (Giknis & Clifford, 2001) (28)indicate that the incidence of mammary adenocarcinoma can rise even up to 58.3% (range = 8.6% - 58.3%; mean = 23.7%). This would lead to the conclusion that the increased incidences of 29% and 32% observed at 500 and 2500 ppm TSM are well within normal variation. More relevant contemporary (covering study initiation dates of 1984-1986) published HCD for Sprague-Dawley rats from the same supplier, breeder location and parental stock (Lang, 1992; McMartin et al, 1992)(30&34) as the animals utilized in the TSM study indicate that the incidence of mammary adenocarcinoma ranges between 7-31.4% (mean 18%). Even these contemporary HCD would still support the conclusion that the increased incidences of 29% and 32% observed at 500 and 2500 ppm TSM are part of normal biological variability in this strain of rats.

Further evidence of the high spontaneous incidence of mammary tumours in Sprague-Dawley rats is the finding that 97% of the control animals in the TSM cancer study had mammary gland hyperplasia, a precursor lesion of mammary tumours, and that incidences of 72%, 69% and 82% were seen at 25, 500 and 2500 ppm. These data indicate that there was significant variation across treated and control groups in the incidence of mammary gland hyperplasia, with no particular trend appearing. This response was consistent with the variation in the incidence of mammary adenocarcinomas observed across groups and the absence of a clear dose-response.

In summary, different strands of evidence lead to the conclusion that the increased incidences of mammary gland adenocarcinoma observed at 500 and 2500 ppm TSM are not treatment-related, but

chance findings in a strain of rats highly susceptible to mammary gland tumourigenesis. On this basis, it can be concluded that TSM is not carcinogenic in Sprague-Dawley rats.

Mouse

In a guideline 18-month carcinogenicity study, TSM was administered to four groups of 80 male and 80 female CD-1 mice (1985, 1987 & 1990)(24). The substance was incorporated in the diet at 0, 25, 750 and 7500 ppm for 18 months. The calculated mean daily intake of TSM was 3.2, 97 and 979 mg/kg bw/day for males and 4.3, 128 and 1312 mg/kg bw/day for females. Haematological parameters and plasma proteins were measured 3, 6, 9, 12 and 18 months after study initiation on 10 mice from each sex and group.

Terminal body weights and mean body weight gains were statistically significantly decreased in the top dose females. No other treatment-related effects were observed at any dose level.

Overall, TSM was not carcinogenic in the mouse up to the MTD in females and up to the limit dose (979 mg/kg bw/d) in males.

4.10.1.2 Carcinogenicity: inhalation

No data are available.

4.10.1.3 Carcinogenicity: dermal

No data are available.

4.10.2 Human information

No data are available.

4.10.3 Other relevant information

No structural alert for oestrogen receptor binding was identified for TSM and its rat and groundwater metabolites (including triazine amine) by a number of *in silico* assessments, including the OECD QSAR Toolbox (v.3.3.5), OASIS TIMES v2.27.16, MedChem Studio v4.0, ADMET Predictor v7.2 and the USEPA rtER Expert System v1. In addition, TSM and its metabolites lacked structural alerts for binding to the dopamine D_2 and D_3 receptors following homology modelling (Salmas *et al*, 2015; Platania *et al*, 2012)(39&38). Furthermore, using MedChem Studio v4.0, no similarities were found between TSM and its metabolites with known dopamine agonists and antagonists.

In an *in vitro E-screen* assay in MCF-7 human breast cancer-derived cells, designed to identify estrogenic compounds, TSM did not show oestrogenic activity over several concentrations ranging from 10^{-10} to 10^{-4} molar by measuring the relative proliferative potency against oestradiol Bitsch *et al.* (2002) (20). It is noted that although the metabolites of TSM were not tested in this assay, human MCF-7 cells are capable of some biotransformation and possess some human metabolism enzymes including some cytochrome P450 enzymes.

4.10.4 Summary and discussion of carcinogenicity

The chronic toxicity and carcinogenicity of TSM were investigated in a rat dietary study and in a mouse feeding study.

In the mouse, TSM was not carcinogenic up to the MTD in females and up to the limit dose (979 mg/kg bw/d) in males.

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-fold 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 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 the mouse.

In addition, QSAR assessments show that TSM and its rat and groundwater metabolites (including triazine amine) have no capability to 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, TSM 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 activities in TSM and its metabolites lends further support to the assertion that the mammary gland adenocarcinomas seen in the TSM 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 TSM is not treatment-related but a chance finding in a strain of rats highly susceptible to mammary gland tumourigenesis. On this basis, it can be concluded that TSM is not carcinogenic to Sprague-Dawley rats.

4.10.5 Comparison with criteria

The carcinogenic potential of TSM has been investigated by the oral route in rats and mice. There is no convincing evidence in these studies that TSM is carcinogenic in experimental animals. Therefore, classification of TSM for carcinogenicity is not warranted.

It is noted that no classification for carcinogenicity was proposed by the TCC&L group (the EU group responsible for advising on C&L of substances at the time) in 1997 for this substance on the basis of the same data.

4.10.6 Conclusions on classification and labelling

Not classified – conclusive but insufficient for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The chronic toxicity and carcinogenicity of TSM 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. TSM 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 TSM 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, TSM 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 TSM and its metabolites lends further support to the assertion that the mammary gland adenocarcinomas seen in the TSM 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 TSM 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 TSM 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 TSM does not have an endocrine tumorigenic mode of action in the rat. One MSCA raised some concern on the possible tumorigenic MoA of TSM 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".

Additional key elements

The information submitted by Industry during public consultation consist of four study reports and two position papers.

Author confidential (2015): Thifensulfuron Methyl (DPX-M6316) Technical: 6 Day Uterotrophic Assay for Detecting Estrogenic Activity and Prolactin Changes in Ovariectomized Rats. GLP. OECD TG 440.

In this study three groups of young adult ovariectomized Crl:CD(SD) rats (15/group) were dosed by oral gavage with a suspension of 0, 150, or 300 mg/kg/d TSM (Lot Nr: DPX-M6316-293) administered in 0.5% methylcellulose for 5 consecutive days. The negative control group received the vehicle only. Two separate ovariectomized positive control groups treated for 5 days via gavage, one group administered 0.1 mg/kg/d of the estrogen receptor agonist 17α-ethynyl estradiol in corn oil (with 1% ethanol) and one administered 2 mg/kg/d of the dopamine (D2) receptor antagonist haloperidol (in 0.5% methylcellulose) were included. Clinical signs, vaginal cytology and body weight (bw) were recorded daily for all five dose groups. Food consumption was recorded on test day 1 and 6. All animals were euthanized ca. 24 hours after the last treatment and uterine weights were collected to assess the ability of the test substance to induce uterine growth and blood was collected from all animals for serum prolactin concentration analysis.

In the TSM treatment groups during the whole study period no mortalities, treatment-related clinical signs, or body weight (bw) effects were seen. Similarly, food consumption was not affected. All animals remained in diestrus for the duration of the study, and at scheduled euthanasia neither gross observations nor any treatment-related effects on uterine weight or effects on serum prolactin levels at any dose level were noted.

In the positive control groups treated with either 17α-ethynyl estradiol or haloperidol, neither mortality nor clinical observations occured. Mean bw gain and mean final bw decreased and were accompanied by decreased food consumption and food efficiency compared to the negative control. On test day 4, all animals treated with 17α-ethynyl estradiol showed cytological markers indicative of either proestrus or estrus. Relative uterine wet weight (to final bw) and blotted weight were increased to 188% and 175% of the negative control, respectively. Serum prolactin levels were increased by 505% compared to negative control in 17α-ethynyl estradiol treated animals. Animals receiving haloperidol remained in diestrus for the duration of the study. No gross observations were noted nor any treatment related effects on uterine weight. Serum prolactin levels were increased by 250% compared to the negative control in haloperidol treated animals.

In conclusion, under the conditions of this study TSM did not induce changes on any parameters consistent with the potential to act as an estrogen receptor agonist in

ovariectomized adult female rats, and did not modulate serum prolactin concentrations. Rats administered 17α-ethynyl estradiol showed effects consistent with an estrogen receptor agonist and animals treated with haloperidol showed effects consistent with a D2 receptor antagonist.

Author confidential (2016): IN-A4098: Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC). GLP. US EPA Health Effects Test Guidelines OPPTS 890.1250.

In this study, the ability of TSM (IN-A4098) to compete with radioligand [^{3}H] 17β-estradiol for binding estrogen receptors (ERs) in rat uteri homogenate was investigated. Uteri from 100 Sprague-Dawley female rats (85 to 100 days of age) ovariectomized seven days prior to being killed were used to prepare the uterine cytosol. The final concentrations of TSM assessed in the binding assays were: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M for all three valid independent runs. All concentrations were tested in replicates of 3. In addition, solvent control tubes (3 replicates) were prepared to assess total binding. These replicates included the radioligand [^{3}H] 17β-estradiol, cytosol (containing the ERs) and solvent but without the competitor 17β-estradiol. The total binding tubes allowed for the identification of maximal binding of [3H]-17β-estradiol. Nonspecific binding was also assessed in replicates of 3 by determining the [^{3}H]-17β-estradiol bound in the presence of 100-fold excess unlabeled 17β-estradiol. The duration of incubation at approximately 4°C was 16-20 hours. A complete concentration response curve for the positive control 17β-estradiol, negative control octyltriethoxysilane and weak positive control 19-norethindrone, was run each time the binding assay was performed.

In the three valid independent runs, the mean specific binding for the test substance was between ≥91% and >98% showing that TSM could not displace the radiolabeled ligand from the receptor at every concentration tested, indicating TSM (IN-A4098) is “not interactive” with the ERs.

Author confidential (2016): IN-A4098: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)). GLP. US EPA Health Effects Test Guidelines OPPTS 890.1300

In this study, the ability of TSM (IN-A4098) to act as an agonist of human estrogen receptor alpha (hERα) using the hERα-HeLa-9903 cell line was investigated. The final concentrations of TSM tested in the transcriptional activation assays were: 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M for both valid independent runs. All concentrations were tested in replicates of 6/plate. In addition, for each concentration, 2 replicates/plate were prepared that incorporated the hERα antagonist ICI 182,780. Replicates incorporating the hERα antagonist allow for the identification of non-specific (i.e., nonhERα-mediated) induction of the luciferase gene. The duration of exposure was 20 - 24 h. A complete concentration response curve for each of four reference compounds (17β-estradiol, 17α-estradiol, corticosterone and 17α-methyltestosterone) was run each time the transcriptional activation assay was performed.

There was no cytotoxicity (≥20% reduction in cell viability) observed with TSM or the controls in any of the valid independent runs. In two independent runs of the transcriptional activation assay, TSM did not result in an increase in luciferase activity at any of the viable concentrations tested.

In conclusion, under the conditions of this study TSM is not an agonist of human estrogen receptor alpha (hERα) in the HeLa-9903 model system.

Author confidential (2015): Thifensulfuron Methyl (DPX-M6316) Technical: Receptor Binding Assay. Non-GLP. Non-GL

The activity of TSM was evaluated in nine radioligand binding assays utilizing human recombinant CHO cell receptors (D1, D2L, D2S, D3, D4.2, D4.4, D4.7, D5, Dopamine Transporter (DAT). Methods employed in this study were adapted from the scientific literature to maximize reliability and reproducibility. Biochemical assay results were reported as the percent inhibition of specific binding or activity. No significant responses (>50% inhibition or stimulation for biochemical assays) were noted in any of the radioligand binding assays. These results show that TSM is unlikely to interact with the dopamine receptor.

Author confidential (2015): In Silico QSAR Evaluation of Thifensulfuron-Methyl (DPX-M6316) and Metabolites for Potential Endocrine and Dopaminergic Activity. Non-GLP. Non-GL

In this position paper, TSM (DPX-M6316), and its rat metabolites and ground water metabolites were evaluated as target molecules for potential endocrine (estrogenic and androgenic) and dopamine (agonist or antagonist) activities using the OECD QSAR Toolbox v3.3.5, OASIS TIMES v2.27.16, MedChem Studio v4.0, and ADMET Predictor v7.2 software programs.

No structural alerts for estrogen receptor binding were found for these target molecules by the OECD QSAR Toolbox for the USEPA rtER Expert System ver1. Furthermore, TSM and its metabolites were labeled “Non binder, without OH or NH2 group” for the Toolbox Estrogen Receptor Binding alert. Models from OASIS TIMES and ADMET Predictor were used to evaluate TSM and its metabolites for androgen and estrogen binding potential. The predictions from the models and alerts indicate that binding to androgen or estrogen receptors is unlikely.

In order to better understand the potential for dopamine receptor binding, the common pharmacophores were investigated. Furthermore, MedChem Studio v4.0 was used to perform similarity analyses on TSM and its metabolites against known dopamine agonists and antagonists. The weight of evidence from the results of these evaluations indicates that the target molecules will not bind to dopamine D1, D2 or D3 receptors.

In summary, the weight of evidence from these *in silico* analyses supports there being no significant concerns with TSM or its metabolites for estrogen- or androgen-mediated activities, or dopamine antagonistic activities.

Author confidential (2015): Thifensulfuron-Methyl: Assessment of Potential Endocrine Activity from the U.S. Environmental Protection Agency (US EPA) Endocrine Disruptor Screening Program (EDSP) for the 21st Century Dashboard. Non-GLP. Non-GL

In this position paper, the information on TSM provided via the EDSP21 Dashboard is summarised. TSM was evaluated in 8 assays, and tested negative for bioactivity in all 8 tests. Of the 18 potential assays for estrogenic activity, TSM tested negative for bioactivity

in 17 of the 18 assays. While TSM tested positive in the ATG_ERE_CIS_up assay, the AC50 (59 uM) occurred at a concentration that was approaching observed cytotoxicity. In addition, TSM tested negative in all of the other ER assays. Of the 4 potential assays for thyroid activity, TSM was evaluated in 3 assays with negative outcomes. Considering the weight of evidence from the EDSP21 assays, this demonstrates that TSM does not interact with the estrogen, androgen, or thyroid signaling systems when evaluated in a wide variety of assay types with a comprehensive range of endocrine-related endpoints.

The available data do not support the carcinogenicity mediated by ED mechanism of TSM. Commenting RAC members do not consider the mammary gland tumors as a consequence of the ED properties of TSM.

Assessment and comparison with the classification criteria

In the mouse carcinogenicity study, TSM 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,	**(14%)**	**(9%)**	**(19%)**	**(24%)**
single+multiple	12/58	6/54	15/52	20/62
Adenosquamous cell	**(21%)**	**(11%)**	**(29%)**	**(32%)**
carcinoma	-	-	1	-

* 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**.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Although this proposal is targeted to carcinogenicity and developmental toxicity, this endpoint has been included as it may provide information to aid interpretation of the developmental toxicity studies.

The potential effects of TSM on fertility and reproductive performance have been investigated in a 2-generation study in rats.

Table 12: Summary table of relevant reproductive toxicity studies – Fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Preliminary 1-generation reproductive toxicity study (included in the report of the 90-day study) No guideline Conducted prior to the implementation of GLP, but QA statement confirming consistency with GLP requirements SD rats (6/sex/group) Dietary administration TSM 93.6 – 95.6% 1984a(35)	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)	As fertility was unexpectedly low in the control animals, no meaningful comparison between reproductive parameters in the treated groups and in the control groups could be performed.

2-generation reproductive toxicity study OECD 416 Not GLP but QA Dietary administration SD rats (20/sex/group) TSM 95.6-98% 1985(22)	0, 25, 500, 2500 ppm (1.8, 34, 175 mg/kg bw/d in males; 2.4, 48, 244 mg/kg bw/d in females)	**2500 ppm (175/244 mg/kg bw/d in males/females)**: *Parental toxicity* ↓bw gain (up to 9%) in F_0 females and F_1 males and females; *Fertility and reproductive performance* No treatment-related effects in both generations; *Offspring toxicity* No treatment-related effects in both generations; **500 and 25 ppm:** No treatment-related effects in both generations NOAEL[$] (parental toxicity) = 500 ppm (34/48 mg/kg bw/d in males/females) NOAEL[$] (reproductive toxicity) = 2500 ppm (175/244 mg/kg bw/d in males/females) NOAEL[$] (offspring toxicity) = 2500 ppm (175/244 mg/kg bw/d in males/females)

[$] As given in the RAR

4.11.1.1 Non-human information

A preliminary one-generation experiment was performed and included in the report of the 90-day rat study (1984a)(35). In this study, the same dose levels of TSM employed in the 90-day study were administered in the diet to groups of 6 male and 6 female Sprague-Dawley rats. However, as after mating, fertility was unexpectedly low in the control animals, no meaningful comparison between reproductive parameters in the treated groups and in the control groups could be performed. Nevertheless, fertility and other reproductive and lactation performance indices in all the test groups were within the range of expected biological variability and comparable among test groups.

In a guideline 2-generation study, TSM was administered to groups of 20 male and 20 female Sprague Dawley rats (1985b)(22). The substance was incorporated in the diet at 0, 25, 500 and 2500 ppm. The calculated mean daily intake of TSM was 1.8, 34 and 175 mg/kg bw and 2.4, 48 and 244 mg/kg bw/day for males and females respectively.

Slight decreased body weights or body weight gains (4 to 9%) were recorded in parental females of the F_0 generation and parental males and females of the F_1 generation, indicating a minimal toxicity of the 2500 ppm dose.

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 pups born, number pups alive, pup weight and number pups weaned were not affected by treatment with TSM. In F_2 selected weanlings the absolute and relative weights of kidneys were slightly decreased (13 to 15%) in the males of the 2500 ppm group, without microscopic changes. These effects were not considered to be of toxicological significance.

Overall, no adverse effects on fertility and reproductive performance or on offspring were observed after continuous treatment of rats during two generations with TSM. Slight adult body weight

effects were observed at 2500 ppm (the highest dietary level tested) suggesting minimal parental toxicity may occur at this dose level.

4.11.1.2 Human information

None available.

4.11.2 Developmental toxicity

The developmental toxicity of TSM has been investigated in standard rat and rabbit studies.

Table 13: Summary table of relevant reproductive toxicity studies - Development

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Developmental toxicity study Mated female SD **rats** (25/group) Gavage administration on GD 7-16 OECD 414 Conducted prior to the implementation of GLP, but QA statement confirming consistency with GLP requirements TSM 95.6% 1984(41)	0, 30, 200, 800 mg/kg bw/d	**800 mg/kg bw/d**: *Maternal effects* ↓bw gain (up to 11%) over dosing period; *Foetal effects* ↓bw (3%)*; Absent renal papilla (5 foetuses/5 litters *vs* 1/1 in controls) – a microscopic evaluation to confirm this observation was not conducted; [HCD 0-3/0-3 foetuses/litters based on macroscopic evaluation only] Small renal papilla (4 foetuses/4 litters *vs* 0/0 in controls)*; [HCD 0-10 foetuses/0-6 litters based on macroscopic evaluation only] Delayed ossification of skull bones (10 foetuses/5 litters *vs* 1/1 in controls); [HCD 0-18 foetuses/0-10 litters] **200 and 30 mg/kg bw/d**: No treatment-related effects; NOAEL$ (maternal toxicity) = 200 mg/kg bw/d NOAEL$ (developmental toxicity) = 200 mg/kg bw/d
Developmental toxicity study Mated female New Zealand **rabbits** (20/group) Gavage administration on GD 7-19 OECD 414 Conducted prior to implementation of GLP, QA statement confirming consistency with GLP requirements TSM 95.4% 1985(42)	0, 22, 158, 511 mg/kg bw/d	**511 mg/kg bw/d**: *Maternal effects* ↓bw gain (58-69%) over dosing period; Bw loss (36 g *vs* a gain of 4 g in controls) during GD 7-9; *Foetal effects* No treatment-related effects; **158 and 22 mg/kg bw/d**: No treatment-related effects NOAEL$ (maternal toxicity) = 158 mg/kg bw/d NOAEL$ (developmental toxicity) = 511 mg/kg bw/d

$ As given in the RAR; *Statistically significant

4.11.2.1 Non-human information

Rat

In a developmental toxicity study conducted in 1983, which conformed to the guidelines available at the time, TSM (in 0.5% methyl cellulose) was administered *via* oral gavage to groups of 25 pregnant female Sprague-Dawley rats at 0, 30, 200, or 800 mg/kg bw/day (doses selected upon a pilot study) from days 7-16 of gestation (1984)(41). The day of mating was designated a day 1 of gestation, and caesarean sections were performed on gestation day 21. Current OECD guideline 414 (consistent with EEC Method B.31) requires administration of the test substance from around the time of implantation until the day prior to caesarean section or gestation days 6-20 with caesarean sections performed on gestation day 21; note that the current guideline and practice is to designate the day of mating as day 0 of gestation.

Maternal body weight gains in the high-dose group remained slightly below control levels (2-11% decreases over the dosing period, not statistically significant; 11% decrease on days 7-9 of gestation) indicating evidence of slight maternal toxicity at 800 mg/kg bw/day.

There was a small (3%) but statistically significant decrease in foetal body weight in the 800 mg/kg bw/day group.

At 800 mg/kg bw/day, the macroscopic observation of size 0 (absent renal papilla) was recorded in 5 foetuses (5 foetuses/5 litters [5/25 litters = 2%] *vs* 1/1 in controls; not statistically significant). The incidence of size 0 renal papilla at the high dose was above the laboratory historical control range of 0/0 – 3 foetuses/3 litters (from 29 studies (with 34 separate control groups) conducted between 1980 and 1989). At the time the study was conducted (and the studies from which the historical control data were obtained), renal papilla size was scored macroscopically on a scale of 0 to 4 (similar to the scale described by Woo and Hoar (1972)(46), without histopathological verification of renal papilla development being undertaken.

In the test facility, and in the industry in general, the method for examining foetal kidney papillary development has evolved sufficiently such that beginning in the years following the current study, it became standard practice to confirm the absence of renal papilla microscopically. It was generally observed that this was a necessary step since apparently “size 0” kidneys were frequently reassigned to “size 1” following microscopic confirmation. Additionally, it should be noted that the foetuses were examined one day prior to what would be viewed as current industry standard, and as a result of missing that last 24 hours of in utero development, it would not be surprising to see an increase in observations reflecting general background variability. This would be especially applicable to the foetal kidney since according to Woo and Hoar (1972)(46) and (2000) (19), renal development occurs late in gestation, and continues into the postnatal period. This is confirmed by Schreuder *et al* (2011)(40) who demonstrated that new born rats and mice are at an early stage of kidney development, with approximately 20% of mature nephrons present at birth, with nephrogenesis continuing until lactation day 7-10. Specifically, (2000) (19) measured the volume of metanephric compartments from embryonic day 14 to day 21 in developing Sprague-Dawley rat foetuses. They demonstrated that 92.4% of the kidney was undifferentiated mesenchyme on embryonic day 14, and by day 21, 46.7 % of the kidney was still undifferentiated.

Similarly, Woo and Hoar (1972)(46) macroscopically evaluated the size of the renal papilla in CD rat offspring collected from untreated dams on gestation days 19, 20, 21, and following delivery (denoted as days 22 and 23) using a dissecting scope. Papilla size was scored according to the following scale: no papilla (size 0), small (+), medium (++), long (+++), and full size (++++). The incidence of offspring with size 0 renal papilla was described in the text of the publication for the

specified gestation days. The incidences of offspring with size +, ++, +++, and ++++ renal papilla were also presented graphically in the publication, and the incidences of all the observations are summarized in the following table:

Gestation Day	No papilla (size 0)[a]	Small (size +)	Medium (size ++)	Long (size +++)[b]	Full size (size ++++)[b]
19	2.1%	50%[a]	38%a	10%	0
20	1.2%	50%[a]	40%b	9%	0
21	1.2%	25%[b]	45%b	27%	2
22 (birth)	0	3%[b]	22%b	50%	25
23 (lactation day 1)	0.6%	0.6%[b]	7%b	45%	47

a) Presented in the text of the publication and in Figure 3 of the publication (Woo and Hoar 1972)(46)

b) Presented on Figure 3 of the publication (Woo and Hoar 1972)(46)

These data illustrate the developmental continuum occurring during late gestation and early lactation in offspring from untreated rats and that in control rats on day 19 of gestation there was a 2.1% incidence of absent renal papilla (higher than the incidence of 2% of absent renal papilla observed at 800 mg/kg bw/day TSM). These controls rats with absent renal papilla developed perfectly normal following delivery. As discussed in the Woo and Hoar (46) study, size 0 renal papilla is a normal developmental variation in a developmental continuum, and is not considered to be consistent with the malformation of agenesis.

Increased frequencies of litters with foetuses having small renal papilla (4 foetuses/4 litters vs 0/0 in controls; statistically significant) and incomplete ossification of the skull (10 foetuses/5 litters vs 1/1 in controls, not statistically significant) were also observed at 800 mg/kg bw/day. It is noted that the incidence of small renal papilla at the top dose was well within the laboratory historical control range of 0/0 – 10/6 (from 33 studies conducted between 1981 and 1989); this would question the relation to treatment of the finding. However, when considering the additional observations of absent renal papilla, a possible (unspecific) effect of treatment at 800 mg/kg bw/day on the development of the renal papilla cannot be completely excluded.

When the incidence of observations of “size 0” and “size 1” renal papillae from the TSM study are combined together, (which would be appropriate given the lack of microscopic confirmation), the combined value for the 800 mg/kg/day group (9 foetuses) falls within the range of test facility historical control data (0-10 foetuses) for size 1 renal papillae. This may indicate that the effects on the renal papilla might even be non-treatment related. It is also noteworthy that during this time of peak foetal development (gestation days 19 through postpartum day 3), there was not likely to be any exposure to the test substance since dosing was completed on gestation day 16, and in the metabolism studies, excretion of TSM was determined to be complete within 72 hours (see section 4.1.3.

Overall, an increase in the incidence of absent renal papilla was observed at the top dose of 800 mg/kg bw/day. Although this increase was above the laboratory historical control data for this

finding, without histological confirmation, it is most likely that in some of the affected foetuses the renal papilla was not completely absent (size 0) but only very small (size 1), as shown by the fact that the combined incidence (9 foetuses) of size 0 (absent) papilla and size 1 (small) papilla were within the laboratory historical control data for size 1 (small) papilla. This may indicate the effects on the renal papilla were not treatment-related or represented at maximum delayed development rather than a permanent malformation. This interpretation is supported by evidence that size 0 renal papilla in the rat as seen in control rats in the Woo & Hoar (1972)(46) study is a transitory and reversible growth retardation of the kidney of no functional significance, and that when the development of the kidney is delayed, the papilla may not form fully due to the rapid growth rate of the renal parenchyma relative to that of the papilla just before parturition (Woo & Hoar, 1972)(46). In addition, it is noted that delayed ossification of the skull and reduced foetal body weight occurred at the top dose, supporting the view that TSM induced delayed development in the rat at the highest dose tested of 800 mg/kg bw/day.

In conclusion, administration of TSM to pregnant rats during the period of organogenesis resulted in evidence of limited maternal effects (small decrease in body weight gain) and some developmental toxicity (delayed development of the renal papilla, delayed ossification and reduced foetal weight) at a dose level of 800 mg/kg bw/day. The observed developmental toxicity appeared to be the consequence of retardation and was seen in the presence of some maternal toxicity. It is most likely this was the unspecific, secondary consequence of the maternal toxicity observed at the high dose of mg/kg bw/day. It is also possible the effects on the renal papilla were not treatment-related at all.

Rabbit

In a guideline developmental toxicity study, TSM (in 0.5% carboxymethyl cellulose) was administered orally *via* gavage to groups of 20 pregnant female New Zealand white rabbits at nominal doses of 0, 30, 200, or 650 mg/kg bw/day (doses selected upon a pilot study) from days 7-19 of gestation (1985)(42). In the pilot study, moderate maternal toxicity (decreases in body weight gain and food consumption) was seen at 600 mg/kg bw/day, but deaths (2/6) and abortions (3/6) were observed at the next dose level of 900 mg/kg bw/day. Four out of 15 test suspensions were analysed at each dose levels: the measured doses were 0, 22, 158 and 511 mg/kg bw.

Body weight gains in the high-dose group were reduced (by 58-69%, not statistically significant; more notably during the first two days of treatment) from days 7 to 20 of gestation (treatment period), indicating evidence of minimal maternal toxicity. At this dose, during days 7-9 of gestation, there was a mean maternal body weight loss of 36 g compared to a gain of 4 g in control animals.

There were no significant differences between the control and experimental groups in pregnancy rate, number of nidations, abortions or total resorption of litters. No effects on the number of live or dead foetuses *per* litter, mean foetal weights or any type of malformations or variations were detected.

In conclusion, TSM did not cause developmental toxicity in rabbits up to an oral dose of 511 mg/kg bw/d at which some maternal toxicity (decreased body weight gain from days 7 to 19, not statistically significant; more notably during the first two days of treatment) occurred.

4.11.2.2 Human information

4.11.3 Other relevant information

No data are available.

4.11.4 Summary and discussion of reproductive toxicity

Fertility

The potential effects of TSM on fertility and reproductive performance were investigated in a rat dietary 2-generation study. In this study, there were no adverse effects on fertility, reproductive performance and offspring up to a dose (175 mg/kg bw/day) causing parental toxicity.

Development

The developmental toxicity potential of TSM was investigated in standard rat and rabbit studies.

There were no adverse effects on development in rabbits up to the top dose of 511 mg/kg bw/day which caused minimal maternal toxicity.

In the rat developmental toxicity study, TSM caused limited maternal toxicity (small decrease in body weight gain) and some degree of developmental toxicity (delayed development of the renal papilla, delayed ossification and reduced foetal weight) at the top dose level of 800 mg/kg bw/day. The observed developmental toxicity appeared to be the consequence of retardation and was seen in the presence of some maternal toxicity. It is most likely this was the unspecific, secondary consequence of the maternal toxicity observed at the high dose of 800 mg/kg bw/day. It is also possible the effects on the renal papilla were not treatment-related at all.

4.11.5 Comparison with criteria

Fertility

The potential effects of TSM on fertility and reproductive performance were investigated in a rat dietary 2-generation study. In this study, there were no adverse effects on fertility, reproductive performance and offspring up to a dose (175 mg/kg bw/d) causing parental toxicity. On this basis, classification of TSM for fertility is not warranted.

Development

The developmental toxicity potential of TSM was investigated in standard rat and rabbit studies.

There were no adverse effects on development in rabbits up to the top dose of 511 mg/kg bw/day which caused minimal maternal toxicity.

In the rat developmental toxicity study, TSM caused limited maternal toxicity (small decrease in body weight gain) and some degree of developmental toxicity (delayed development of the renal papilla, delayed ossification and reduced foetal weight) at the top dose level of 800 mg/kg bw/day. The observed developmental toxicity appeared to be the consequence of retardation and was seen in the presence of some maternal toxicity. It is most likely this was the unspecific, secondary

consequence of the maternal toxicity observed at the high dose of 800 mg/kg bw/day. It is also possible the effects on the renal papilla were not treatment-related at all.

When comparing these findings in the rats with the criteria, the following conclusions can be drawn:

Category 1A (known human reproductive toxicant) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to TSM and an adverse effect on development.

Category 1B (presumed human reproductive toxicant) is also not appropriate as *there is no clear evidence of an adverse effect on development in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects.* The delayed development of the renal papilla, if any, observed in the rat is considered to be the secondary, non-specific consequence of the maternal toxicity seen at the high dose of 800 mg/kg bw/day.

Category 2 (suspected human reproductive toxicant) is also not appropriate because *there is no evidence of an adverse effect on development in experimental animals that is considered not to be the secondary, non-specific consequence of other toxic effects.* The delayed development of the renal papilla, if any, observed in the rat is considered to be the secondary, non-specific consequence of the maternal toxicity seen at the high dose of 800 mg/kg bw/day.

Therefore, classification of TSM for developmental toxicity is not warranted. It is noted that no classification for developmental toxicity was proposed by the TCC&L group (the EU group responsible for advising on C&L of substances at the time) in 1997 for this substance on the basis of the same data.

4.11.6 Conclusions on classification and labelling

Not classified – conclusive but insufficient for classification

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The potential effects of TSM on fertility and reproductive performance were investigated in a rat dietary 2-generation study. The animals were dosed with 0, 25, 500, 2500 ppm TSM 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) TSM 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 TSM for fertility is not warranted.

Development

The developmental toxicity potential of TSM was investigated in one standard rat and one rabbit study.

The rabbits were dosed with 0, 22, 158 or 511 mg/kg bw/d TSM 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, TSM 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 TSM). 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 TSM 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”.

Additional key elements

The available data submitted by Industry during public consultation consists of one study report and one position paper.

Author confidential (2015): Thifensulfuron Methyl (DPX-M6316) Technical: Developmental Reproducibility Toxicity Study in Rats. GLP. OECD TG 414.
Groups of 22 time mated females Crl:CD(SD) rats were administered 0 or 800 mg/kg/day TSM from GD 6 through GD 20 via gavage. During the in-life phase of the study, body weights, food consumption, and clinical observations before and after dosing were collected on a daily basis. All dams were euthanized on GD 21; the gross necropsy included an examination and description of uterine contents including counts of *corpora lutea*, implantation sites, resorptions, and live and dead fetuses. The liver and kidneys were collected and weighed from all females surviving to scheduled euthanasia. Maternal blood was also collected from all surviving adult females and processed to serum for possible future analysis. All live fetuses were examined externally and following euthanasia, fresh visceral examination of the fetal kidneys only was performed.

At 800 mg/kg/day, there were no adverse test substance-related effects on any maternal or fetal endpoint. Specifically, there were no alterations observed in the fetal kidneys; all fetal kidneys appeared normal at the fresh visceral examinations.

Under the conditions of this study which was conducted using a contemporary exposure protocol (day of mating is designated as day 0 of gestation, with caesarean sections performed on gestation day 21), the findings that were reported previously (small or absent renal papilla) were not reproduced.

Author confidential (2015): Thifensulfuron methyl: Dosimetry Assessment for the Female Spraque-Dawley Rat During Gestation. Non-GLP. Non-GL.
In this position paper, the comparative dosimetry of the developmental and reproduction studies in rats with TSM is assessed. The disparity in renal papillary findings between the developmental and reproductive studies could be the result of the lower dose used in the feeding study (approximately 220 mg/kg/day compared to 800 mg/kg/day in the developmental study). In the developmental study, dosing was discontinued on GD15 so that by GD19—the beginning of the rapid phase of renal papillary development—maternal blood concentrations were predicted to decline to less than 0.5 µg/g. In contrast, the continuous dosing protocol of the reproduction study resulted in a predicted blood concentration on GD19 and throughout gestation and lactation of 4.4 µg/g. Thus, while the dose level (800 mg/kg/day) in the developmental study was approximately 4 times higher than that in the reproduction study, the differing dosing protocols, along with elimination kinetics of the test material, result in an approximately 10-fold higher predicted systemic exposure in the reproduction study compared to the developmental study. These results support the conclusion that the observation of small or absent renal papilla in the developmental toxicity study with TSM were not test substance-related but resulted from the high variability in renal papillary development in late gestation rat fetuses.

The following documents were also taken into account in the formulation of RAC opinion:

-The RAR 08 vol3 B6 Tox

-EFSA opinion on TSM (EFSA Journal 2015;13(7):4201)

-The new studies were received by DuPont in early 2016 and made available during the public consultation.

In the Conclusion on the peer review of the pesticide risk assessment of the active substance TSM EFSA (EFSA Journal 2015;13(7):4201), the position on developmental toxicity on TSM was the following:

"The developmental toxicity potential was discussed during the experts' meeting: the majority of the experts considered that the findings on the renal papilla in rats suggest that classification as 'Reproductive toxicant category 2' would be required for TSM.

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 TSM.

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

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

Developmental

Two standard developmental toxicity studies (one with rats and the other with rabbits) on TSM 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) Mean % affected per litter	135/25 38.7%	114/22 39.0%	145/23 48.0%	163/24 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 TSM (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**.

4.12 Other effects

4.12.1 Non-human information

No data are available.

4.12.1.1 Neurotoxicity

There are no indications from the existing database that TSM has effects on the nervous system. Therefore, no specific neurotoxicity studies have been conducted.

4.12.1.2 Immunotoxicity

No data are available.

4.12.1.3 Specific investigations: other studies

No data are available.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Information on the environmental fate and behaviour and ecotoxicity of thifensulfuron-methyl is taken mainly from the original Draft Assessment Report (DAR) for approval of the substance as a pesticide under Directive 91/414/EEC (DAR, 1996) and also from the Renewal Assessment Report according to Regulation (EU) 1107/2009 (RAR, July 2014, updated Feb. 2015). The list of agreed endpoints from the previous DAR assessment is included in the European Commission's 'Review Report for the active substance thifensulfuron-methyl' (Thifensulfuron-methyl, SANCO/7577/VI/97-final, 12 December 2001). At the time of writing this draft CLH Report on thifensulfuron-methyl, the RAR has been considered in EFSA peer review and an EFSA Conclusion and updated list of endpoints for thifensulfuron-methyl has been published (EFSA Journal 2015;13(7):4201).

Note that separate dossiers were received from two Notifiers for the renewal of approval of thifensulfuron-methyl (TSM), i.e. Du Pont de Nemours and the 'EU TSM AIR 2 Task Force' (comprising Rotam Agrochemical Europe and Cheminova AS). For the purposes of the pesticide assessment and this CLH Report both dossiers were combined and studies from both sources have been used. Of these, only the endpoints considered relevant to the environmental classification of thifensulfuron-methyl have been included in this CLH Report. Some studies have been included even though there are questions over their reliability - these are highlighted.

5.1 Degradation

The information on environmental degradation of thifensulfuron-methyl is summarised in Table 14 and in more detail further below. The environmental fate and behaviour studies were conducted using one or both radiolabelled forms of thifensulfuron-methyl ([thiophene-2-^{14}C]-thifensulfuron-methyl and [triazine-2-^{14}C]-thifensulfuron-methyl). The ^{14}C-radiolabels were placed in the most stable ring positions of thifensulfuron-methyl as indicated in Figure 1. Radiochemical purity was always greater than 96%.

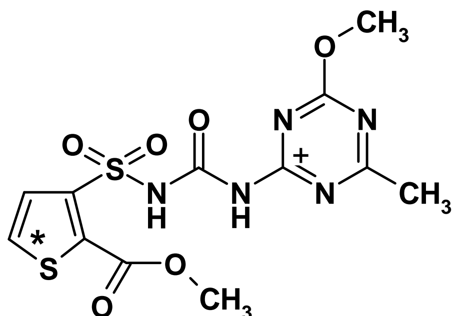


Figure 1: Positions of radiolabels in thifensulfuron-methyl
* Denotes [thiophene-2-^{14}C]thifensulfuron-methyl
+ Denotes [triazine-2-^{14}C]thifensulfuron-methyl

Table 14: Summary of relevant information on degradation

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>Aqueous hydrolysis as a function of pH; performed according to:
U.S. EPA 161-1 (1982), OPPTS 835.2120 (2008), SETAC Europe (1995) and OECD 111
To GLP</td>
<td>Hydrolysis DT_{50}s at various temperatures in buffered solutions.
<table>
<tr><th>pH</th><th>Temp (°C)</th><th>DT_{50} (days)</th></tr>
<tr><td>4</td><td>20
30
50</td><td>6.3
1.9
0.2</td></tr>
<tr><td>7</td><td>20
30
50</td><td>199
65
4.0</td></tr>
<tr><td>9</td><td>20
30
50</td><td>23.4
6.5
0.6</td></tr>
</table>
Hydrolysis rates were highly pH and temperature dependant.</td>
<td>Study performed to GLP - with no significant deviations from guidelines; considered reliable.
Study results were calculated using simple first-order kinetics.</td>
<td>Wardrope (2011)(47)</td>
</tr>
<tr>
<td>Aqueous hydrolysis as a function of pH; performed according to OECD 111 and to GLP.</td>
<td>Tier 2 study conducted at 25°C in buffered solutions at pH 4, 7 and 9; hydrolysis DT_{50}s were:
pH 4: 2.4 days
pH 7: 137 days
pH 9: 7.1 days
Hydrolysis rates were highly pH dependant.</td>
<td>Study performed to GLP - with no significant deviations from guideline; considered reliable.
DT_{50}s calculated using simple first-order kinetics.</td>
<td>Simmonds and Buntain (2012)(48)</td>
</tr>
<tr>
<td>Aqueous photolysis in sterile buffer solutions, performed according to US EPA, 161-2; not to GLP</td>
<td>Photolysis in sterilised buffer solutions at pH 5, 7 and 9 at 25ºC in dark or light (summer sunlight at 34o N and 285-2800 nm). DT_{50}s in light were:
pH 5: 98 hours
pH 7: 125 hours
pH 9: 97 hours</td>
<td>Study was not performed to GLP but was otherwise reliable with no significant deviations from guideline.
DT_{50}s calculated using simple first-order kinetics.</td>
<td>Ryan (1986)(49)</td>
</tr>
<tr>
<td>Aqueous photolysis in natural water, performed according to JMAFF guideline 12 Nohsan no. 8147 and to GLP</td>
<td>Photolysis in pH 7 buffered natural water at 25ºC in dark or light (equivalent to 30 days of natural sunlight at midday at 41o N):
DT_{50} = 0.5 days
Quantum yield of direct phototransformation in water at $\Sigma > 290$ nm = 0.037 molecules/ photon.</td>
<td>Study performed to GLP - with no significant deviations from guideline; considered reliable.</td>
<td>Lentz (2001)(50)</td>
</tr>
</table>

Aqueous photolysis in sterile buffer solutions, performed according to OECD 316 and to GLP	Photolysis in pH 7 buffered natural water at 25ºC in dark or light (equivalent to 18.2 days natural sunlight at 30-50°N): DT_{50} = 0.32-0.67 days (7.7-16.2 hours) Quantum yield of direct phototransformation in water at Σ > 290 nm = 0.044 molecules/ photon.	Study performed to GLP - with no significant deviations from guideline; considered reliable.	Oddy (2012)(51)
Ready biodegradation - modified Sturm test, performed according to: EU 92/69/EEC C.4-C, OECD 301Band to GLP	Minimal biodegradation (1%) by the end of the study (Day 29), conducted at 19.8-22.9ºC and pH 7.3-7.6. Substance considered to be not readily biodegradable under the conditions of this test	Study performed to GLP - with no significant deviations from EU guideline; considered reliable	Barnes, 2000(52)
Aerobic water/sediment degradation simulation test study to SETAC guideline and subsequent kinetic analysis acc. to FOCUS procedures	Studied in two systems at 20°C in the dark for 182 days. Whole system DT_{50} = 21-27 days; <4 and <9% mineralisation for thiophene and triazine moieties; bound residues <18%	Study performed to GLP - with no significant deviations from guideline; considered reliable; DT_{50}s calculated using first-order kinetics.	Spare (2000)(53)
Desk study and further analysis of Spare (2000) above. Conducted to FOCUS guidance , GLP not required	Further refinement of the results from the above study derived single first order whole system degradation DT_{50} values of 18.2 days for the Town Park water-sediment system and 26.1 days for the Red Oak water-sediment system	Analyses performed with no significant deviations from guidance; considered reliable	van Beinum, and Beulke (2006)(54)
Aerobic water/sediment degradation simulation test study to OECD 308 guideline and to GLP.	Studied in two systems at 20°C in the dark for 104 days. Whole system DT_{50} = 17.6-32.3 days; <3% mineralisation and <10% bound residues	Study performed to GLP - with no significant deviations from guideline; considered reliable; DT_{50}s calculated using simple first-order kinetics.	Simmonds (2012)(55)

5.1.1 Stability in water (abiotic degradation)

5.1.1.1 Aqueous hydrolysis

Four studies are available relating to the sterile aqueous hydrolysis of thifensulfuron-methyl, of these only three are considered reliable in the 2015 RAR. The study ref. AMR 224-84 by M.K. Koeppe and B.C. Rhodes (1984) included in the original DAR (1996) was no longer considered reliable in the RAR and so has not been included here. It has been superseded by the following three studies:

Study 1
Report: (47) Wardrope, L. (2011); Hydrolysis of [^{14}C]-DPX-M6316 (thifensulfuron-methyl) as a function of pH
DuPont Report No.: DuPont-30225
Guidelines: U.S. EPA 161-1 (1982), OPPTS 835.2120 (2008), SETAC Europe (1995), OECD 111 (2004) **Deviations:** None
Testing Facility: Charles River Laboratories, Tranent, Scotland, UK
Testing Facility Report No.: 809364
GLP: Yes
Certifying Authority: Department of Health (UK)

Study summary:
The hydrolysis of [^{14}C]-thifensulfuron-methyl in sterile aqueous buffered solutions at pH 4 (phthalate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer) and at 20, 30 and 50°C was studied in the dark for up to 30 days. The test item concentration was 5.0 μg/mL with acetonitrile (0.13%) as a co-solvent. Samples were analysed by LSC (Liquid Scintillation Counting) and HPLC (High Performance Liquid Chromatography). Identification of parent and significant hydrolysis products was by co-chromatography and the identifications confirmed using LC-MS (Liquid Chromatography Mass Spectrometry) analysis. The limit of quantification (LOQ) for both radiolabelled forms was <1% AR. Total recovery of radioactivity ranged from 95.13-104.11%.

Hydrolysis of thifensulfuron-methyl was pH and temperature dependant. At lower temperatures the rate of hydrolysis was significantly less than at higher temperatures. The pH dependency of the rate of hydrolysis was in the order pH 4 >pH 9 >>pH7. The first-order hydrolytic DT_{50} values of thifensulfuron-methyl were 6.3, 1.9 and 0.2 days in pH 4 buffer incubated at 20, 30 and 50°C, respectively. The first-order DT_{50} values of thifensulfuron-methyl were 199.0, 65.0 and 4.0 days in pH 7 buffer incubated at 20, 30 and 50°C, respectively. The first-order DT_{50} values of thifensulfuron-methyl were 23.4, 6.5 and 0.6 days in pH 9 buffer incubated at 20, 30 and 50°C, respectively.

At pH 4 at all temperatures, the major transformation products detected were a polar product, IN-A5546, IN-A4098, IN-L9226, and IN-RDF00 at maximum concentrations of 56.36% (50°C), 93.73% (50°C), 54.11% (50°C), 11.86% (30°C) and 31.85% AR (20°C), respectively. At pH 7 the major transformation products detected were IN-A5546, IN-L9223, IN-A4098, and IN-L9225 at maximum concentrations (observed at 50ºC) of 16.50%, 90.90%, 90.50% and 6.71% AR, respectively. At pH 9 the major transformation products detected were IN-L9223, IN-A4098, and IN-L9225 at maximum concentrations of 23.56% (observed at 50°C), 88.64% (50°C), 74.61% (50°C) and 70.05% AR (30°C), respectively.

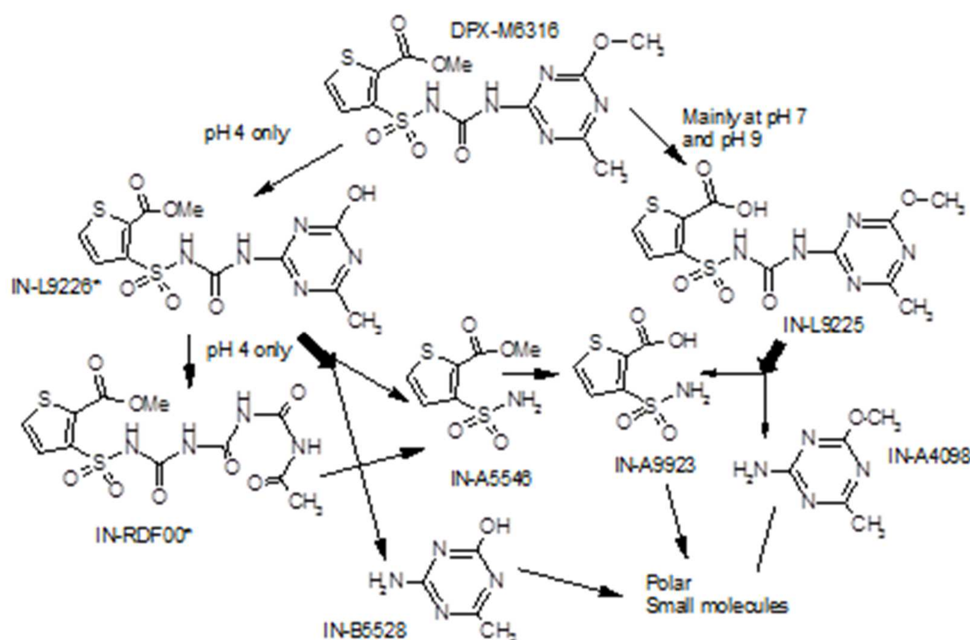
During the EFSA peer review, further information was provided by DuPont on the unidentified polar metabolite which appeared to be formed at levels significantly exceeding 10% applied radioactivity (AR) in the hydrolysis study. This has now been identified as IN-B5528 (see Wardrope, 2014 in RAR, not reported here) although further polar fractions remain unidentified, these may be accounted for in part by what the thifensulfuron-methyl Task Force identify as IN-F5475 (see Simmonds and Buntain, 2012 (48) below). Further information on the aquatic risk posed by this unidentified fraction has been requested in the EFSA Peer Review Conclusion (2015), however this is not considered to impact on the aquatic hazard classification of thifensulfuron-methyl itself.

Table 15: Hydrolytic DT_{50} and rate constants for thifensulfuron-methyl

Analyte	pH	Temperature (°C)	DT_{50} (days)	k (day-1)	r2	Method of calculation
Thifensulfuron-methyl	4	20	6.3	0.109	0.993	Simple first-order
		30	1.9	0.367	0.997	Simple first-order
		50	0.2	3.145	0.998	Simple first-order
	7	20	199	0.003	0.603	Simple first-order
		30	65	0.011	0.881	Simple first-order
		50	4.0	0.173	0.992	Simple first-order
	9	20	23.4	0.030	0.973	Simple first-order
		30	6.5	0.106	0.997	Simple first-order
		50	0.6	1.133	0.999	Simple first-order

A proposed hydrolytic degradation pathway for thifensulfuron-methyl is outlined below.

Figure 2: Proposed degradation pathway of thifensulfuron-methyl (DPX-M6316) under hydrolytic conditions



Study 2

Report: (48) M. Simmonds, I. Buntain (2012) (48) [^{14}C]-Thifensulfuron-methyl: Hydrolysis in sterile buffer at pH 4, 7 and 9. Battelle UK Ltd. [Cheminova A/S]
Unpublished report No.: WB/10/008 [CHA Doc. No. 260 TIM]
Guidelines: OECD 111 **Deviations:** None
GLP: Yes (certified laboratory)

Study summary:
This hydrolysis study was submitted by the 'Thifensulfuron-methyl Task Force', it was briefly evaluated in the RAR (2015) and considered acceptable. The hydrolysis of thifensulfuron-methyl was studied in the dark in sterile aqueous buffered solutions at pH 4 (sodium acetate), pH 7 (tris

(hydroxymethyl) methylamine) and pH 9 (sodium tetraborate) at a nominal concentration of 1 mg/L. To fully elucidate the pathway for hydrolytic degradation two radiolabelled forms of the test item were employed; [thiophene-2-^{14}C]-thifensulfuron-methyl and [triazine-2-^{14}C]-thifensulfuron-methyl.

A Tier 1 study was conducted at pH 4, 7 and 9 at 50°C. Duplicate samples for each pH value were analysed at zero time and after 5 days incubation. The aqueous solutions were analysed directly by LSC and HPLC. The overall recovery of radioactivity was good and within the range 98.3-105.5% of applied radioactivity (AR). Extensive degradation of thifensulfuron-methyl was observed at all pH and thus a Tier 2 study was triggered.

The Tier 2 study was conducted at pH 4, 7 and 9 at 25°C. Duplicate samples were analysed over 30 days of incubation and then again directly by LSC and HPLC. The overall recovery of radioactivity was good and within the range of 94.3-105.8% AR.

At pH 4 hydrolysis of thifensulfuron-methyl was extensive with the levels of the parent molecule dropping to ca 50% AR after only 2 days and to < 1% by 30 days. Six individual degradates were detected at levels >10% AR over the duration of the study; IN-L9226 (max 13.6% AR, day 3), IN-RDF00 (2-ester-3-triuret, max 34.0% AR, day 30), IN-A5546 (max 64.2% AR, day 30), thiophene urea (IN No. unknown, max 9.9% AR, day 14), IN-A4098 (max 26.1% AR, day 14) and IN-F5475 (methyl triazine diol, max 33.2% AR, day 30). Two additional unidentified products were detected at maximum levels of 5-6% AR.

At pH 7 hydrolysis of thifensulfuron-methyl was much less extensive than at pH 4 with the parent molecule still representing ca 87% AR after 30 days. No individual degradates were detected at levels >10% AR although two were found at >5% AR; IN-A5546 (max 7.6% AR, day 30) and IN-A4098 (max 5.9%, day 30).

At pH 9 hydrolysis of thifensulfuron-methyl at pH 9 was again extensive with the levels of the parent molecule dropping to ca 50% AR after 7 days and to <3% by 30 days. Three individual degradates were detected at levels >10% AR over the duration of the study; IN-L9225 (max 79.8% AR, day 30), IN-L9223 (max 16.8% AR, day 30) and IN-A4098 (max 12.4% AR, day 30).

DT_{50} values for the hydrolytic degradation of thifensulfuron-methyl at 25°C were reported in this study to be 2.4 days, 137 days and 7.1 days at pH 4, 7 and 9 respectively.

5.1.1.1 Aqueous photolysis

Study 1

Report: (49) Ryan D.L. (1986). The photodegradation of [Thiophene-2-^{14}C] DPX-M6316 and [Triazine-2-^{14}C] DPX-M6316 in water.
Report No.: AMR 511-86
Guidelines: US EPA, Pesticide Assessment Guidelines: Environmental Fate 161-2.
Deviations: None
Test facility: DuPont de Nemours, Agricultural Products Research Division Experimental Station Wilmington, Delaware, U.S.A.
GLP: No

This aqueous photolysis study by Ryan (1986) (49) was included in the original thifensulfuron-methyl DAR from 1996. In the subsequent 2014 renewal dossier received from DuPont it was

proposed that the study partially meets current guidelines, with the only deviation being that it was not conducted to GLP. In the DuPont submission this study has also been supported by the study of Lenz (2001) (5) and Umstaetter (2006) - see below. In the Task Force dossier this study has been superseded by the study of Oddy (2012) (51) see further below. For completeness, details of the Ryan (1986) (49) study are included here as apart from not being conducted to GLP it was otherwise found to be acceptable.

Study summary:
[Thiophene-2-^{14}C]thifensulfuron-methyl or [triazine(U)-^{14}C]thifensulfuron-methyl (radiochemical purity greater than 98%) and [thiophene-2-^{13}C]thifensulfuron-methyl (purity 97%) were dissolved at 10 ppm in sterile buffer solution (acetonitrile <0.5%) at pH 5, 7 or 9 and kept at 25°C in either darkness or exposed to the equivalent of summer sunlight (285-2800 nm) at Wilmington, USA (34° North). Large amounts of photo-degradation products were generated for spectral analysis by irradiating 320 ppm solutions of thifensulfuron-methyl six inches (15.24 cm) under a bank of six fluorescent sun lamps for 42 hours. It was noted that thifensulfuron-methyl does not absorb after 310 nm. $^{14}CO_2$ was trapped and analyses of degradation products were performed by TLC (Thin Layer Chromatography), HPLC, MS (Mass Spectrometry) and NMR (Nuclear Magnetic Resonance) for 14 days. First-order reaction kinetics were assumed for the decline of thifensulfuron-methyl over time.

The resulting mass balance was in the range 93-114 % and pH values were stable. In darkness, thifensulfuron-methyl was significantly degraded at pH 5 and 9. In light, degradation was enhanced at every pH (see Table 16). When corrected for hydrolysis, the photolysis rate was independent of pH in the pH range 5-9 (117-129 hours). Major degradation products were triazine amine (14%), triazine urea (11%) and methyl-3-(4-methoxy-6-methyl-1,3,5,-triazin-2-yl-amino)-2-thiophene carboxylate (7%). A large number of minor compounds were detected, each at <4%. Detection of $^{14}CO_2$ indicated extensive breakdown of the thiophene ring.

Table 16: Thifensulfuron-methyl photo-degradation kinetics from Ryan (1986) (49)

	Linear DT_{50} (hours)		
	pH 5	pH 7	pH 9
Darkness	608	4400	381
Sunlight	98	125	97

Study 2
Report: (50) Lentz, N.R. (2001); Photodegradation of thifensulfuron-methyl in natural water by simulated sunlight
DuPont Report No.: DuPont-6047
Guidelines: Japanese Guideline 12 Noshan No. 8147 **Deviations:** None
Testing Facility: Ricerca, LLC, Concord, Ohio, USA
Testing Facility Report No.: 013515-1
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted by DuPont to provide further information in support of the original photolysis study from the DAR (Ryan, 1986) (49). The purpose of Lentz (2001) (50) was to determine the degradation rate and quantum yield of thifensulfuron-methyl in natural water and pH 7 buffer under constant irradiation. Several known degradants were identified by co-

chromatography with known standards, including IN-V7160, IN-A5546, IN-L9225 and IN-L9226, however these were not reported in detail. Later desk-based Dupont position papers by Umstaetter (2006) and Sharma, A.K. (2014) provide further examination of the results from Lentz (2001) (50) and the other photolysis studies to further identify these photolytic degradants. This is not of significance to the classification of the parent compound and so these studies are not reported here; they are included in the RAR (2015).

Study summary:
The aqueous phototransformation of [^{14}C]-thifensulfuron-methyl was studied in sterile natural water (collected from Lums Pond, New Castle County, Delaware) and sterile buffer at pH 7 and 25 ± 1°C for 15 days. The initial test item concentration was 4.67-4.90 µg/L and the study was conducted under artificial irradiation (Suntest XLS+, Enhanced Model benchtop xenon exposure system, 290 nm cut-off). This was stated to be equivalent to at least 30 days of natural sunlight at midday, Painesville Ohio, USA (41° North). Samples were analysed directly by high-performance liquid chromatography with radiochemical flow detection (HPLC-RAD) to determine the distribution of radioactivity. The quantum yield of thifensulfuron-methyl was calculated using chemical actinometry to be $\Phi = 0.037$. Assuming first order kinetics, the DT_{50} value in both irradiated solutions (natural water and in pH 7 buffer) was calculated to be 0.5 days. In the dark controls samples thifensulfuron-methyl degraded with a DT_{50} value of 126 days.

The mass balance of radioactivity for [thiophene-2-^{14}C]-thifensulfuron-methyl in sterile natural water and sterile pH 7 buffer ranged from 97.1 to 102.3% and 96.6 to 103.6%, respectively. The mass balance ranged from 100.0 to 106.5% and 99.1 to 105.6% for [triazine-2-^{14}C]thifensulfuron-methyl in sterile natural water and sterile pH 7 buffer, respectively. [Thiophene-2-^{14}C] thifensulfuron-methyl photodegraded rapidly to IN-A5546 plus polar compounds in natural water and in pH 7 buffer within 2 days. After 7 days of irradiation no single degradation product could be identified and most of applied radioactivity consisted of the polar fraction. [Triazine-2-^{14}C] thifensulfuron-methyl photodegraded rapidly to IN-V7160 polar compounds and one unidentified transient metabolite in natural water and in pH 7 buffer within 2 and 7 days, respectively.
Further examination of the degradants by Umstaetter (2006) and Sharma, A.K. (2014) indicated that the unknown degradant was likely to be the same photoproduct identified in AMR-511-86 (Ryan, 1996) (49) as methyl-2-(4-methoxy-6-methyl-1,3,5-triazin-2-yl-amino)-3-thiophene-carboxylate (IN-D8858).

Study 3

Report:	(51) Oddy A. (2012). [^{14}C]-Thifensulfuron-methyl: Aqueous Photolysis and Quantum Yield Determination in Sterile Buffer Solution. Battelle UK Ltd [Cheminova A/S], Unpublished report No.: WB/10/009 [CHA Doc. No.284 TIM]
Guidelines:	OECD Guideline 316: Phototransformation of Chemicals in Water – Direct Photolysis (October 2008). **Deviations:** None
GLP:	GLP compliance statement and quality assurance statement supplied.

This study was submitted by the TSM Task Force for the purpose of renewal and has been evaluated by the UK RMS and CA and considered acceptable. Further discussion on the identity of photolytic degradants found in Oddy (2012) is provided in the position paper by Sharma, A.K. (2014) - which is not included here but can be found in the RAR (2015).

Study summary:
The photolysis of thifensulfuron-methyl in an aqueous environment was investigated in accordance with the two tiered approach described in OECD guideline 316. A Tier 1 theoretical screen was first

performed to estimate the maximum possible direct photolysis rate constant and corresponding DT_{50} value for thifensulfuron-methyl under varying pH acetate buffers (pH 4, 7 and 9). The UV absorbance of the thifensulfuron-methyl in each buffer solution was measured between 295 and 380 nm. The spectral data were used to estimate the maximum photolysis rate constant at each pH for thifensulfuron-methyl at 40° latitude for the summer season.

The test substance was found to have a molar decadic absorption coefficient >10 in a range of wavelengths ≥290 nm (297.5-320.0 nm) across the pH values. DT_{50} values for the three pHs (4, 7, and 9) were estimated to be 0.06, 0.10 and 0.09 days respectively.

From this screening test it was determined that thifensulfuron-methyl would be predicted to undergo direct photolysis and that a full experimental study was required. In the Tier 2 study, the photolysis of [thiophene-2-^{14}C]-thifensulfuron-methyl (radiolabel purity 96.8%) and [triazine-2-^{14}C]-thifensulfuron-methyl (radiolabel purity 96.8%) in aqueous buffer solution (0.01 M phosphate, pH 7) was investigated. The study was conducted under sterile conditions at 25±2ºC, with continuous irradiation under artificial sunlight provided by a xenon arc lamp with filters to cut off any radiation below 290 nm.

The study was conducted using a Heraeus Sun Test (CPS+) apparatus and irradiation was continued for a period of 168 hours (7 days; equivalent to 18.2 days natural sunlight at 30-50°N) by which time >90% of the applied thifensulfuron-methyl had degraded and the formation and decline of major transformation products had been established. Samples and vessel rinses were analysed by LSC, followed by HPLC and selected LC-MS. Full methodological details are given in the RAR.
For the irradiated samples the overall mean recoveries at 168 hours were 96.1% AR (range 88.2-100.5% AR) for the thiophene label and 99.1% AR (range 94.5-101.9% AR) for the triazine label. The corresponding figures for the non-irradiated samples were 98.6% AR (range 97.0-101.1% AR) for the thiophene label and 99.7% AR (range 98.0-101.0% AR) for the triazine label.

In the thiophene labelled samples the parent compound decreased rapidly from >97% AR at time zero to <5% AR after 72 hours, reaching <1% AR by the end of the irradiation period (168 hours). In the triazine labelled samples the parent compound decreased rapidly from >98% AR at time zero to <5% AR after 72 hours, reaching ca 1% AR by the end of the irradiation period.

There were concurrent increases in a large number of polar and non-polar degradates. A major non-polar degradant from both radiolabels was identified by LC-MS to be thiophenyl triazinyl amine which reached a maximum 12.2-14.3% AR and then declined to <1% AR by the end of the study. For the triazine label IN-A4098 was found at a maximum of 16.8% AR and IN-V7160 which reached a maximum 19.4% AR. Several minor non-polar degradates (each at <10% AR) were also detected. The polar fractions consisted of a multitude of individual degradates, each at <5.2% AR.
In the non-irradiated samples, no significant degradation of the parent compound was seen for either label over the duration of the experiment with limited (<3% AR) formation of other degradants.

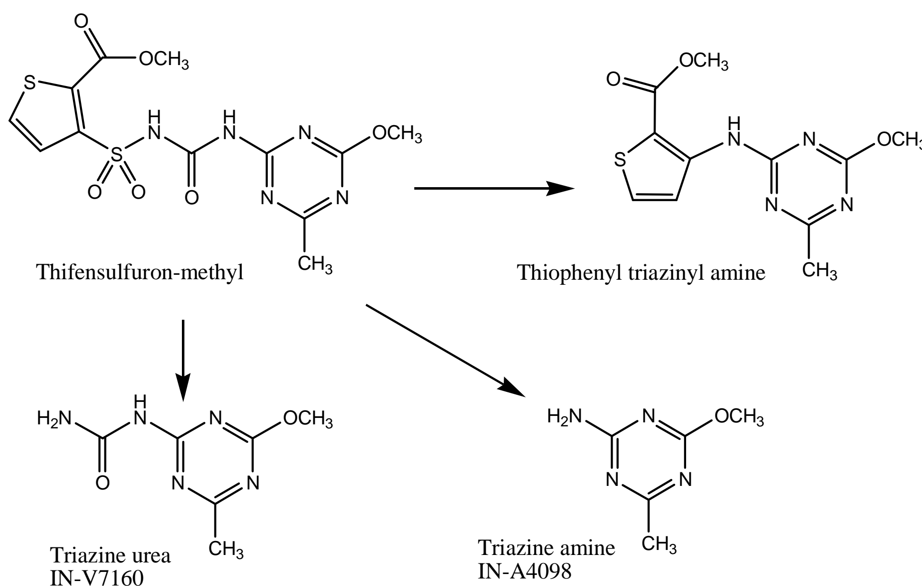
The quantum yield for thifensulfuron-methyl in aqueous solution at pH 7 was found to be 0.044.
The DT_{50} for thifensulfuron-methyl in natural sunlight was calculated to be between 0.32 and 0.67 days (7.7-16.2 hours) according to the FOCUS guidance document on degradation kinetics and assuming first order kinetics (see Table 17). A photolytic degradation pathway was also proposed, see Figure 3:

Table 17: Degradation rate of thifensulfuron-methyl using Single First Order (SFO) kinetics for irradiated samples

System	Chi^2 error	DT_{50} in suntest	DT_{50} in natural sunlight*
Irradiated – thiophene label	3.6615	6.2 hours (0.26 days)	16.2 hours (0.67 days)
Irradiated – triazine label	9.9058	3.0 hours (0.12 days)	7.7 hours (0.32 days)

*corrected for 1 suntest day equivalent to 2.6 days natural sunlight at 30-50ºN

Figure 3: Proposed photolytic degradation pathway of thifensulfuron-methyl



5.1.2 Biodegradation

Report: (52) Barnes, S.P. (2000); DPX-M6316 assessment of ready biodegradability by modified Sturm test
DuPont Report No.: DuPont-4373
Guidelines: EEC Method C.4-C. (1992), OECD 301 B (1992) **Deviations:** None
Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, UK
Testing Facility Report No.: DPT 533/003580
GLP: Yes
Certifying Authority: Department of Health (U.K.)

This study was submitted by the TSM Task Force for the purpose of renewal under pesticides legislation, it has been evaluated by the UK RMS (RAR, 2015) and CA and is considered reliable.

Study summary:
The ready biodegradability of thifensulfuron-methyl (DPX-M6316, 99.7% pure) was examined using the CO_2 Evolution Test (Modified Sturm Test) OECD 301B. Treatments included the test item at a concentration of 30 mg/L; two control treatments containing only the inoculum; one positive control treatment containing inoculum plus reference standard (sodium benzoate) and one

toxicity control treatment containing the inoculum plus test item and reference standard. All the treatments were prepared with inoculum from a secondary effluent treatment plant receiving predominantly domestic sewage.

Test mixtures were aerated for 29 days with carbon dioxide (CO_2) free air. The CO_2 released by each treatment was trapped and determined at the end of the test. Full methodology is provided in the RAR (2015).

The pH of each test and control mixture was between 7.4 and 7.5 at the start of the test and 7.3 to 7.6 at the end. The rate of air-flow during the test ranged from 40 to 80 mL/minute. Temperature ranged from 19.8 to 22.9°C over the test period.
Sodium benzoate had biodegraded by 64% at Day 7 and 86% by Day 29 in the absence of thifensulfuron-methyl meeting the validity criteria of the test. The treatment containing both sodium benzoate and thifensulfuron-methyl had biodegraded by 66% at Day 7 showing that thifensulfuron-methyl was not inhibitory at this concentration.

The cumulative CO_2 production by treatments containing only thifensulfuron-methyl was negligible and had achieved, at most, 1% of the theoretical value (TCO_2, 110.1 mg CO_2) by the end of the test on Day 29. Based on the pass levels (60% bio-degradation in the 10-day window period) thifensulfuron-methyl cannot be considered as readily biodegradable since a 1% degradation was achieved during the test period of 29 days.

5.1.2.1 Biodegradation estimation

Not provided or required.

5.1.2.2 Screening tests

Not provided or required.

5.1.2.3 Simulation tests

Natural water/sediment studies

Four water/sediment studies were included in the original 1996 thifensulfuron-methyl DAR and subsequent Addenda, these were:
- Lewis W. and Carter L. G. (1986, report no. AMR 540-86),
- Matla Y. A., Muttzall P. I. and Vonk J. W. (1991, report no. TNO R91/256)
- Muttzall P. I. and Vonk J. W. (1992, report no. TNO R91/255)
- Spare W. C. (2000, report no. 1206)

In the subsequent submission and assessment for renewal of thifensulfuron-methyl under pesticides legislation (RAR, 2015) it was proposed that the first three studies do not meet current guidelines, should not be relied upon and have been superseded by Spare (2000) (53) and those of van Beinum and Beulke (2006) (54) and Simmonds (2012) (55) submitted for renewal (see below). Pertinent details of the study by Spare (2000) (53) and the two new studies are therefore included here, more detailed methodology and reporting is available in the RAR (2015).

Study 1

Report: (53) Spare W.C. (2000), Degradability and fate of thifensulfuron methyl in the aerobic environment (water/sediment system). Revision 1
DuPont Testing Facility Report No.: DuPont-1206 RV1
Guidelines: SETAC guideline **Deviations:** None
GLP: Yes
Certifying Authority: Department of Health (U.K.)

This study was submitted by the TSM Task Force for the purpose of renewal under pesticides legislation, it has been evaluated by the UK RMS (RAR, 2015) and CA and is considered to be reliable.

Study summary:
[Thiophene-2-^{14}C] and [triazine-2-^{14}C] thifensulfuron-methyl were applied at 1 mg/l to 2 water sediment systems (50 g sediment + 200 ml water). Characteristics of the water sediment systems are given in the table below. Incubation was at 20°C for 182 days. Volatiles were trapped. Water and sediment phases were analysed separately. Sediment was extracted, extracts were concentrated and analysed by HPLC and TLC. Unextracted residue was determined by combustion. The water phase was directly analysed by HPLC and TLC.

Table 18: Characteristics of water sediment systems

		Middletown, MD, Red Oak Sream	Middletown, MD, Town Park Pond
Sediment	Texture	Loamy sand	Loam
	sand %	83	43
	silt %	16	46
	clay %	1	11
	OM %	1.1	2.6
	pH	7.1	7.2
Water	pH	7.6	7.8

There was a full recovery of all applied radioactivity. Mineralization was low : <4 % for the thiophene moiety and < 9 % for the triazine moiety after 182 d. Bound residues were <18 % for both moieties. Extractable residue in sediment was <15 % and no compound exceeded 10 % (<8 % each). Most of the applied radioactivity was found in water. The major metabolites derived from the thiophene moiety were IN-L9225 (thifensulfuron acid) max. 54 % after 70-100 d, IN-JZ789 (O-desmethyl thifensulfuron acid) max. 18 % after 70 d and IN-L9223 (2-acid-3-sulfonamide) max. 39 % after 182 d. The major metabolites derived from the triazine moiety were IN-L9225 max. 55 % after 100 d, IN-JZ789 max. 10 % after 84 d, IN-V7160 (triazine urea) max. 25 % after 182 d) and IN-A4098 (triazine amine) max. 19 % after 182 d. The metabolites IN-L9226 (O demethyl thifensulfuron-methyl) and IN-W8268 (thiophene sulfonimide) were detected in small amounts. For thifensulfuron-methyl, DT_{50} and DT_{90} values were calculated to be respectively 21 - 27 d and 70 - 89 d in water, and 21 - 27 d and 71 - 91 d in whole system using first order kinetics.

It was concluded that thifensulfuron-methyl is significantly degraded in water sediment systems. Degradation occurs by hydrolysis to the acid derivative IN-L9225 (max. 55 % after 70-100 d) further degraded to IN-JZ789 (max. 21 % after 125 d) by O-demethylation. Cleavage of the sulfonylurea bridge leads to IN-L9223 (2-acid-3-sulfonamide, max. 39 % after 182 d) and IN-V7160 (triazine urea, max. 25 % after 182 d) and IN-A4098 (triazine amine, max. 19 % after 182 d). No major compounds were found in sediment. Thifensulfuron-methyl was poorly mineralised (<4 % for the thiophene moiety and <9 % for the triazine moiety after 182 d) and bound residues were <18 % for both moieties.

Study 2

Report: (54) van Beinum, W., Beulke, S. (2006); Calculation of degradation endpoints from water-sediment studies for thifensulfuron-methyl (DPX-M6316) and its metabolites
Testing Facility and DuPont Report No.: DuPont-18745
Guidelines: FOCUS (2005) **Deviations:** None
Testing Facility: Central Science Laboratory, Sand Hutton, York, UK
GLP: No
Certifying Authority: Not applicable

This desk study was provided in support of renewal of thifensulfuron-methyl and to further derive rate and route of degradation information from the original experimental study by Spare (2000) (53). It has been evaluated by the UK RMS (RAR, 2015) and CA and is considered to be reliable.

Study summary:

Degradation endpoints from two water-sediment systems (Town Park Pond and Red Oak Stream - see Spare W.C. (2000) (53)) with [^{14}C]-thifensulfuron-methyl (thiophene label and triazine label) were derived in accordance with FOCUS guidance for the parent compound and its major metabolites IN-L9225, IN-JZ789, IN-L9223, IN-V7160, and IN-A4098. A maximum of four kinetic models were fitted to the concentrations of the parent compound or the degradants in the water phase, the sediment phase and the whole system from maximum accumulation onwards. A model that considers parent degradation in the water and sediment and exchange between the two compartments was fitted to water and sediment data simultaneously.

DT_{50} values for thifensulfuron-methyl in the Town Park water-sediment system were 16.5 days in the water column, 10.6 days in the sediment, and 16.8 days in the total system. Dissipation was somewhat slower in the Red Oak water-sediment system, with DT_{50} values of 23.5 days in the water column, 25.3 days in the sediment phase, and 23.4 days in the total system. DT_{50} values for degradants were reported but are not considered further for classification of the parent substance.

Table 19: Summary of persistence endpoints for thifensulfuron-methyl

	System	DT_{50} (days)	DT_{90} (days)	Chi2 error %	Kinetic Model
Water	Town Park	16.5	63.1	3.1	FOMC
	Red Oak	23.5	89.7	3.2	FOMC
Sediment	Town Park	10.6	87.7	5.9	HS
	Red Oak	25.3	97.4	5.9	FOMC
Total System	Town Park	16.8	63.8	1.5	DFOP
	Red Oak	23.4	90.4	1.9	HS

Single first order (SFO) degradation DT_{50} values for thifensulfuron-methyl derived for use in FOCUS surface water modelling were 18.2 days for the whole Town Park water-sediment system (chi^2 error % = 3.9) and 26.1 days for the whole Red Oak water-sediment system (chi^2 error % = 3.2).

Study 3

Report: (55) Simmonds M. (2012) [^{14}C]-Thifensulfuron-methyl: Degradation and retention in two water-sediment systems. Battelle UK Ltd [Cheminova A/S],
Unpublished report No.: WB/10/010 [CHA Doc. No. 285 TIM]
Guidelines: OECD 308 **Deviations:** None
GLP: Yes (certified laboratory)

This water/sediment study was provided in support of renewal of thifensulfuron-methyl by the TSM Task Force. It has been evaluated by the UK RMS (RAR, 2015) and CA and is considered to be reliable.

Study summary:
The route and rate of degradation of [thiophene-2-^{14}C]-thifensulfuron-methyl (98.8% pure) and [triazine-2-^{14}C]-thifensulfuron-methyl (99.4% pure) has been investigated in two water-sediment systems: Swiss Lake water-sediment system, pH (H_2O) 7.4 and Calwich Abbey Lake system, pH (H_2O) 8.3, for 104 days in darkness at 20°C. The ratio of water to sediment was 1:4. Details of each sediment system are provided below:

Table 20: Physicochemical parameter of the water/sediment systems

System	Swiss Lake				Calwich Abbey Lake			
	Before Start		At End		Before Start		At End	
Flask number	86825	86826	86825	86826	86833	86834	86833	86834
Water phase								
Total OC (µg/L)	8.0	-	-	-	3.9	-	-	-
pH	7.6	7.3	6.5	6.75	8.25	8.35	7.31	7.09
Oxygen content (mg/L)	7.2	7.1	6.2	6.1	7.4	6.9	5.9	6.3
Redox potential (mV)	80	51	419	283	80	50	391	104
Sediment								
Redox potential (mV)	-168	-566	-373	-454	-512	-406	-589	-512
C.E.C (meq/100g)	3.3				10.1			
pH	6.0				7.4			
OC (%)	0.95	-	-	-	5.0	-	-	-
Microbial biomass (µgC/g dry sediment)	161		136		838		786	
% Clay	4	-	-	-	8	-	-	-
% Silt	7	-	-	-	59	-	-	-
% Sand	89	-	-	-	33	-	-	-
UK Classification	Sand				Silt loam			

Thifensulfuron-methyl was applied to the water surface at an initial water concentration of 0.08 mg/L. Sampling was undertaken at various intervals over 104 days after application. The water was analysed for radioactive content by liquid scintillation counting (LSC). Sediment samples were extracted and quantified by LSC. Sediment residues were air dried and residues were further extracted via fractionation into humin, humic acids and fulvic acids followed by quantification of radioactivity by LSC. The test substance and potential degradants were identified in extracts by either HPLC, LC-MS or LSC. Overall recoveries were good for all systems with mean values for each system ranging from 98.3 to 100.1% of applied radioactivity (AR) and individual recoveries all being within 93.2% to 103.7% AR.

In the total system, thifensulfuron-methyl steadily degraded in both systems, declining to levels of between 2.8% and 11.6% AR over the course of the study. The dissipation of thifensulfuron-methyl from the water phase and degradation in the total system was evaluated according to the FOCUS guidance document on degradation kinetics using the most appropriate model for the best fit to the data set. The results were calculated from the thiophene and triazine labels combined as replicates and are presented in the table below.

The maximum degree of volatile formation was low in both systems, ranging from 1.8% to 2.6% AR in both labels and both systems at the end of the study.
In the thiophene-labelled systems, the major degradants observed were IN-L9225 (max. 52.7% AR), IN-L9223 (max 24.3% AR) and IN-JZ789 (max. 15.5% AR). Other minor metabolites were formed, none of which achieved >10% AR at any time point.
In the triazine-labelled systems, the major metabolites observed were IN-L9225 (max. 49.6% AR), IN-A4098 (max. 20.0% AR) and IN-JZ789 (max. 13.1% AR). Other minor metabolites were formed, none of which achieved >10% AR at any time point.
Kinetic evaluations were also carried out on degradants but these are not considered further here for classification of the parent substance.

Table 21: Summary of DT_{50} values for thifensulfuron-methyl in water sediment systems.

Water-sediment system	Thifensulfuron-methyl	
	Kinetic model	DT_{50} (days)
Swiss Lake (water)	SFO	32.0
Swiss Lake (total system)	SFO	32.3
Calwich Abbey (water)	SFO	17.3
Calwich Abbey (total system)	SFO	17.6

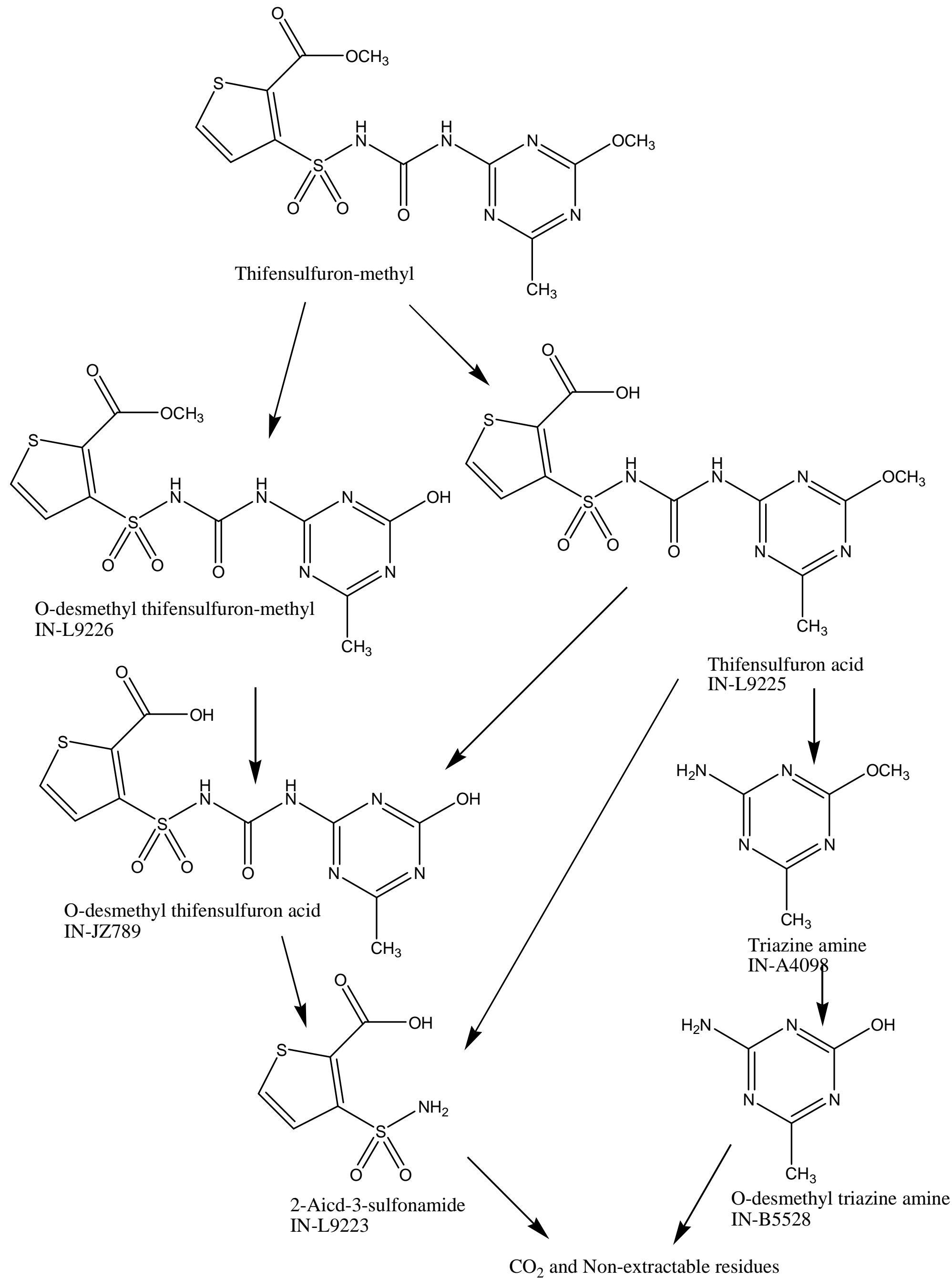
SFO = single first order

In conclusion [^{14}C]-thifensulfuron-methyl was found to steadily degrade in natural water/sediment systems incubated under aerobic conditions at 20 ºC with total system degradation DT_{50} values of 32.3 days and 17.6 days for the Swiss Lake and Calwich Abbey systems respectively. The mean was 25 days. Degradation was predominately in the water phase and the applied radioactivity dissipated gradually from water to the sediment to form bound residues (≤10% AR) and minor amounts of carbon dioxide/mineralisation (<3% AR).

Overall calculation of whole system degradation half-lives

The above water/sediment study results were considered together in the RAR (2015) to derive $DegT_{50}$ values for modelling purposes. For thifensulfuron-methyl, combining the acceptable water/sediment study data from the two Applicants resulted in four contrasting systems being tested. The geometric mean of the four acceptable whole system values (i.e. 18.2, 26.1, 32.3 and 17.6 d) was calculated to be 22.8 d and this value has been used in exposure modelling. In addition the following degradation pathway in natural water/sediment systems was proposed.

Figure 4: Proposed degradation pathway for thifensulfuron-methyl in water/sediments systems



5.1.3 Summary and discussion of degradation

Abiotic degradation

In a reliable aqueous hydrolysis study (Wardrope, 2011) (47) thifensulfuron-methyl was determined to be hydrolytically stable at certain pH and temperatures but to also degrade rapidly at others. At the more environmentally realistic temperature tested 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. In a second reliable hydrolysis study conducted at 25°C (Simmonds and Buntain, 2012) (48) hydrolysis DT_{50}s were 2.4, 137 and 7.1 days at pH 4, 7 and 9 respectively - again showing high variability and pH dependence. As the hydrolysis half life is not consistently <16 days for all environmentally relevant pH, thifensulfuron-methyl screens as ‘not rapidly degradable’.

Aqueous photolysis is envisaged to contribute significantly to the degradation of thifensulfuron-methyl in certain natural water systems (probably combined with hydrolysis). Based on the results of the reliable studies by Ryan (1986) (49) Lentz (2001) (50) and Oddy (2012) (51) photolysis half lives <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, it is not felt that photolysis alone is sufficiently consistent to determine thifensulfuron-methyl as being ‘rapidly degradable’.

Biotic degradation

In a reliable OECD 301B ready biodegradation study (Barnes, 2000) (52) conducted at pH 7.3 to 7.6 and 19.8 to 22.9°C, no substantive degradation of thifensulfuron-methyl (1%) was observed over 29 days. According to the criteria requiring ≥60% of the theoretical CO_2 production within 10 days of achieving 10% biodegradation, thifensulfuron-methyl is considered to be ‘not readily biodegradable’ under the conditions of this test.

Reliable aerobic water/sediment studies are available from the study by Spare (2000) (53) - with a further analysis of results from this by van Beinum and Beulke (2006) (54) and also from Simmonds (2012) (55). The two experimental studies were conducted at 20°C in the dark for 104-182 days. Whole system degradation DT_{50}s for thifensulfuron-methyl were calculated to be 17.6-32.3 days depending upon the system studied and calculation method. 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 sediment was low in all 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 metabolites (>10%) occurred in sediment. However, as the aquatic toxicity of these is less than for parent thifensulfuron-methyl (see Annex 1), they are not considered further in relation to the hazard classification of the parent substance.

Overall, 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). Neither is it transformed sufficiently rapidly into entirely non-classifiable degradants. Consequently, thifensulfuron-methyl is considered to be ‘not rapidly degradable’ for the purposes of classification under the CLP Regulation.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 22: Summary of relevant information on adsorption/desorption

Method	Results	Remarks	Reference
Absorption/desorption *via* batch equilibrium method according to OECD 106, OPPTS 835.1230 and SETAC guidelines. To GLP	Adsorption and desorption properties of [^{14}C]-thifensulfuron-methyl investigated in five soils. Koc ranged from 10-128 (mean 53.4); K_{FOC} ranged from 9-86 (mean 41.4) (mL/g)	Conducted at 13 or 20ºC (based on stability). Soil pH 4.8-7.6, organic carbon 0.8-3.0%. Minor deviations but otherwise reliable and to GLP	Bell (2011) (56)
Absorption/desorption *via* batch equilibrium method according to OECD 106 and to GLP	Adsorption and desorption properties of [^{14}C]-thifensulfuron-methyl investigated in four soils. Koc ranged from 3.1-8.4 (mean 5.9); (mL/g)	Conducted at 20ºC. Soil pH 5.1-7.3, organic carbon 1.3-3.9%. Study reliable and to GLP	Simmonds and (2012)(57)

A large number of adsorption/desorption studies are reported in RAR (2015), most are on degradants of thifensulfuron-methyl and these are not considered further here for classification of the parent substance. Of the studies on thifensulfuron-methyl itself, one original study from the 1996 DAR (Priester, T. M., 1985) has since been considered unreliable by the UK RMS and is superseded by two later studies submitted for renewal by DuPont and the TSM Task Force (i.e. Bell, 2011(56) and Simmonds and (2012)(57), therefore Priester (1985) is not included here.

Study 1

Report: (56) Bell, S. (2011); Absorption/desorption of [^{14}C]-DPX-M6316 (thifensulfuron-methyl) *via* batch equilibrium method

DuPont Report No.: DuPont-30563

Guidelines: OECD 106 (2000), OPPTS 835.1230 (2008), SETAC (1995); **Deviations:** No significant deviations

Testing Facility: Charles River Laboratories (UK), Tranent, Scotland, UK

Testing Facility Report No.: 809469

GLP: Yes

Certifying Authority: Department of Health (U.K.)

This adsorption/desorption study was provided in support of renewal of thifensulfuron-methyl by DuPont. It has been evaluated by the UK RMS (RAR, 2015) and CA and despite some minor deviations it is considered to be reliable.

Study summary:

The adsorption and desorption properties of [^{14}C]-thifensulfuron-methyl (radiochemical purity 98.9%) were investigated in five soils from USA, Germany, Spain, and France (pH range 4.8 to 7.6, organic carbon range 0.8 to 3.0%). These included a loamy sand, clay, clay loam, loam and sandy

loam. A summary of the physical and chemical properties of the soils is provided in the RAR (2015).

The definitive adsorption experiment was performed using the batch equilibration method on the soils at five concentrations (ranging from nominal concentrations of 0.05-5.00 µg/mL) of the test substance in 0.01 M $CaCl_2$. Mixing and equilibration with soils was conducted for either 24 or 4 hours and at either 20 or 13°C dependant on the soil tested, this was due to the instability of the thifensulfuron-methyl in certain soil mixtures. Radioactivity of resulting samples was determined by LSC and identification was by reverse phase HPLC. Overall recovery of radioactivity was determined at the highest test concentration for all soils and mean recoveries ranged from 92.97% to 106.71%. The test substance was stable during the adsorption phase of the experiment. Full methodological details and results are provided in the RAR (2015).

The adsorption coefficients K_d, K_{om}, and K_{oc} were calculated and reported for each soil at each concentration of the test substance. The key Freundlich adsorption isotherm parameters are reported below.

Table 23: Freundlich adsorption isotherm parameters for thifensulfuron-methyl from Bell (2011) (56)

Soil type	OC%	Soil pH ($CaCl_2$)	Kd (g/g)	Koc (mg/g)	Kf	Kfoc	1/n	R^2
Loamy Sand (Sassafras)	0.81	4.8	0.76	94	0.6660	82	0.9023	0.9959
Clay (Lleida)	1.74	7.6	0.17	10	0.1551	9	0.9826	0.9687
Clay Loam (Drummer)	2.96	5.7	3.78	128	2.5468	86	0.8211	0.8211
Loam (Gross-Umstadt)	1.39	6.6	0.3	21	0.2679	19	0.9599	0.9599
Sandy Loam (Nambsheim)	2.03	7.3	0.29	14	0.2164	11	0.8389	0.8389
Arithmetic mean				53.4	0.7705	41.4	0.901	-
pH dependence, yes or No			No					

Thifensulfuron-methyl was tested at 20 ± 2°C for Sassafras, Drummer and Gross-Umstadt soils and at 13 ± 0.5°C for the Lleida and Nambsheim soils.

Study 2

Report: (57) (2012) [^{14}C]-Thifensulfuron-methyl: Adsorption to and desorption from four soil. Battelle UK Ltd. [Cheminova A/S], Unpublished report No.: WB/10/007 [CHA Doc. No. 259 TIM]

Guidelines: OECD Guideline for the Testing of Chemicals, “Adsorption - Desorption Using a Batch Equilibrium Method”, Method 106, January 2000; **Deviations:** None

GLP: **Yes.** GLP practice statement and QA statement supplied. GLP certified laboratory.

This adsorption/desorption study was provided in support of renewal of thifensulfuron-methyl by the TSM Task Force. It has been evaluated by the UK RMS (RAR, 2015) and CA and is considered to be reliable.

Study summary:

In this adsorption/desorption study, four UK soils were used to assess the adsorption behaviour of [triazine-2-^{14}C]- thifensulfuron-methyl (purity 99.4%) in soil. The soils used were a sandy loam, silt loam, loam and a clay loam ranging from pH 5.1-7.3 and an organic carbon content (OC) of 1.3-

3.9%. The soils were considered to exhibit sufficient variation in soil characteristics for the purposes of the adsorption experiment. A summary of physical and chemical properties of the soils is provided in Table B.8.197 in the RAR (2015).

Preliminary studies were carried out to check for adsorption to testing tubes, to determine any background radioactivity in the soil, to determine the soil:solution ratio to be used and also the appropriate adsorption and desorption times to ensure that the test item remained stable for the duration of the definitive test. Due to the instability of [^{14}C]-thifensulfuron-methyl observed, the definitive test was conducted at 20°C for a limited time period of up to 2 hours, followed by a 1 hour desorption time.

Treatment was conducted in triplicate with each soil at 20°C. The soils were mixed and equilibrated for 16 hours with a 0.01M $CaCl_2$ solution at a 1:1 ratio prior to adding 1 mL of the treatment solution containing 1.0, 0.33, 0.1, 0.03 or 0.01 mg/L [^{14}C]-thifensulfuron-methyl. The tubes were shaken for 2 hours at which time point tubes were centrifuged and the supernatants were analysed by LSC. Soils samples also were extracted with resulting supernatants analysed by LSC followed by identification using HPLC. A similar procedure was conducted after a 1 hour desorption cycle with calcium chloride solution. The recoveries of radioactivity were quantified, with all recoveries within the acceptable range of 90-110% of applied radioactivity. Full methodological details and results are provided in the RAR (2015).

For all soils there was a good correlation and linear relationship between the soil and solution concentration for all soils tested. The Kf values ranged from 0.08 m/L in the sandy loam to 0.33 m/L in the loam soil. The corresponding values for Koc ranged from approximately 3 mL/g in the clay loam to 8 mL/g in the loam, with a mean value of 6 mL/g. The values of 1/n for the desorption were similar to those obtained for the adsorption for each soil and ranged from 0.96 in the silt loam to 1.04 in the clay loam. The Freundlich exponents displayed linearity with 1/n values ranging from 0.95 to 1.01, thus indicating little change between the amount adsorbed onto the soil and the amount in solution through the concentration range tested.

Table 24: Adsorption/desorption constants and correlation coefficients for thifensulfuron-methyl in soil

Soil type	OM %	OC %	pH*	Adsorption				Desorption			
				K_f (ml/g)	K_{oc} (ml/g)	1/n	R^2	K_f (ml/g)	K_{ocdes} (ml/g)	1/n	R^2
Long woods	2.2	1.3	7.3	0.08	6.0	0.967	0.999	0.14	10.7	1.002	0.999
Farditch	6.0	3.5	5.9	0.22	6.2	0.952	1.000	0.54	15.4	0.961	1.000
Kenslow	6.8	3.9	5.1	0.33	8.4	0.949	0.999	0.58	14.9	0.994	0.999
Lockington	4.8	2.8	5.5	0.09	3.1	1.012	0.998	0.29	10.5	1.039	0.997
Mean	-	-	-	0.18	5.9	0.970	0.999	0.39	12.9	0.999	0.999

Kf = Freundlich coefficient
R^2 = Correlation coefficient squared
Koc = Desorption coefficient for organic carbon
* pH (0.01M $CaCl_2$)
Kocdes = desorption coefficient for organic carbon

Overall consideration of adsorption/desorption data

The above study results were considered together in the RAR (2015) to derive combined data on sorption in nine contrasting soils. No obvious correlation existed between soil sorption and other soil properties such as soil pH. Considering the data set as a whole, there was no clear correlation between sorption (Kf) and soil organic carbon content. However the UK RMS considered that some of this may have been due to the fact that across the nine soil types and two studies, equilibrium times varied from 2 to 24 hours and incubation temperatures varied from 13 to 20°C. Considering the four soils tested by the Task Force, where both equilibrium time and temperature were consistent, a clear correlation between sorption and organic carbon was observed. On this basis it was considered valid, mainly for modelling purposes, to normalise sorption for organic carbon content and hence derive a median Kfoc of 9 mL/g and an arithmetic mean 1/n of 0.932.

Given the range of K_{oc} values seen in these studies, thifensulfuron-methyl would be expected to have a 'high' to 'very high' mobility in soil (according to the ASTM International Classification scale) and it would likely stay in the water phase and not dissipate to sediments - as confirmed in the water/sediment studies provided in Section 5.1.2.3.

5.2.2 Volatilisation

Neither thifensulfuron-methyl nor any of its principal degradation products have significant volatility. The vapour pressure of thifensulfuron-methyl is 5.2×10^{-9} Pa at 20°C. Also, the Henry's law constant of thifensulfuron-methyl is less than 3×10^{-2} Pa-m^3/mol, suggesting little potential for volatilisation in the environment. Henry's law constants below 3×10^{-2} Pa-m^3/mol indicate that the compound is less volatile than water and can be considered essentially non-volatile.

In addition a study on the 'Photodegradation oxidative degradation of thifensulfuron-methyl' in the air (Schmuckler M.E., 1999) is reported in the RAR (2015). This was conducted to U.S. EPA 796.3900 (1992) and an OECD guideline on Photochemical Oxidative Degradation in the Environment (1987a, 1988a). The half-life of thifensulfuron-methyl for reaction in the gas phase in the troposphere based on average daily air concentrations of hydroxyl radicals (12-hour day, 1.5×10^6 OH radicals per cm^3) was determined to be 41.425 hours (3.5 days). The overall OH rate constant was 3.0984×10^{-12} cm^3/molecule^{-sec}. Whilst the half-life is greater than the 2 day trigger indicating a potential for long range transport, this theoretical risk was discounted due to the rapid degradation in water and soil, low vapour pressure and high solubility coupled with low application rates.

5.2.3 Distribution modelling

Not submitted or required

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Table 25: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water EEC method A 8 (shake flask) To GLP	log K_{ow} = 0.0253 at pH 5 log K_{ow} = -1.65 at pH 7 log K_{ow} = -2.10 at pH 9 All at 25°C	Valid study to GLP. For modelled Log K_{ow} values for degradants see Table 26 below	Huntley and Edgar, 2000(13) (see Table 8, Section 1.3)
Fish flow-through bioconcentration study conducted to OECD 305E and GLP	Bluegill sunfish (*Lepomis macrochirus*) exposed to 5 mg/L [thiophene-2-^{14}C] thifensulfuron-methyl for 28 days + 14 days depuration. Measured whole fish bioconcentration factor (BCF): <0.8. Study conducted at 20-22°C	Reliable study to GLP	Larkin (1984)(58)

5.3.1.1 Bioaccumulation estimation

The log K_{ow} of thifensulfuron-methyl is -1.65 (at 25 °C, pH 7 ref. Section 1.3). Hence, an assessment of its potential for bioaccumulation is not triggered. In the RAR (2015) Log K_{ow} values for major environmental degradants have also been modelled using up to four different methods based on the degradants' structure (The Biobyte programme, The SRC (Syracuse Research Corporation) program, EpiSuite, and the SciTegic programme). The results of these analyses were considered acceptable by the RMS and are summarised below.

Table 26: Predicted Log K_{ow} values of major degradants as modelled by different software (denoted in brackets)

Thifensulfuron-methyl degradant	Octanol/water partition co-efficient (log K_{ow} calculation method)
IN-A4098	0.18 (Sci Tegic) 1.26 (SRC) 0.33 (BioByte)
IN-L9225	0.78 (Sci Tegic) 1.32 (SRC) 1.56 (BioByte) 1.26-1.32 (EpiSuite)
IN-L9226	0.78 (Sci Tegic) 0.22 (SRC) 0.75 (BioByte) 0.22-0.89 (EpiSuite)
IN W8268	0.31(Sci Tegic) 0.27 (SRC) 0.64 (BioByte) 0.27 (EpiSuite)
IN JZ789	0.55 (Sci Tegic) 0.26 (SRC) 1.31 (BioByte) 0.26 (EpiSuite)
IN-L9223	0.12 (Sci Tegic) 0.07 (SRC) 0.2 (BioByte) 0.07 (EpiSuite)
IN-V7160	-0.16(Sci Tegic) 0.89 (SRC) 0.37(BioByte)

For further details on each of the degradants, see Annex 1. All Log K_{ow} values are below the CLP trigger of 4 (highest modelled value is 1.32), indicating that the potential of bioaccumulation is very low for the parent substance and also for its degradants.

5.3.1.2 Measured bioaccumulation data

Although not required, due to the low Log K_{ow} of thifensulfuron-methyl, a fish bioconcentration study on the substance is available and was included in the original DAR (1996) and repeated in the RAR (2015). As the CLP Log K_{ow} trigger for concern is not met, only brief details of this study are reported below:

Report: (58) Larkin J.C. (1984); DPX-M6316 [Thiophene-2-^{14}C] Flow-through Bioconcentration Study with Bluegill Sunfish, *Lepomis machrochirus*
DuPont Report No.: AMR 182-84
Guidelines: OECD 305E **Deviations:** minor
GLP: Not to GLP

Study summary:
In a fish bioconcentration study conducted to OECD guideline 305E (with minor deviations), bluegill sunfish, *Lepomis macrochirus* were exposed to [thiophene-2-^{14}C]thifensulfuron-methyl (radiochemical purity >98%) at a nominal concentration of 5 mg/L. No GLP statement was included in the report, however the study was otherwise considered to be reliable in the thifensulfuron-methyl DAR.

The flow-through test design consisted of a 28-day exposure phase followed by a 14-day depuration phase. Water temperature was maintained at 20-22°C and fish were sampled at regular intervals during the exposure and depuration periods. Water and tissue samples were analysed for radioactivity and bioconcentration factor (BCF) were calculated. There is no indication that lipid or growth correction was conducted, however this is not considered to have significantly affected the results. The calculated whole fish BCF was <0.8 on all sampling days during exposure. Throughout the study, no bioconcentration of ^{14}C residues from [thiophene-2-^{14}C]thifensulfuron-methyl occurred in bluegill sunfish.

5.3.2 Summary and discussion of aquatic bioaccumulation

As the log K_{ow} of thifensulfuron-methyl is -1.65 and less than the CLP trigger of 4, this is not envisaged to affect its aquatic hazard classification. The same is apparent for any major degradants of thifensulfuron-methyl. The low bioaccumulation potential was confirmed in an experimental study on bluegill sunfish (Larkin, 1984) (58) where the whole fish bioconcentration factor (BCF) was <0.8 and substantially less than the CLP BCF trigger of 500.

5.4 Aquatic toxicity

The thifensulfuron-methyl RAR (2015) includes a very large number of aquatic ecotoxicity studies submitted by DuPont and the TSM Taskforce on thifensulfuron-methyl as well as its degradants and certain formulations. Only the acute and chronic studies on technical thifensulfuron-methyl which are relevant for hazard classification purposes are reported here. Some higher tier studies have been submitted for refined risk assessment, including some containing sediment, variable exposure durations and temperatures and with recovery phases. These are non-standard for hazard classification but are included below where they may still be of some relevance. The aquatic toxicity endpoints for degradants are summarised briefly in Annex 1. Since thifensulfuron-methyl is considered ‘not rapidly degradable’ from a CLP hazard perspective and degradants are not as toxic as the parent substance, these are not considered further in relation to the aquatic hazard classification of thifensulfuron-methyl.

A summary of the aquatic toxicity of thifensulfuron-methyl is given in Table 27. The lowest potentially relevant acute and chronic endpoints for hazard classification for each trophic group are highlighted in **bold**.

Further relevant details of the aquatic toxicity studies are included in the sections following the Table, as well as in Section B.9.2.1 of the RAR (2015).

Table 27: Summary of information on the aquatic toxicity of thifensulfuron-methyl

Study type and duration	Species	Endpoint	Toxicity value	Reference
Fish				
Acute toxicity	**Rainbow trout (*Oncorhynchus mykiss*)** **Bluegill sunfish (*Lepomis macrochirus*)**	**96 h LC_{50}**	**>100 mg a.s/L (nom[$])**	**(1983 a & b) (59)(60)**
Prolonged toxicity (fish juvenile growth test)	**Rainbow trout (*Oncorhynchus mykiss*)**	**21 d NOEC**	**250 mg a.s/L (mm)**	**(1991) (61)**
Aquatic invertebrates				
Acute toxicity	**Water flea (*Daphnia magna*)**	48 h EC_{50}	**470 mg a.s/L (mm)**	**Wetzel (1986) (62)**
Acute toxicity			>970 mg a.s/L (mm)	Hutton (1989a) (63)
Acute toxicity	***Chironomous riparius* larvae (no sediment phase of study)**		**>100 mg a.s/L (nom)**	**Juckeland (2012) (64)**
Chronic toxicity	**Water flea (*Daphnia magna*)**	**21 d NOEC**	**100 mg a.s/L (mm)**	**Hutton (1989b) (65)**
Algae				
Acute and chronic toxicity	*Pseudokirchneriella subcapitata*	24-48 h E_rC_{50} 120 h NOE_rC	17 mg a.s/L (nom[$]) 5 mg a.s/L (nom[$])	Douglas and Handley (1987) (66)
	Pseudokirchneriella subcapitata, Skeleonema costatum, ***Navicula pelliculosa****, Anabaena flos-aquae*	120 h EC_{50} 120 h NOEC *P. subcapitata*	>0.0157 mg a.s/L (i.m) 0.0157 mg a.s/L (i.m)	**Hicks (1995) (67)**
		120 h EC_{50} 120 h E_rC_{50} 120 h NOEC *A. flos-aquae*	>0.0263 mg a.s/L (i.m) >0.0263 mg a.s/L (i.m) 0.0263 mg a.s/L (i.m)	
		168 h E_bC_{50} **24-48 h E_rC_{50}** **168 h NOEC** ***N. pelliculosa***	>0.0173 mg a.s/L (i.m) **0.00162 mg a.s./L (mm)[#]** **0.00116 mg a.s./L (mm)[#]**	
		120 h EC_{50} 120 h NOEC *S. costatum*	>0.0175 mg a.s/L (i.m) 0.0175 mg a.s/L (i.m)	
	Anabaena flos-aquae	72 h EC_{50} 96 h E_rC_{50} 72-96 h NOE_rC	0.742 mg a.s/L (mm) 0.825 mg a.s/L (mm) <0.59 mg a.s./L (mm)	Boeri, Magazu and Ward (1999) (68)

Aquatic macrophytes				
Acute and chronic toxicity	**Duckweed (*Lemna minor*)**	14 d EC_{50} **14 d E_rC_{50}** **14 d NOE_rC**	0.0013 mg a.s/L (nom$^{\$}$) **0.002 mg a.s/L (nom$^{\$}$)** **0.0005 mg a.s/L (nom$^{\$}$)**	**Douglas and Handley (1988) (69)**
Acute and chronic toxicity	**Duckweed (*Lemna gibba*)**	14-day EC_{50} **14 d E_rC_{50}** **14 d NOE_rC**	0.000866 mg a.s./L (mm) **0.00087 mg a.s./L (mm)** **0.00023 mg a.s./L (mm)**	**Kannuck and Samel (1995) (70)**
Acute and chronic - 7 d variable exposure duration + recovery*	Duckweed (*Lemna gibba*)	4 d E_rC_{50} 4 d NOEC	0.0032 mg a.s./L (nom) 0.00014 mg a.s./L (nom)	Porch, Kendall and Krueger (2011a) (71)
Acute and chronic toxicity	**Duckweed (*Lemna gibba*)**	**7 d E_rC_{50}** **7 d NOE_rC**	**0.0011 mg a.s./L (mm)** **0.00037 mg a.s./L (mm)**	**Arnie *et al.* (2015) (77)**
Acute and chronic toxicity	*Ceratophyllum demersum*	14 d E_rC_{50} 14 d NOE_rC	32.15 mg a.s/L (mm) <2.4 mg a.s/L (mm)	Hoberg (2011a) (72)
	Elodea canadensis	14 d E_rC_{50} 14 d NOE_rC	0.0217 mg a.s/L (mm) <0.058 mg a.s/L (mm)	Hoberg (2011b) (73)
	Myriophyllum aquaticum	14 d E_rC_{50} 14 d NOE_rC	0.1871 mg a.s/L (mm) <0.22 mg a.s/L (mm)	Hoberg (2011c) (74)
	Vallisneria americana	**14 d E_rC_{50}** 14 day NOE_rC	**0.0011 mg a.s/L (mm)** <0.00025 mg a.s/L (mm)	**Hoberg (2011d) (75)**
	Myriophyllum spicatum	14 d E_rC_{50} 14 d NOE_rC	0.0516 mg a.s/L (mm) <0.20 mg a.s/L (mm)	Hoberg (2011e) (76)

Studies, species and endpoints used in the aquatic hazard classification have been highlighted in **bold**.
nom = endpoint based on nominal concentrations; nominal endpoints marked $^{\$}$ were not verified.
mm = endpoint based on mean measured concentrations
i.m = endpoint based on initial measured concentrations
* This higher tier *Lemna* study was considered to be valid, however it is not considered to be suitable for use in hazard classification, although it does provide further assurance about the choice of classification endpoints.
[#] Note, for this most sensitive species only, these geometric mean measured growth rate endpoints have been tentatively calculated from the initial measured and terminal LOD concentrations and the estimated initial measured ErC50 and NOErC for *N. pelliculosa* of 0.0159 mg a.s/L and 0.00815 mg a.s./L respectively.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The following two acute fish studies were originally evaluated in the thifensulfuron-methyl DAR (1996) and again included in the RAR (2015), however their reliability has been questioned in the RAR and ESFA Conclusion (2015). This is discussed further below.

Study 1
Report: (1983a)(59); 96-hour LC_{50} of INM-6316-20 to rainbow trout
DuPont and Testing Facility Report No.: HLR 503-83
Guidelines: Not given
GLP: Not to GLP

Study summary:

This study on the acute toxicity of thifensulfuron-methyl (INM-6316-20) to rainbow trout *Oncorhynchus mykiss* was conducted 1983 and included in the original DAR (1996). Thifensulfuron-methyl (purity 95.6%) was tested at nominal concentrations of 0, 0.1, 1, 10 and 100 mg/l (the substance was solubilised in water adjusted to pH 9). A NaOH solvent control (pH 9) was included, there were 2 test vessels of 15 L maintained in a water bath at 12°C per test concentration. There were 5 fish per test chamber with mean length4.3 cm and mean wet weight 1.89 g at dosing (total of 10 fish per concentration). Mortality counts were made every 24 hours for 96 hours. pH was measured in the control and in the low, medium and high test concentrations and the beginning and end of the test and ranged from 7.0-8.0. It is not clear whether temperature was monitored throughout the test.

No mortality was observed on any of the 4 days of the study. Therefore, the nominal 96 hour LC_{50} for bluegill sunfish exposed to thifensulfuron-methyl was reported to be >100 mg/L and the nominal no observable effect concentration (NOEC) was 100 mg/L.

No GLP statement was included and the test guideline used was not specified. The study met the principles of OECD guideline 203, however there were no apparent recording of sub-lethal effects, or analysis of actual concentrations of thifensulfuron-methyl in test solutions. The lack of confirmation of test concentrations in this static study could affect its validity, particularly as thifensulfuron-methyl was initially dissolved at a high pH of 9 (and pH continued at 7-8) which is known to increase its rate of hydrolysis (see Section 5.1.1.1).

Study 2

Report: (1983b)(60); 96-hour LC_{50} of INM-6316-20 to bluegill sunfish

DuPont and Testing Facility Report No.: HLR 509-83

Guidelines: Not given

GLP: Not to GLP

Study summary:

This study on the acute toxicity of thifensulfuron-methyl (coded as INM-6316-20) to bluegill sunfish *Lepomis macrochirus* was conducted 1983 and included in the original DAR (1996). Thifensulfuron-methyl (purity 95.6%) was tested at nominal concentrations of 0, 0.1, 1, 10 and 100 mg/l (the substance was solubilised in water adjusted to pH 9). A NaOH solvent control (pH 9) was included, there were 2 test vessels of 15L maintained in a water bath at 22°C per test concentration. There were 5 fish per test chamber with mean length4.3 cm and mean wet weight 1.89 g at dosing (total of 10 fish per concentration). Mortality counts were made every 24 hours for 96 hours. pH was measured in the control and in the low, medium and high test concentrations and the beginning and end of the test and ranged from 7.0-8.0. It is not clear whether temperature was monitored throughout the test.

No mortality was observed on any of the 4 days of the study. Therefore, the 96 hour LC_{50} and no observable effect concentration (NOEC) for bluegill sunfish exposed to thifensulfuron-methyl was reported to be >100 mg/l.

No GLP statement was included and the test guideline used was not specified. The study met the principles of OECD guideline 203, however there were no apparent recording of sub-lethal effects, or analysis of actual concentrations of thifensulfuron-methyl in test solutions. The lack of confirmation of test concentrations in this static study could affect its validity, particularly as thifensulfuron-methyl was initially dissolved at a high pH of 9 (and pH continued at 7-8) which is known to increase its rate of hydrolysis (see Section 5.1.1.1).

Conclusion regarding acute toxicity to fish

The above two acute fish toxicity studies by (1983) (59) and (60) are old and pre-GLP, they have a number of deviations from a modern OECD 203 test, most importantly there was no measurement of actual test concentrations in test media during these static studies. It cannot therefore be confirmed that exposure was maintained within 80-120% of nominal concentrations - on which the endpoints were based. The Applicant considers that other studies suggest thifensulfuron-methyl is adequately stable in water, however these are sometimes flow-through or semi-static studies of varying durations. Nevertheless, it is not expected that the herbicide thifensulfuron-methyl would pose a high acute hazard to fish compared with that for algae and higher aquatic plants, so further acute vertebrate testing on fish would not be warranted. The acute aquatic hazard assessment will focus on algae and plants.

5.4.1.2 Long-term toxicity to fish

Report: (1991)(61); Flow-through, 21-day toxicity of DPX-M6316-100 (technical) to rainbow trout (*Oncorhynchus mykiss*)
DuPont Report No.: HLR 321-91
Guidelines: OECD 204 (1984). **Deviations:** None
GLP: Yes

This fish juvenile growth test on *Oncorhynchus mykiss* was originally evaluated in the thifensulfuron-methyl DAR (1996).

Study summary: Rainbow trout (*O. mykiss*) were exposed to technical thifensulfuron-methyl (DPX 6316-100, purity 98%) at nominal concentrations of 0, 19, 38, 75, 150 and 300 mg/L (the substance was solubilised in water adjusted to pH 9). Ten fish were used per treatment group. Test solutions were delivered intermittently to 10 L glass exposure chambers (temperature 13°C). Fish were exposed to the test substance for 21 days. This was a flow-through test design and the volume of the chamber was exchanged six times daily. Concentrations of thifensulfuron-methyl in test solutions were determined by HPLC. Analysis of variance and Barlett's test were used as statistical methods.

Mean measured concentrations of thifensulfuron-methyl in test solutions were 16, 33, 52, 110, and 250 mg/L (69-87% of nominal), endpoints were expressed in terms of mean measured concentrations. No mortality was observed, the 7, 14 and 21-day LC_{50}s were therefore greater than 250 mg/L (the highest measured test concentration that could be achieved because of solubility limitations). No significant differences in length, weight of fish or sub lethal effects were observed. The 21-day NOEC for juvenile rainbow trout exposed to thifensulfuron-methyl was therefore reported to be a measured 250 mg/L.

Additional information on prolonged toxicity to fish

A second valid and GLP compliant flow-through 21-day OECD 204 test was submitted on a formulated product of thifensulfuron-methyl, i.e. ‘Harmony’, which contains 75% w/v thifensulfuron methyl (see Pierson K.B., 1991, included in the RAR, 2015). There was no mortality and 21-day NOECs for fish length, weight and sub lethal behavioural effects were each 200 mg formulation/L respectively - which was equivalent to a measured 156 mg thifensulfuron-methyl/L.

Although tested using a formulated product, this gives some further assurance that the active substance poses a low acute and chronic hazard to fish.

Conclusion regarding chronic toxicity to fish

The above 21-day studies were performed to OECD 204, which was originally a 14-day prolonged acute test guideline measuring only mortality of relatively insensitive life stages. However, the submitted tests were extended to 21 days and included observations on fish length, weight and sub-lethal behavioural effects. Normally it is preferred that at least a 28-day study (e.g. OECD 215 juvenile fish growth test) is available for chronic fish classification - and OECD 204 has since been deleted from the OECD library. However, in this case, given that sub-lethal assessments were undertaken and thifensulfuron-methyl (although not 'rapidly degradable') is also not bioaccumulative or especially persistent in water - the available prolonged studies are considered sufficient to indicate a low chronic toxicity of thifensulfuron-methyl to fish. Further true chronic fish studies would not be warranted. Thifensulfuron-methyl is a herbicide and rather than conduct a surrogate chronic hazard assessment based on the (low but also uncertain) acute toxicity to fish, it is proposed to focus instead on its chronic effect on algae and plants.

5.4.2 Aquatic invertebrates

5.4.2.1 Acute/short-term toxicity to aquatic invertebrates

Study 1
Report: Wetzel, J.W. (1986); *Daphnia magna* static acute 24 and 48-hour EC50 of INM-6316-20
DuPont Report No.: HLR 258-86
Guidelines: U.S. EPA 1600/4-85/012, (1985) **Deviations:** Mostly minor
GLP: Statement of compliance included with the report.

This study on the acute toxicity of thifensulfuron-methyl to aquatic invertebrates was originally evaluated in the DAR (1996) and included again in the RAR (2015), however its reliability has been questioned in the RAR and ESFA Conclusion (2015). This is discussed further below.

Study summary:
The acute toxicity of technical thifensulfuron methyl (97.1% pure) to *Daphnia magna* was assessed according to U.S. EPA test guideline 1600/4-85/012 (1985). Nominal test concentrations of thifensulfuron-methyl (coded as INM-6316-20) were 0, 100, 130, 180, 240, 320, 420, 560, 750 and 1000 mg/L. A total of 20 daphnids was used per test concentrations. The water temperature was maintained at 20°C and test solutions were not aerated. The test was a static design and lasted for 48 hours. Observations of immobility were made at 24 and 48 hours. The EC_{50}s were calculated by probit analysis.

Water samples were analysed directly by reverse phase HPLC on days 0, 1 and 2 of the study. The mean concentrations taken over days 0, 1 and 2 were 131, 167, 229, 301, 394, 506, 667, 887 and 1190 mg/L, respectively. These represented 118-131% of the nominals. Based on mean measured test concentrations, the 24-hour EC_{50} was 650 mg/L (95% confidence intervals (CI) 590-740 mg/L). Themean measured 48-hour EC_{50} was 470 mg/L (95% CI 420-530 mg/L). The acute 48-hour mean measured NOEC was 167 mg/l (based on immobility).

This 1986 study partially meets the current guideline (OECD 202). Concerns were reported in the RAR about a lack of clear measurement of test substance concentrations in test media throughout

the test, however this appears to have been performed satisfactorily. Endpoints are based on mean measured concentrations. In any case, the Applicant (DuPont) considers that reconduct of this test is unlikely to yield a significantly different result since results of an additional study to address this endpoint (Hutton, 1989 see below (63)) also suggest thifensulfuron methyl is adequately stable in water.

Study 2
Report: (63) Hutton, D.G., 1989a; Static acute 48-hour EC_{50} of IN-M6316-25 to fed *Daphnia magna*
DuPont and Testing Facility Report No.: HLR 95-89
Guidelines: OECD 202, U.S. EPA 72-2. **Deviations:** None
Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for the purpose of renewal under pesticides legislation and is included in the RAR (2015).

Study summary:
The acute toxicity of technical thifensulfuron-methyl (97.0% pure) to fed *Daphnia magna* was determined in an unaerated, static, 48-hour test. The static test was conducted in accordance with OECD 202 and U.S. EPA Guideline E, 72-2. Due to limited solubility and stability of thifensulfuron-methyl in the accepted aquatic testing solvents, NaOH solutions were used to increase the pH of the stock solution to 9, increasing the solubility of the test material. Treatments consisted of a dilution water control, a sodium hydroxide solution control and nine nominal concentrations of 100, 130, 180, 240, 320, 420, 560, 750 and 1000 mg thifensulfuron-methyl/L (because of solubility limitations). Test solutions were measured at test initiation and at termination to verify stability of the test item. The corresponding mean measured concentrations were 84, 120, 170, 220, 310, 410, 550, 720 and 970 mg thifensulfuron-methyl/L. There were 5 daphnids (<24 hours old) per test chamber with four replicates per test concentration and control. Daphnids were fed during the test. Immobility and sub-lethal (behavioural) observations were made every 24 hours. Temperature was maintained at 19.5-19.8°C and pH was 7.1-8.8.

Some undissolved material was present at 750 and 1000 mg/L. Despite this, nominal concentrations were maintained at ±20% and the presence of undissolved material was not seen to impact on daphnids. The highest concentration causing no immobility was 410 mg thifensulfuron-methyl/L and the lowest concentration causing 100% immobility was >970 mg thifensulfuron-methyl/L, the highest concentration tested. The 48-hour EC_{50} for *Daphnia magna* based on immobility and mean, measured concentrations was >970 mg thifensulfuron-methyl/L, the highest concentration tested.

Study 3
Report: (64) Juckeland, D. (2012). Acute toxicity of Thifensulfuron-methyl technical to *Chironomus riparius* in a 48-hour static test. (TSM)
Sponsor: Cheminova A/S.
Report No: 296 TIM.
Guidelines: OECD 235 (2011). **Deviations:** None
Test facility: BioChem Agrar
Test facility report number: 11 10 48 045 W

GLP: Yes

This study was submitted for the purpose of renewal under pesticides legislation and is included in the RAR (2015). This was an acute test on chironomid larvae without sediment, rather than the usual chronic study. However, the UK RMS and CA consider it reliable and of potential use in acute aquatic hazard assessment.

Study summary:
A 48-hour static acute toxicity test was performed according to OECD 235 guideline (2011) in order to evaluate the acute effects of thifensulfuron-methyl (97.4% pure) on larvae of *Chironomus riparius* at a single limit test concentration of 100 mg/L. Larvae were 1st instar (approximately 48 hours after hatching). There were 4 control and test concentration replicates each containing 5 individual larvae. Each glass beaker contained 10 mL of M4 test medium. There was no feeding of larvae and no aeration. Temperature was maintained at 18.6-20°C; pH was 7.74-7.75 at test initiation and 7.55-7.61 at test termination; the photoperiod was 16 h light:8 h dark; oxygen concentration was 8.61-8.75. Analytical verification took place at test initiation and test termination in the test concentrations and the control vessels. Numbers of immobilised *Chironomus* larvae (including any abnormal behaviour) were assessed after 24 and 48 hours.

All validity criteria were met for this study. The number of immobilised larvae in the control was ≤15% (0%). A potassium chloride toxic standard was tested in a separate but identically performed study and EC_{50} values were within the expected range of toxicity. At test initiation, the analytical verification showed that the concentrations of the thifensulfuron-methyl technical were 97% of nominals; at termination concentrations were 110% of nominals. As concentrations were maintained within ± 20% of the nominal, results are based on nominal concentrations.

No immobilisation or other abnormal behavioural effects on larvae were observed at 24 or 48 hours. Therefore the acute nominal EC_{50} value for immobilisation of *C. riparius* larvae after 48-hours exposure to thifensulfuron-methyl was considered to be >100 mg/L; the acute nominal NOEC was determined to be 100 mg/L.

5.4.2.1 Long-term toxicity to aquatic invertebrates

Report: (65) Hutton, D.G. (1989b); Chronic toxicity of IN-M6316-25 to *Daphnia magna*
DuPont Report No.: HLR 70-89
Guidelines: OECD 202 (1984), U.S. EPA 72-4 (1988). **Deviations:** None
Testing Facility: Haskell Laboratory for toxicology and industrial medicine
GLP: Yes

This study was evaluated in the original thifensulfuron-methyl DAR (1996) and also included in the RAR (2015). The study is considered to be reliable.

Study summary:
The chronic toxicity of technical thifensulfuron methyl ('IN-M6316-25', 97.0% pure) to *Daphnia magna* was determined under semi-static conditions over 21 days according to OECD 202 (1984) and U.S. EPA 72-4 (1988) guidelines. *Daphnia* were exposed at nominal concentrations of 0, 42, 64, 99, 152, 235 and 350 mg/L. A total of 40 daphnids was used per test concentrations. The parent *Daphnia* were transferred to fresh test solutions three times per week, at which time survival and reproduction data were collected. Concentrations of thifensulfuron methyl were measured in new

and old media by HPLC. At test conclusion, the lengths of surviving adult *Daphnia* were measured and numbers of young produced were determined. The water temperature was 20°C and test solutions were not aerated.

Mean measured concentrations of thifensulfuron-methyl were 40, 66, 100, 150, 240 and 340 mg/L and were within ±20% of nominals. The NOECs for growth reproduction and survival were 100, 150 (based on 1st day of reproduction) and 340 mg/L based on measured concentrations.
Based on growth, the most sensitive parameter, the overall measured 21-day NOEC was 100 mg thifensulfuron-methyl/L.

Conclusions regarding acute and chronic toxicity to aquatic invertebrates

In the original thifensulfuron-methyl DAR (1996) a 48-hour acute EC_{50} of 470 mg/L was proposed (Wetzel, 1986). This was based on mean measured concentrations ranging from 131-1190 mg/L and the study is considered of use in hazard classification.

For renewal, DuPont have submitted a further acute study with *Daphnia* exposed to thifensulfuron-methyl. The study by Hutton (1989a) (63) proposes a 48 hour EC_{50} of >970 mg/L (based on mean measured concentrations). This was based on a study with concentrations ranging from 84-970 mg/L. Immobility was observed in this study at the 3 highest test concentration but these effects did not affect 50%. It has been noted by the pesticide RMS that *Daphnia* used in this study were fed, the OECD study guideline (point 19) states that the *Daphnia* should not be fed during the study. It is not thought that this deviation is sufficient to invalidate the study as otherwise good adherence to current methods was demonstrated. As such this study can be considered suitable for use in hazard assessment.

Additionally an acute *Chironomous* study has been submitted at renewal. This reliable study did not include a sediment life phase for *Chironomous* larvae and therefore has been considered along with the other acute aquatic invertebrate studies. The resulting 48-hour acute measured EC_{50} of >100 mg/L provides some information for classification (indicating a low hazard) but is not itself an accurate endpoint.

It is noted that the endpoint of >970 mg/L from Hutton (1989a) (63) is a factor of 2 greater than the endpoint derived from the Wetzel, 1986 study (470 mg/L). Therefore, preference should be given to the acute study where the daphnia were not fed. Although in EFSA peer review, concerns were expressed regarding the Wetzel study, in the absence of a more modern study it is the most sensitive and accurate acute invertebrate endpoint available. Therefore the **EC_{50} of 470 mg/L** is chosen for the acute hazard classification for aquatic invertebrates.

With regards to chronic toxicity to aquatic invertebrates, a 21-day semi-static study on *Daphnia magna* is available (Hutton, 1989b) (65). Test concentrations were maintained within ±20% of nominals and based on growth, the most sensitive parameter, the overall measured 21-day NOEC was 100 mg thifensulfuron-methyl/L. The study is reliable and this endpoint is used for chronic hazard classification for aquatic invertebrates.

5.4.3 Toxicity to algae

Study 1
Report: (66) M.T. Douglas and J.W. Handley (1987); The algistatic activity of DPX-M6316-26
DuPont Report No.: 6316/ME7
Guidelines: OECD 201, U.S. EPA 122-2. **Deviations:** None
Testing Facility: Huntingdon Research Centre Ltd
GLP: Yes

This study was evaluated in the original thifensulfuron-methyl DAR (1996) and also included in the RAR (2015). The reliability of the study was questioned in the RAR and EFSA Conclusion (2015), principally due to the lack of measurement and confirmation of test concentrations throughout the study.

Study summary:
The chronic toxicity of technical thifensulfuron methyl (94.4% pure) to *Pseudokirchneriella subcapitata* (tested as *Selenastrum capricornutum*) was determined according to US EPA guideline 122-2 and OECD 201. Algae were exposed under static conditions at nominal concentrations of 0, 1.25, 2.5, 5.0, 10.0 and 20 mg thifensulfuron-methyl/L. The initial concentration of *P. subcapitata* was 1.5 x 10^5 cells/50 ml and they were incubated with the test compound under continuous illumination and aeration at 24°C for 120 hours. Growth rate was measured and cell densities were recorded at test initiation and termination. The concentration resulting in a 50% inhibitory effect on cell density (E_bC50) at 72 or 120 hours and the median concentration resulting in 50% inhibition of growth (E_rC50) were determined, along with the overall NOEC (concentration at which no inhibition of growth or occurrence of abnormalities were detected). Only the E_rC50 and NOE_rC are used for hazard classification and these are reported below. At the end of the exposure phase, samples of affected algae were placed in fresh growth media to see if thifensulfuron-methyl was algitoxic or algistatic.

Analytical data on concentrations of the test substance in the test media were not reported. This deviation made the validity of the study questionable although the test solution preparation was well described. The resulting E_rC50 (at 24-48 hours) for *P. subcapitata* was therefore based on nominal concentrations of thifensulfuron-methyl and was calculated to be 17 mg/L. A growth rate endpoint covering longer durations was not reported. The 120-hour nominal NOEC was determined to be 5.0 mg/L, it is presumed this related to growth as well as other effects and also to shorter durations. Regrowth of inhibited cultures indicated that under these test conditions the test substance is algistatic to *P. subcapitata* and not algicidal.

Study 2
Report: (67) Hicks, S.L. (1995); Thifensulfuron methyl (DPX-M6316): Influence on growth and reproduction of four select algal species
DuPont Report No.: AMR 2890-93
Guidelines: U.S. EPA-FIFRA 122-2 & 123-2 **Deviations:** None
Testing Facility: ABC Laboratories, Inc. and DuPont Haskell Laboratory, Columbia, Missouri, USA and Newark, Delaware, USA
Testing Facility Report No.: #41475
GLP: Yes

This study was evaluated in the original thifensulfuron-methyl DAR (in a 2000 addendum to the 1996 DAR) and also included in more detail in the RAR (2015).

Study summary:
This study was conducted to determine the effects of thifensulfuron-methyl (purity 99.63%) on the growth and reproduction of two freshwater algae *Pseudokirchneriella subcapitata* (tested as *Selenastrum capricornutum*) and *Anabaena flos-aquae*, one freshwater diatom (*Navicula pelliculosa*) and one marine diatom (*Skeletonema costatum*). Along with untreated controls, *P. subcapitata* and *Skeletonema costatum* were exposed to single nominal concentrations of 13.8 µg thifensulfuron-methyl/L. *Anabaena flos-aquae* was exposed to a nominal concentrations ranging from 1.4 to 22.4 µg thifensulfuron-methyl/L. *Navicula pelliculosa* was exposed to nominal concentrations of 0.865 to 13.8 µg thifensulfuron-methyl/L. Each test concentration, the untreated controls, and the abiotic control were tested as four replicates. The organisms were exposed for 5 days under static condition, without test medium renewal, with the exception of *Navicula pelliculosa* which was exposed for 7 days without test medium renewal.

Test temperatures ranged from 20-24°C and pH from 7.2-8.2. Full details of the initial cell populations, growth media, test conditions, photoperiod and light intensity for each species are given in the RAR (2015). Test solutions were measured on Day-0 and at termination to verify stability of the test item. Cell counts were recorded approximately 0, 1, 2, 3, 4 and 5 days after test initiation as well as 6 and 7 days for *Navicula pelliculosa*.

Analytical verification of the test solutions was performed on samples collected at test initiation and termination. Thifensulfuron methyl could not be detected in most of the test solutions (<limit of detection (LOD)), including the abiotic stability blanks, at the end of each exposure period. The exception was the *Skeletonema costatum* study in which the measured level of thifensulfuron methyl was stable throughout the exposure. The study report states that this is due to the active substance being a photosensitive compound and as such, concentrations may have been stable in the *Skeletonema costatum* study because the solutions were exposed to light for a shorter duration of the day (16 hours) rather than the continuous illumination that the other species received.

The following analytical results and effects endpoints (based on initial measured rather than mean measured concentrations) were reported for each species:

- *Pseudokirchneriella subcapitata*

The 0-hour (initial) measured concentration for the nominal 13.8 µg a.s./L test solution was 15.7 µg a.s./L, representing 114% of nominal. The 120-hour measured concentration was <0.841 µg a.s./L (<LOD).
120-hour NOEC = 15.7 µg a.s./L (≡ 0.0157 mg/L)
120-hour EC_{50} estimated to be >15.7 µg a.s./L (≡ >0.0157 mg/L)

- *Anabaena flos-aquae*

The 0-hour (initial) measured concentrations for the nominal 1.40, 2.80, 5.60, 11.2 and 22.4 µg a.s./L test solutions were 1.03, 2.04, 5.13, 12.0 and 26.3 µg a.s./L, representing 73-117% of nominal. The 120-hour measured concentration were all <0.029 µg a.s./L (<LOD).
120-hour NOEC = 26.3 µg a.s./L (≡ 0.0263 mg/L)
120-hour EC_{50} estimated to be >26.3 µg a.s./L (≡ >0.0263 mg/L)
E_rC_{50} (0-120 h) estimated to be >26.3 µg a.s./L (≡ >0.0263 mg/L)

- *Navicula pelliculosa*

The 0-hour (initial) measured concentrations for the nominal 0.865, 1.73, 3.45, 6.90 and 13.8 µg a.s./L test solutions were 0.767, 1.31, 2.09, 8.15 and 17.3 µg a.s./L, representing 61-125% of nominal. The 120-hour measured concentrations were all <0.165 µg a.s./L (<LOD).

168-hour NOEC = 8.15 µg a.s./L (≡ **0.00815 mg/L**) – see also recalculated mean measured values below.
168-hour EC_{50} estimated to be >17.3 µg a.s./L (≡ >0.0173 mg/L)
E_bC_{50} (0-168 h) estimated to be >17.3 µg a.s./L (≡ >0.0173 mg/L)
E_rC_{50} (24-48 h) = 15.9 µg a.s./L (95% CL: <0 - >17.3 µg a.s./L) (≡ **0.0159 mg/L**) – see also recalculated mean measured values below.

- *Skeletonema costatum*

The 0-hour (initial) measured concentration for the nominal 13.8 µg a.s./L test solution was 17.5 µg a.s./L, representing 127% of nominal. The 120-hour measured concentration was 14.5 µg a.s./L, which represents 105% of nominal.
120-hour NOEC = 17.5 µg a.s./L (≡ 0.0175 mg/L)
120-hour EC_{50} estimated to be >17.5 µg a.s./L (≡ >0.0175 mg/L)

Overall the most sensitive of the species tested under these conditions was the freshwater diatom *Navicula pelliculosa* with a 48 h E_rC50 of 15.9 µg a.s./L (≡ 0.0159 mg a.s/L) and a NOEC over 168 hours (7-days) of 8.15 µg a.s./L (≡ 0.00815 mg a.s/L). In the absence of other information from the study, this NOEC is considered to have included growth rate effects (i.e. NOE_rC) as well as to cover shorter timescales. Ideally, to give a true indication of the toxicity of a substance, results for hazard classification should be based on mean measured test concentrations over the duration of the studies rather than initial measured. For all but the *Skeletonema costatum* study, in which thifensulfuron methyl was stable throughout the exposure, initial measured endpoints were used which could underestimate intrinsic toxicity.

The Applicant (DuPont) has commented that the loss of thifensulfuron methyl from the culture medium over 168-hours was likely a result of the photosensitivity of the molecule and resulting degradation under the algal test conditions. Similar degradation was noted in the abiotic controls. They believe that the calculation of average values, specifically geometric means, is not appropriate with regard to thifensulfuron methyl due to the photosensitivity of the molecule and resulting rapid degradation. Any effect noted in *Navicula pelliculosa* is likely a result of rapid onset of effect. Calculation of the geometric means for the NOEC and E_rC_{50} are theoretically possible however. For *N. pelliculosa*, assuming the 0-hour measured concentration of 8.15 µg/L (the basis for the 168-h NOEC) and the LOD of 0.165 µg/L as the test termination measured concentration, the resulting NOEC based on a geometric mean of these equals 1.16 µg/L. The 24 - 48-h E_rC_{50} based on the initial measured concentration of thifensulfuron methyl equalled 15.9 µg/L. Using the LOD of 0.165 µg/L as the test termination measured concentration, the resulting 24 - 48-hr geometric mean measured E_rC_{50} equals 1.62 µg/L. Ideally given the rapid dissipation and effects, there would be analysis during these exposure periods for more accurate determination of the mean measured effect concentrations at different time-points. However, in the absence of any more accurate values, the lowest mean measured 'acute' E_rC_{50} and 'chronic' NOE_rC endpoints from the Hicks (1995)(67) study are considered to be 1.62 µg/L (≡ 0.00162 mg/L) and 1.16 µg/L (≡ 0.00116 mg/L) respectively for *N. pelliculosa*.

Study 3
Report: (68) Boeri, R.L., Magazu, J.P., Ward, T.J. (1999); Thifensulfuron methyl technical: growth and reproduction test with the freshwater alga, *Anabaena flos-aquae*
DuPont Report No.: DuPont-2378
Guidelines: U.S. EPA / OPPTS 850.5400 (1996) **Deviations:** None
GLP: Yes

This study was evaluated in the original thifensulfuron-methyl DAR (in a 2000 addendum to the 1996 DAR) and also included in the RAR (2015) although in no more detail as the endpoints were not the most sensitive for algae compared with those subsequently submitted for renewal and they were not subsequently used.

Study summary:
This study was conducted to determine the effects of thifensulfuron-methyl (purity 99.7%) on growth of the green alga, *Anabaena flos-aquae* over 96 hours exposure in static conditions at nominal concentrations of 0, 0.64, 1.3, 2.5, 5.0 and 10.0 mg/L.

The study was conducted according to guideline OPPTS 850.5400 (1996) and the Notifier considers that the study also meets the current guideline (OECD 201). Test temperatures ranged from 20-24ºC and pH from 7.2-8.2. Test solutions were measured on Day-0 and at termination to verify stability of the test item. Mean measured concentration were 0, 0.59, 1.22, 2.27, 4.59 and 9.09 mg/L, i.e. 91-94% of nominals.

The following results were reported (expressed as mean measured concentrations of thifensulfuron-methyl):
EC50 - 96 h = 0.84 mg a.s./L (number of cells)
EC50 - 72 h = 0.742 mg a.s./L (number of cells)
E_rC50 - 96 h = 0.825 mg.a.s./L (growth rate)
E_rC50 - 72 h = 1.03 mg a.s./L (growth rate)

Without further information and since effects on growth occurred at even the lowest tested concentration, the 72-96 h mean measured NOE_rC based on growth rate is considered to be <0.590 mg thifensulfuron-methyl/L (extracted from the original study report).

5.4.4 Toxicity to higher aquatic plants/macrophytes

Study 1
Report: (69) Douglas, M.T and Handley, J, W. (1988). An assessment of the inhibitory effect of DPX-M6316-26 technical on the growth of duckweed (*Lemna minor*) (DuPont). April 1989.
DuPont Report No: DPT 186C/881591
Guidelines: Draft OECD guideline for testing chemicals 'Duckweed, static growth inhibition test' December 1981; EPA pesticide assessment guidelines 122-2 and 123-2. **Deviations:** None
Test facility: Huntingdon Research centre, Cambridge, UK
GLP: Yes

This study was evaluated in the original thifensulfuron-methyl DAR (1996) and also included in more detail in the RAR (2015). The reliability of the study was questioned in the RAR and EFSA Conclusion (2015), principally due to the lack of measurement and confirmation of test concentrations throughout the study.

Study summary:
The effects of technical thifensulfuron-methyl (95.4% pure) on duckweed (*Lemna minor*) were assessed according to a then draft OECD guideline and US EPA guidelines 122-2 and 123-2, as well as to GLP. *L. minor* was exposed for 14 days to nominal concentrations of 0, 0.5, 1, 2, 4 and 8 µg thifensulfuron-methyl/L, a solvent control was also included. Duckweed with 10-15 fronds (2-3 fronds/plant) were exposed at each concentration, there were 5 plants per replicate and 3 replicates/concentration (30-45 fronds in total). The study was conducted at a temperature of 21°C and pH of 5.5, in a semi-static system where the test substance was replenished every other day. There was however, no reported analytical verification of test concentrations. Although preparation of the test solution was well described, this deviation has made the validity of the study questionable. Frond counts were recorded at each medium renewal day and at test termination. The EC_{50} value was determined by fitting a logistic curve to the data. All test concentrations and the control were then incubated for a further 7 day 'recovery period' at which point there was a final frond count.

No abnormalities with *L. minor* were detected in any of the control or test cultures at 0.5, 1.0 and 2.0 µg/L. However chlorosis of the fronds was observed after 7 day exposure to 8.0 µg/L and after 9 days at 4.0 µg/L. After 11 days, chlorosis and a blackening of the fronds was observed at both 4.0 and 8.0 µg/L. These effects were observed to be more pronounced after 14 days exposure. The 14-day EC_{50} (frond number) and the NOEC for *Lemna minor* were a nominal 1.3 µg/L (95% CL: 1.1-1.5) and 0.5 µg/L thifensulfuron-methyl, respectively. The 14-day E_rC_{50} was subsequently calculated via exponential modelling to be 2.01 µg/L (≡ 0.002 mg/L). No specific growth rate NOE_rC was provided; endpoints derived for frond number are typically lower that those derived from growth rate, therefore a conservative NOErC is considered to be the same as for other parameters at 0.5 µg/L (≡ 0.0005 mg/L). *L. minor* exposed at concentrations of 2 µg/L and less showed complete recovery during a 7 day recovery period.

Study 2
Report: (70) Kannuck, R.M., Samel, A., 1995: Thifensulfuron-methyl (DPX-M6316): Influence on growth and reproduction of *Lemna gibba* G3 (DuPont)
DuPont Report No.: AMR 2982-94
Guidelines: U.S. EPA 123-2 **Deviations:** None
Testing Facility: DuPont Stine-Haskell Research Center, Newark, Delaware, USA
Testing Facility Report No.: AMR 2982-94
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The reliability of the study was subsequently questioned in the RAR and EFSA peer review, principally due to uncertainty regarding the accuracy of the methods used to confirm test concentrations (see comments below).

Study summary:
The effect of technical thifensulfuron-methyl (99.63% pure) on duckweed (*Lemna gibba* G3) was determined in a static, 14-day test. The test was conducted in accordance with U.S. EPA-FIFRA, Guideline 123-2. Treatments consisted of an untreated control, an abiotic (stability) control and six nominal concentrations of 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 µg thifensulfuron-methyl/L nutrient media. The initial population was 3-5 plants with 3-5 fronds each per replicate, 5 replicates per

control/treatment. Frond counts were made approximately every other day and biomass was measured at the beginning and end of the test. The test temperature was maintained at 24.5-26.0°C, the pH was 7.5-7.63 at test initiation and 8.62-9.32 at test termination; a 24 hour photoperiod was used (4049-5582 lux). To assess recovery of the *Lemna gibba* G3 after the initial 14-day exposure period, fronds were counted after 0, 2, 4, 7, 9, 11 and 14 days (study day 14, 16, 18, 21, 23, 25 and 28).

Active substance content of solutions was determined on Day 0 and Day 14 via ELISA assay (enzyme-linked immunosorbant assay) and validated via HPLC - see comments on reliability below. Recoveries of thifensulfuron-methyl were 51-97% of nominals at initiation and 14-39% by Day-14. At the request of the pesticide RMS, updated endpoints were provided based on geometric mean measured concentrations. The corresponding mean measured concentrations were reported to be 0.226, 0.553, 0.884, 1.329, 1.786 and 2.189 µg thifensulfuron-methyl/L.

Endpoints were subsequently determined using mean measured concentrations: Based on frond count, the 14-day EC_{50} was 0.866 µg thifensulfuron-methyl/L and the NOEC was 0.226 µg thifensulfuron-methyl/L. Based on biomass, the 14-day E_bC_{50} was 1.05 µg thifensulfuron-methyl/L and the NOEC was 0.226 µg thifensulfuron-methyl/L. A 14-day E_rC_{50} growth rate endpoint based on front count, was subsequently determined to be 0.87 µg a.s./L (≡ 0.00087 mg a.s/L). No specific growth rate NOE_rC was determined so it is considered to conservatively be the same as for other parameters at 0.226 µg/L (≡ 0.00023 mg a.s/L). Further methodological and reporting details on this study are given in the RAR (2015) - section B.9.2.1.1, p 50-58.

Based on a recovery test, in which exposed fronds at each concentration exhibiting ≥50% growth inhibition were placed in fresh untreated media for 14 days, thifensulfuron-methyl was reported to be phytostatic to *Lemna gibba* at ≤0.884 µg /L and phytocidal at ≥1.329µg thifensulfuron-methyl/L.

Comments on reliability and relevance of Kannuck and Samel (1995) (70):
Although conducted to an older guideline, the reported methodology shows reasonable adherence to the current OECD guideline 221. Control fronds did not show visible abnormalities and frond number increased by approx 9.6 times over the first 7 day exposure period (>7x as required by OECD 221), therefore growth in the test system was adequately demonstrated. In the RAR (2015) concern was however expressed over the reliability of the ELISA and HPLC methods used to analyse for the stability of thifensulfuron-methyl and determine mean measured concentrations. Both methods were deemed to have insufficient resolution and not be specific enough to thifensulfuron-methyl - they were likely to have picked up degradation products also. During the subsequent EFSA peer review process and in the final EFSA Conclusion (2015) it was proposed to discount the whole Kannuck and Samel (1995) (70) study. Since the original *Lemna minor* study by Douglas and Handley (1988) (69) (Study 1 above) was also considered unreliable in EFSA peer review and the relevance of Porch et al. (2011) (71) (Study 3 below) for risk assessment was discounted, the next lowest aquatic macrophyte endpoint for *Vallisneria americana* (see Study 7 below) was used in the interim for risk assessment. In their 2015 Conclusion, EFSA have stated that a further reliable *Lemna* toxicity study is still required in order to conclude on the aquatic risk assessment for thifensulfuron-methyl, This has since been submitted and is evaluated below as study 4.

Study 3
Report: (71) Porch, J.R., Kendall, T.Z., Krueger, H.O. (2011a); Thifensulfuron-methyl (DPX-M6316) technical: A 7-day, variable exposure duration toxicity test with duckweed (*Lemna gibba* G3) (DuPont)
DuPont Report No.: DuPont-30629
Guidelines: U.S. EPA OPPTS 850.4400 (1996), OECD 221 (2006) **Deviations:** None
Testing Facility: Wildlife International, Ltd., Easton, Maryland, USA
Testing Facility Report No.: 112A-328
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). It is a non-standard higher tier study combining variable duration exposure and recovery phases and its relevance for hazard classification is uncertain. However, it is reported below for completeness.

Study summary:
The toxicity of technical thifensulfuron-methyl (98.2% pure) to duckweed *Lemna gibba* G3 was determined after various lengths of exposure in a 7-day test. The test was based on U.S. EPA Series 850 - Ecological Effects Test Guidelines, OPPTS Number 850.4400 and OECD Guideline 221. Treatments consisted of four exposure intervals (12, 24, 48 and 96 hours) each with six nominal concentrations. Test concentrations varied with the exposure interval and ranged from 0.043 to 658 µg a.s./L (secondary stock solution), along with an untreated control. There were 4 plants with 3 fronds each per replicate. At the end of each exposure interval, plants were moved from the test solution to untreated 20x AAP nutrient medium for the remainder of the 7 day test period.
Full methodology, reporting and details of the test concentration for each duration are given in the RAR (2015).

Temperature was 22.9-25.5°C during the whole exposure period. The pH was measured as 7.7-8.1 at test initiation only. Plants were kept under a 24-hr photoperiod (4560-5720 lux). Test concentrations were measured on Day 0 (new) and at the end of the last 96-hour exposure period to verify stability of the test item. Frond counts and any visual observations of frond health were made on Days 0, 3, 5 and 7. Biomass was determined at the completion of the 7-day test. Growth rates were determined on Day 7 and were based on frond count and biomass.

Test concentrations were measured on Day 0 (new) and at the end of the last 96-hour exposure period to verify stability of the test item. The concentrations tested appeared to relate to the 12-hour exposure period plus the secondary stock solution, it is not clear if/how all of the various exposure concentrations and durations were measured. Those concentrations analysed were 94-105% of nominals over 96 hours. As the test item was stated to be stable throughout the exposure period, results were based on nominal concentrations. The 7-day EC_{50} values, based on nominal thifensulfuron-methyl concentrations were as follows:

Table 28: Nominal *Lemna gibba* effects endpoints from variable duration exposure test with thifensulfuron-methyl

Exposure duration (hrs)	Overall NOEC (µg a.s./L)	E_bC_{50} (µg a.s./L)		E_yC_{50} (µg a.s./L)		E_rC_{50} (µg a.s./L)	
		Frond number	Biomass (dry wt)	Frond number	Biomass (dry wt)	Frond number	Biomass (dry wt)
12	5.3	175	387	149	304	632	>658
24	1.6	17.3	112	14.9	87.6	>198	>198
48	0.48	4.1	12.4	3.5	10.1	>59.3	>59.3
96	0.14	0.48	1.3	0.45	1.1	3.2	>17.8

Comments on reliability and relevance of Porch, Kendall and Krueger (2011)(71):
In the RAR (2015) the pesticide RMS considered the study to have met the validity criterion regarding control average growth rate in the current OECD guideline 221 with regard to frond number (at least 0.275/day) as well as a lack of any visual toxicity in control fronds. Environmental conditions of the study were also maintained approximately within recommended ranges. As such this study is considered reliable.

This was however a non-standard study intended for use in higher tier risk assessment; it featured variable duration exposure followed by recovery in clean test media also for the variable duration of the remaining 7 days. It is also not clear that concentrations and exposure were maintained under all of the test conditions that were investigated.

Although shorter than the usual 7 or 14 day studies for *Lemna*, the longest exposure duration (96 hour / 4 day) E_rC_{50} for frond number of 3.2 µg a.s./L (≡ 0.0032 mg/L) and overall 4 day NOEC of 0.14 µg a.s./L(≡ 0.00014 mg/L) could be considered of some relevance to hazard assessment in support of the chosen endpoints. The results from this study also very clearly indicate a pattern of decreasing EC_{50} with increasing exposure duration.

Note: A further non-standard study on the toxicity of thifensulfuron-methyl to *Lemna gibba* G3 was submitted for renewal and evaluated in the RAR (2015), i.e.: 'Porch, J.R., Kendall, T.Z., Krueger, H.O. (2011b); Thifensulfuron-methyl (DPX-M6316) technical: A 16-day toxicity test on duckweed (*Lemna gibba* G3) with exposure during dormancy' (Report No.: DuPont-30630). This 16-day higher tier risk assessment study replicated exposure during a period of temperature-induced dormancy (down to 8°C) to evaluate potential effects from thifensulfuron-methyl exposure during colder seasons. This was followed by a two-day clearance period in untreated medium and an increased temperature of 24°C to stimulate the resumption of rapid growth. Although the test was conducted based on modifications of standard U.S. EPA/OPPTS and OECD (221) guidelines, it is not considered sufficiently standard or suitable for use in hazard classification, therefore it is not considered further here.

Study 4
Report: (77) Arnie, J. R., Chafey, K. W., Bodle, E. S., Porch, J. R. (2015). Thifensulfuron Methyl (DPX-M6316) Technical: A 7-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba* G3) (DuPont). November 2015.
DuPont Report No: DuPont-44981
Guidelines: U.S. EPA, Series 850 - Ecological Effects Test Guidelines: OCSPP Guideline 850.4400 (2012); OECD Guideline for the Testing of Chemicals: Guideline 221 (2006).
Deviations: None.
Test facility: Wildlife International, Easton, Maryland, U.S.
Testing Facility Project No.: 112P-257

GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was recently submitted following production of the thifensulfuron-methyl RAR (Feb. 2015 revision). This was partly in response to the concern identified in the EFSA 'Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl' (EFSA Journal 2015;13(7):4201) in which it was considered that insufficient reliable data were available to finalise assessment of the risk to aquatic organisms from thifensulfuron-methyl. The submission of this study also serves to clarify the aquatic hazard classification of thifensulfuron-methyl since *Lemna* was one of the most sensitive taxa identified in earlier testing.

Study summary:
The effects of technical thifensulfuron-methyl (98.2% pure) on duckweed (*Lemna gibba* G3) were assessed according U.S. EPA/OCSPP Guideline 850.4400 (2012) which meets the requirements of OECD Guideline 221 (2006); the study was also conducted to GLP. *L. gibba* was exposed for 7 days under semi-static test conditions to nominal concentrations of 0.020, 0.051, 0.13, 0.32, 0.80 and 2.0 µg thifensulfuron-methyl/L. A blank nutrient medium control and a single replicate abiotic (stability) control were also included. Due to any remaining solvent (acetonitrile) in flasks being evaporated under a gentle stream of nitrogen for five minutes, no solvent control was included. Duckweed with an average 3 fronds/plant were exposed at each concentration, there were initially 4 plants per replicate and 4 replicates per concentration and blank control. Two additional replicates were included in each treatment and blank control group for use in a 7-day recovery test (in clean medium following the 7-day exposure period) to determine whether effects were phytostatic or phytocidal.

The study was conducted at a temperature of 24 ± 2ºC, a light intensity of 4,590 to 5,740 lux and a pH of 8.0 to 8.9. Renewal of the test medium occurred on days 3 and 5. Measured concentrations of thifensulfuron methyl were determined from each test concentration and blank control at test initiation, from new and old solutions at each renewal and at test termination (day 7). Samples were analyzed by HPLC with tandem mass spectrometric detection (LC/MS/MS). Frond counts were recorded from all replicates on test days 0, 3 and 5. Frond count, biomass and corresponding yields and growth rates were also determined from the same replicates at test termination. Any chlorosis, necrosis or other frond abnormalities were also noted. The EC_{50} values were determined using non-linear regression and comparison of treatment and control groups using analysis of variance and Dunnett's t-test.

The measured concentrations of thifensulfuron methyl ranged from 90.8 to 107% of nominal concentrations. Although these were within 80-120% of nominals, endpoints were based on the geometric mean measured concentrations of 0.020, 0.053, 0.14, 0.37, 0.84 and 2.2 µg a.s./L. Validity criteria in relation to frond doubling time were met. The percentage growth inhibition relative to the blank control was expressed in terms of frond count, frond count yield, biomass, biomass yield and growth rates for biomass and frond count.

Following 7 days at mean measured concentrations of 0.020, 0.053, 0.14, 0.37, 0.84 and 2.2 µg a.s./L there was 3, -14, 1, 7, 62 and 84% inhibition based on frond count, respectively. Chlorosis, necrosis or other abnormal growth were reported in 15% of fronds at 0.37 µg a.s./L and 56% at 0.84 µg a.s./L. Only the frond count growth rate endpoints required for hazard classification of thifensulfuron-methyl are reported here - and these were as follows:

0-7 day mean measured E_rC_{50} = 1.1 µg a.s./L (≡ 0.0011 mg a.s/L) (95% CL: 1.0 to 1.3 µg a.s./L).
0-7 day mean measured NOE_rC = 0.37 µg a.s./L (≡ 0.00037 mg a.s/L).

Although not relevant for classification, it was reported from the recovery test, that the effects of thifensulfuron methyl on *Lemna gibba* are expected to be reversible (phytostatic) at concentrations ≤ 0.84 µg a.s./L and irreversible (phytocidal) at concentrations ≥ 2.2 µg a.s./L.

Comments on reliability and relevance of Arnie *et al.* (2015) (77):
This recently submitted test on *Lemna gibba* is potentially of use, not only in addressing EFSA's requirements but for classification purposes. The study was performed to GLP and according to guideline without significant deviation; it was also well reported, with clear endpoints. It is therefore considered fully reliable and suitable for use in hazard classification.

Study 5
Report: (72) Hoberg, J.R. (2011a); Thifensulfuron-methyl (DPX-M6316) technical - growth inhibition of the aquatic macrophyte *Ceratophyllum demersum* (DuPont)
DuPont Report No.: DuPont-30626
Guidelines: None given but the methodology described is similar to the then proposed draft OECD guideline for testing the rooted aquatic macrophyte *Myriophyllum* spp.
Deviations from proposed protocol: None
Testing Facility: Smithers Viscient, Wareham, Massachusetts, USA
Testing Facility Report No.: 97.6529
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The study contained sediment, however natural water/sediment studies evaluated at Section 5.1.2.3 above indicate that thifensulfuron-methyl stays predominantly in the water phase and does not partition. Although not on a standard aquatic macrophyte test species, the study is therefore considered of potential relevance to hazard classification and so is summarised below.

Study summary:
The toxicity of technical thifensulfuron-methyl (98.2% pure) to the non-rooted aquatic macrophyte, coontail weed (*Ceratophyllum demersum*), was determined under static conditions in a 14-day exposure test. The study was conducted with six nominal concentrations of 0.34, 1.0, 3.3, 10, 32, and 100 mg thifensulfuron-methyl/L. A dilution water control and an abiotic stability control were included. Five replicates with 3 plants per replicate were initiated for each test substance concentration and the dilution water control. Each 1-liter test chamber (replicate) contained 0.80 L of the test solution, stocked with 3 plants with shoot lengths of 5 cm each. Each 1-liter test chamber (replicate) also contained one plastic pot with 5 cm of sediment and four slow-release fertiliser pellets. Sediment comprised 5% peat, 75% sand and 20% clay, with pH adjusted to 7.0 with $CaCO_3$ as according to OECD 218. A single test vessel containing no *C. demersum,* fertiliser or sediment was initiated at the highest test concentration for the abiotic stability control. Shoot length and shoot dry weight were assessed at initiation, on Day 7 and at exposure termination (Day 14). On days 0 and 14 analytical samples were taken from each test solution and the active substance content was determined via HPLC. Further methodological and reporting details are given in the RAR (2015).

The test vessels were maintained at a temperature of 22-26°C, pH was 7.7 to 9.8 throughout the exposure period and the photoperiod was 16-hour light/8 hour dark (5700-7600 lux). Mean, measured concentrations ranged from 69-88% of nominal concentration and were 0.24, 0.73, 2.3, 8.1, 25 and 88 mg a.s./L. The Day 14 measured concentration of the 100 mg a.s./L abiotic control was 86% of the nominal concentration.

At termination, chlorosis, necrosis, fragmentation of plants and one plant death were reported at various levels in different test concentrations, full details are given in the RAR (2015). Chlorosis and fragmentation were reported at the lowest measured concentration of 0.24 mg a.s./L. Mean shoot lengths did not appear to be significantly affected after 14 days exposure to thifensulfuron-methyl. There was however a concentration-dependant inhibition of mean shoot dry weight which ranged from 6 to 47% (at 88 mg/L) relative to the control. Based on these results and since no concentration tested resulted in ≥50% inhibition, the 14-day EC_{50} value for dry shoot weight was estimated to be >88 mg a.s./L, the highest mean measured concentration tested.

All tested concentrations of thifensulfuron-methyl resulted in adverse effects after 14 days exposure. At the lowest tested concentration 14/15 plants were observed as chlorotic with increasing levels of necrosis seen at higher concentrations. The 14-day mean measured NOEC for phytotoxicity in *Ceratophyllum demersum* was therefore determined to be <0.24 mg/L. No specific growth rate NOE_rC was given but given the effects seen, this is also conservatively assumed to be <0.24 mg/L. An estimate of the 14-day mean measured E_rC_{50} based on reduction in shoot growth rate was subsequently calculated to be 32.15 mg thifensulfuron-methyl/L.

Comments on reliability and relevance of Hoberg (2011a) (72):
The overall quality of the submitted report is considered to be good, with methodology and results clear and detailed. Although no guideline currently exists for testing this species, the methodology described is similar to the then draft OECD guideline for testing *Myriophyllum* spp. The lack of visual damage and good shoot growth (approx 6 x) in the control over 14 days indicates a suitable test design. The average specific growth rate in the control for main shoot length was calculated by the RMS to be 0.0236 d^{-1}. The test substance was initially stable within 80-120% of nominals but declined after 14 days, however results were based on mean measured concentrations and overall the study is considered to be reliable.

Study 6
Report: (73) Hoberg, J.R. (2011b); Thifensulfuron-methyl (DPX-M6316) technical - growth inhibition of the aquatic macrophyte *Elodea canadensis* (DuPont)
DuPont Report No.: DuPont-30628
Guidelines: None given but the methodology described is similar to the then proposed draft OECD guideline for testing the rooted aquatic macrophyte *Myriophyllum* spp.
Deviations from proposed protocol: None
Testing Facility: Smithers Viscient, Wareham, Massachusetts, USA
Testing Facility Report No.: 97.6528
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The study contained sediment, however natural water/sediment studies evaluated at Section 5.1.2.3 above indicate that thifensulfuron-methyl stays predominantly in the water phase

and does not partition. Although not on a standard aquatic macrophyte test species, the study is therefore considered of potential relevance to hazard classification and so is summarised below.

Study summary:
The toxicity of technical thifensulfuron-methyl (98.2% pure) to the rooted aquatic macrophyte, *Elodea canadensis* was determined under static conditions in a 14-day exposure test. The study was conducted with six nominal concentrations of 0.098, 0.39, 1.6, 6.3, 25 and 100 mg thifensulfuron-methyl/L. A dilution water control and an abiotic (stability) control were included. Five replicates, each containing one pot with 3 plants, were initiated for each test substance concentration and the dilution water control. Each 4-liter test chamber (replicate) contained a pot holding 3 cm of sediment, planted with three plants with shoot lengths of approximately 7 cm each. A single test vessel containing no *E. canadensis* or sediment was initiated at the highest test concentration for the abiotic control. Sediment comprised 5% peat, 75% sand and 20% clay, with pH adjusted to 7.0 with $CaCO_3$ as according to OECD 218. Shoot length and shoot dry weight were assessed at initiation, on Day 7 and at exposure termination (Day 14). On days 0 and 14 analytical samples were taken from each test solution and the active substance content was determined via HPLC. Further methodological and reporting details are given in the RAR (2015).

The test vessels were maintained at a temperature of 22-25°C, pH was 7.7 to (a notably high) 11 throughout the exposure period and the photoperiod was 16-hour light/8 hour dark (4900-8600 lux). Initial measured concentrations ranged from 99-100% of nominals but declined over the 14 days such that mean measured concentrations were 0.058, 0.22, 0.98, 4.0, 16 and 75 mg a.s./L (57-75% of nominals). The Day 14 measured concentration of the 100 mg a.s./L abiotic control was 84% of nominal.

At test termination no morphological abnormalities were observed in the blank control. Increasing levels of necrosis were however seen at all treatment levels. Low levels of mortality and chlorosis was observed amongst some plants at higher treatment levels. Following 14 days exposure to thifensulfuron-methyl, mean shoot length among plants in the blank control was 11 cm. The mean shoot lengths among treated plants ranged from 8.3 to 9.2 cm and reductions were seen at all concentrations. Mean shoot dry weight for the blank control plants on day 14 was 0.0752 g, an increase of 3.8 times from Day 0. The increase in dry weights for treated plants ranged from 2.2 to 3.2 times. Since no concentration tested resulted in ≥50% inhibition, the 14-day EC_{50} value for dry weight was estimated to be >75 mg a.s./L the highest mean measured concentration tested.

All tested concentrations of thifensulfuron-methyl resulted in adverse effects after 14 days exposure. At the lowest tested concentration 20% of plants were observed to be necrotic on day 14 with increasing levels of effects seen at higher concentrations. The 14-day mean measured NOEC for *Elodea canadensis* for phytotoxicity was therefore determined to be <0.058 mg/L. An estimate of the 14-day mean measured E_rC_{50} based on reduction in shoot growth rate was subsequently calculated to be 0.0217 mg thifensulfuron-methyl/L (i.e. less than the lowest concentration tested). No specific growth rate NOE_rC was given but given growth effects seen in all treatments and the low value of the estimated E_rC_{50}, this is also proposed to be <0.058 mg/L.

Comments on reliability and relevance of Hoberg (2011b) (73):
The overall quality of the submitted report is considered to be good, with methodology and results clear and detailed. Although no guideline currently exists for testing this species, the methodology described is similar to the then draft OECD guideline for testing *Myriophyllum* spp. The lack of visual damage and good shoot growth (approx 4 x) in the control over 14 days indicates a suitable test design. The average specific growth rate in the control for main shoot length was calculated by

the RMS to be 0.0469 d^{-1}. The test substance was initially stable within 80-120% of nominals but declined after 14 days, however results were based on mean measured concentrations and overall the study is considered to be reliable – although unable to determine accurate growth rate endpoints.

Study 7

Report: (74) Hoberg, J.R. (2011c); Thifensulfuron-methyl (DPX-M6316) technical - growth inhibition of the aquatic macrophyte *Myriophyllum aquaticum* (DuPont)
DuPont Report No.: DuPont-30627
Guidelines: None given but the methodology described is similar to the then proposed draft OECD guideline for testing *Myriophyllum* spp.
Deviations from proposed protocol: None
Testing Facility: Smithers Viscient, Wareham, Massachusetts, USA
Testing Facility Report No.: 97.653
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The study contained sediment, however natural water/sediment studies evaluated at Section 5.1.2.3 above indicate that thifensulfuron-methyl stays predominantly in the water phase and does not partition. Although not on a standard aquatic macrophyte test species, the study is therefore considered of potential relevance to hazard classification and so is summarised below.

Study summary:
The toxicity of technical thifensulfuron-methyl (98.2% pure) to the aquatic macrophyte *Myriophyllum aquaticum* under static conditions was determined in a 14-day exposure test. The study was conducted with six nominal concentrations of 0.34, 1.0, 3.3, 10, 32 and 100 mg thifensulfuron-methyl/L. A dilution water control and an abiotic (stability) control were included. Five replicates, with 3 plants, were initiated for each test substance concentration and the dilution water control. Each 4-liter test chamber (replicate) contained a pot holding 3 cm of sediment, planted with three plants with exposed shoot lengths of approximately 7 cm each. A single test vessel containing no *M. aquaticum* or sediment was initiated at the highest test concentration for the abiotic control. Sediment comprised 5% peat, 75% sand and 20% clay, with pH adjusted to 6.6 with $CaCO_3$ as according to OECD 218. Shoot length and shoot dry weight were assessed at initiation, on Day 7 and at exposure termination (Day 14). On days 0 and 14 analytical samples were taken from each test solution and the active substance content was determined via HPLC. Further methodological and reporting details are given in the RAR (2015).

The test vessels were maintained at a temperature of 24-25°C, pH was 7.9 to a high 9.6 throughout the exposure period and the photoperiod was 16-hour light/8 hour dark (4600-7700 lux). Initial measured concentrations ranged from 91-97% of nominals but declined over the 14 days such that mean measured concentrations were 0.22, 0.65, 2.2, 6.6, 22 and 77 mg a.s./L (63-68% of nominals). The Day 14 measured concentration in the 100 mg a.s./L abiotic control was 81% of nominal.

At test termination no morphological abnormalities were observed in the blank control or plants exposed to 0.22 mg a.s./L. At higher concentrations, increasing apical bud damage, chlorosis and (from 2.2 mg/L) necrosis was observed after 14 days. A 77 mg a.s./L 12/15 plants were observed as necrotic on Day 14. Following 14 days exposure to thifensulfuron-methyl, mean shoot length among plants in the blank control was 9.9 cm. The mean shoot lengths among treated plants ranged

from 6.8 to 8.5 cm. Mean shoot dry weight for the blank control plants on day 14 was 0.0489 g, an increase of 2.9 times from Day 0. The increase in dry weights for treated plants ranged from 1.6 to 2.4 times. Since no concentration tested resulted in ≥50% inhibition, the 14-day E_bC_{50} value for dry weight was estimated to be >77 mg a.s./L the highest mean measured concentration tested.

Based on effects seen at 0.65 mg/L and above, the 14-day mean measured NOEC for observed phytotoxicity in *Myriophyllum aquaticum* was determined to be 0.22 mg thifensulfuron-methyl/L. An estimate of the 14-day mean measured E_rC_{50} based on reduction in shoot growth rate was subsequently calculated to be 0.1871 mg /L (i.e. less than the lowest concentration tested). A specific growth rate NOE_rC was not given but given growth effects seen in all treatments and the calculated E_rC_{50}, this is proposed to be <0.22 mg/L.

The Applicant has subsequently questioned the growth rate endpoint estimated in the RAR and EFSA Conclusion. Since no thifensulfuron methyl concentration resulted in ≥50% inhibition of dry shoot weight, the 14-day E_bC_{50} was estimated to be >77 mg a.s./L, the highest mean measured concentration tested. Assuming this shoot weight E_bC_{50} was greater than the highest dose tested, they are uncertain how the E_rC_{50} for shoot growth can be below the lowest concentration tested. Based on the lack of effects at 0.22 mg/L and the shoot dry weight E_bC_{50} of >77 mg a.s./L they have proposed that the normally higher NOE_rC should conservatively be estimated to also be 0.22 mg/L.

Comments on reliability and relevance of Hoberg (2011c) (74):
The overall quality of the submitted report is considered to be good, with methodology and results clear and detailed. A new OECD test guideline (No. 239) is available for *Myriophyllum* spp. and the methodology described is similar to the draft available at the time. The lack of visual damage and good shoot growth (approx 3 x) in the control over 14 days indicates a suitable test design. The average specific growth rate in the control for main shoot length was calculated by the RMS to be 0.0341 d^{-1}. The test substance was initially stable within 80-120% of nominals but declined after 14 days, however results were based on mean measured concentrations and overall the study is considered to be reliable. The discrepancy over the E_rC_{50} value determined in the RAR and EFSA Conclusion and by the Applicant will be highlighted to the pesticide RMS, however the UK CA will retain the EFSA agreed E_rC_{50} for now and therefore also considers the NOE_rC for *Myriophyllum aquaticum* to be <0.22 mg/L. As these are not the pivotal endpoints for hazard classification (or risk assessment), this choice currently makes no substantive difference.

Study 8

Report: (75) Hoberg, J.R. (2011d); Thifensulfuron-methyl (DPX-M6316) technical - growth inhibition of the aquatic macrophyte *Vallisneria americana* (DuPont)
DuPont Report No.: DuPont-30624
Guidelines: None given but the methodology described is similar to the then proposed draft OECD guideline for testing the rooted aquatic macrophyte *Myriophyllum* spp.
Deviations from proposed protocol: None
Testing Facility: Smithers Viscient, Wareham, Massachusetts, USA
Testing Facility Report No.: 97.6531
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The study contained sediment, however natural water/sediment studies evaluated at

Section 5.1.2.3 above indicate that thifensulfuron-methyl stays predominantly in the water phase and does not partition. Although not on a standard aquatic macrophyte test species, the study is therefore considered of potential relevance to hazard classification and so is summarised below.

Study summary:
The toxicity of thifensulfuron-methyl to the rooted aquatic monocot macrophyte *Vallisneria americana* (known as eelgrass, tape grass or water parsley), was determined under static conditions over a 14-day exposure period. The study was conducted with seven nominal concentrations of 0.00045, 0.0021, 0.0094, 0.043, 0.19, 0.88 and 4.0 mg thifensulfuron-methyl/L. A dilution water control and an abiotic (stability) control were included. Five replicates, with 3 plants, were initiated for each test substance concentration and the dilution water control. Each 4-liter test chamber (replicate) contained a pot holding 3 cm of sediment, planted with three plants reared from tubers/winter buds) with exposed shoot lengths of approximately 16 cm each. A single test vessel containing no *V. americana* or sediment was initiated at the highest test concentration for the abiotic control. Sediment comprised 5% peat, 75% sand and 20% clay, with pH adjusted to 7.4 with $CaCO_3$ as according to OECD 218. Shoot length and shoot dry weight were assessed at initiation, on Day 7 and at exposure termination (Day 14). On days 0 and 14 analytical samples were taken from each test solution and the active substance content was determined via HPLC. Further methodological and reporting details are given in the RAR (2015).

The test vessels were maintained at a temperature of 23-25°C, pH was 7.7 to a high 10 throughout the exposure period and the photoperiod was 16-hour light/8 hour dark (4500-7400 lux). Initial measured concentrations very close to nominals (>92%) but declined over the 14 days such that mean measured concentrations were 0.00025, 0.0011, 0.0046, 0.021, 0.098, 0.41 and 1.9 mg a.s./L (47-55% of nominals). Levels in the lowest four concentrations were <LOQ by Day-14. Based on recommendations in the OECD 'Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures' (2000), one-half the LOQ was used to calculate the mean, measured concentration when the measured concentration was below detectable limits at test termination. The Day 14 measured concentration in the 4.0 mg a.s./L abiotic control was 65% of nominal.

At test termination no morphological abnormalities were observed in the blank control or plants exposed to 0.00025 and 0.021 mg a.s./L. Leaf curl was observed among six and four plants exposed at 0.0011 and 0.0046 mg a.s./L respectively but was not considered to be adverse. No mortality was observed in any of the treatment levels tested. Chlorosis was observed among all plants exposed to the 0.098, 0.41 and 1.9 mg a.s./L. Following 14 days exposure to thifensulfuron-methyl, mean shoot length among plants in the blank control was 31.9 cm. The mean shoot lengths among treated plants ranged from 18.7 cm at the second highest concentration to 27.6 cm at the lowest. Mean shoot dry weight for the blank control plants on day 14 was 0.1986 g, an increase of 3.4 times from Day 0. The increase in dry weights for treated plants ranged from 2.1 times at the three highest concentrations to 2.9 times at the lowest. Since no concentration tested resulted in ≥50% inhibition, the 14-day E_bC_{50} value for dry weight was estimated to be >1.9 mg a.s./L the highest mean measured concentration tested.

Based on chlorosis seen at 0.098 mg/L and above, the 14-day mean measured NOEC for observed phytotoxicity in *Vallisneria americana* was determined to be 0.021 mg thifensulfuron-methyl/L. An estimate of the 14-day mean measured E_rC_{50} based on reduction in shoot growth rate was subsequently calculated to be 0.0011 mg/L. A specific growth rate NOE_rC was not given but given the calculated E_rC_{50} and based on 13.5% reduction in shoot length and 15% reduction in dry weight at the lowest concentration, the mean measured NOE_rC is proposed to be <0.00025 mg thifensulfuron-methyl/L.

Comments on reliability and relevance of Hoberg (2011d) (75):
The overall quality of the submitted report is considered to be good, with methodology and results clear and detailed. Although no guideline currently exists for testing this species, the methodology described is similar to the then draft OECD guideline for *Myriophyllum* spp. The lack of visual damage and good shoot growth (approx 3.5 x) in the control over 14 days indicates a suitable test design. The average specific growth rate in the control for main shoot length was calculated by the RMS to be 0.0778 d^{-1}. The test substance was initially stable within 80-120% of nominals but declined after 14 days, however results were based on mean measured concentrations and overall the study is considered to be reliable – although clear growth rate endpoints were not initially determined.

Study 9
Report: (76) Hoberg, J.R. (2011e); Thifensulfuron-methyl (DPX-M6316) technical - growth inhibition of the aquatic macrophyte *Myriophyllum spicatum* (DuPont)
DuPont Report No.: DuPont-30625
Guidelines: None given but the methodology described is similar to the then proposed draft OECD guideline for testing *Myriophyllum* spp.
Deviations from proposed protocol: None
Testing Facility: Smithers Viscient, Wareham, Massachusetts, USA
Testing Facility Report No.: 97.6532
GLP: Yes
Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

This study was submitted for renewal of thifensulfuron-methyl and evaluated in the detail in the RAR (2015). The study contained sediment, however natural water/sediment studies evaluated at Section 5.1.2.3 above indicate that thifensulfuron-methyl stays predominantly in the water phase and does not partition. The study is therefore considered of potential relevance to hazard classification and so is summarised below.

Study summary:
The toxicity of technical thifensulfuron-methyl (98.2% pure) to the aquatic macrophyte *Myriophyllum spicatum* was determined under static conditions in a 14-day exposure test. The study was conducted with five nominal concentrations of 0.34, 1.0, 3.3, 32 and 100 mg thifensulfuron-methyl/L. A dilution water control and an abiotic (stability) control were included. Five replicates, with 3 plants, were initiated for each test substance concentration and the dilution water control. Each 4-liter test chamber (replicate) contained a pot holding 3 cm of sediment with exposed shoot lengths of approximately 7 cm each. A single test vessel containing no *M. spicatum* or sediment was initiated at the highest test concentration for the abiotic control. Sediment comprised 5% peat, 75% sand and 20% clay, with pH adjusted to 6.6 with $CaCO_3$ as according to OECD 218. Shoot length and shoot dry weight were assessed at initiation, on Day 7 and at exposure termination (Day 14). On days 0 and 14 analytical samples were taken from each test solution and the active substance content was determined via HPLC. Further methodological and reporting details are given in the RAR (2015).

The test vessels were maintained at a temperature of 23-25°C, pH was 7.7 to a high 10 throughout the exposure and the photoperiod was 16-hour light/8 hour dark (4400-7500 lux). Initial measured concentrations very close to nominals (>91%) but declined over the 14 days such that mean

measured concentrations were 0.20, 0.59, 2.1, 22 and 75 mg a.s./L (59-75% of nominals). The Day 14 measured concentration in the 100 mg a.s./L abiotic control was 63% of nominal.

At test termination no morphological abnormalities were observed in the blank control. A total of 1, 2 and 9 dead plants were observed in the 0.20, 2.1 and 75 mg a.s./L treatment levels, respectively. Significant necrosis and apical bud damage was observed (in a non-concentration related way) in plants exposed to 0.20 mg/L and above. Following 14 days exposure to thifensulfuron-methyl, mean shoot length among plants in the blank control was 12.8 cm. The mean shoot lengths among treated plants ranged from 8.5to 9.2 cm, with plants at all concentrations being affected. Mean shoot dry weight for the blank control plants on day 14 was 0.0733 g, an increase of 3.7 times from Day 0. The increase in dry weights for treated plants ranged from 0.4 to 2.4 times (inhibited by 34% in the lowest concentration to 89% at the highest). The 14-day E_bC_{50} value was determined to be 0.94 mg a.s./L with an upper 95% confidence interval of 11 mg a.s./L. A lower 95% confidence interval could not be calculated.

Based on 53% necrosis and apical bud damage seen at 0.2 mg/L, the 14-day mean measured phytotoxicity NOEC for *Myriophyllum spicatum* was determined to be <0.2 mg thifensulfuron-methyl/L. An estimate of the 14-day mean measured E_rC_{50} based on reduction in shoot growth rate was subsequently calculated to be 0.0516 mg /L (less than the lowest concentration tested). A specific growth rate NOE_rC was not given but based on the E_rC_{50} and significant reductions in shoot length and dry weight at the lowest concentration, the mean measured NOE_rC is also assumed to be <0.2 mg/L.

Comments on reliability and relevance of Hoberg (2011e) (76):
The overall quality of the submitted report is considered to be good, with methodology and results clear and detailed. A new OECD test guideline (No. 239) is available for *Myriophyllum* spp. and the methodology described is similar to the draft available at the time. The lack of visual damage and good shoot growth (approx 3.7 x) in the control over 14 days indicates a suitable test design. The average specific growth rate in the control for main shoot length was calculated by the RMS to be 0.0552 d^{-1} (greater than the finalised guideline requires). The test substance was initially stable within 80-120% of nominals but declined after 14 days, however results were based on mean measured concentrations and overall the study is considered to be reliable – although clear growth rate endpoints were not initially determined.

5.4.5 Other aquatic organisms (including sediment)

A study is available on the acute toxicity of thifensulfuron-methyl to the free-swimming 1st instar larvae of the midge *Chironomus riparius*. Unlike most sediment-dweller tests, this was an acute study only and did not include emergence or a sediment phase and so it is considered relevant for use in acute hazard assessment for the aqueous compartment. As it was not a sediment test, it has therefore been included above with other acute aquatic invertebrate tests in Section 5.4.2.1.

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

Abiotic and biotic degradation

Hydrolysis of thifensulfuron-methyl is highly variable and influenced by both temperature and pH. At the more environmentally realistic temperature tested of 20°C, hydrolysis DT_{50}s were 6.3 days at pH 4, 199 days at pH 7 and 23.4 days at pH 9. In a second hydrolysis study conducted at 25°C hydrolysis DT_{50}s were 2.4, 137 and 7.1 days at pH 4, 7 and 9 respectively - again showing high variability and pH dependence. As the hydrolysis half life is not consistently <16 days for all environmentally relevant pH, thifensulfuron-methyl screens as 'not rapidly degradable'.

Aqueous photolysis studies indicate that under certain environmental condition, rapid photolysis of thifensulfuron-methyl can occur. However, in typical turbid European natural surface waters, particularly at higher latitudes and outside of summer periods, photolysis is not expected to be such a significant or consistent route of degradation.

In a ready biodegradation study no substantive degradation of thifensulfuron-methyl was observed over 29 days (1%). Thifensulfuron-methyl is therefore considered to be 'not readily biodegradable'.

In aerobic natural water/sediment systems, thifensulfuron-methyl was found to stay predominantly in the water phase with little dissipation to sediment. Whole system degradation DT_{50}s were calculated to be 17.6-32.3 with a geomean DT_{50} across 4 systems of 22.8 days. Mineralisation rates were low at <3 to <9%. A large number of mainly hydrolysis and photolysis degradants were isolated, some at >10% of applied radioactivity. However, as the aquatic toxicity of these is less than for parent thifensulfuron-methyl (see Annex 1) they are not considered further in relation to hazard classification of the parent substance.

Overall, 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). Neither is it transformed sufficiently rapidly into entirely non-classifiable degradants. Consequently, thifensulfuron-methyl is considered to be 'not rapidly degradable' for the purposes of classification under the CLP Regulation.

Bioaccumulation

Thifensulfuron-methyl has a log K_{ow} at pH 7 of -1.65 which is below the CLP trigger of 4 indicating a low potential for bioaccumulation. This was confirmed in an experimental study on bluegill sunfish where the whole fish bioconcentration factor (BCF) was <0.8 and substantially less than the CLP BCF trigger of 500. Overall, bioaccumulation of thifensulfuron-methyl will not impact on its chronic aquatic hazard classification or M-factor.

Aquatic toxicity

Available toxicity endpoints for aquatic organisms are summarised above in Table 27, the relevance of these for hazard classification is considered further below.

Discussion on acute and chronic classification endpoints for fish:

Two acute fish toxicity studies on rainbow trout and bluegill sunfish are available (1983 a&b) (59&60). These each gave 96 hour LC_{50}s of >100 mg thifensulfuron-methyl/L. During the recent EFSA peer review of thifensulfuron-methyl, these studies were considered unreliable since they did not include analysis of test concentrations and endpoints were based on nominals. Whilst this is agreed, other evidence, including from prolonged toxicity tests and from thifensulfuron-methyl formulation studies on fish, suggests that the herbicide thifensulfuron-methyl is of low acute toxicity to fish. The above LC_{50}s are therefore considered to be sufficiently accurate to indicate that thifensulfuron-methyl does not require classification regarding its acute toxicity to fish.

No 'true' chronic toxicity study on fish is available, however a prolonged 21-day study has been submitted on rainbow trout using thifensulfuron-methyl and also a 75% w/v formulation. These investigated sub-lethal parameters including fish length, weight and behavioural effects but not reproductive effects. The 21-day measured NOECs were each >100 mg thifensulfuron-methyl/L (250 mg/L actual). Although not 'rapidly degradable', thifensulfuron-methyl is not especially persistent or bioaccumulative in aquatic systems, therefore the available prolonged studies are considered sufficient to indicate a low chronic toxicity and that thifensulfuron-methyl does not require classification regarding its chronic toxicity to fish.

Discussion on acute and chronic classification endpoints for aquatic invertebrates:

Two acute toxicity studies on *Daphnia magna* are available. One early study by Wetzel (1986) proposed a 48 hour EC_{50} of 470 mg/L based on mean measured concentrations. The other *D. magna* study (Hutton (1989a) (63) gave a 48 hour EC_{50} >970 mg/L based on mean measured concentrations, however this included feeding of daphnids which is not standard in acute tests but which was not thought to have significantly affected the result. Another reliable acute invertebrate study is available on 1st instar *Chironomous riparius* larvae (with no sediment included), this gave a nominal 48 hour EC_{50} >100 mg/L. Considering all of the studies together, the acute EC_{50} for invertebrates is likely to exceed 100 mg thifensulfuron-methyl/L and so no acute aquatic hazard classification would be required on this basis.

One reliable chronic study on *Daphnia magna* is available (Hutton, 1989b) (65), this gave a measured 21-day NOEC of 100 mg thifensulfuron-methyl/L which also indicates a low chronic hazard and no need for chronic classification for aquatic invertebrates.

Discussion on acute and chronic classification endpoints for algae:

A number of studies are available on the toxicity of thifensulfuron-methyl to algae/diatoms. These cover four species with *Pseudokirchneriella subcapitata* and *Anabaena flos-aquae* tested twice. The studies provide short-term EC_{50}s for 'acute' hazard classification and NOECs for chronic classification. Where available, endpoints relating to growth rate (i.e. E_rC_{50} and NOE_rC) are preferred for hazard classification. The study on *Pseudokirchneriella subcapitata* from the original 1996 DAR (Douglas and Handley,1987) (66) both based on initial measured concentrations. These endpoints have been tentatively recalculated based on mean measured concentrations as an E_rC_{50} of 0.00162 mg/L and a NOEC of 0.00116 mg/L. Although the *N. pelliculosa* NOEC covers a longer than usual duration for algal tests and it is not clear that it is also based on growth rate, it would be the lowest precautionary algal endpoint to use for chronic classification. These are still not the lowest acute and chronic classification endpoints however – see those for macrophytes below.

Discussion on acute and chronic classification endpoints for aquatic macrophytes:

As expected, this sulfonylurea herbicide thifensulfuron-methyl is most toxic to aquatic macrophytes. 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. No consideration of surrogate approaches is required.

Studies are available on its effect on seven plant species, including two *Lemna* and two *Myriophyllum* species, which are now standard test organisms for herbicides under EU pesticides legislation. These studies provide short-term EC_{50}s for 'acute' hazard classification and NOECs for 'chronic' classification. As with algae, endpoints relating to growth rate (E_rC_{50} and NOE_rC) are preferred for hazard classification where available. Of the species tested, *Lemna gibba* appears from the studies by Kannuck and Samel (1995) (70) and Arnie *et al.* (2015) (77) to be most sensitive, with acute E_rC_{50}s of 0.00087 to 0.0011 mg a.s./L and chronic NOE_rCs of 0.00023 to 0.00037 mg a.s./L, both based on mean measured concentrations over 14 or 7 days. During EFSA peer review of thifensulfuron-methyl, the reliability of the Kannuck and Samel study was called in to question 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 (see discussion at Section 5.4.4, 'Study 2'). The original DAR 1996 *Lemna minor* study by Douglas and Handley (1988) (69) was also considered unreliable in EFSA peer review since it did not include analytical verification of test concentrations (unvalidated nominals were used). The higher tier study on *Lemna gibba* by Porch *et al*. (2011) (71) which made use of variable exposure and recovery durations is also discounted for risk (and hazard) assessment. However, the recently submitted Arnie *et al.* (2015) study on *L. gibba* is considered fully reliable and relevant.

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) (75) for interim risk assessment. These were an acute 14-day E_rC_{50} of 0.0011 mg/L and a chronic 14-day NOE_rC of <0.00025 mg a.s./L (this is revised downwards by the UK CA from that proposed in the RAR) both based on mean measured concentrations. The 2015 EFSA Conclusion meanwhile stated that further data (especially a reliable *Lemna* study on thifensulfuron-methyl) were still required to assess and conclude on the risk to aquatic organisms from the active substance. This has now been submitted in the form of the Arnie *et al.* (2015) study on *L. gibba* (77).

The UK CA 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 a.s./L). The *Lemna gibba* study by Kannuck and Samel (1995) (70) may well also not be entirely accurate in its determination of measured concentrations (although it was otherwise performed and reported reliably). Therefore, for aquatic hazard classification, it is proposed to use the new reliable endpoints from the study by Arnie *et al* (2015). These are..:

For 'acute' hazard classification; the measured ***Lemna gibba*** **7-day E_rC_{50} of 0.0011 mg/L** - which is the same as the 14-day measured E_rC_{50} for *V. americana*, slightly lower than the potentially unreliable 14-day nominal E_rC_{50} for *L. minor* (0.002 mg/L) but slightly higher than the potentially unreliable 14-day measured E_rC_{50} for *L. gibba* (0.00087 mg/L) from Kannuck and Samel (1995).

For the 'chronic' hazard classification; the measured ***Lemna gibba*** **7-day NOE_rC of 0.00037 mg/L** - which is slightly higher than the imprecise 'less than' NOE_rC of <0.00025 mg/L proposed for *V.*

americana and the potentially unreliable 14-day 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-day nominal NOE_rC for *Lemna minor* (0.0005 mg/L).

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

As discussed in Section 5.5, it is proposed to use the reliable measured 7-day E_rC_{50} for *Lemna gibba* of 0.0011 mg/L (from Arnie *et al*. (2015)(77))for acute aquatic hazard classification. This is in the range 0.001 mg/l $< L(E)C_{50} \leq$ 0.01 mg/l, and so thifensulfuron-methyl should be classified under CLP as:

Acute Category 1 with an acute M-factor of 100

Also as discussed in Section 5.5, it is proposed to use the reliable 7-day NOE_rC for *Lemna gibba* of 0.00037 mg/L (from Arnie *et al.*(2015)(77) for chronic aquatic hazard classification. This is in the range 0.0001 mg/l $< NOEC \leq$ 0.001 mg/l, and since thifensulfuron-methyl is 'non-rapidly degradable' according to CLP criteria, it should be classified as:

Chronic Category 1 with a chronic M-factor of 100

In conclusion..:

Aquatic Acute category 1; H400: Very toxic to aquatic life
Acute M-factor = 100
Aquatic Chronic category 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M-factor = 100

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

TSM 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 TSM 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 TSM screens as stable to hydrolysis.

Aqueous photolysis is envisaged to contribute significantly to the degradation of TSM 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 TSM 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 TSM 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 TSM 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 TSM 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 TSM 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 TSM, 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 TSM is ultimately degraded (>70%) within 28 days (equivalent to a degradation half-life of <16 days). Consequently, the DS considered TSM as not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

The log Kow of TSM 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 TSM, 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 TSMand 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 TSM as having a low potential for bioaccumulation for the purpose of classification.

Aquatic Toxicity

The ecotoxicological tests results for TSM from available acute and chronic studies are summarised in the following table and sections. Only the valid acute and chronic studies on TSM 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 TSM 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 TSM.

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)
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 TSM 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 TSM formulation studies on fish, suggests that the herbicide TSMis 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 TSM, the Kannuck and Samel study was considered unreliable due to concerns that the ELISA and HPLC methods used to measure TSMwere 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 TSM) 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 TSMas:

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} ≤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 TSMas 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 TSMdoes 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 TSMis ultimately degraded (>70%) within 28 days (equivalent to a degradation half-life of <16 days). Consequently, TSMis considered to be not rapidly degradable for the purposes of classification under the CLP Regulation.

Aquatic Bioaccumulation

TSMhas 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 TSMis 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 TSMhas 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 TSMis 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 TSMis a 7-day chronic NOE_rC=0.00037 mg/L, based on mean measured concentrations for aquatic plants (*Lemna gibba*).

Conclusion on classification

TSMis 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 TSMshould 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.**

6 OTHER INFORMATION

No other relevant information available.

7 REFERENCES

All references are taken from the Renewal Assessment Report (RAR)

Thifensulfuron-methyl - Volume 3, Annex B.2: Physical and Chemical Properties – July 2014

Thifensulfuron-methyl - Volume 3, Annex B.6 : Toxicology and Metabolism – July 2014

Thifensulfuron-methyl - Volume 3, Annex B.8 : Environmental fate and behaviour

Thifensulfuron-methyl - Volume 3, Annex B.9 : Ecotoxicology

Physico-chemical properties

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8 ANNEXES

Annex 1: Information on environmental degradants of thifensulfuron-methyl

Table 1: Environmentally relevant degradant codes and locations

Substance	Compartments of relevance
Thifensulfuron-methyl (a.s.)	Soil, surface water
IN-A4098 (triazine amine) AKA CGA 150829, AE F059411	Soil, surface water, groundwater
IN-A5546 (2-thiophenecarboxylic acid, 3-(aminosulfonyl)-methyl)	Soil, surface water
IN-JZ789 (O-desmethyl thifensulfuron acid)	Soil, surface water, groundwater
IN-L9223 (2-acid-3-sulfonamide)	Soil, surface water, groundwater
IN-L9225 (thifensulfuron acid)	Soil, surface water, groundwater
IN-L9226 (O-desmethyl thifensulfuron-methyl)	Soil, surface water
IN-V7160 (triazine urea)	Soil, surface water
IN-W8268 (thiophene sulphonimide)	Soil, surface water, groundwater
2-acid-3-triuret	Soil, surface water, groundwater
IN-RDF00	Surface water
IN-B5528	Surface water
IN-D8858	Surface water

Environmental fate studies have been conducted on the degradants stated below:

IN-L9225/TH-A (thifensulfuron acid)

IN-L9223/TP-SA-A (2-acid-3-sulfonamide)

IN-JZ789 (O-desmethyl thifensulfuron acid)

IN-V7160/TA-U (triazine urea)

IN-A4098/MM-TA (triazine amine; 2-amino-4-methoxy-6-methyl-1,3,5-triazine) also referred to as CGA 150829, AE F059411

IN-W8268/TP-SI (thiophene sulfonimide)

IN-A5546

IN-L9226/DM-TH (O-desmethyl thifensulfuron-methyl; hydroxy-TM)

2-acid-3-triuret

IN-B5528

Aquatic toxicity data on degradants of thifensulfuron-methyl are included in the following Table:

Table 2: Aquatic toxicity data on degradants of thifensulfuron-methyl

Duration and test compound	Species	Endpoint	Toxicity value	Reference
Fish				
Acute toxicity IN-L9225	Rainbow trout *(Oncorhynchus mykiss)*	96 hr LC50	>1.0 mg/L (mm)	(1999)
Acute toxicity IN-L9225 (DuPont)			>120 mg/L (mm)	(2001)
Acute toxicity IN-L9223			>1.1 mg/L (mm)	(1999)
Acute toxicity IN-JZ789			>0.94 mg /L (mm)	(1999)
Acute toxicity IN-V7160			>1.0 mg /L (mm)	(1999)
Acute toxicity IN-A4098 (DuPont)			>200 mg/L (nom)	(1988)
Acute toxicity IN-A4098 (DuPont)			>0.93 mg/L (mm)	(1999)
Acute toxicity IN-W8268 (DuPont)			>115 mg/L (mm)	(2000)
Aquatic invertebrates				
Acute toxicity IN-L9225	Water flea *(Daphnia magna)*	48 hr EC_{50}	> 0.044 mg/L (nom)	Hutton, D.G (1989)
Acute toxicity IN-L9225			>0.8 mg/L (mm)	Samel, A (1999)
Acute toxicity IN-L9225 (DuPont)			>130 mg/L (mm)	Samel, A. (2001)
Acute toxicity IN-L9223			>1.2 mg/L (mm)	Samel, A (1999)
Chronic toxicity IN-L9223 (TSM)		NOEC	31 mg/L (nom)	Vinken, R., Wydra, V. (2007a)
Chronic toxicity IN-L9223 (DuPont)			13 mg/L (mm)	Samel, A. (2000)
Acute toxicity IN-JZ789		48 hr EC_{50}	>1.1 mg/L (mm)	Hoke, R.A (1999)
Acute toxicity			>1.3 mg/L (mm)	Samel, A (1999)

Duration and test compound	Species	Endpoint	Toxicity value	Reference
IN-V7160				
Chronic toxicity IN-V7160 (TSM)		NOEC	31 mg/L (nom)	Vinken, R., Wydra, V. (2007b)
Chronic toxicity IN-V7160 (DuPont)			11 mg/L (mm)	Hoke, R.A. (2001)
Acute toxicity IN-A4098 (DuPont)		48 hr EC_{50}	>99 mg/L (mm)	Samel, A. (1999)
Acute toxicity IN-A4098 (DuPont)			>100 mg/L (nom)	Heusel, R., Weller, O., Gosch, H. (1998)
Chronic toxicity IN-A4098 (TSM)		NOEC	32 mg/L (nom)	Grade, R., Wydra, V., Moll, M. (2006)
Chronic toxicity IN-A4098 (DuPont)			97 mg/L (mm)	Samel, A. (1999)
Acute toxicity IN-W8268 (DuPont)		48 hr EC_{50}	>125 mg/L (mm)	Samel, A. (2000)
Algae				
Toxicity IN-L9225	Green microalgae *(Pseudokirchneriella subcapitata)*	EC_{50} (72 h)	>1.02 mg/L (mm)	Sloman T.L (1999)
Toxicity IN-L9225 (DuPont)		E_bC_{50} (72 h)	33.4 mg/L (nom) cell density	Sloman, T.L. (2001)
		E_rC_{50} (72 h)	36.5 mg/L (nom)	
Toxicity IN-L9223		EC_{50} (72 h)	>1.3 mg/L (mm)	Sloman T.L (1999)
Toxicity IN-JZ789			>1.28 mg/L (mm)	Sloman T.L (1999)
Toxicity IN-V7160		EC_{50} (72 h)	>11 mg/L (mm)	Sloman T.L (1999)
Toxicity IN-A4098 (TSM)		E_bC_{50} and E_rC_{50} (72 h)	>100 mg/L (nom)	S. Pawlowski, V. Wydra (2006a)
Toxicity IN-A4098 (DuPont)			>100 mg/L (nom)	Heusel, R., Weller, O., Gosch, H. (1998)
Toxicity IN-A4098 (DuPont)			>10 mg/L (nom)	Sloman, T.L. (1999)
Toxicity IN-A4098 (DuPont)	*Scenedesmus subspicatus*	E_bC_{50} and E_rC_{50} (72 h)	> 90 mg/L (nom)	Rufli, H. (1987)

Duration and test compound	Species	Endpoint	Toxicity value	Reference
Toxicity IN-W8268 (TSM)	Green microalgae (*Pseudokirchneriella subcapitata)*	E_bC_{50}, E_rC_{50} and E_yC_{50} (72 h)	>100 mg/L (nom)	Vinken, R., Wydra, V. (2007c)
Toxicity IN-W8268 (DuPont)		E_bC_{50} (72 h)	29.9 mg/L (nom) cell density	Sloman, T.L. (2000) (submitted for renewal)
		E_rC_{50} (72 h)	31.6 mg/L (nom)	
Toxicity IN-L9226 (TSM)		E_bC_{50}, E_rC_{50} and E_yC_{50} (72 h)	>89 mg/L (nom) based on 89.1% purity	Vinken, R., Wydra, V. (2007d)
Toxicity IN-A5546 (DuPont)		E_bC_{50} (72 h)	48 mg/L (mm)	Hoberg, J.R. (2007)
		E_rC_{50} (72 h)	>110 mg/L (mm)	
Toxicity 2-acid-3-triuret (TSM)		E_yC_{50} and E_rC_{50} (72 h)	>100 mg/L (nom)	Falk S. (2012)
Aquatic macrophytes				
Toxicity IN-L9225	Duckweed *(Lemna gibba)*	14 d EC_{50} E_rC_{50}	>1 mg/L (mm) >1 mg/L (mm)	Sloman T.L, Leva, S.E (1997)
Toxicity IN-L9225 (DuPont)		14 d EC_{50} 14 d ErC_{50}	36.76 mg/L (mm) 82.2 mg/L (mm)	Boeri, R.L., Wyskiel, D.C., Ward, T.J. (2001)
Toxicity IN-L9223		14 d EC_{50} E_rC_{50}	>1 mg/L (nom) >1 mg/L (nom)	Sloman T.L, (1999)
Toxicity IN-L9223 (DuPont)		14 day E_bC_{50} and EC_{50} E_rC_{50}	>172.1 mg/L (nom) >172.1 mg/L (nom)	Sloman, T.L. (2001b)
Toxicity IN-JZ789		14 d EC_{50} E_rC_{50}	>1 mg/L (nom) >1 mg/L (nom)	Sloman T.L, (1999)
Toxicity IN-JZ789 (DuPont)		14 day E_bC_{50} and EC_{50} E_rC_{50}	>100 mg/L (nom)	Sloman, T.L. (2001a)
Toxicity IN-V7160		14 d EC_{50} E_rC_{50}	>10 mg/L (nom) >10 mg/L (nom)	Sloman T.L, (1999)
Toxicity IN-V7160 (DuPont)		14 day E_bC_{50} and EC_{50} E_rC_{50}	>100 mg/L (nom) >100 mg/L (nom)	Sloman, T.L. (2001c)
Toxicity IN-A4098 (TSM)		7-day EC_{50}	>100 mg/L (nom)	S. Pawlowsky, V. Wydra (2006b)
Toxicity IN-A4098 (DuPont)		14 day E_bC_{50} and EC_{50} E_rC_{50}	>10 mg/L (nom)	Sloman, T.L., Leva, S.E. (1998)
Toxicity IN-A4098 (DuPont)		7 day E_rC_{50} 7 day E_bC_{50}	>100 mg/L (nom)	Sowig, P. (2002)

Duration and test compound	Species	Endpoint	Toxicity value	Reference
Toxicity IN-W8268 (TSM)		7-day E_yC_{50} 7 day E_rC_{50}	30.3 mg/L (nom) >100 mg/L (nom)	Vinken R., V. Wydra (2007e)
Toxicity IN-W8268 (DuPont)		14 day E_bC_{50}	>100 mg/L (nom)	Sloman, T.L. (2000)
		14 day EC_{50} E_rC_{50}	39.5 mg/L (nom) >100 mg/L (nom)	
Toxicity IN-L9226 (TSM)		7-day E_yC_{50} 7 day E_rC_{50}	0.17 mg/L (mm) 0.31 mg/L (mm) (endpoints corrected for 89.1% purity)	Vinken, R., Wydra, V. (2007f)
Toxicity IN-L9226 (DuPont)		14-day EC_{50} (all parameters)	>37.5 mg/L (mm)	Sloman T.L. (2004)
Toxicity IN-A5546 (DuPont)		7-day E_yC_{50} 7 day E_rC_{50} 7 day E_bC_{50}	>40.4 mg/L (mm)	Sloman T.L. (2006)
Toxicity 2-acid-3-triuret (TSM)		7-day E_yC_{50} 7 day E_rC_{50}	>100 mg/L (nom)	Weber K. (2012)
Toxicity IN-B5528 (Dupont)		7-day E_yC_{50} 7 day E_rC_{50} 7 day E_bC_{50}	>119.52 mg/L (nom)	Chandrasehar, G (2010)

A number of endpoints are greater than values. The Applicant has stated that because of limited material availability, the original studies were conducted with rather low maximum concentrations. The highest possible maximum concentration was used to ensure that safety of the metabolite was adequately demonstrated. As there was no mortality, immobility, or inhibition above 50% seen in these studies, the RMS deemed it acceptable to use these > endpoints as limit values.