CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: TRIFLUSULFURON-METHYL

EC Number: **not allocated**

CAS Number: 126535-15-7

Index Number: **not allocated**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance (The evaluated variant is the triflusulfuron-methyl)

Table 1. Substance identity:

Substance name:	***Triflusulfuron-methyl***
EC number:	***Not allocated***
CAS number:	126535-15-7
Annex VI Index number:	***Not allocated***
Degree of purity:	***≥960 g/kg***
Impurities:	See confidential annex

1.2 Harmonised classification and labelling proposal

Table 2. The current Annex VI entry and the proposed harmonised classification: triflusulfuron-methyl

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	**Carc. 2-H351** Aquatic Acute 1 – H400 M-factor (acute): 100 Aquatic Chronic 1 – H410 M-factor (chronic): 10	**Carc. Cat.3 R40** N; R50/53 Specific Concentration Limits: C≥0.25% N; R50-53 0.025%≤C<0.25% N; R51-53 0.0025%≤C<0.025% R52-53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	**Carc. 2-H351** Aquatic Acute 1 – H400	**Carc. Cat. 3; R40** N R50/53 Specific Concentration

	M-factor (acute): 100 Aquatic Chronic 1 – H410 M-factor (chronic): 10	Limits: C≥0.25% N; R50-53 0.025%≤C<0.25% N; R51-53 0.0025%≤C<0.025% R52-53

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

Table 3. Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Conclusive but not sufficient for classification
2.2.	Flammable gases	None		None	Not adequate
2.3.	Flammable aerosols	None		None	Not adequate
2.4.	Oxidising gases	None		None	Not adequate
2.5.	Gases under pressure	None		None	Not adequate
2.6.	Flammable liquids	None		None	Not adequate
2.7.	Flammable solids	None		None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None		None	No data
2.9.	Pyrophoric liquids	None		None	Not adequate
2.10.	Pyrophoric solids	None		None	No data
2.11.	Self-heating substances and mixtures	None		None	Not adequate
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	No data
2.13.	Oxidising liquids	None		None	Not adequate
2.14.	Oxidising solids	None		None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None		None	Not adequate
2.16.	Substance and mixtures corrosive to metals	None		None	Data not sufficient for classification
3.1.	Acute toxicity - oral	None		None	Data conclusive but not sufficient for classification
	Acute toxicity - dermal	None		None	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	None		None	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None		None	Data conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None		None	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Data lacking
3.4.	Skin sensitisation	None		None	Data conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None		None	Data conclusive but not

					sufficient for classification
3.6.	Carcinogenicity	**Carc. 2-H351**	**None**	None	
3.7.	Reproductive toxicity	None		None	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None		None	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None		None	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard			None	Not evaluated
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1 – H400** **Aquatic Chronic 1 – H410**	100 (acute) 10 (chronic)	None	
5.1.	Hazardous to the ozone layer	-		None	No data

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

Labelling:

Signal word: Warning
Hazard statements: H351 (Suspected of causing cancer); H410
Precautionary statements: not harmonised
Pictograms: GHS09, GHS08

Proposed notes assigned to an entry: none

Table 4. Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	None		None	Conclusive but not sufficient for classification
Oxidising properties	None		None	Conclusive but not sufficient for classification
Flammability	None		None	Conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	None		None	No other physic-chemical properties tested
Thermal stability	None		None	Data lacking, inconclusive but not sufficient for classification
Acute toxicity	None		None	Data conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	None		None	Data conclusive but not sufficient for classification
Repeated dose toxicity	None		None	Data conclusive but not sufficient for classification
Irritation / Corrosion	None		None	Data conclusive but not sufficient for classification
Sensitisation	None		None	Data conclusive but not sufficient for classification
Carcinogenicity	**Carc. Cat.3 R40**	**None**	None	
Mutagenicity – Genetic toxicity	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – fertility	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – development	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	Data conclusive but not sufficient for classification
Environment	N, R50/53	C≥0.25% N; R50-53 0.025%≤C<0.25% N; R51-53 0.0025%≤C<0.025% R52-53	None	

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

Labelling:
Indication of danger: N, Xn
R-phrases: R40, R50-53
S-phrases: S36/37, S60, S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Trimethylsulfuron-methyl is not listed in the Annex I of the 67/548/EC Directive.

No registration is available on triflusulfuron methyl on 20/11/2011 (PPP substance considered as registered – REACH art 15).

2.2 Short summary of the scientific justification for the CLH proposal

The data presented here, in particular Leydig cell hyperplasia and adenomas observed in male rats after Triflusulfuron-methyl treatment, which seems to act as a weak aromatase inhibitor, inducing a decrease in blood estradiol and a subsequent disruption of the hypothalamic-pituitary-testis axis, a relevant mechanism to human justify warranting a classification as a Cat 2 H351.
Toxicity studies for algae and aquatic plants EC50s at concentrations ≤ 1 mg/L were observed. In addition, triflusulfuron-methyl is not readily biodegradable although it is unlikely for the substance to bioaccumulate in aquatic organisms (log Kow < 3). As a consequence and according to the CLP Regulation, triflusulfuron-methyl should be classified as R50-53 (Aquatic Acute 1 – Aquatic Chronic 1). Based on the toxicity data for *Lemna gibba* (ErC50 = 0.0035 mg/L and NOEC = 0.00127 mg/L) M-factors of 100 (acute) and 10 (chronic) are also proposed.

2.3 Current harmonised classification and labelling

No current harmonised classification in Annex VI of CLP.

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

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

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2.4 Current self-classification and labelling

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

Triflusulfuron-methyl is currently classified with Carc. 2-H351 at the national level.

A notification in the classification and labelling inventory reports a classification Eye Irrit 2, Aquatic Acute 1 and Aquatic Chronic 2.

2.4.2 Current self-classification and labelling based on DSD criteria

Triflusulfuron-methyl is currently classified with Xn Carc. Cat. 3 R40 at the national level. Triflusulfuron-methyl is currently labelled at the national level with S2: « keep out of the reach of children », S36/37 « wear suitable protective clothing and gloves » and S46: « if swallowed, seek medical advice immediately and show this container or label ».

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Triflusulfuron-methyl is currently not classified according to Annex VI of CLP.

Triflusulfuron-methyl is an active substance in the meaning of Directive 91/414/EEC. In accordance with Article 36(2) of the CLP Regulation, Triflusulfuron-methyl shall be subjected to harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points.

Part B.

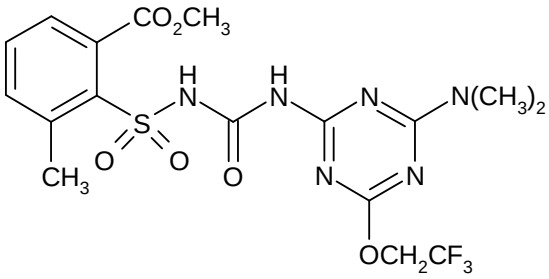
SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

The variant triflusulfuron-methyl is evaluated below

Table 5. Substance identity; triflusulfuron-methyl

EC number:	Not allocated
EC name:	Triflusulfuron-methyl
CAS number (EC inventory):	Not allocated
CAS number:	126535-15-7
CAS name:	methyl 2-[[[[[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]-3-methylbenzoate
IUPAC name:	methyl 2-[4-dimethylamino-6-(2,2,2-trifluoroethoxy)–1,3,5-triazin-2-ylcarbamoylsulfamoyl]-*m*-toluate
CLP Annex VI Index number:	Not allocated
Molecular formula:	$C_{17}H_{19}F_3N_6O_6S$
Molecular weight range:	492.43
Structural formula:	

Note: DAR refers to triflusulfuron. However, all of the data evaluated refer to triflusulfuron-methyl. Therefore CLH Report & dossier is presented for triflusulfuron-methyl. No data allows to draw a conclusion on triflusulfuron.

1.2 Composition of the substance

Table 6. Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Triflusulfuron-methyl		≥ 960 g/kg	
impurities		see confidential annex	

Current Annex VI entry:

No harmonised classification

Table 7. Impurities (non-confidential information)

Impurities are confidential. See confidential annex.

Table 8. Additives (non-confidential information)

none

Current Annex VI entry:

No harmonised classification

1.2.1 Composition of test material

The purity of the test substance is given in each test descritpion when appropriate.

1.3 Physico-chemical properties

Table 9. Summary of physico - chemical properties of triflusulfuron-methyl

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance	White crystalline solid. (PGAI 98.9%) and white powdery crystalline solid (TGAI.)	Moore, 2002 Dupont 5776	observation
Melting/freezing point	159 °C – 162 °C (purity 98.9 %)	Moore, 2002 Dupont 5775	Measured
Boiling point	No boiling was measured (decomposition of active substance occurs at the temperature above melting point)	Moore, 2002 Dupont 5775	Measured
Relative density	*Density: 1.481 ±0.001g/cm^3 at 20° ±0.8 ° C (n=3)*	Huntley, 2001 Dupont 6280	Expressed as density. Measured
Vapour pressure	The vapour pressure at 20°C was 1.01 x 10^{-5} Pa	Ravi, 2010 Dupont-27588 revision 1	Measured using a > 95 % purity test item.
Henry's law	*At pH 5 H=1.31 10^{-3} Pa.m^3.mol^{-1}* *At pH 7 H=1.91 10^{-5} Pa.m^3.mol^{-1}* At pH 9 H=4.52 10^{-7} Pa.m^3.mol^{-1}	*Hirata, 2009* Dupont-28975	calculation
Surface tension	67.92 mN/m at 4.465 mg/L at 20°C Purity : 98.9%	Hammond 1999 Dupont-2280	measured
Water solubility	Purity 98.6% (flask method) PH 3: 0.0011 g/L at 25 °C PH 5: 0.0038 g/L at 25 °C PH 7: 0.26 g/L at 25 C PH 9: 11 g/L at 25 °C	Moore, Schmuckler, 1997 AMR 4571-97	Measured (flask method)
Partition coefficient n-octanol/water	pH 5: 2.3 at 25 °C pH 7: 0.96 at 25 °C pH 9: -0.066 at 25 °C purity : 95.6% (this purity is slightly below the minimum purity of 96 % which is not expected to modify significantly the results)	Rhodes, and Cooke, 1992 AMR 1984-91	Measured (HPLC determination of the aqueous and octanol phase)
Flash point	triflusulfuron-methyl is a solid with a melting point is <40°C. The active substance is not expected to have a flash point at ambient temperature so no other data required	-	-
Flammability	The test material (95.6 % purity) failed to propagate a flame so the active substance can be considered as not flammable TGAI (95.6%). (this purity is slightly below the minimum purity of 96 % which is not expected to modify significantly the results)	Gravell, 1995 AMR 3028-94	measured
Explosive properties	No explosive properties (95.6%) (this purity is slightly below the minimum purity of 96 % which is not expected to modify significantly the results)	Gravell, 1995 AMR 3028-94	measured
Self-ignition temperature	not relevant as the active substance is not a liquid and does not have a melting point below 40°C		
Oxidising properties	Not oxidizing (structural interpretation)	Gravell, 1995 AMR 3028-94	Expert statement

Granulometry	no data	-	
Stability in organic solvents and identity of relevant degradation products	No data on stability but solubility is given below		
Dissociation constant	pKa: 4.4 at 25 °C	Rhodes, and Cooke, 1983 AMR 1983-91	measured
Viscosity	Not relevant. Moreover triflusulfuron is a solid	-	-
Solubility in organic solvent	Purity : 95.6%, at 25 °C Acetone : 120 g/L, Acetonitrile : 80 g/L, Chloroform : 160 g/L, Ethyl acetate : 27 g/L, n-hexane : <0.0016g/L, Methanol 7.0 g/L, Dichloromethane 580 g/L, Octan-1-ol : 0.026 g/L Toluene : 2.0 g/L	Rhodes, and Cooke, 1992 AMR 1981-91	measured
UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)	purity of the test item: 98.9% *UV/Vis spectra in acidic, neutral or basic solutions:* *(ε in mol^{-1} cm^{-1})* *λ (nm) Acidic Neutral Basic* *228 $4.03x10^4$ N/A N/A* *235 N/A $5.17x10^4$ $5.17x10^4$* 291 $9.81x10^2$ $4.74x10^2$ $4.81x10^2$	Moore, 1999 Dupont 2560	measured
Storage stability at 25°C and 5°C	no data		
Granulometry	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

Two manufacturing processes are mentioned in the report . One was used to develop the active substance and used in initial field trials and some toxicological studies (“primary process”) but not used anymore. The other MP is the main process to manufacture the active substance and used today (“actual process”).
Details are given in the confidential part (Annex I to the CLH report).

2.2 Identified uses

Triflusulfuron-methyl (variant of triflusulfuron) is an herbicide to be used in agriculture under field conditions. In the EU dossier, deposited representative uses are on Sugar and fodder beets

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10. Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A10 (flammability)	negative	Results are negative, no classification	Gravell, 1995 AMR 3028-94
UN-Bowes-Cameron-Cage test Q (auto-flammability)	negative	Results are negative, no classification	Gravell, 1995 AMR 3028-94
EEC A14 (explosivity)	negative	Results are negative, no classification	Gravell, 1995 AMR 3028-94
Structural interpretation (oxidizing properties)	negative	no classification is proposed	Gravell, 1995 AMR 3028-94

3.1 Explosive properties

Triflusulfuron-methyl is a stable organic substance. None of these components or grouping are associated with explosive hazards. All are stable groupings in high oxidation states. Moreover, a study from Gravell, 1995 (AMR 3028-94) using EEC A14 method has been performed indicating no explosive properties for triflusulfuron-methyl test item.

3.2 Flammability

Triflusulfuron-methyl is an organic compound. This material is not likely to undergo self heating under bulk storage conditions and is unlikely to auto-ignite. Moreover, in the Gravell's study (1995), triflusulfuron-methyl was shown not to be highly flammable using EEC A10 method. In the same study, auto-ignition has not been observed (see Table 9)

The determination of flash point is not relevant because the active substance is a solid.

Triflusulfuron-methyl can be given as not flammable.

3.3 Oxidising potential

Oxidising compounds are materials that can easily transfer oxygen to other compounds i.e. they contain weakly bound oxygen, for example NO3 and peroxides. Bound oxygen must also become available through a low energy degradation route with a low energy of activation. The oxygen in Triflusulfuron-methyl is bound in stable structural groupings with strong oxygen bonds. The decomposition temperature of Triflusulfuron-methyl is around 160°C. Triflusulfuron-methyl can therefore be considered stable under the conditions of oxidation.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

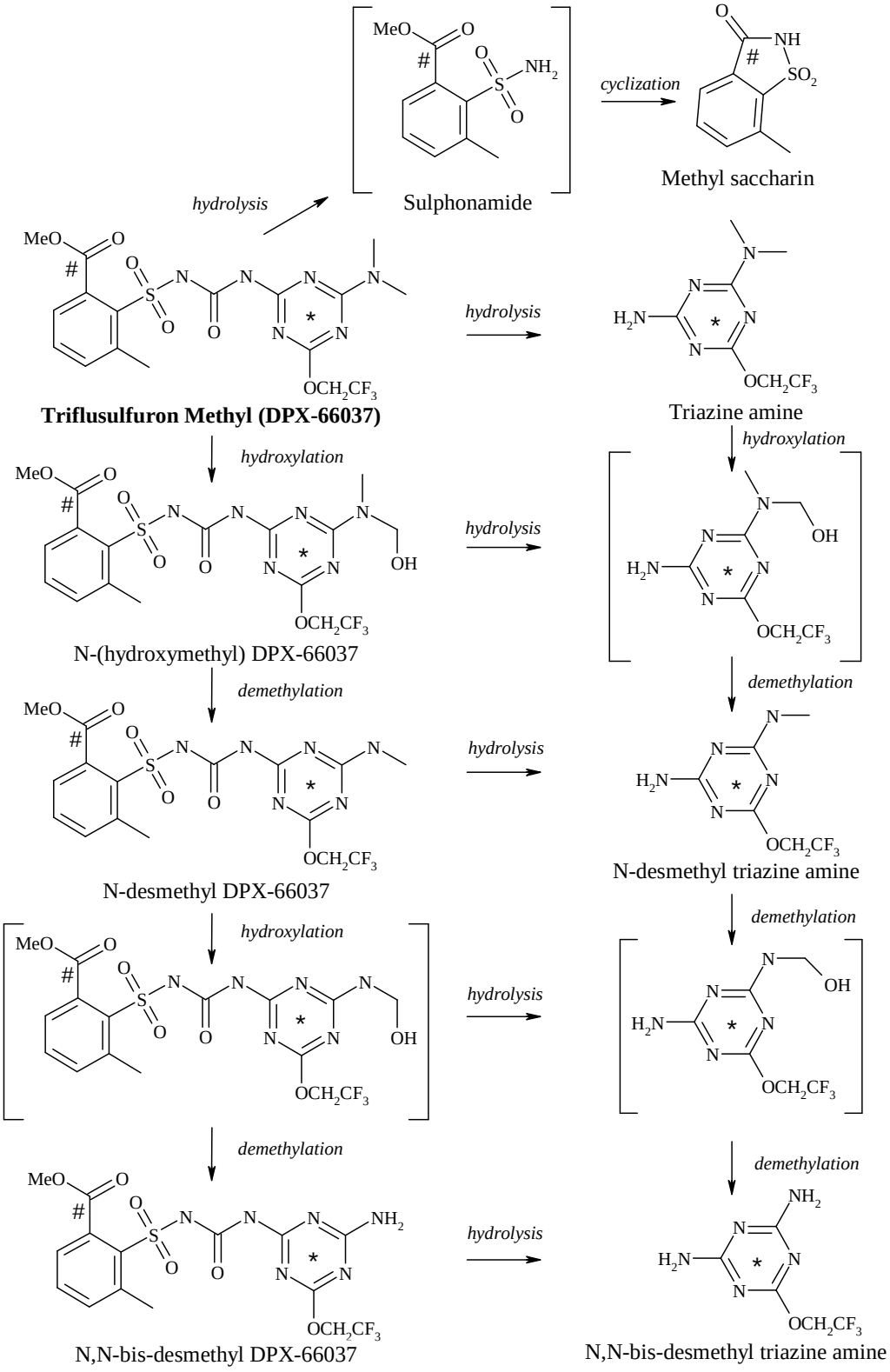
Following oral dosing in rats, [^{14}C]-Triflusulfuron-methyl was rapidly absorbed and excreted both in urine and faeces (> 80% and > 90% of the administered dose was excreted respectively in 48 and 72 hours in urine and faeces). Female rats appeared to excrete slightly more residues in urine than male rats. The difference in urine was offset by a greater excretion of residues in the faeces of male rats than female rats.

The oral absorption of the low dose of Triflusulfuron-methyl in the rat was estimated to be 63.5 and 79.5% of the dose, in males and females respectively, based on the radioactivity recovered in the urine and the carcass. The notifier has estimated that the mean absorption of Triflusulfuron-methyl in the rat was 87% (84-92%) of the administered low dose, based on the sum of ^{14}C metabolites in the liver, urine and faeces (assuming all faeces metabolites were derived from liver metabolism).
Although the biliary elimination of the radioactivity was not measured and the possible metabolism of Triflusulfuron-methyl in the gastrointestinal tract was not studied, the high similarity between the metabolite profiles in the faeces and in the urine or liver was consistent with the notifier's proposal.

Tissue clearance was rapid with less than 2.5% remaining in all tissues at 120 hours. Clearance was slower from liver and blood than from other tissues. There was no evidence of alteration in the pattern of excretion or tissue distribution of radioactivity after several daily administrations of the compound.

Triflusulfuron-methyl was extensively metabolised especially when administered at low doses in the rat. The major biotransformation pathways for Triflusulfuron-methyl were hydroxylation/demethylation on the triazine ring and cleavage of the sulphonylurea bridge. No qualitative difference was noticed in the metabolism of the compound in the male and female rats. Considering the percentage of the metabolites identified and the metabolic pathway proposed, it can be concluded that the metabolism of Triflusulfuron-methyl in rats was well understood.

Proposed metabolic pathway for [^{14}C]-triflusulfuron-methyl in the rat:



4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Following oral administration in rats, Triflusulfuron-methyl is rapidly absorbed and excreted in urine and faeces. Triflusulfuron-methyl did not accumulate in the body and was extensively metabolised, especially when administered at low doses in the rat. The major biotransformation pathways for Triflusulfuron-methyl were hydroxylation/demethylation of the triazine ring and cleavage of the sulphonylurea bridge.

4.2 Acute toxicity

Table 11. Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral, rat EEC Method B.1	LD_{50} administered in methylcellulose > 5000 mg/kg b.w.	Purity: 98.7% "actual process"	Clouzeau J. (1992)
Oral, rat OECD 401	LD_{50} administered in corn oil > 5000 mg/kg b.w.	Purity: 95.6% "primary process"	Sarver J.W. (1991a)
Oral, rabbit OECD 401	LD_{50} administered in methylcellulose > 5000 mg/kg b.w.	Purity: 95.6% "primary process"	Sarver J.W. (1991b)
Inhalation, rat OECD 403	4-h LC_{50} > 5.1 mg/L (nose only)	Purity: 95.6% "primary process"	Panepinto A.S. (1991)
Percutaneous, rat EEC Method B.3	LD_{50} > 2000 mg/kg b.w.	Purity: 98.7% "actual process"	Clouzeau J. (1992)
Percutaneous, rabbit OECD 402	LD_{50} > 2000 mg/kg b.w.	Purity: 95.6% "primary process"	Sarver J.W. (1991)

4.2.1 Non-human information

4.2.1.1 *Acute toxicity: oral*

Reference: Clouzeau J. (1992)
GLP: Yes
Guidelines: EEC Method B.1

Single dose of the test substance (66037-59, actual process, purity 98.7% in methylcellulose 0.5%) was administered by oral gavage to groups of 5 male and 5 female fasted Sprague Dawley rats at 5000 mg/kg body weight. Rats were weighed and observed during a 14-day recovery period and submitted to gross pathological examination.

No mortalities were observed. Hypokinesia was observed in 6 animals at 2 hours post-dosing and hypokinesia combined with piloerection was observed in 8 animals at 4-hours post-dosing. All rats appeared normal by day 2 or earlier and throughout the remainder of the study. There was no test substance-related body weight loss observed during the study. No test substance-related gross lesions were observed at necropsy.

The oral LD_{50} of triflusulfuron-methyl (actual process), administered in methylcellulose, in rats was greater than 5000 mg/kg b.w. for both male and female rats.

Reference: Sarver J.W. (1991a)
GLP: Yes
Guidelines: OECD 401

Single dose of the test substance (66037-24, primary process, purity 95.6% in Corn oil) was administered by oral gavage to groups of 5 male and 5 female fasted Sprague Dawley rats at 5000 mg/kg body weight. Rats were weighed and observed during a 14-day recovery period and submitted to gross pathological examination.

One male rat was found dead from a dosing injury on test day 3. Clinical signs most often observed in male and female rats included lethargic behaviour, hunched posture, and ocular and nasal discharges. One male rat had moderate weight loss (8% of fasted body weight) one day after dosing. No test substance-related gross lesions were observed at necropsy.

The oral LD_{50} of triflusulfuron-methyl (primary process), administered in corn oil, in rats was greater than 5000 mg/kg b.w. for both male and female rats.

Reference: Sarver J.W. (1991b)
GLP: Yes
Guidelines: OECD 401

Single dose of the test substance (66037-24, primary process, purity 95.6% in methylcellulose 0.25%) was administered by oral gavage to groups of 5 male and 5 female fasted New Zealand white rabbits at 5000 mg/kg body weight. Rabbits were weighed and observed during a 14-day recovery period and submitted to gross pathological examination.

No mortality was observed. No clinical signs of toxicity were observed. One male rabbit had moderate weight loss (6% of the previous body weight) on test day 5. No test substance-related gross lesions were observed at necropsy.

The oral LD_{50} of triflusulfuron-methyl (primary process), administered in methylcellulose, in rabbits was greater than 5000 mg/kg b.w. for both males and females.

4.2.1.2 *Acute toxicity: inhalation*

Reference: Panepinto A.S. (1991)
GLP: Yes
Guidelines: OECD 403

Groups of 5 male and 5 female Sprague Dawley rats were exposed, nose only during a 4-hour period, to 5.1 mg/L of the test substance (66037-28, primary process, 94.9%). Test atmospheres were generated by suspension of triflusulfuron-methyl particulates in air. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing. Rats that were found dead or survived the observation period were examined for gross pathological changes. The main exposure parameters were as follows: flow rate, 30-35 L/min; analytical concentration, 5.1 ± 2.2 mg/L; mass median aerodynamic diameter, 2.3 µm; particles < 3 µm, 64%.

No mortalities were observed. Upon removal of the rats from the test chambers, nasal discharge was observed in 2 of 5 male rats. Hyperactivity and/or hypersensitivity were first observed in all rats on test day 7 or 8 but were absent in all but 1 female on the following day. Additionally, 1 female rat exhibited abnormal

mobility on test day 8 that had subsided by test day 9. Two other female rats exhibited muscle fasciculations of the head on test day 7 that resolved by test day 8. All surviving animals appeared normal by test day 9 or earlier and throughout the remainder of the study. One day following exposure, 8 rats exhibited weight loss ranging from 0.4 to 5.5% of initial body weight. By the end of the recovery period, all rats had regained the lost weight and exhibited normal patterns of weight gain. No test substance-related gross lesions were observed at necropsy.

The acute inhalation LC_{50} for Triflusulfuron-methyl (primary process) was greater than 5.1 mg/L of air for 4 hours (nose only) for both male and female rats.

4.2.1.3 *Acute toxicity: dermal*

Reference: Clouzeau J. (1992)
GLP: Yes
Guidelines: EEC Method B.3

Single dose of the test substance (66037-59, actual process, purity 98.7% moistened in water) was applied to the shaved, intact skin of 5 male and 5 female Sprague Dawley rats at a dose of 2000 mg/kg. The application site was occluded for 24 hours after which the test substance was removed. The rats were observed for clinical signs, body weight effects, and mortality for up to 14 days following application. All rats were examined for gross pathological changes.

No mortality, clinical signs of toxicity, or dermal irritation were observed in any of the rats. In addition, there were no test substance-related body weight effects or gross lesions.

The dermal LD_{50} of triflusulfuron-methyl (actual process) in rats was greater than 2000 mg/kg b.w. for both male and female rats.

Reference: Sarver J.W. (1991)
GLP: Yes
Guidelines: OECD 402

Single dose of the test substance (66037-24, primary process, purity 95.6% moistened in dimethyl phtalate) was applied to the shaved, intact skin of 5 male and 5 female New Zealand white rabbits at a dose of 2000 mg/kg. The application site was occluded for 24 hours after which the test substance was removed. The rabbits were observed for clinical signs, body weight effects, and mortality for up to 14 days following application. All rabbits were examined for gross pathological changes.

No mortalities were observed. No clinical signs of toxicity were observed. Slight to moderate erythema was observed in most rabbits 1 day after application of the test substance. By 4 days after application, no dermal irritation was observed in any of the rabbits. There were no test substance-related body weight effects noted. No test substance-related gross lesions were observed at necropsy.

The dermal LD_{50} of triflusulfuron-methyl (primary process) in rabbits was greater than 2000 mg/kg b.w. for both males and females.

4.2.1.4 *Acute toxicity: other routes*

No data available

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

Triflusulfuron-methyl (actual or primary process) has low acute toxicity via the oral, dermal or inhalation routes (rat and rabbit oral LD_{50} > 5000 mg/kg bw, rat 4-h nose only LC_{50} > 5.1 mg/L, rat and rabbit dermal LD_{50} > 2000 mg/kg bw) .

4.2.4 Comparison with criteria

The oral LD50 lie above the classification cut-off of 2000 mg/kg under both Directive 67/548/EEC and regulation (EC) 1272/2008; therefore no classification is proposed.

The dermal LD50 lie above the classification cut-off of 2000 mg/kg under both Directive 67/548/EEC and regulation (EC) 1272/2008; therefore no classification is proposed.

The inhalation LC50 lies above the classification cut-off of 5 mg/L/4h under both Directive 67/548/EEC and regulation (EC) 1272/2008; therefore no classification is proposed.

4.2.5 Conclusions on classification and labelling

These data indicate that no classification is required regarding acute toxicity under either Directive 67/548/EEC or the CLP Regulation.

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

No specific target organ toxicity identified after single exposure.

These data indicate that no classification is required under either Directive 67/548/EEC or the CLP Regulation.

4.4 Irritation

4.4.1 Skin irritation

Table 12. Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation, rabbit EEC Method B.4.	Not irritating	Purity: 98.7% “actual process”	Clouzeau J. (1992)
Skin irritation, rabbit, OECD 404	Not irritating	Purity: 95.6% “primary process”	Sarver J.W. (1992)

4.4.1.1 *Non-human information*

Reference: Clouzeau J. (1992)
GLP: Yes

Guidelines: EEC Method B.4

0.5 g of the moistened test substance (66037-59, actual process, purity 98.7%) was applied on the shaved skin of 3 male New Zealand rabbits for 4 hours under a semi-occlusive dressing. One, 24, 48 and 72 hours after application, the test sites were evaluated for erythema, oedema and other evidence of dermal effects.

No dermal irritation was observed in 2 animals. Erythema (score of 1) was observed in 1 animal at 1 and 24 hours but was resolved by 48 hours. There were no test substance-related body weight effects or clinical signs noted.

Under the conditions of this study, the test compound (actual process) was classified as a non-irritant to rabbit skin.

Reference: Sarver J.W. (1992)
GLP: Yes
Guidelines: OECD 404

0.5 g of the moistened (with dimethyl phtalate) test substance (66037-24, primary process, purity 95.6%) was applied on the shaved skin of 6 male New Zealand rabbits for 4 hours under a semi-occlusive dressing. One, 24, 48 and 72 hours after application, the test sites were evaluated for erythema, oedema and other evidence of dermal effects.

No oedema or erythema was observed in any of the animals. There were no test substance-related body weight effects or clinical signs noted.

Under the conditions of this study, the test compound (primary process) was classified as a non-irritant to rabbit skin.

4.4.1.2 *Human information*

No data available

4.4.1.3 *Summary and discussion of skin irritation*

After a 4-hour skin exposure of Triflusulfuron-methyl (actual or primary process) to New Zealand rabbits under semi-occlusive dressing, no skin irritation was observed in any rabbit. Triflusulfuron-methyl is not a skin irritant.

4.4.1.4 *Comparison with criteria*

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified irritant for the skin if it causes significant inflammation which persists for at least 24 hours after an exposure period of up to four hours on the rabbit. That is to say that the mean value of the scores for either erythema and eschar formation or oedema formation calculated over all the animals tested is 2 or more or if the mean value of 2 or more calculated for each animal separately has been observed in two or more animals. Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time.

2) Criteria in the CLP classification:

A substance shall be classified as irritant in category 2 if in at least 2 of 3 tested animals mean value for erythema/eschar or for oedema is between 2.3 and 4.0 from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions. If inflammation persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling, substance shall be also considered as irritant.

3) Comparison with criteria:

Here, mean scores 24 to 72 hours for erythema and oedema were below the criteria for classification and labelling.

4.4.1.5 *Conclusions on classification and labelling*

In this context, Triflusulfuron-methyl does not support classification for skin irritation under either directive 67/548/EEC or CLP regulation criteria.

4.4.2 Eye irritation

Table 13. Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation, rabbit EEC Method B.5.	Not irritating	Purity: 98.7% "actual process"	Clouzeau J. (1992)
Skin irritation, rabbit, EEC Method B.5.	Not irritating	Purity: 95.6% "primary process"	Sarver J.W. (1991)

4.4.2.1 *Non-human information*

Reference: Clouzeau J. (1992)
GLP: Yes
Guidelines: EEC Method B.5

100 mg of the undiluted test substance (66037-59, actual process, purity 98.7%) was introduced in the left eye of 3 male New Zealand rabbits. Eyes remained unwashed. 1, 24, 48 and 72 hours after application, the rabbits were examined for evidence of eye irritation.

The test substance produced corneal opacity (score of 1) in 1 rabbit, conjunctival redness (scores of 1 or 2) in 3 rabbits, conjunctival chemosis (scores of 1 or 2) in 3 rabbits, and discharge (score of 1) in 3 rabbits. No signs of irritation were present at 48 hours. There were no test substance-related body weight effects or clinical signs noted.

Under the conditions of this study, the test compound (actual process) does not classify as an eye irritant.

Reference: Sarver J.W. (1991)
GLP: Yes
Guidelines: EEC Method B.5

56 mg (0.1 mL) of the undiluted test substance (66037-24, primary process, purity 95.6%) was introduced in the left eye of 6 male New Zealand rabbits (5 males and 1 female). Eyes remained unwashed. 1, 24, 48 and 72 hours after application, the rabbits were examined for evidence of eye irritation.

The test substance produced conjunctival redness (score of 1) in 6 rabbits, and discharge (score of 3) in 1 rabbit. No signs of irritation were present at 48 hours. There were no test substance-related body weight effects or clinical signs noted.

Under the conditions of this study, the test compound (primary process) does not classify as an eye irritant.

4.4.2.2 *Human information*

No data available

4.4.2.3 *Summary and discussion of eye irritation*

After application Triflusulfuron-methyl (actual or primary processs) in the eyes of male and female New Zealand rabbits, transient and slight ocular reactions were observed, including conjunctival redness, conjunctival chemosis and discharge. At 48 hours, no signs of irritation were observed in any rabbit.

Triflusulfuron-methyl is not an eye irritant.

4.4.2.4 *Comparison with criteria*

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified as a substance which could provoke irritating to eyes if it causes, when applied to the eye of the animal, significant ocular lesions which occur within 72 hours after exposure and which persist at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test have any of the following values:

- Cornea opacity equal to or greater than 2 but less than 3,
- iris lesion equal to or greater than 1 but not greater than 1.5,
- redness of the conjunctivae equal to or greater than 2.5,
- oedema of the conjunctivae (chemosis) equal to or greater than 2.

Or if 3 animals are tested, if the lesions on 2 or more animals, are equivalent to any of the above values axcept that for iris lesion the value should be equal to or greater than 1 but less than 2 and for redness of the conjunctivae the value should be equal to or greater than 2.5.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

2) Criteria in the CLP classification :

A substance shall be classified as a substance which could induce reversible eye irritation, classified in Category 2 (irritating to eyes), if when applied to the eye of an animal, a substance produces:

- At least in 2 of 3 tested animals, a positive response of:

  Corneal opacity ≥ 1 and/or

  Iritis ≥ 1 and/or

  Conjunctival redness ≥ 2 and/or

Conjunctival oedema ≥ 2

Calculated as the mean scores following grading at 24, 48, and 72 hours after instillation of the test material, and which fully reverse within an observation period of 21 days.

3) Comparison with criteria:

Here, mean scores 24 to 72 hours for corneal opacity, iritis, conjunctival redness and conjunctival oedema were below the criteria for classification and labeling.

4.4.2.5 *Conclusions on classification and labelling*

In this context, Triflusulfuron-methyl does not support classification for eye irritation under either directive 67/548/EEC or CLP regulation criteria.

4.4.3 Respiratory tract irritation

4.4.3.1 *Non-human information*

No data available

4.4.3.2 *Human information*

No data available

4.5 Corrosivity

Triflusulfuron-methyl is not a corrosive substance.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14. Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Skin sensitization, guinea-pigs OECD 406	Not skin sensitizer	Purity: 98.7% "actual process" The study was only found indicative as no concurrent control was used.	Clouzeau J. (1992)
Skin sensitization, guinea-pigs OECD 406	Not skin sensitizer	Purity: 95.6% "primary process"	Armondi S. (1991/1994)

4.6.1.1 *Non-human information*

Reference: Clouzeau J. (1992)
GLP: Yes
Guidelines: OECD 406
The study was only found indicative, as no concurrent positive control was used.

The dermal sensitisation potential of triflusulfuron-methyl (66037-59, actual process, purity 98.7%) was evaluated by the Magnusson-Kligman Maximisation method in 10 male and 10 female Hartley albino guinea pigs. Groups of 5 males and 5 females served as controls. No concurrent positive control group was included in the test. During a 10-day induction phase (Day 1-10), the test substance was administered by intradermal injection (Day 1) and by cutaneous application (Day 8). For the intradermal induction phase, animals received 3 injections (0.1 mL) in the scapular region: Freund's adjuvant (50% in 0.9% saline); either paraffin oil (vehicle controls) or a 1% solution of the test substance (determined in a range finding study) in vehicle (treated group); and a 50/50 (v/v) mixed solution of Freund's adjuvant with vehicle (control group) or with the test substance (treated group). One day prior to cutaneous induction, local irritation was induced at the cutaneous induction site using 0.5 mL of 10% sodium laurylsulphate in vaseline. For cutaneous induction (Day 8), 0.5 mL of vehicle (control group) or 500 mg of the test substance in its original form (determined in a range finding study) was applied to the scapular region using a dry compress and an occlusive dressing. After approximately 48 hours, dressings were removed, and one hour later skin reactions were recorded. In the cutaneous challenge phase (Day 22), both treated and control groups received 500 mg of the test substance in its original form and 0.5 mL of vehicle. Test substance and vehicle were administered in opposite posterior flanks using a dry compress and hypoallergenic dressing. Dressings were removed after 24 hours and cutaneous reactions were scored 24 and 48 hours after removal of the dressing.

On Day 10, after removal of the dressing from the test site, necrosis was observed in all animals for both the control and treated groups at the intradermal injection sites. There were no cutaneous responses noted at the topical induction test sites. No cutaneous reactions were observed 24 and 48 hours post exposure at the challenge sites. There were no test substance-related clinical signs or body weight effects observed.

Under the experimental conditions used (Magnusson and Kligman assay), the test compound was not considered as a skin sentitiser to guinea pigs.

Reference: Armondi S. (1991/1994)
GLP: Yes
Guidelines: OECD 406

The dermal sensitisation potential of triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was evaluated by the Magnusson-Kligman Maximisation method in 10 male and 10 female Duncan Hartley albino guinea pigs. Based on a range-finding study, twenty animals were intradermally induced on Day 1 with 0.1 mL of a 1.5% w/v suspension of triflusulfuron-methyl (determined in a range finding study) with or without Freund's Complete Adjuvant. Similarly, 3 male and 3 female guinea pigs were treated with 0.1 mL of 0.1% suspension of DNCB, which served as a positive control. An additional group of 10 male and 10 female guinea pigs was treated with 0.9% saline and served as the vehicle control group. Six days after the intradermal induction phase, all test sites were re-clipped and pre-treated with 10% sodium lauryl sulfate in petrolatum. The test substance (25% w/v, determined in a range finding study), vehicle control, or positive control article suspensions were applied 24 hours later. Two weeks following the topical induction phase, the test and vehicle control animals were challenged on the clipped right and left flanks with 0.2 mL 0.9% saline and 0.2 mL of the test article (25% w/v), respectively. The positive control animals were treated similarly with 0.1% DNCB on the left flank and 0.9% saline on the right flank. Approximately 24 and 48 hours after the challenge phase, the test sites were evaluated for signs of sensitisation. Very faint redness (usually nonconfluent, score of 0.5) was not considered a positive dermal reaction. Scores of 1 (faint redness, usually confluent) or greater were required to be indicative of sensitisation.

During the challenge phase, no signs of erythema were observed in the test substance-treated animals at 25% at 24 or 48 hours. No signs of erythema were observed in the negative control animals challenged with the test substance at 25%. Slight patchy mild redness to severe erythema and oedema was observed in the positive control animals.

Under the experimental conditions used (Magnusson and Kligman assay), the test compound was not considered as a skin sentitiser to guinea pigs.

4.6.1.2 *Human information*

No data available

4.6.1.3 *Summary and discussion of skin sensitisation*

Under the conditions of the two M & K tests, Triflusulfuron-methyl did not induce any positive response in guinea pigs. Triflusulfuron-methyl is not a skin sensitizer.

4.6.1.4 *Comparison with criteria*

Considering that negatives results were obtained from the two animal tests, no classification is required for Triflusulfuron-methyl under either Directive 67/548/EEC or the CLP Regulation (including the criteria defined in the 2nd ATP).

4.6.1.5 *Conclusions on classification and labelling*

Triflusulfuron-methyl is not a skin sensitizer to guinea-pig in the maximisation tests and therefore no classification is warranted.

4.6.2 Respiratory sensitisation

No data available

4.7 Repeated dose toxicity

Table 15. Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral			
90-day feeding study in Sprague Dawley rats 0, 100, 2000, 10000 and 15000 ppm corresponding to 0, 6.2, 127, 646 and 965 mg/kg b.w. in males and 0, 7.54, 150, 774 and 1070 mg/kg b.w. in females OECD 408	At 2000ppm in M and F: ↓ body weights, ↓ body weight gains, ↓ food efficiency, ↑ mean relative liver weights (without histological changes), and regenerative anemia. At 15000 ppm: renal tubular atrophy in M and F (marked only in F), testicular atrophy/degeneration and oligospermia, ↓ testicular weights. NOAEL for Triflusulfuron-methyl (synthetized via the primary process) was 100 ppm (6.2 mg/kg b.w.) for M and (7.54 mg/kg b.w) for F	Purity: 95.8% "primary process"	Biegel L.B. (1993)
90-day feeding study in Sprague Dawley rats 0, 100, 2000, 10000 and 15000 ppm corresponding to 0, 6.56, 133, 658 and 1036 mg/kg b.w. in males and 0, 7.71, 153, 783 and 1124 mg/kg b.w. in females OECD 408	At 2000 ppm in M and F: ↓ body weighs, ↓ body weight gains, ↓ food efficiency, ↑ mean relative liver weights (without histological changes), and haemolytic anemia. At 10000 ppm, in both sexes, renal hemosiderosis was correlated to the haemolytic process of red blood cells. NOAEL for Triflusulfuron-methyl (synthetized via the actual process) was 100 ppm (6.56 mg/kg b.w.) for M and (7.71 mg/kg b.w) for F	Purity: 98.7% "actual process"	Biegel L.B. (1992)
90-day feeding study in CD1 mice 0, 50, 750, 3750 and 7500 ppm corresponding to 0, 7.13, 116, 569 and 1164 mg/kg b.w. in males and 0, 11.8, 166, 817 and 1799 mg/kg b.w. in females (based material purity of 98.2%) OECD 408	No test substance related effects on the incidence of clinical signs of toxicity or mortality, body weights, food consumption or food efficiency for M or F at any dietary concentration. There were no test substance related effects on haematology parameters. ↑ mean absolute and relative liver weights in 3750 and 7500 ppm in M and F and at 750 ppm in M with ↑ incidence of centrilobular hepatocellular hypertrophy. NOAEL for Triflusulfuron-methyl (synthetized via the primary process) was 50 ppm	Purity: 91.9% "primary process" (the purity decreased between the beginning (94%) and the completion (81%) of the study. The actual intake of Triflusulfuron-methyl by mice might be lower due to the degradation of the active substance during the study and to the instability of the test material in food at the lowest concentration. Then, the correspondence	Mebus C.A. (1991)

Method	Results	Remarks	Reference
	(7.13 mg/kg b.w.) for M and 750 ppm (166 mg/kg b.w) for F	between ppm and mg/kg b.w could not be accurately determined.	
90-day feeding study in Beagle dogs 0, 100, 4000 and 8000 ppm corresponding to 0, 3.9, 146 and 268 mg/kg b.w. in males and 0, 3.7, 160 and 261 mg/kg b.w. in females OECD 409	At 4000 and 8000 ppm: - enlargement of the liver in M and F with an ↑ incidences of pigmented sinusoidal macrophages, bile stasis only in F, -testicular atrophy characterized by aspermatogenesis (aspermia in epididymis), decrease in thickness of the seminiferous tubules and cytoplasmic vacuolation of the germinal epithelium. - at 8000 ppm in M and F: hypercellularity of bone marrow consistent with the regenerative nature of the haematological effects NOAEL for Triflusulfuron-methyl (synthetized via the primary process) was 100 ppm (3.9 mg/kg b.w.) for M and (3.7 mg/kg b.w) for F	Purity: 95.6% "primary process"	Atkinson J.E. (1991)
1-year feeding study in Beagle dogs 0, 35, 875, and 3500 ppm corresponding to 0, 0.99, 26.9 and 111.8 mg/kg b.w. in males and 0, 1.2, 27.7 and 93.9 mg/kg b.w. in females OECD 452	At 3500 ppm : ↓ red blood cells, haematoglobin and haematocrit in M and F, ↑liver weight (+36% in M and +35% in F) with minimal centrilobular hepatocellular hypertrophy ↑ alkaline phosphatase only in M NOAEL = 875 ppm in M (26.9 mg/kg b.w) and F (27.7 mg/kg b.w)	Purity: 95.6% "primary process	Auletta C.S. (1993)
Dermal			
21-day dermal study in New Zealand White Rabbits 0, 50, 300 or 1000 mg/kg b.w/day OECD 410	No evidence of systemic toxicity. NOAEL = 1000 mg/kg b.w/day in M and F	Purity: 95.6% "primary process" EU guidelines requires 28 days. This study was conducted for only 21 days	MacKenzie S.A. (1993)

4.7.1 Non-human information

4.7.1.1 *Repeated dose toxicity: oral*

Reference: Biegel L.B. (1993)
GLP: Yes
Guidelines: OECD 408

Technical grade triflusulfuron-methyl (66037-8, primary process, purity 95.8%) was administered continuously via dietary administration to separate groups of Sprague Dawley rats (10/sex) at concentrations of 0, 100, 2000, 10000 and 15000 ppm for 90 days. The estimated mean daily intake of triflusulfuron-methyl was 0, 6.2, 127, 646 and 965 mg/kg body weight for males and 0, 7.54, 150, 774 and 1070 mg/kg body weight for females. Clinical signs were recorded daily, food consumption and body weight were recorded weekly. Ophthalmological examinations were performed during the pre-test and prior to the final sacrifice. Blood samples were collected for haematology and clinical chemistry after 53 and 90 days of feeding. Urine examinations were carried out on the same period. All animals were necropsied and selected organs weighed and a range of tissues were taken. ANOVA and Fisher's exact test were used as statistical methods.

The analyses of diet formulations indicated that the compound was homogeneously distributed in the food and had an acceptable stability when the diets were kept refrigerated, or at room temperature for < 7 days (especially in the low dose). Statistically significant changes induced by the treatment are summarized in table 4.7.1.1-1.

Table 4.7.1.1-1 – Effects of triflusulfuron-methyl (primary process) after a 90-day feeding exposure to rats

	Control		**100 ppm**		**2000 ppm**		**10000 ppm**		**15000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body weight			-	-	↓ (9)	↓ (16)	↓ (30)	↓ (29)	↓ (40)	↓ (35)
Body weight gain			-	-	↓ (16)	↓ (40)	↓ (52)	↓ (67)	↓ (72)	↓ (82)
Food consumption			-	-	-	↓ (10)	↓ (23)	↓ (19)	↓ (34)	↓ (31)
Food efficiency			-	-	↓ (12)	↓ (35)	↓ (38)	↓ (59)	↓ (57)	↓ (73)
Haematology										
RBC			-	-	↓	↓	↓	↓	↓	↓
Haemoglobin			-	-	↓	↓	↓	↓	↓	↓
Haematocrit			-	-	↓	-	↓	↓	↓	↓
Reticulocytes			-	-	↑	-	↑	↑	↑	↑
Biochemistry										
Glucose			-	-	-	-	↓	↓	↓	↓
Total proteins					↓	-	↓	-	↓	-
Organ weights										
Liver (relative)			-	-	-	↑ (24)	-	↑ (32)	↑ (13)	↑ (34)
Testis (absolute) Testis (relative)			- -		- -		- ↑ (46)		↓ (32) ↑ns(14)	
Gross observation										
Small testes					-		-		↑	
Histology										
Testes (atrophy/degeneration, oligospermia)	1/10		0/10		2/10		3/10		10/10	
Kidney Haemosiderosis Renal tubular cell atrophy			-	-	↑	↑	↑	↑	↑ 1/10	↑ 9/10

↑: increase. ↓: decrease. – no changes. ( ): % changes from concurrent controls.

The test compound induced decreases in body weight and body weight gain in both genders for doses ≥ 2000 ppm. If decreased food consumption was responsible for the decrease in body weight, decreased food efficiency was also a contributing factor. The haematological findings were considered to be indicative of a compound related anaemia. Increases in mean and relative reticulocytes counts might indicate that a regenerative bone marrow response took place and that the observed anaemia was independent of the nutritional stress. It appeared that the changes in erythrocyte morphology were the result of a direct effect of the test compound on the erythrocyte membrane, which in turn causes the erythrocytes to he removed from circulation more rapidly by the spleen (extra vascular haemolysis). The pattern of organ weight changes observed in this study (decreased absolute weights and increased relative weights) was consistent with observations reported with chronic underfeeding in the rat, except for relative liver weights [chronic underfeeding in the rat usually causes decrease in the relative liver weight in rats having decrease in total body weights (Scharer, K., Toxicology, 7:45-56, 1977)]. The increase in the mean relative liver weights in the 2000, 10000 (females) and 15000 (both genders) concentration levels suggests there is a compound-related effect on liver (without histopathological changes). The only treatment-related gross observation was small testes in the 15000 ppm group. This observation correlated with the histological observation of testicular atrophy and decreased absolute testicular weight. Testicular atrophy might result from the poor nutritional status, based on published data (Wright, J.R., pp 218-225, in Monographs on Pathology of Laboratory Animals, Genital System, T.C. Jones, U. Mohr, and R.D. Hunt, eds. Springer-Verlag Berlin Heidelberg, 1987) and on the findings of a previous 14-day oral toxicity study conducted at a dose level of 1500 mg/kg/day in which no testicular effects were recorded (the study was not available). Renal tubular epithelial cell atrophy was observed in the high-dose groups. The absence of necrosis and regeneration suggests that the atrophy in the kidney was due to the nutritional status of rats in the 15000 ppm group. Renal hemosiderosis was present in both male and female rats at doses ≥ 2000 ppm. This lesion was interpreted as a compound-related effect and is probably the result of the haemolytic anaemia.

Under the conditions of this study, administration of triflusulfuron-methyl (synthesised by the primary process) to male and female rats resulted in sub-chronic toxicity at dietary concentrations of 2000 ppm or greater. Toxicity was manifest in the form of decreased body weights, decreased body weight gains, decreased food efficiency, increased mean relative liver weights (without histological changes), and regenerative anaemia. Renal tubular epithelial atrophy, testicular atrophy/degeneration and oligospermia, and decreases in testicular weights were observed at dietary concentrations of 15000 ppm and were most likely attributable to poor nutrition. Renal hemosiderosis was correlated to the haemolytic process of red blood cells. The NOAEL for male and female rats was 100 ppm triflusulfuron-methyl corresponding to 6.2 and 7.5 mg/kg b.w. in males and females, respectively.

Reference: Biegel L.B. (1992)
GLP: Yes
Guidelines: OECD 408

Technical grade triflusulfuron-methyl (66037-59, actual process, purity 98.7%) was administered continuously via dietary administration to separate groups of Sprague Dawley rats (10/sex) at concentrations of 0, 100, 2000, 10000 and 15000 ppm for 90 days. The estimated mean daily intake of triflusulfuron-methyl was 0, 6.56, 133, 658 and 1036 mg/kg body weight for males and 0, 7.71, 153, 783 and 1124 mg/kg body weight for females. Clinical signs were recorded daily, food consumption and body weight were recorded weekly. Ophthalmological examinations were performed during the pre-test and prior to the final sacrifice. Blood samples were collected for haematology and clinical chemistry after 53 and 95 days of feeding. Urine examinations were carried out on the same period. All animals were necropsied and selected organs weighed and a range of tissues were taken. ANOVA and Fisher's exact test were used as statistical method.

The analyses of diet formulations indicated that the compound was homogeneously distributed in the food and had an acceptable stability when the diets were kept refrigerated, or at room temperature for < 7 days (especially in the low dose). Statistically significant changes induced by the treatment are summarized in table 4.7.1.1-2.

Table 4.7.1.1-2 – Effects of triflusulfuron-methyl (actual process) after a 90-day feeding exposure to rats

	Control		100 ppm		2000 ppm		10000 ppm		15000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body weight			↑(10)	-	↓ns (7)	-	↓ (12)	↓ (14)	↓ (19)	↓ (17)
Body weight gain			-	-	↓ns (11)	-	↓ (18)	↓ (27)	↓ (28)	↓ (33)
Food consumption			-	-	-	-	-	↓ (13)	↓ (10)	↓ (17)
Food efficiency			-	-	↓ (10)	-	↓ (12)	↓ (15)	↓ (20)	↓ (21)
Haematology										
RBC			-	-	↓	↓	↓	↓	↓	↓
Haemoglobin			-	-	↓	↓	↓	↓	↓	↓
Haematocrit			-	-	↓	↓	↓	↓	↓	↓
Reticulocytes			-	-	↑	-	-	↑	↑	↑
Lymphocytes			-	-	-	-	↑	-	↑	-
Organ weights										
Liver (relative)			-	-	-	↑ns (11)	-	↑ (33)	↑ (20)	↑ (28)
Histology										
Kidney Haemosiderosis			-	-	-	-	↑	↑	↑	↑

↑: increase. ↓: decrease. –: no changes. ( ): % changes from concurrent controls. Ns: not statistically significant

The test compound induced statistically significant decreases in body weight and body weight gain in both genders for doses ≥ 10000 ppm. If decreased food consumption was responsible for the decrease in body weight, decreased food efficiency was also a contributing factor. The haematological findings (minimal changes at 2000 ppm) were considered to be indicative of a compound related haemolytic anaemia (increases in mean and relative reticulocytes counts). Mild lymphocytosis occurred in the 10000 and 15000 ppm male groups. These leukocytic alterations were slightly more pronounced at the 53-day sampling time than at the 95-day sampling time, and were considered as probably a secondary result of inflammatory or immunologic response to the haemolysis. The pattern of organ weight changes observed in this study (decreased absolute weights and increased relative weights) was consistent with observations reported with chronic underfeeding in the rat, except for relative liver weights. The increase in the mean relative liver weights in the 2000 (female, not statistically significant), 10000 (females) and 15000 (both genders) concentration levels suggests there is a compound-related effect on liver (without histological changes). Renal hemosiderosis was present in both male and female rats at doses ≥ 10000 ppm. This lesion was interpreted as a compound-related effect and is probably the result of the haemolytic anaemia.

The effects (growth, haemolytic anaemia, liver) induced by the test compound (actual process) in this study was less pronounced than those observed in the previous study with the test compound produced by the primary process. Moreover, the effects observed on testes in the first study were not found in the present study. Leucocytosis in the 10000 and 15000 males groups was recorded in only one study.

Under the conditions of this study, administration of triflusulfuron-methyl (synthesised by the actual process) to male and female rats resulted in sub-chronic toxicity at dietary concentrations of 2000 ppm or greater. Toxicity was manifest in the form of decreased body weights, decreased body weight gains, decreased food efficiency, increased mean relative liver weights (without histological changes), and haemolytic anaemia. Renal hemosiderosis was correlated to the haemolytic process of red blood cells. The NOAEL for male and female rats was 100 ppm triflusulfuron-methyl corresponding to 6.6 and 7.7 mg/kg b.w. in males and females, respectively.

Reference: Mebus C.A. (1991)
GLP: Yes

Guidelines: OECD 408
The study was found acceptable. However, the actual intake of triflusulfuron-methyl by mice might be lower due to the degradation of the active substance during the study and to the instability of the test material in food at the lowest concentration (see below). Then the correspondence between ppm and mg/kg b.w. could not be accurately determined.

Technical grade triflusulfuron-methyl [66037-14, primary process, purity 91.9% (the purity of the test substance decreased between the beginning (94%) and the completion (81%) of the study)] was administered continuously via dietary administration to separate groups of CD1 mice (15/sex) at concentrations of 0, 50, 750, 3750 and 7500 ppm for 90 days. The estimated mean daily intake of triflusulfuron-methyl (based material purity of 98.2%) was 0, 7.13, 116, 569 and 1164 mg/kg body weight for males and 0, 11.8, 166, 817 and 1799 mg/kg body weight for females. Clinical signs were recorded daily, food consumption and body weight were recorded weekly. Ophthalmological examinations were performed during the pre-test and prior to the final sacrifice. Blood samples were collected (10 mice/sex/group) for haematology after 47 and 90 days of feeding. All animals were necropsied and selected organs weighed and a range of tissues were taken. ANOVA and Fisher's exact test were used as statistical method.

The analyses of diet formulations indicated that the compound was homogeneously distributed in the food and had an acceptable stability when the diets were kept refrigerated, or at room temperature for < 7 days for the concentrations ≥ 750 ppm. The 50 ppm food samples were less stable at room temperature and the concentrations decreased to about 50% of the nominal after 4 days. Statistically significant changes induced by the treatment are summarized in table 4.7.1.1-3.

Table 4.7.1.1-3 – Effects of triflusulfuron-methyl (primary process) after a 90-day feeding exposure to rats

	Control		**50 ppm**		**750 ppm**		**3750 ppm**		**7500 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Organ weights										
Liver (relative)			-	-	↑ns (6)	-	↑ (14)	↑ (11)	↑ (28)	↑ (20)
Histology										
Liver Centrilobular hypertrophy	0/15	0/15	0/15	0/15	5/15	0/15	11/15	9/15	14/15	14/15

↑: increase. ↓: decrease. –: no changes. ( ): % changes from concurrent controls. Ns: not statistically significant

There were no test substance-related effects on the incidence of clinical signs of toxicity or mortality, body weights, food consumption or food efficiency for male or female mice at any dietary concentration. There were no test substance related effects on haematology parameters. Mean absolute and relative liver weights were significantly increased in 3750 and 7500 ppm male and female mice and in the 750 ppm male group. Microscopic examination revealed that both male and female mice had increased incidence of centrilobular hepatocellular hypertrophy. The incidence of centrilobular hypertrophy corresponded with increased liver weights and likely represents microsomal enzyme induction and hyperplasia of endoplasmic reticulum.

Under the conditions of this study, the NOAEL of triflusulfuron-methyl (synthesised via the primary process) was 50 ppm for male and 750 ppm for female mice. The NOAEL was based on increased liver weights and centrolobular hepatocellular hypertrophy.

Reference: Atkinson J.E. (1991)
GLP: Yes
Guidelines: OECD 409

Technical grade triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered continuously via dietary administration to separate groups of Beagle dogs (4/sex) at concentrations of 0, 100,

4000 and 8000 ppm for 90 days. The estimated mean daily intake of triflusulfuron-methyl was 0, 3.9, 146, and 268 mg/kg body weight for males and 0, 3.7, 160 and 261 mg/kg body weight for females. Clinical signs were recorded daily, food consumption and body weight were recorded weekly. Ophthalmological examinations were performed during the pre-test and prior to the final sacrifice. Blood samples were collected for haematology and clinical chemistry after 45 and 90 days of feeding. All animals were necropsied and selected organs weighed and a range of tissues were taken. ANOVA (parametric and nonparametric) was used as statistical methods.

The analyses of diet formulations indicated that the compound was distributed homogeneously in the diet, was stable if stored at room temperature for 0, 4, or 7 days, or if stored frozen for 0, 4, 7, or 14 days, and was at expected concentrations at cage-site. Statistically significant changes induced by the treatment are summarized in table 4.7.1.1-4.

Table 4.7.1.1-4 – Effects of triflusulfuron-methyl (primary process) after a 90-day feeding exposure to dogs

	Control		**100 ppm**		**4000 ppm**		**8000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality								2/4
Body weight			-	-	-	-	↓ (25)	↓ (15)
Body weight gain			-	-	-	-	↓ (84)	↓ (57)
Haematology								
RBC			-	-	-	-	↓	↓
Haemoglobin			-	-	-	-	↓	↓
Haematocrit			-	-	-	-	↓	↓
Reticulocytes			-	-	-	-	↑	↑
Biochemistry								
SGOT/SGPT (d 45)			-	-	↑	-	↑	↑
Alkaline phosphatase					-	-	↑	↑
Organ weights								
Liver Absolute relative			-	-	 ↑ (28) ↑ (30)	 ↑ (42) ↑ (42)	 ↑ (28) ↑ (60)	 ↑ (36) ↑ (55)
Testis Absolute Relative			-	-	 ↓ns(25) ↓ns(23)		 ↓ (64) ↓ (45)	
Gross observation								
Liver enlarged	0/4	0/4	0/4	0/4	4/4	4/4	3/4	2/4
Testis small	0/4		0/4		2/4		4/4	
Epididymis small	0/4		0/4		0/4		2/4	
Histology								
Liver Sinusoidal macrophages: brown pigment Bile stasis	 1/4 0/4	 0/4 0/4	 0/4 0/4	 0/4 0/4	 3/4 0/4	 4/4 3/4	 3/4 0/4	 4/4 4/4
Testis Tubular atrophy Vacuolation germinal epithelium	 0/4 0/4		 0/4 0/4		 2/4 0/4		 3/4 2/4	
Epididymis Aspermatogeneis/Oligospermia Cell debris	 0/4 0/4		 0/4 1/4		 2/4 4/4		 4/4 3/4	
Bone marrow: hypercellularity	0/4	0/4	0/4	0/4	0/4	0/4	2/4	4/4

↑: increase. ↓: decrease. – no changes. ( ): % changes from concurrent controls.

Two high dose female dogs were sacrificed moribund. Treatment related effects were loss body weight, food consumption and severe anaemia. Animals in the 8000 ppm groups exhibited little to no body weight gain during the study while weight gains in the 100 and 4000 ppm dose groups were comparable to controls. An

initial decrease in food consumption was observed in the highest dose groups and was likely due to poor palatability, which was overcome after the first month on test.

The haematological changes (8000 ppm) were considered to be indicative of a compound related haemolytic anaemia. Mild lymphocytosis (not statistically significant) occurred in the 4000 and 8000 ppm groups. Mild transient increases in blood SGOT/SGPT and slight increases in alkaline phosphatase were indicative of moderate hepatotoxicity of the test compound at the highest dose level.

Organ weight changes considered to be treatment related were seen in the liver (increased) and testis (decreased) in the mid and high dose groups. Kidney relative weight was also increased (41%) in the 8000 ppm female group.

Test substance-related changes seen at gross post-mortem examination were enlargement of the liver in the 4000 and 8000 ppm males and females, small testes in the 4000 and 8000 ppm males and small epididymis in the 8000 ppm males. In the liver, microscopically, increased incidences of pigmented sinusoidal macrophages occurred in the 4000 and 8000 ppm male and female groups relative to controls. Bile stasis, characterised by the accumulation of brown pigment in the bile cannaliculi, was seen in the 4000 and 8000 ppm females. In the 4000 and 8000 ppm males, testicular atrophy was noted. This finding was characterised by aspermatogenesis (aspermia in the epididymis), decrease in thickness of the seminiferous tubules and cytoplasmic vacuolation of the germinal epithelium. The hypercellularity of bone marrow, seen in the 8000 ppm males and females, was consistent with the regenerative nature of the haematological effects.

Under the conditions of this study, administration of triflusulfuron-methyl (synthesised by the primary process) to male and female dogs resulted in sub-chronic toxicity at dietary concentrations of 4000 ppm or greater. Toxicity was manifest in the form of liver and testicular effects at the two highest doses. Regenerative anaemia and decreased body weight gain were observed at 8000 ppm. The NOAEL for male and female dogs was 100 ppm triflusulfuron-methyl corresponding to 3.9 and 3.7 mg/kg b.w. in males and females, respectively.

Reference: Auletta C.S. (1993)
GLP: Yes
Guidelines: OECD 452

Technical grade triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered continuously via dietary administration to separate groups of Beagle dogs (5/sex) at concentrations of 0, 35, 875 and 3500 ppm for 1 year. The estimated mean daily intake of triflusulfuron-methyl was 0, 0.99, 26.9, and 111.8 mg/kg body weight for males and 0, 1.2, 27.7 and 93.9 mg/kg body weight for females. Clinical signs were recorded daily, food consumption and body weight were recorded weekly. Ophthalmological examinations were performed during the pre-test and prior to the final sacrifice. Blood samples were collected for haematology and clinical chemistry in the pre-test period and after 3, 6, 9 and 12 months of feeding. Urinalysis was performed in the pre-test period and after 3, 6, 9 and 12 months of feeding. All animals were necropsied and selected organs weighed and a range of tissues were taken. ANOVA (parametric and nonparametric) was used as statistical methods.

The analyses of diet formulations indicated that the compound was distributed homogeneously in the diet, was stable if stored at room temperature for 7 days. Some 35 ppm diet samples were found slightly out of the limits (± 20% of nominal). Statistically significant changes induced by the treatment are summarized in table 4.7.1.1-5.

Table 4.7.1.1-5 – Effects of triflusulfuron-methyl (primary process) after a 1-year feeding exposure to dogs

	Control		**35 ppm**		**875 ppm**		**3500 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality							1/5	1/5
Haematology								

RBC			-	-	-	-	↓	↓
Haemoglobin			-	-	-	-	↓	↓
Haematocrit			-	-	-	-	↓	↓
Biochemistry								
Alkaline phosphatase					-	-	↑ (50)	-
Organ weights								
Liver relative			-	-	↑ ns (15)	-	↑ (36)	↑ (35)
Histology								
Liver Centrolobular hepatocellular hypertrophy (minimal)	0/5	0/5	0/5	0/5	0/5	0/5	3/5	2/5

↑: increase. ↓: decrease. – no changes. ( ): % changes from concurrent controls.

One male and one female in the 3500 ppm group were sacrificed *in extremis* on test days 151 and 99, respectively. The cause of death in the male dog appeared to be acute haemorrhage in the pleural cavity and gastrointestinal tract and thymus necrosis. The cause of death in the female dog was pulmonary bacterial infection. Thus, the aetiology of these early deaths could not be linked mechanistically to compound administration, although a compound-related effect could not be ruled out. Haematology evaluations revealed a slight anaemia in the 3500 ppm males and females that was most pronounced at month 3. Mean serum alkaline phosphatase values for 3500 ppm males at all intervals were increased (25-50%) when compared to controls. Mean liver weight parameters in 3500 ppm males and females were greater than mean control values. These effects were consistent with the microscopic observation of minimal centrilobular hepatocellular hypertrophy observed in these treatment groups. The slight increased (not statistically significant) liver weight in the 875 ppm male group might be treatment related but was not accompanied with any histological changes.

The NOAEL in the 1-year feeding study in dogs was 875 ppm (26.9 and 27.7 mg/kg/day for males and females, respectively). This NOAEL was based on changes in red cell mass parameters and liver enzymes (alkaline phosphatase) at 3500 ppm. Increased liver weight and corresponding microscopic liver hypertrophy were also present at 3500 ppm.

4.7.1.2 *Repeated dose toxicity: inhalation*

No data available

4.7.1.3 *Repeated dose toxicity: dermal*

Reference: MacKenzie S.A. (1993)
GLP: Yes
Guidelines: OECD 410. Although EU guideline requires 28 days, this study was conducted for only 21 days.

Technical grade triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was moistened with deionised water and applied to the shaved, intact dorsal skin of 5 male and 5 female New Zealand White rabbits (5/sex/dose) for 21 daily (consecutive) applications. The rabbits were exposed to the test substance for 6 hours per day. Exposure doses were 0, 50, 300, or 1000 mg/kg/day. Evaluated parameters included body weight, body weight gain, food consumption, food efficiency, evaluations for dermal irritation, clinical signs, clinical pathology (pre-test period and prior to final sacrifice), organ weights, and gross and microscopic pathology. ANOVA (parametric and nonparametric) was used as statistical methods.

Exposure to the test compound produced no statistically significant, treatment-related changes in mean body weight, body weight gain, food consumption and food efficiency. Slight or mild erythema was observed in animals in all groups (including controls). These effects were considered responses to the mechanical

irritation resulting from the testing procedure. No signs of systemic toxicity were observed. No compound-related effects were observed in haematology, clinical chemistry, or gross or microscopic pathology. Minimal to mild inflammation and acanthosis of the skin were observed in some rahbits in treated and control groups; these findings were attributed to the testing procedure. No target organ was identified.

In a 21-day dermal toxicity study, triflusulfuron-methyl produced no evidence of systemic toxicity at daily dosages of 50, 300, and 1000 mg/kg of body weight. The NOAEL was the high dosage, 1000 mg/kg/day, in both male and female rabbits.

4.7.1.4 *Repeated dose toxicity: other routes*

No data available

4.7.1.5 *Human information*

No data available.

4.7.1.6 *Other relevant information*

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4.7.1.7 *Summary and discussion of repeated dose toxicity*

Oral route:

The short-term toxicity of Triflusulfuron-methyl was evaluated in rats (90-day), mice (90-day), dogs (90-day and 1-year), after oral exposure. All of the studies were considered acceptable.

The targets organs of orally administered Triflusulfuron-methyl were primarily liver and red blood cells in rats and dogs, and liver in mice., rat and dog. Mice were relatively less sensitive than rats or dogs to Triflusulfuron-induced toxicity, as effects were limited to liver in this species.

Body weight gains were particularly impaired at the highest dose levels in rats (10000 and 15000 ppm) and in dogs (8000 ppm). Food efficiency was reduced at the same dose levels in the rats.

Liver weight was increased in all species, without any histological modification in rats, with centrilobular hypertrophy in mice and with some indication of moderate hepatotoxicity in dogs at 8000 ppm (elevated blood enzymes). Increased liver weight and corresponding microscopic liver hypertrophy were also present at 3500 ppm in the 1-year dog study.

The haematological changes in rats and dogs were consistent with a compound relative moderate to slight haemolytic anaemia at 10000-15000 ppm in rats and 3500-8000 ppm in dogs. Anaemia was occasionally accompanied by liver or kidney hemosiderosis.

Additionally, testicular atrophy/degeneration accompanied by oligospermia and decrease in the thickness of seminiferous tubules was observed in one 90-day rat study and the 90-day dog study. They were not recorded in the other 90-day rat study and in the 1-year dog study. It should be noticed that the testicular effects were observed at doses that significantly impaired the growth rate and were likely secondary to marked effects on body weight gain of treated animals. In a 90-day rat study statistically decreased testicular weights, and increased incidence of testicular atrophy/degeneration and oligospermia were observed in rats fed 15000 ppm Triflusulfuron-methyl. The mean body weight for this group of male rats was decreased by 40% when compared to the control group. This type of testicular effect has previously been reported to be caused by poor nutrition in the rat. Therefore, in light of the marked decrease in body weight, body weight gain, as well as the statistically significant decrease in food consumption, it was concluded that the observed

testicular effects in this study were attributed to general toxicity and not a direct compound-related effect In another 90-day rat study, in male rats fed 15000 ppm Triflusulfuron-methyl, only a 20% decrease in body weight and no testicular effects were observed, supporting the conclusion that the testicular effects were primarily due to general toxicity. In the 90-day study in dogs with Triflusulfuron-methyl, the testicular effects noted at 8000 ppm could be most likely the result of significantly decreased body weight gains in sexually immature animals. Testicular changes at 4000 ppm were equivocal at best, and the absence of testicular effects in dogs administered 3500 ppm for one year strongly suggests that the findings noted at 4000 ppm in the 90 day study could be not compound-related. Thus, based on consideration of the results of both the 90-day and 1-year studies, Triflusulfuron-methyl should not be considered a primary testicular toxin in dogs. "The report also concludes that, based on testicular weights and histology, compound-related effects were also present at 4000 ppm. However, there were no statistically significant effects on testes weights and with the exception of one animal (which had bilateral testicular atrophy, see below), the testicular weights of individual animals in the 4000 ppm were similar to-controls. Microscopically, bilateral testicular atrophy was present in 1 dog. The weight of evidence strongly suggests that this change is unrelated to compound exposure since only 1 of 4 dogs was affected, testicular atrophy is known to occur spontaneously in beagle dogs, and, perhaps most importantly, no testicular changes were present in dogs fed diets containing 3500 ppm of Triflusulfuron-methyl for 1 year. In the epididymis, minimal accumulation of cell debris in the epididymis was present in all dogs in the 4000 ppm group. However, this change was not dose-related, was associated with corroborative testicular changes in only one dog, and is a common finding in immature animals. Thus, this epididymal change is most likely attributable to the immaturity of the test species."

Two different methods have been used to produce the technical test compound. Similar target organs were observed in 90-day feeding studies in rats with Triflusulfuron-methyl synthesised by the primary or actual coupling processes. However, body weight and food consumption decrements were less severe in the study using the actual-process-derived technical material, particularly at high dose levels, and effects likely to be linked to general toxicity (testicular effects) were not reproduced.

Inhalation exposure:

No data.

Dermal exposure:

Triflusulfuron-methyl was not toxic by the dermal route in the rabbits at doses of up to 1000 mg/kg/day.

4.7.1.8 *Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD*

Oral route:

Based on the result of short-term (90-day and 1-year) oral studies, the NOAELs for triflusulfuron-methyl were based primarily on decreases in body weight parameters, decreases in red cell mass parameters, and/or effects on liver weights and liver enzymes. Organ weight and microscopic changes occurring secondary to body weight (testis) and haematology effects were also noted, particularly at higher doses. Mice were relatively less sensitive than rats or dogs to triflusulfuron-induced toxicity, as effects were limited to liver hypertrophy.

In the 90-day studies, these effects on liver and/or red blood cell parameters were observed from 127 mg/kg bw/d in rats, 146 mg/kg bw/d in dogs and 116 mg/kg bw/d in mice. In the 1-year dog study, these effects appeared from 94 mg/kg bw/d in females and 112 mg/kg bw/d in males.

Inhalation route:

No data.

Dermal route:

No adverse effects were observed at or below 1000 mg/kg bw in the available study (21-day exposure).

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

Rationale for classification as R48/22 (Danger of serious damage to health by prolonged exposure):

The 67/548/EEC criteria for classification as R48/22 are as follow:
Substances are classified as R48/22 when significant serious damage (clear functional disturbance or morphological change which has toxicological significance), is likely to be caused by repeated or prolonged exposure by an appropriate route, in a 90-day repeated-dose study conducted in experimental animals at a dose ≤ 50 mg/kg/d. When interpreting the results of a sub-acute (28-days) toxicity test, this value should be increased approximately three fold.

Consequently, as the observed changes of liver and red blood cells in rats and dogs and liver in mice are observed at doses > 50 mg/kg b.w/d, Triflusulfuron-methyl does not require classification for sub-chronic toxicity.

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

No classification is considered necessary for repeated exposure.

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

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

The same repeated dose toxicity findings are considered relevant for classification as STOT RE. See 4.7.1.8.

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

Rationale for classification as STOT-RE:

The CLP criteria for classification as STOT-RE are as follow:
“Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed in a 90-day repeated-dose study conducted in experimental animals within the guidance value ranges of 10-100 mg/kg/d.

Consequently, as the observed changes of liver and red blood cells in rats and dogs and liver in mice in the 90-day studies are observed at doses > 100 mg/kg b.w/d, Triflusulfuron-methyl does not require classification for sub-chronic toxicity.

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

No classification is considered necessary for repeated exposure.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 16. Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Gene mutation Ames test *S typhimurium* (TA97 TA98, TA100, TA1535) OECD 471	Negative	Purity 91.9% "primary process"	Reynolds V.L. (1991)
Gene mutation Ames test *S typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) OECD 471	Negative	Purity : 98.7% "actual process"	Molinier B. (1992)
Gene mutation CHO/HPRT assay OECD 476	Negative	Purity : 95.6% "primary process"	Rickard L.B. (1991)
DNA damage and repair *In vitro UDS assay* OECD 482	Negative	Purity : 95.6% "primary process"	Bentley K.S. (1991)
Chromosome aberration *In vitro* Mammalian Chromosomal Aberration (human lymphocytes) OECD 473	Negative without metabolic activation (3h exposure) Positive with metabolic activation (≥ 1700 µg/mL)	Purity : 95.6% "primary process"	Bentley K.S. (1991)
Chromosome aberration *In vitro* Mammalian Chromosomal Aberration (human lymphocytes) OECD 473	Negative without metabolic activation (3h exposure) Positive with metabolic activation (≥ 1850 µg/mL)	Purity : 98.7% "actual process"	Bentley K.S. (1992)
Chromosome aberration *In vitro* Mammalian Chromosomal Aberration (human lymphocytes) OECD 473	Negative (24 to 48 h exposure without metabolic activation). Negative with metabolic activation	Purity : 98.7% "actual process"	Molinier B. (1992)
Chromosome aberration *In vivo* mouse micronucleus test (oral route) OECD 474	Negative	Purity : 95.6% "primary process"	Gerber K.M. (1991)
Chromosome aberration *In vivo* mouse micronucleus test (oral route) OECD 474	Negative	Purity : 98.7% "actual process"	Molinier B. (1992)
Chromosome aberration *In vivo* spermatogonial chromosome aberration OECD 486	Negative	Purity : 98.7% "actual process"	Gudi R. (1997)

4.9.1 Non-human information

4.9.1.1 *In vitro data*

Mutagenicity evaluation in S. typhimurium (Ames test)

Reference: Reynolds V.L. (1991)
GLP: Yes
Guidelines: OECD 471

Triflusulfuron-methyl (66037-14, primary process, purity 91.9%, in DMSO) was used. *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 were used for testing. Rat liver S-9 fraction was prepared from the livers of rats, which received an i.p. injection of Aroclor 1254. Positive controls included 2-aminoanthracene (1-2 µg/plate) with all strains, sodium azide (2 µg/plate) with TA1535 and TA100, acridine (2 µg/plate) with TA97, 2-nitrofluorene (25 µg/plate) with TA98. The negative control was DMSO. Mutagenicity tests were performed in the absence and the presence of S9. After a preliminary assay (0.1 to 5000 µg/plate) to define the concentrations to be used for the mutagenicity study, the test substance was tested on two independent assays. The method used was the direct plate incorporation method. The concentrations of the test substance were 50, 100, 250, 500, 1000 and 3000 µg/plate. The test substance was classified as positive when the average number of revertants in any strain at any test substance concentration was at least 2 times greater than the average number of revertants in the concurrent negative control and occurred in a positive dose-response relationship ($p \leq 0.05$).

The test substance was toxic at concentrations ≥ 3000 µg/plate for the TA 100 strain with and without S9 mix. The average number of revertants at all concentrations of the test substance was similar to concurrent negative controls in studies both with and without metabolic activation. The assays were validated by the responses of the positive controls.

Under the conditions of this test, triflusulfuron-methyl (primary process) was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay (Ames test).

Reference: Molinier B. (1992)
GLP: Yes
Guidelines: OECD 471

Triflusulfuron-methyl (66037-59, actual process, purity 98.7%, in DMSO) was used. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were used for testing. Rat liver S-9 fraction was prepared from the livers of rats, which received an i.p. injection of Aroclor 1254. Positive controls included 2-anthramine (1-2 µg/plate) with all strains, sodium azide (1 µg/plate) with TA1535 and TA100, 9-aminoacridine (50 µg/plate) with TA1537, 2-nitrofluorene (0.5 µg/plate) with TA1538 and TA 98. The negative control was DMSO. Mutagenicity tests were performed in the absence and the presence of S9. After a preliminary assay (10 to 5000 µg/plate) to define the concentrations to be used for the mutagenicity study, the test substance was tested on two independent assays. The methods used were: the direct plate incorporation method for the 2 assays without S9 mix and for the first assay with S9 mix, and the pre-incubation method (1 h, 37°C) for the second assay with S9 mix. The concentrations of the test substance were 62.5, 125, 250, 500 and 1000 µg/plate. The test substance was classified as positive when the average number of revertants in any strain at any test substance concentration was at least 2 times greater than the average number of revertants in the concurrent negative control and occurred in a positive dose-response relationship ($p \leq 0.05$).

The test substance was toxic at concentrations ≥ 2500 µg/plate for the TA 100 strain with and without S9 mix: no colonies were observed but the bacterial lawn was normal. At 1000 µg/plate, the test substance was slightly toxic as seen by a decrease in the number of revertants. At lower concentrations, no toxicity was

observed. The average number of revertants at all concentrations of the test substance was similar to concurrent negative controls in studies both with and without metabolic activation. The assays were validated by the responses of the positive controls.

Under the conditions of this test, triflusulfuron-methyl (actual process) was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay (Ames test).

Mutagenicity evaluation in mammalian cells (CHO/HGPRT assay)

Reference: Rickard L.B. (1991)
GLP: Yes
Guidelines: OECD 476

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%, in DMSO) was tested in the CHO/HGRT mutation assay with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Both an initial and an independent repeat mutagenicity assay were conducted, with concurrent evaluation of test substance toxicity. Duplicate flasks of exponentially growing CHO-K_1-BH_4 cells were exposed to the test substance at concentrations of 0, 100, 500, 1000, 1500, and 2000 µg/mL. The cells were exposed for 18-19 hours in the non-activated, and for 5 hours in the activated test systems. The highest concentration level was set based on solubility and culture medium pH limitations, and the need to limit the concentration of the organic solvent to 1% (v/v) in the treatment medium. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-Thioguanine (TG, 2-amino-6-mercaptopurine)-resistant phenotype. Ethyl methanesulfonic acid (62.7 µg/mL) and 9,10-dimethyl-1,2-benzanthracene (3.9 µg/mL) were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of ≤ 50% of the concurrent vehicle controls. The assay was considered positive when the mutant frequency of one or more of the concentrations tested was significantly greater than that of the solvent control, where significance was judged at the 95% level of confidence (Student's t test). Additionally, there should be a significant positive correlation between the mutant frequency and increasing concentrations of the test substance, where significance was judged at the 99% level of confidence.

Severe cytotoxicity was observed at the 2000 µg/mL concentration level in both the non-activated and S9 activated trials. Plating efficiency was similar to negative controls for dose levels of triflusulfuron-methyl ranging from 100 to 1500 µg/mL (not shown). No statistically significant increases in mutant frequency occurred at any of the test substance concentrations, and no positive dose-response relationship was present in either the non-activated or activated test system (table 4.9.1.1-1).

Table 4.9.1.1-1- *In vitro* mammalian gene mutation: Summary of mutagenicity findings

Compound	**Conc. µg/mL**	**Mutation Frequency without Activation ***		**Mutation Frequency with S9 Activation ***	
		Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
Triflusulfuron methyl	*0*	*2.15*	*0.6*	*0*	*4.1*
	100	*3.7*	*0.65*	*1.85*	*8.8*
	500	*8.65*	*1.15*	*8.55*	*0*
	1000	*5.8*	*0*	*3.55*	*5.55*
	1500	*2.45*	*1.3*	*0.7*	*8.25*
	2000	*Ne*	*Ne*	*Ne*	*Ne*
EMS	*62.7*	*131.9§*	*82.9§*	*Ne*	*Ne*
DMBA	*3.9*	*Ne*	*Ne*	*150.95§*	*82.7§*

* mutants per 1 x 10^6 surviving cells defined as: total mutant colonies/(number of selection dishes x cloning efficiency x 2 x 10^5 cells) x 10^6; values represent the average of duplicate flasks. EMS = ethyl methanesulfonate. DMBA = 9,10-dimethyl-1,2-benzanthracene. Ne = not evaluated due to toxicity. § statistically significant ($p \leq 0.05$; Student's t test)

Under the conditions of this test, triflusulfuron-methyl (primary process) was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* mammalian cell (CHO/HGPRT) mutation assay.

Mutagenicity evaluation in the in vitro unscheduled DNA synthesis in isolated rat hepatocytes

Reference: Bentley K.S. (1991)
GLP: Yes
Guidelines: OECD 482

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%, in DMSO) was tested in the Unscheduled DNA Synthesis (UDS) assay using primary cultures of rat hepatocytes. The concentrations tested were 0, 50, 100, 500, 1000, 1500, and 2000 µg/mL. The highest dose level was set based on solubility and culture medium pH limitations, and the need to limit the concentration of the organic solvent to 1% (v/v) in the treatment medium. Two independent assays were performed. 2-Acetylaminofluorene (0.02 and 0.2 µg/mL) and DMSO served as positive and vehicle controls, respectively. Hepatocyte cultures were exposed to the test substance (or the positive or vehicle control) along with 5 µCi/mL ^{3}H-thymidine/mL, for approximately 18 hours. Cytotoxicity was assessed based on lactate dehydrogenase (LDH) concentration in the culture medium and by microscopic examination of hepatocyte cultures of fixed and stained cells. Twenty-five cells from each culture, two cultures from each concentration were scored individually by measuring the area of the silver grains over the nucleus and the area of the grains in two or more nucleus-sized regions in the cytoplasm immediately adjacent to the nucleus. Areas of the grains were converted to grain counts through the use of a factor that was determined for each slide. Cytoplasmic grain counts were subtracted from the nuclear grain counts to determine the net nuclear grains (NNG) of each cell. The mean NNG and standard error of the mean NNG were calculated for each slide. The mean NNG from all slides of the same test concentration were averaged to determine the UDS response for that treatment in each trial. A test substance was considered positive if the average UDS response for any concentration of the test substance from both trials was +5 NNG or more and this increase was at least 3 standard deviations above the control response or statistically significant at $p \leq 0.01$. There should also be a statistically positive correlation ($p \leq 0.05$) between increasing concentrations of test substance and the average UDS response in the absence of a negative correlation between test concentration and average cytoplasmic grains.

Cytotoxicity, determined by elevation of LDH activity in the medium, was not observed at any concentration. However, upon visual inspection of the cells on the slides, signs of toxicity were apparent at 1500 and 2000 µg/mL. At these concentrations, the cells were not scorable. None of the concentrations of the test substance caused a significant increase in the mean net nuclear grain counts when compared to negative controls. Consequently the percent of cells in repair was not calculated. The responses of the positive control validated the assays.

In vitro treatment of primary cultures of hepatocytes from male rats with triflusulfuron-methyl at concentrations up to the highest non cytotoxic dose of 1000 µg/ml showed no induction of UDS (DNA repair).

In vitro mammalian cytogenicity test - clastogenicity in human lymphocytes

Reference: Bentley K.S. (1991)
GLP: Yes

Guidelines: OECD 473. Cells were exposed to the test substance for 3 hours only, in the absence of metabolic activation.

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%, in DMSO) was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Cytotoxicity was evaluated based on a cell cycle delay assessment (i.e., determination of the average generation time, AGT) at test substance concentrations of 0, 0.1, 0.5, 0.8, 1.0, 1.2, 1.5, and 2.0 (limit of solubility) mg/mL.
Three independent clastogenicity trials were conduced. Concentration levels in the non-activated and S9-activated Trial 1 of the chromosome aberration assays were 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/mL. The non-activated and S9-activated Trial 2 was conducted at concentrations of 0, 0.1, 1.0, 1.5, and 2.0 mg/mL concentration levels. Based on the results of the first 2 trials, an additional S9-activated Trial 3 was conducted at the 0, 1.5, 1.7, 1.85, and 2.0 mg/mL concentration levels. Supplemental scoring of cells from the first-division metaphase/cytotoxicity trial in conjunction with the first S9 activated trial was also conducted. Mitomycin C (0.35 µg/mL) and cyclophosphamide (10 µg/mL) were used as the positive control for the non-activated and S9 activated test systems, respectively. Cells were treated for approximately 3 hours, and metaphase cells were harvested approximately 24 hours following the initiation of treatment. Colcemid was present during the final 2-3 hours to arrest cells in metaphase. Cells were evaluated for toxicity (mitotic inhibition), and then 100 metaphase cells per dose level were evaluated for structural chromosome aberrations. The test substance was considered to induce a positive response if the percentage of cells with aberrations was increased, as compared to the negative (solvent) control, at a minimum of one test concentration ($p \leq 0.05$; Fisher's Exact test), and there was a statistically significant dose-related increase in the percent abnormal cells ($p \leq 0.01$; Cochran-Armitage test).

AGT was increased after metabolic activation for the concentration of triflusulfuron-methyl of 2000 µg/mL. The chromosome aberration data are summarized in table 4.9.1.1-2. Under non-activated conditions, no statistically significant increases or concentration-related trends in structural chromosome aberrations were observed in either Trial 1 or Trial 2 at any concentration level. It should be noticed that the cells were exposed to the test compound only for 3 hours. In Trial 1 with S9 activation, no statistically significant increases or concentration-related trends in chromosome aberrations were observed, although cytotoxicity (as indicated by depression of mitotic index) was observed at 2 mg/mL. In Trial 2, a statistically significant increase in chromosome aberrations was seen at the 2.0 mg/mL concentration, which was also highly cytotoxic. This positive result was confirmed by supplemental scoring of cells from the first-division metaphase cytotoxicity trial conducted in conjunction with Trial 1, where a statistically significant increase ($p < 0.05$, Fisher's exact test) in cells with chromosome aberrations was observed in the 2.0 mg/mL dose group as compared with the negative control group (not shown). In Trial 3, which was conducted only with S9 activation, statistically significant and concentration-related increases in structural chromosome aberrations were observed at the 1.7, 1.85, and 2.0 mg/mL concentration levels. In this trial, cytotoxicity was again noted at the 2.0 mg/mL concentration level. Numerical chromosome aberrations were not evaluated in any of the trials.

Table 4.9.1.1-2 - Summary of chromosome aberration induced in vitro by triflusulfuron-methyl (primary process)

Triflusulfuron-methyl Dose (mg/mL)	Exposure Time (hrs)	S9 Activation	Average Mitotic Index[a]	Percent of cells with structural aberrations[b]
TRIAL 1				
DMSO	**3**	**-**	**6.4**	**0**
0.1	**3**	**-**	**8.6**	**2**
0.5	**3**	**-**	**9.3**	**1**
1.0	**3**	**-**	**9.0**	**0**
1.5	**3**	**-**	**7.4**	**0**
2.0	**3**	**-**	**7.6**	**0**
MMC	**3**	**-**	**6.0**	**20***
DMSO	**3**	**+**	**7.7**	**0**
0.1	**3**	**+**	**10**	**0**
0.5	**3**	**+**	**11**	**0**
1.0	**3**	**+**	**9.4**	**0**
1.5	**3**	**+**	**9.6**	**1**
2.0	**3**	**+**	**2.1**	**3**
CP	**3**	**+**	**6.4**	**20 ***
TRIAL 2				
Untreated	**3**	**-**	**6.7**	**0**
DMSO	**3**	**-**	**7.5**	**1**
0.1	**3**	**-**	**6.0**	**0**
1.0	**3**	**-**	**5.8**	**2**
1.5	**3**	**-**	**5.6**	**0**
2.0	**3**	**-**	**3.9**	**3**
MMC	**3**	**-**	**6.2**	**20 ***
Untreated	**3**	**+**	**8.2**	**0**
DMSO	**3**	**+**	**8.6**	**2**
0.1	**3**	**+**	**9.2**	**0**
1.0	**3**	**+**	**7.0**	**0**
1.5	**3**	**+**	**6.0**	**0**
2.0	**3**	**+**	**3.7**	**11***
CP	**3**	**+**	**5.6**	**11 ***
TRIAL 3				
DMSO	**3**	**+**	**9.5**	**5**
1.5	**3**	**+**	**7.4**	**11**
1.7	**3**	**+**	**8.0**	**14***
1.85	**3**	**+**	**6.4**	**15***
2.0	**3**	**+**	**3.8**	**17***
CP	**3**	**+**	**6.0**	**30***

* statistically significant ($p \leq 0.05$; Fisher's Exact test). MMC = 0.35 µg/mL mitomycin C. CP = 10 µg/mL cyclophosphamide. (a): 500 cells evaluated per dose group. (b): 100 metaphase cells evaluated per dose group

Triflusulfuron-methyl (primary process) was negative for the induction of chromosome aberrations in the non-activated test system, but positive in the S9-activated test system. Under the conditions of this assay, triflusulfuron-methyl was found to be positive in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes. The absence of clastogenic potential without activation should be confirmed after a prolonged treatment time (20 hours).

Reference: Bentley K.S. (1992)
GLP: Yes
Guidelines: OECD 473. Cells were exposed to the test substance for 3 hours only, in the absence of metabolic activation.

Triflusulfuron-methyl (66037-59, actual process, purity 98.7%, in DMSO) was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Two independent clastogenicity trials were conduced. Concentration levels in the non-activated and S9-activated trials were 0, 0.5, 1.5, 1.7, 1.85, and 2.0 mg/mL based on the information and data of the earlier chromosome aberration assay (study 775-91, B.6.4.4.1). Mitomycin C (0.35 µg/mL) and cyclophosphamide (10 µg/mL) were used as the positive control for the non-activated and S9 activated test systems, respectively. Cells were treated for approximately 3 hours, and metaphase cells were harvested approximately 19-20 hours following the initiation of treatment. A second harvest time, approximately 24 hours after the first (43-hours post treatment), was also included in the second trial, but not evaluated since positive results were obtained in the concurrently-treated activated

cultures initially harvested 20 hours post-treatment. Colcemid was present during the final 2-3 hours to arrest cells in metaphase. Cells were evaluated for toxicity (mitotic inhibition), and then 100 metaphase cells per dose level were evaluated for structural chromosome aberrations. The test substance was considered to induce a positive response if the percentage of cells with aberrations was increased, as compared to the negative (solvent) control, at a minimum of one test concentration ($p \leq 0.05$; Fisher's Exact test), and there was a statistically significant dose-related increase in the percent abnormal cells ($p \leq 0.01$; Cochran-Armitage test).

The chromosome aberration data are summarized in table 4.9.1.1-3. Under non-activated conditions, no statistically significant increases or concentration-related trends in structural chromosome aberrations were observed in either trial. In both trials with S9 activation, cytotoxicity was observed at 2.0 mg/mL, as evident by mitotic index depression. Statistically significant ($p \leq 0.05$) increases in the numbers of cells with chromosome aberrations were also seen at that concentration. No statistically significant increases in the number of cells with structural chromosome aberrations were evident at concentration levels of ≤ 1.85 mg/mL in either activated trial based upon pair-wise analysis. However, by trend analysis, a statistically significant ($p \leq 0.01$) concentration-related increase in the number of cells with structural chromosome aberrations was noted in the first activated trial at 1.85 mg/mL. This increase was not reproduced in the second trial.

Table 4.9.1.1-3 - Summary of chromosome aberration induced in vitro by triflusulfuron-methyl (actual process)

Triflusulfuron-methyl dose (mg/mL)	Exposure time (hrs)	S9 activation	Average mitotic index[a]	Percent of cells with structural aberrations[b]
TRIAL 1				
DMSO	**3**	**-**	**9.2**	**2**
0.5	**3**	**-**	**9.3**	**1**
1.5	**3**	**-**	**10.9**	**1**
1.7	**3**	**-**	**8.4**	**2**
1.85	**3**	**-**	**8.5**	**5**
2.0	**3**	**-**	**8.6**	**6**
MMC	**3**	**-**	**9.0**	**36***
DMSO	**3**	**+**	**9.8**	**1**
0.5	**3**	**+**	**9.4**	**2**
1.5	**3**	**+**	**8.7**	**0**
1.7	**3**	**+**	**6.6**	**4**
1.85	**3**	**+**	**4.3**	**6**
2.0	**3**	**+**	**3.2**	**12***
CP	**3**	**+**	**2.8**	**45 ***
TRIAL 2				
DMSO	**3**	**-**	**6.8**	**3**
0.5	**3**	**-**	**6.0**	**2**
1.5	**3**	**-**	**7.1**	**5**
1.7	**3**	**-**	**6.2**	**4**
1.85	**3**	**-**	**5.5**	**4**
2.0	**3**	**-**	**5.4**	**3**
MMC	**3**	**-**	**7.0**	**48 ***
DMSO	**3**	**+**	**7.5**	**1**
0.5	**3**	**+**	**8.3**	**2**
1.5	**3**	**+**	**6.0**	**0**
1.7	**3**	**+**	**5.2**	**3**
1.85	**3**	**+**	**3.0**	**4**
2.0	**3**	**+**	**2.0**	**9***
CP	**3**	**+**	**3.4**	**35 ***

* statistically significant ($p \leq 0.05$; Fisher's Exact test). MMC = 0.35 µg/mL mitomycin C. CP = 10 µg/mL cyclophosphamide. (a): 500 cells evaluated per dose group. (b): 100 metaphase cells evaluated per dose group

Triflusulfuron-methyl (actual process) was negative for the induction of chromosome aberrations in the non-activated test system, but positive in the S9-activated test system at a concentration of 2 mg/mL, a concentration that also produced cytotoxicity. Under the conditions of this assay, triflusulfuron-methyl was found to be positive in the *in vitro* mammalian cytogenetics test using human peripheral blood lymphocytes.

The absence of clastogenic potential without activation should be confirmed after a prolonged treatment time (20 hours).

Reference: Molinier B. (1992)
GLP: Yes
Guidelines: OECD 473

Triflusulfuron-methyl (66037-59, actual process, purity 98.7%, in DMSO) was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Two independent clastogenicity trials were conduced. Concentration levels in the first non-activated and S9-activated chromosome aberration assays were 0, 12.5, 25, 50, 100, 200, and 400 µg/mL. 400 µg/mL was the limit of solubility of the test compound (in this study, the final concentration of DMSO in the medium was limited to approximately 0.2%, against 1% in both previous studies). Based on the cytotoxicity assessment, three concentration levels and controls were evaluated for clastogenicity: 50, 100, and 200 µg/mL in the non-activated assay, and 100, 200, and 400 µg/mL in the S9 activated assay. In the non-activated repeat chromosome aberration assay, the concentration levels were 50, 100, and 200 µg/mL, and in the S9 activated assay the concentration levels were 100, 200, and 400 µg/mL. Mitomycin C (0.2 µg/mL) and cyclophosphamide (50 µg/mL) were used as the positive control for the non-activated and S9 activated test systems, respectively. In the non-activated tests, the cells were exposed to the test substance for 24 hours in the first assay, and for 24 and 48 hours in the repeat test. In the S9 activated tests, cells were exposed to the test substance and the S9 mix for 2 hours, after which the cells were rinsed and incubated with fresh culture medium until harvest after 22 or 46 hours. Colcemid was present during the final 2-3 hours to arrest cells in metaphase. Cells were evaluated for toxicity (mitotic inhibition), and then 200 metaphase cells per dose level were evaluated for structural chromosome aberrations. Numerical chromosome aberrations were evaluated only at the 48-hour time period. The test substance was considered to induce a positive response if, at one or more concentrations, it induced a reproducible and statistically significant increase in the incident of aberrant cells. This increase should exceed the normal range of the historical negative control data.

Cytotoxicity, as indicated by a reduction of the mitotic index, was observed at 400 µg/mL under non-activated conditions only after the 24-hour exposure time used in the first non-activated test. With S9 activation, the mitotic index was similar to that of the controls. In the repeat test, the mitotic index was slightly reduced (by 31%) at 200 µg/mL under non-activated conditions only. However, these slides showed reduced quality, indicating some toxicity. With S9 activation the mitotic index was similar to that of the controls in the repeat test. At the 48-hour sampling time, the mitotic indices were similar to that of the controls for all concentration levels, with and without S9 activation. No statistically significant increases or concentration-related trends in structural and numerical chromosome aberrations were observed in any test at any sampling time under non-activated or S9 activated conditions.

Triflusulfuron-methyl (actual process) was negative for the induction of chromosome aberrations in both the non-activated and S9 activated test systems. Under the conditions of this assay (highest concentrations tested 200-400 µg/ml, 24-48 hours of exposure to the test compound without metabolic activation), triflusulfuron-methyl was found to be negative in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes.

4.9.1.2 *In vivo data*

Mutagenicity evaluation in the in vivo micronucleus test in the mouse

Reference: Gerber K.M. (1991)
GLP: Yes
Guidelines: OECD 474

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%, in corn oil) was administered orally (single applications) at 0, 1250, 2500 or 5000 mg/kg body weight to groups of 5-6 male and 5-6 female CR1-CD mice. The positive control groups were treated with single oral dose of 40 mg/kg cyclophosphamide. Negative controls and animals treated with the highest dose of triflusulfuron-methyl were sacrificed 24, 48 and 72 hours after administration for bone marrow sampling. Bone marrows were collected from the remaining dosed groups and the positive controls after 24 hours. A total of 1000 polychromatic erythrocytes (PCEs) per animal were counted for the presence of micronuclei. The ratio of PCEs to NCEs was evaluated. The data were transformed (arcsine square-root function) prior to statistical one-way analysis of variance (ANOVA). The dose groups were compared using each animal as an experimental unit.

Within approximately 3-4 hours of dosing, lethargy and/or abnormal gait were observed in most mice treated with the 5000 mg/kg dose. By 24-28 hours post-treatment, 1 male and 1 female were found dead, and approximately 75% of the male mice and 50% of the female mice were still slightly lethargic. Within 48 hours of dosing, another male mouse was found dead. With the exception of ruffled fur and soft stool in some animals, all remaining animals in the 5000 mg/kg dose group appeared normal at 48 hours. In the intermediate and low dose groups, lethargy was observed within 3-4 hours of dosing in all animals treated with 2500 mg/kg, and in 40% of the males and females treated with 1250 mg/kg. Forty percent of the male mice and 60% of the female mice in this dose group exhibited abnormal gait. All animals in the 2500 and 1500 mg/kg dose groups appeared to recover within 24 hours of treatment. Statistically significant mean body weight loss was observed in the 5000 mg/kg treated males at the 48-hour sampling time. No significant weight loss or decreased weight gain occurred in the females or in the other treated groups at any sacrifice interval as compared to concurrent vehicle controls.
There were no statistically significant increases in the MNPCE frequency in male or female mice at any sampling time at any dose level tested. Statistically significant depressions (40 to 45%) in the proportion of PCEs among 1000 NCEs were observed at 48-hours in the 5000 mg/kg males and females. Significant increases in MNPCEs were noted in the positive controls.

Triflusulfuron-methyl (primary process) did not induce an increase in micronuclei in bone marrow cells of male or female mice and is considered negative in this *in vivo* assay, at acute oral doses up to 5000 mg/kg b.w.

Reference: Molinier B. (1992)
GLP: Yes
Guidelines: OECD 474

Triflusulfuron-methyl (66037-59, actual process, purity 98.7%, in 0.5% methylcellulose) was administered orally (single application) at 0 or 5000 mg/kg body weight to groups of 10 male and 10 female Swiss OF1 mice. The positive control groups (5 males and 5 females) were treated with single oral dose of 40 mg/kg cyclophosphamide. Five mice/sex/group were sacrificed after 24 hours or 48 hours (24 hours only for the CP group) and bone marrow smears were prepared. A total of 2000 polychromatic erythrocytes (PCEs) per animal were counted for the presence of micronuclei. The ratio of PCEs to NCEs was evaluated. The test substance was considered positive if there was a statistically significant increase (Kastenbauam and Bowen test; $p \leq 0.05$ for males and female combined) in the mean number of MNPCEs for at least one of the sampling times when compared to the vehicle group, and if this increase was double the number of MNPCEs of the historical control data.

No clinical signs of toxicity or mortality were observed in either males or females after treatment with the test substance. In the test substance treated groups, the mean values of MNPCEs were similar to those of their respective control groups. There were no statistically significant increases in the mean MNPCEs frequency at any sampling time. No statistically significant depressions in the proportion of PCEs among 1000 NCEs were observed among the test substance treated animals at any sampling time. Statistically significant increases in MNPCEs were noted in the CP (positive control) group, confirming the sensitivity of the test system.

Triflusulfuron-methyl (actual process) did not induce an increase in micronuclei in bone marrow cells of male or female mice and is considered negative in this *in vivo* assay, at an acute oral dose of 5000 mg/kg b.w.

Mutagenicity evaluation in the in vivo spermatogonial chromosome aberration test in the mouse

Reference: Gudi R. (1997)
GLP: Yes
Guidelines: OECD 483

Triflusulfuron-methyl (66037-59, actual process, purity 98.7%, in corn oil) was evaluated for its ability to induce structural chromosome aberrations in spermatogonial cells of CR1:CD®–1(ICR)BR male mice. The animals (8/sex/group) received the test substance orally (single dose) at concentrations of 0, 1250, 2500, and 5000 mg/kg b.w. The positive control group was treated by a single intraperitoneal injection with Mitomycin C at dose levels of 1.75 and 2.5 mg/kg. Animals were sacrificed at 24 or 48 hours (control and test substance groups only) following dosing. Only one dose level (1.75 mg/kg) of the positive control was selected for cytogenetic analysis 24 hours after treatment. Four to five hours prior to sacrifice, each animal received colchicine by a single i.p. injection of 4.0 mg/kg body weight to arrest dividing cells in metaphase. Following sacrifice, spermatogonial cells were collected from testis, treated with hypotonic solution, fixed, smeared on slide and stained with Giemsa. Slides were evaluated in 5 animals/group/time point. A total of 500 cells per animal were evaluated to assess toxicity based on the average number of mitoses (mitotic index). A total of 500 cells per group were evaluated for structural chromosome aberrations. Only cells with 38-42 chromosomes were judged acceptable for scoring. The number of cells with aberrations in treated groups was compared to solvent control using the Fisher's exact test. The Cochran-Armitage trend test was used to test for evidence of a dose response. The test substance was considered to induce a positive response when the number of aberrant cells was significantly increased in a dose responsive manner relative to the vehicle control. A significant increase at the high dose only with no dose-response was considered suspect, and an increase at any other dose with no dose-response was considered equivocal.

A reduction in the rate of body weight gain was observed at the 2500 mg/kg dose level, and a loss in body weight was observed at the 5000 mg/kg dose level. Clinical signs observed within 4 hours following dose administration included ataxia and lethargy in mice at all dose levels. On day one following dose administration, clinical signs included lethargy and diarrhoea at the 1250 and 2500 mg/kg dose levels. A dose response decrease in the mitotic index was observed at both sampling points, with an average 56% and 54% reduction observed at the top dose at the 24- and 48-hour sacrifice times, respectively. No statistically significant increases in structural chromosome aberrations were observed at any dose level at either sacrifice time. The positive control groups were significantly different from the negative control group for all evaluated endpoints.

Triflusulfuron-methyl (actual process) did not induce structural chromosome aberrations in spermatogonial cells of male mice and was considered non clastogenic for germ cells in this *in vivo* test at doses up to 5000 mg/kg b.w.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Triflusulfuron-methyl was negative for mutagenicity and/or genotoxic potential in bacterial cultures, cultured mammalian cells (CHO cells), and primary rat hepatocytes (unscheduled DNA synthesis). Triflusulfuron-methyl was positive for chromosome aberration in 2 out of 3 *in vitro* assays in human lymphocytes. These positive responses occurred only at high *in vitro* concentrations (≥ 1700 µg/mL), in the presence of a metabolic activation system. In addition, three *in vivo* assays for chromosome aberrations (2 mouse micronucleus assays and a mouse spermatogonial assay) were all negative.

The weight of evidence from the complete battery of *in vitro* and *in vivo* genetic toxicology studies conducted with Triflusulfuron-methyl indicates no genotoxic potential.

4.9.5 Comparison with criteria

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified in category 3 for mutagenic endpoint if the substance causes concern for man owing to possible mutagenic effects. There would be evidence from appropriate mutagenicity studies, but this is insufficient to place the substance in category 2. Positive results are needed in assays showing mutagenic effects or other cellular interaction relevant to mutagenicity, in somatic cells in mammals *in vivo*. The latter especially would normally be supported by positive results from *in vitro* mutagenicity assays.
The following methods are considered as appropriate:

- In vivo somatic cell mutagenicity assays:
  - Bone marrow micronucleus test or metaphase analysis
  - Metaphase analysis of peripheral lymphocytes,
  - Mouse coat colour spot tesy
- In vivo somatic cell DNA interaction assys:
  - Test for SCEs in somatic cells,
  - Test for UDS in somatic cells,
  - Assay for the (covalent) binding of mutagen to somatic cell DNA,
  - Assay for DNA damage, e.g. by alkaline elution, in somatic cells.

2) Criteria in the CLP classification:

A substance shall be classified in category 2 for germ cell mutagenicity endpoint if the substance causes concern for humans owing to the possibility that they may induce heritable mutation in the germ cells of humans. This classification is based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
  - Somatic cell mutagenicity tests in vivo, in mammals (mammalian bone marrow chromosome aberration test, mouse spot test or mammalian erythrocyte micronucleus test); or
  - Other in vivo somatic cell genotoxicity test (UDS or SCE assay) which are supported by positive results from in vitro mutagenicity assays (in vitro mammalian chromosome aberration test, in vitro mammalian cell gene mutation test or bacterial reverse mutation test).

3) Comparison with criteria:

Triflusulfuron-methyl was positive for chromosome aberration in 2 out of 3 *in vitro* assays in human lymphocytes. These positive responses occurred only at high *in vitro* concentrations (≥ 1700 µg/mL), in the presence of a metabolic activation system.
However, Triflusulfuron-methyl was negative for mutagenicity and/or genotoxic potential in bacterial cultures, cultured mammalian cells (CHO cells), and primary rat hepatocytes (unscheduled DNA synthesis).
In addition, three *in vivo* assays for chromosome aberrations (2 mouse micronucleus assays and a mouse spermatogonial assay) were all negative.

The weight of evidence from the complete battery of *in vitro* and *in vivo* genetic toxicology studies conducted with Triflusulfuron-methyl indicates no genotoxic potential.
-

4.9.6 Conclusions on classification and labelling

No classification is required for triflusulfuron-methyl under either Directive 67/548/EEC or the CLP Regulation.

4.10 Carcinogenicity

Table 17. Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
2-year dietary carcinogenicity study in Sprague Dawley rat (0, 10, 100, 750 or 1500 ppm corresponding to 0, 0.406, 4.06, 30.6 and 64.5 mg/kg b.w for males and 0, 0.546, 5.47, 41.5 and 87.7 mg/kg b.w. for females OECD 453	At 750 and 1500 ppm :decreases in body weight, food consumption and circulating erythrocyte mass and increased incidences of interstitial cell (Leydig cells) hyperplasia and adenoma in male rats. In female rats, no neoplastic lesions were observed.	Purity: 95.6% "primary process" Due to poor survival in this study, it was necessary to terminate after 22 months, as opposed to the normal 24 months.	Biegel L.B. (1993)
18-month dietary long term/carcinogenicity study in CD-1 mouse 0, 10, 150, 2500 or 7000 ppm corresponding to 0, 1.37, 20.9, 349 or 1024 mg/kg b.w. for males and 0, 1.86, 27.7, 488 or 1360 mg/kg b.w. for females OECD 451	At 2500 and 7000 ppm: decreases in mean body weight gain and elevated liver weights and hepatic cytochrome P-450 content in both males and females, as well as microscopic findings: increased hepatic foci of cellular alteration and the presence of intrahepatocellular erythrocytes. Slightly increased incidences of hepatocellular adenomas were also present in males.	Purity: 95.6% "primary process"	Initial report: Biegel L.B. (1993) Supplement to intial report Makovec G.T. (1995)

4.10.1 Non-human information

4.10.1.1 *Carcinogenicity: oral*

The long term toxicity and carcinogenicity of Triflusulfuron-methyl were evaluated in Sprague Dawley rats and in CD-1 mice. Based on the findings in the rat study, a series of mechanistic *in vivo* and *in vitro* studies were undertaken to define the mechanism of interstitial cell tumour genesis.

- Rat study

Reference: Biegel L.B. (1993)
GLP: Yes
Guidelines: OECD 453
Deviations: Only 10 rats/sex/group were used for haematological evaluations and/or sacrificed at one year; ovaries were not weighed. Due to the poor survival in this study, it was necessary to terminate the study after 22 months, as opposed to the normal 24 months.

In the rat study, Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered to groups of 62 male and 62 female Sprague Dawley rats. The substance was incorporated in the diet at 0, 10, 100, 750 and 1500 ppm for 22 months (24 months at the beginning). The doses were based on a previous 90-day feeding study in the rat. The corresponding mean daily intake in function of body weight was 0, 0.406, 4.06, 30.6 and 64.5 mg/kg in males and 0, 0.546, 5.47, 41.5 and 87.7 mg/kg b.w. for females.
Due to the poor survival in this study, it was necessary to terminate the study after 22 months, as opposed to the normal 24 months. The percent survival of male rats was 44, 42, 31, 41, and 54% for the 0, 10, 100, 750, and 1500 ppm groups, respectively, after being fed Triflusulfuron-methyl for 670 days. The female rats had

percent survival rates of 38, 44, 37, 35, and 46% for the 0, 10, 100, 750, and 1500 ppm groups, respectively, after being fed Triflusulfuron-methyl for 670 days. This poor survival rate was considered to be not compound-related.

A decreased body weight (up to – 16%) in the 750 (transiently) and 1500 ppm dose groups was observed.
A moderate (up to -17%), compound-related decrease in circulating erythrocyte mass was observed in male rats fed dietary concentrations of 750 and 1500 ppm Triflusulfuron-methyl. These changes were evident at the 3, 6, 12 and 18-month sampling times. No compound-related haematological alterations were observed in female rats.
A compound-related statistically significant increase (+13%) in mean liver weight occurred in the 1500 ppm female rats in the 12-month interim sacrifice. There were no microscopic findings associated with this change.
A slight increase (+ 5-6%, not statistically significant) in mean absolute testes weight occurred in the 1500 ppm males at the final sacrifice. Additionally, mean relative testes weight of the 1500 ppm males was statistically increased (+ 23-28%). Mean relative testes weight of the 750 ppm males was not statistically increased (1-year treatment, + 14%). The increase in absolute testes weight may have been the result of compound-related interstitial cell hyperplasia. However, the increase in relative testes weights was primarily due to the decrease in mean final body weight of the 1500 ppm males.
There were no compound-related gross or microscopic effects noted at the 12-month interim sacrifice. Compound-related microscopic effects were observed in the testes of male rats fed 750 and 1500 ppm Triflusulfuron-methyl and in the sciatic nerve of both male and female rats fed 1500 ppm for 22 months (table 4.10.1.1-1).

Table 4.10.1.1-1 - Two-Year feeding study in rats: Triflusulfuron-methyl-induced microscopic effects

Triflusulfuron-methyl (ppm):	**0**	**10**	**100**	**750**	**1500**
Males:					
Testes					
Number examined	*51*	*46*	*47*	*50*	*51*
Adenoma, interstitial cell	*0*	*2*	*1*	*7**	*7**
Hyperplasia, interstitial cell	*10*	*7*	*11*	*18**	*27**
Sciatic nerve					
Number examined	*51*	*45*	*47*	*50*	*51*
Myelin/axon degeneration	*42 (40min, 2mil)*	*38 (38min)*	*40 (38min, 1mil, 1mod)*	*40 (36min, 3mil, 1mod)*	*46 (35min, 9mil, 2mod)*
Females:					
Sciatic nerve					
Number examined	*48*	*51*	*48*	*48*	*49*
Myelin/axon degeneration	*25 (25min)*	*31 (31min)*	*32 (32min)*	*33 (32min, 1mil)*	*42* (30min, 12mil)*

*Statistically significant (p ≤0.05). Grade of lesions: min=minimal, mil=mild, mod=moderate.

In the testes, there was an elevated incidence of both interstitial cell (Leydig cell) adenoma and hyperplasia in male rats fed 750 and 1500 ppm Triflusulfuron-methyl. Most Leydig cell lesions were not observed until late in the study. The incidence of Leydig cell hyperplasia for male rats being fed 0, 10, 100, 750, and 1500 ppm was 19.6, 15.2, 23.4, 36.0, and 52.9%, respectively, and the incidence of Leydig cell adenomas was 0, 4.3, 2.1, 14.0, and 13.7%, respectively, with adenomas being defined as a lesion greater in size than three cross sections of seminiferous tubules.

A statistically significant increase in the incidence of myelin/axon degeneration of the sciatic nerve was observed in 1500 ppm females. Although an increased incidence in this lesion was not apparent in males, there was an increase in lesion severity in 1500 ppm males. The effect in both males and females was primarily an increase in lesions graded as mild (table 4.10.1.1-1). Furthermore, almost all of the lesions graded as mild occurred in rats examined at the final sacrifice.

The sciatic nerve lesions noted in this study are likely due to an exacerbation, by some unknown mechanism, of the spontaneous lesion seen commonly in the aging rat. The mild nature of this observation, the significantly high background incidence among ageing control rats, its absence in other species tested for chronic toxicity, and the absence of neurological effects in the long term study suggest that Triflusulfuron-methyl is not a neurotoxin. Moreover, most substances toxic to the peripheral nervous system have a diffuse effect and lead to symmetrical polyneuropathy. However, in the present study, lesions occurred only in the sciatic nerve. Sections of optic nerve, present with the eye, were free of lesions as were sections of the spinal cord. Rear limb skeletal muscle innervated by the sciatic nerve was also unaffected, and there was no clinical evidence of a peripheral neuropathy at any time during the study. Further, one would expect that lesions caused by substances that are directly toxic to the nerve would appear sooner than at the end of a two-year feeding study.

The NOAEL for Triflusulfuron-methyl in the 2-year feeding study in rats was 100 ppm (4.06 mg/kg/day) for males and 750 ppm (41.5 mg/kg/day) for females. In male rats, this NOAEL was based on decreases in circulating erythrocyte mass and increased incidences of interstitial cell (Leydig cells) hyperplasia and adenoma in groups fed at 750 or 1500 ppm. In female rats, triflusulfuron was not carcinogenic, and the NOAEL was based on body weight effects and increased incidence of sciatic nerve degeneration at 1500 ppm.

- Mice

Reference: Initial report: Biegel L.B. (1993) - Supplement to initial report: Makovec G.T. (1995)
GLP: Yes
Guidelines: OECD 451

In the 18-month long term/carcinogenicity study in mouse, Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered to groups of 110 male and 110 female CD-1 mice. The substance was incorporated in the diet at 0, 10, 150, 2500 or 7000 ppm for 18 months. The calculated mean daily intake of Triflusulfuron-methyl was 0, 1.37, 20.9, 349 or 1024 mg/kg body weight for males and 0, 1.86, 27.7, 488 or 1360 mg/kg body weight for females. After approximately 2 weeks and 3 and 12 months of feeding, cell proliferation was evaluated in the livers of five mice from each group. At these time points, additional mice from each group were sacrificed and evaluated for hepatic peroxisomal beta-oxidation activity and hepatic cytochrome P-450 content.

A slight decrease of body weight gain was seen at 7000 ppm in both sexes (-11 to –13%).
The liver cell proliferation indices of the 7000 ppm male and female mice were not affected by test substance administration at any of the time points evaluated (2 weeks and 3 and 12 months). There were statistically significant elevations (+46 to +81%) in the total hepatic microsomal cytochrome P-450 content in 7000 ppm males and females at two weeks and in 7000 ppm females at 3 months. Although not statistically significant,

the hepatic cytochrome P-450 content was also elevated in 7000 ppm males at 3 months. There were no effects on cytochrome P-450 at the 12-month time point. There were no test substance-related changes in hepatic beta oxidation activity at any of the time points evaluated.
Absolute and relative liver weights were increased relative to controls in the 2500 (+8 to +10%) and 7000 ppm (+26 to +30%) male and female groups. The increases in liver weight correlated with the elevated hepatic cytochrome P-450 activity in the 7000 ppm mice. Other organ weights for both males and females were comparable to controls.
Compound-related non-neoplastic changes occurred in the liver of male mice fed 2500 ppm and in male and female mice fed 7000 ppm. Increased incidences of hepatic foci of cellular alteration were present in 2500 and 7000 ppm males and females, although this increase was statistically significant only in the female groups. Additionally, there was an increased incidence of intra-hepatocellular erythrocytes in 2500 ppm males and in 7000 ppm males and females (males: 2500 ppm, 5/80; 7000 ppm, 21/80; and females: 7000 ppm, 9/81). This lesion consisted of randomly distributed hepatocytes containing intact erythrocytes. Affected hepatocytes varied in size from normal to three or four times normal. Erythrocytes appeared to be free in the hepatocellular cytoplasm and varied in number from a few to too-numerous-to-count. The hepatocyte nucleus was often displaced to the cell periphery but appeared intact. Lesion severity was graded minimal in all affected livers except for three graded mild and one graded moderate in the 7000 ppm males. The pathogenesis and toxicological significance of this change is unclear. All other microscopic changes occurred in the liver of male mice fed 7000 ppm and included necrosis of individual hepatocytes and increased pigment accumulation in Kupffer cells (Table 4.10.1.1-2).

Slightly increased incidences of hepatocellular adenomas were present in 2500 and 7000 ppm males (Table 4.10.1.1-2). These increases were statistically significantly relative to controls by the Cochran-Armitage trend test. This increase in adenomas also resulted in an increase in the combined incidence of adenomas and carcinomas in the 7000 ppm male group, even though incidences of carcinomas in this group were actually less than in controls. Background incidences of hepatocellular tumours are high in male mice of this strain in chronic studies. The increases in hepatocellular adenomas in 2500 and 7000 ppm males were within laboratory historical control ranges, were not statistically significant by the Fishers exact test, and were not associated with increased incidences of hepatocellular carcinomas. Similarly, a small increase in hepatocellular adenomas (and thus combined adenomas and carcinomas) was present in females fed 2500 ppm. This increase was not dose-related as only one hepatocellular tumour occurred in the 7000 ppm female group.

Table 4.10.1.1-2– 18-month feeding study in mice: Triflusulfuron-methyl-induced microscopic effects

MALES

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

FEMALES

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

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

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

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

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

In conclusion, the NOAEL for Triflusulfuron-methyl was 150 ppm (20.9 mg/kg b.w in males and 27.7 mg/kg b.w in females), this is based on the decrease in mean body weight gain and elevated liver weights and hepatic cytochrome P-450 content in both males and females fed 7000 ppm test article, as well as microscopic findings: increased hepatic foci of cellular alteration and the presence of intrahepatocellular erythrocytes in the mice fed 2500 and 7000 ppm. Slightly increased incidences of hepatocellular adenomas were also present in the 2500 and 7000 ppm male groups.

4.10.1.2 *Carcinogenicity: inhalation*

No studies are available

4.10.1.3 *Carcinogenicity: dermal*

No studies are available

4.10.2 Human information

No data available

4.10.3 Other relevant information

Mechanistic studies with triflusulfuron-methyl in relation to the increase incidences of Leydig cells hyperplasia and adenoma recorded in the 2-year feeding study in rats were assessed.

Hormone analysis in the serum of male rats fed with Triflusulfuron-methyl for 1 year.

Serum had been collected from rats from the chronic 2-year study of Triflusulfuron-methyl, at the 1-year interim sacrifice. Hormone levels (testosterone, estradiol, LH, FSH, and prolactin) were measured in the serum of 10 male rats per treatment group. Radio-immunoassays utilising commercially available kits were used for these hormonal measurements. The results were statistically analysed using (i) an ANOVA, Dunnett's test (assuming normality and homogeneity of variances) and the test for linear trend over groups,

and (ii) normality and homogeneity of variances were verified (Shapiro-Wilk and Leveae test) and when the requirement was not satisfied, Kruskal-Wallis and Dunn's multiple comparisons were done. A one-sided Jonckheere's trend test was also used.
Using statistical analysis (i), in rats fed 750 and 1500 ppm Triflusulfuron-methyl (table 4.10.3-1) there were statistically significant trends of increasing testosterone levels (165 and 189% of control, respectively} and FSH levels (113 and 134% of control, respectively) and a statistically significant trend of decreasing estradiol levels in rats fed 750 and 1500 ppm to 32 and 53% of control, respectively. There were no statistically significant effects on. serum LH or prolactin levels. Using statistical analysis (ii), an additional statistically significant trend of increasing LH concentration was observed in rats fed 1500 ppm Triflusulfuron-methyl. There were no alterations in hormonal levels in rats fed 10 and 100 ppm Triflusulfuron-methyl for 1 year (both statistical analysis).

Table 4.10.3-1 - Summary of hormone levels from male rats fed Triflusulfuron-methyl for 1-year

Conc. (ppm)	Testosterone ng/mL	Estradiol pg/mL	Prolactin ng/mL	LH ng/mL	FSH ng/mL
0	*0.888*	*4.852*	*1.741*	*0.182*	*6.742*
10	*0.810*	*4.183*	*2.766*	*0.155*	*6.197*
100	*0.966*	*3.179*	*1.831*	*0.178*	*7.279*
750	*1.467*	*1.534**	*1.362*	*0.179*	*7.594*
1500	*1.677**	*2.574**	*1.602*	*0.210**	*9.028**

*Statistically significant trend (Jonckheere's trend test) $p < 0.05$

The results of the hormone (estradiol, testosterone, FSH, LH, prolactine) analysis in the serum of rats fed triflusulfuron for 1 year (study HLR 3-93) might support the following mechanism for the test compound, found elsewhere to be an aromatase inhibitor: Triflusulfuron-methyl reduces the serum estradiol levels; this reduction in estradiol results in a perturbation of the negative feedback control of LH and FSH leading ultimately to the observed Leydig cell hyperplasia and increase in adenoma formation (in the 750 and 1500 ppm treated groups). Moreover, there were no alterations in hormonal levels in rats fed 10 and 100 ppm Triflusulfuron-methyl for 1 year and these concentrations did not produce Leydig cell lesions when fed to rats for 2 years.

Two-week oral study in the rat with high doses of Triflusulfuron-methyl.

Male Sprague Dawley rats (10/group) were administered doses of 0, 1000, 1500, and 2000 mg/kg/day of Triflusulfuron-methyl (66037-24, primary process, purity 95.6%). A control group, which was pair-fed to the 2000 mg/kg/day group, was included in addition to the *ad libitum* control group. This study also included 10 additional rats in the *ad libitum* control and 2000 mg/kg/day groups; these rats were injected with human chronic gonadotrophin (hCG) 1 hour prior to sacrifice. All rats were weighed daily and the weights were used to adjust the dose volume for each day of dosing. At the termination of the study, livers, accessory sex glands (weighed together), and testes were weighed and serum and testicular interstitial fluid were collected for hormonal measurements (testosterone, estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin). Hepatic peroxisomes were prepared for beta-oxidation activity measurements, and hepatic microsomes were prepared for measurement of total cytochrome P450 content and aromatase activity. Radio-immunoassays utilising commercially available kits were used for the hormonal measurements. Data were analysed by ANOVA, Dunnett's test, Cochran Armitage test for trend or nonparametric procedure where necessary.

Oral doses of 1000, 1500, and 2000 mg/kg/day caused statistically significant decreases in body weight (11 to 13%), body weight gain (74 to 96%), food consumption (15 to 25%), and food efficiency (71 to 96%) when compared to *ad libitum* control. Test substance-related, statistically significant decreases in absolute

and relative accessory sex gland weights (androgen- and body weight- dependent) were observed in all dose groups. Relative testis weight was slightly increased as well as relative liver weight (Table 4.10.3-2).

Table 4.10.3-2 – Effect of a 2-week administration of Triflusulfuron-methyl on body and organ weights

	Pair fed control	1000 mg/kg	1500 mg/kg	2000 mg/kg
Final body weight	↓1(13)*	↓(11)*	↓(13)*	↓(13)*
Liver weight Absolute relative	 ↓(19)* ↓(7)	 ↓(6) ↑(6)	 ↓(5) ↑(10)*	 ↓(4)# ↑(10)*#
Testis weight Absolute Relative	 - ↑(10)	 - ↑(15)*	 - ↑(9)	 - ↑(14)*
Accessory sex glands Absolute Relative	 ↓(6)* ↓(2)	 ↓(26)* ↓(17)*	 ↓(35)* ↓(25)*	 ↓(30)*# ↓(20)*#

↓: decrease, ↑: increase, -: no statistically significant change, ( ): % from *ad libitum* control, *: statistically significant from *ad libitum* control, #: statistically significant from per fed control

Hormone analysis revealed no alterations in serum or interstitial fluid testosterone concentrations. Serum estradiol levels were statistically decreased to 31.9, 13.6, and 13.4% (13.6%, of pair-fed control) of *ad libitum* control in the 1000, 1500, and 2000 mg/kg/day dose groups, respectively. Interstitial fluid estradiol levels were unremarkable. Although not statistically significant in the 1000, 1500 and 2000 mg/kg/day groups, LH levels were slightly elevated to 133, 113, and 160% of control, respectively, FSH levels were elevated to 141, 141, and 122% of control, respectively, and prolactin levels were elevated to 128, 137, and 150% (250, pair fed) of control, respectively. Inter-individual variations were high. In hCG-stimulated rats dosed with 2000 mg/kg/day, serum testosterone level of the treated rats was statistically elevated to 192% of control and the serum estradiol level was statistically decreased to 24% of control. The serum LH, FSH, and prolactin and interstitial fluid testosterone and estradiol levels of hCG-stimulated rats were not statistically different from that of the control group. Measurements of hepatic beta-oxidation (peroxisome proliferation) and total cytochrome P450 content were unremarkable. Hepatic microsomal aromatase activity (cytochrome P450 isoenzyme responsible for the conversion of testosterone to estradiol) was not statistically significantly altered by administration of the test substance. Inter-individual variations were high, particularly in the control groups.

In a short-term study (2 weeks) in which high doses of Triflusulfuron-methyl were administered to rats, a significant decrease in serum estradiol levels was the most prominent effect of the test substance on hormonal levels. Serum testosterone concentrations were only increased after hCG challenge suggesting that the test compound enhances testosterone synthesis when the biosynthesis pathway is stimulated. LH, FSH and prolactin concentrations in serum were only slightly elevated (not statistically significant) after treatment. The decreased serum estradiol levels in the absence of altered testosterone levels in non-stimulated rats suggest that the test substance might acts to inhibit activity of the cytochrome P450 enzyme, aromatase, responsible for the transformation of testosterone to estradiol. However the activity of aromatase in the liver was not found modified after a short-term exposure to Triflusulfuron-methyl.

***In vitro* biochemical studies with Triflusulfuron-methyl**

In the *in vitro* biochemical studies, the following parameters were evaluated

1. The cytochrome P450 spectra characteristics produced by the test substance were observed spectrophotometrically after incubation with hepatic microsomes from phenobarbital-induced rats;
2. The ability of the test substance (at concentrations from 0.01 to 0.5 mM) to inhibit ammonium perfluorooctanoate (C8)-induced hepatic microsomal aromatase activity was measured. This assay measured the release of tritiated-water from androstan-4-ene-3,17-dione [1-beta-^{3}H] as an indicator of aromatase activity;

3. The direct effect of the test substance, at concentrations from 0.1 to 1,000 μM, on isolated and cultured Leydig cells was assessed. The ability of the Leydig cells to produce testosterone, estradiol, and progesterone was measured in the presence and absence of hCG.

The test substance produced a Type II binding spectra with a peak at 435 nm and a trough at 415 nm. Ligands producing type II binding spectra are known to inhibit cytochrome P450 isozymes (for example, aminoglutethimide, which inhibits the cholesterol side chain cleavage and aromatase by interacting with cytochrome P450 isozymes, produce a type II binding spectra);
Triflusulfuron-methyl inhibited C8-induced aromatase activity in a dose-dependent manner. The estimated value for aromatase inhibition in C8-induced rat liver microsomes was, IC_{50} of 173.6 μM. Compared to other aromatase inhibitors, this value demonstrates a weak inhibitory response by Triflusulfuron-methyl *in vitro* (table 4.10.3-3).
Statistically significant decreases in estradiol synthesis (from – 31 to – 67%) by isolated Leydig cells were present at the two highest *in vitro* concentrations tested (100 and 1000 μM.). This decrease occurred in both hCG-stimulated and non-stimulated Leydig cells. A statistically significant increase in testosterone production (+ 99%) by non-hCG-stimulated Leydig cells was present at 1000 μM. Progesterone production was not affected. These data indicate that the compound does not inhibit testosterone biosynthesis but suggest that it inhibits the ability of Leydig cells to aromatize testosterone to estradiol.

Table 4.10.3-3 - Effect of Triflusulfuron-methyl on hepatic microsomal aromatase activity *in vitro*

Concentration (mM)	**Aromatase activity (fmol/min)**
0	*14025.54*
0.01	*12595.05**
0.02	*12093.99**
0.05	*10981.64**
0.1	*9325.72**
0.2	*7695.15**
0.5	*5463.81**

* Statistically significant difference from control by Dunnett's test, $p < 0.05$.

The *in vitro* studies (cytochrome P450 binding spectrum, hepatic aromatase activity and *in vitro* synthesis of testosterone, estradiol and progesterone by isolated Leydig cells) conducted with Triflusulfuron-methyl indicate that the compound is a likely aromatase inhibitor via a type II binding to cytochrome P450 and then inhibits the transformation of androgens to oestrogens.

To determine if oral gavage administration of low levels of Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) would induce biologically significant hormonal alterations in male rats, male Sprague Dawley rats (15 per group) were dosed daily by oral gavage with 0, 0.1, 0.5, or 5.0 mg Triflusulfuron-methyl/kg body weight/day in a methyl cellulose/Tween 80 vehicle for 28 days. Doses were selected based on results from the 2-year study in rats. Serum was prepared from the collected blood and frozen until analysed for serum hormone concentrations (estradiol, testosterone, LH, FSH, and prolactin).
Since homogeneity of the test substance in gavage suspensions were found questionable at the beginning of the study, the vehicle was modified during the study. No clinical signs of toxicity were observed over the course of the study. There were no test substance-related effects on body weights, food consumption, or food efficiency. There were no statistically significant effects on any organ weights measured. There were no test substance related effects on serum testosterone, LH, FSH, or prolactin over the course of the study. In addition, administration of Triflusulfuron-methyl was not associated with a decrease in serum estradiol, the expected response to an aromatase inhibitor, at any concentration or time point evaluated. Serum estradiol

concentrations increased over the dosing period in all groups including controls except in the 5.0 mg/kg/day group (the biological meaning of these results is however not clear, table 4.10.3-4).

Table 4.10.3-4 – Effects of Triflusulfuron-methyl on serum estradiol concentrations (pg/mL) in rats

	Control	0.1 mg/kg	0.5 mg/kg	5.0 mg/kg
Pre-study	5.905 ± 0.462	5.122 ± 0.577	4.802 ± 0.581	5.043 ± 0.340
Week 2	8.483 ± 0.426	7.218 ± 0.701	7.267 ± 0.682	6.348 ± 0.492
Week 4	10.005 ± 0.665	9.477 ± 0.884	8.160 ± 0.620	7.365 ± 0.487
Recovery	6.859 ± 0.999	-	-	6.541 ± 0.727

The NOAEL of Triflusulfuron-methyl in this study was 5.0 mg/kg/day based on the absence of biologically significant effects on serum hormone concentrations at any time point evaluated.

4.10.4 Summary and discussion of carcinogenicity

The long term toxicity and carcinogenicity of Triflusulfuron-methyl were evaluated in rats and mice. They were completed with appropriate mechanistic *in vivo* and *in vitro* studies. All studies were found acceptable and suitable to assess the oncogenic potential in rodents.

In the rat study, Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was incorporated in the diet at 0, 10, 100, 750 and 1500 ppm for 22 months. The NOAEL for Triflusulfuron-methyl in this long-term feeding study in rats was 100 ppm (4.06 mg/kg/day) for males and 750 ppm (41.5 mg/kg/day) for females. In male rats, this NOAEL was based on decreases in body weight, food consumption and circulating erythrocyte mass and increased incidences of interstitial cell (Leydig cells) hyperplasia and adenoma in groups fed at 750 or 1500 ppm. In female rats, Triflusulfuron-methyl was not carcinogenic, and the NOAEL was based on body weight effects and increased incidence of sciatic nerve degeneration at 1500 ppm (this effect was also observed in the male rat at the same dose level but was not statistically significant).

Oncogenic effects of Triflusulfuron-methyl in rats were limited to the males and consisted of increased incidence of interstitial (Leydig) cell adenomas at dietary concentrations of 750 ppm and above. Because Triflusulfuron-methyl has been shown to be non-genotoxic in a battery of genotoxicity tests, a series of *in vitro* and *in vivo* mechanistic studies were undertaken to define the mechanism of interstitial cell tumour genesis. These studies suggested that Triflusulfuron-methyl is a weak aromatase inhibitor *in vitro* (hepatic microsomes and cultured Leydig cells) via a type II binding to cytochrome P450. Although, hepatic aromatase activity was not significantly decreased *in vivo* for doses up to 2000 mg/kg b.w. administered to rats for 14 days, a significant decrease in serum estradiol levels was the most prominent effect of the test substance on hormonal levels in short-term or long term rat studies. A trend to increasing LH, FSH and testosterone levels was also observed in rat blood in the 1-year study, for dietary concentrations of Triflusulfuron-methyl inducing increased incidences of interstitial (Leydig) cell adenomas. The following possible mechanism of oncogenicity of Triflusulfuron-methyl in rat can be proposed from experimental *in vitro* and *in vivo* findings: the decrease in serum estradiol after treatment with Triflusulfuron-methyl results in a perturbation of the negative feedback control of LH and FSH. This perturbation is manifested as elevated levels of serum LH and FSH and results in an increase in serum testosterone. The long-term hypersecretion of LH is ultimately responsible for the observed increases in Leydig cell hyperplasia and adenoma formation. Disruption of the hypothalamic-pituitary-testis axis is a well known mechanism of Leydig cell hyperplasia and adenoma formation in the rat by non-genotoxic compounds. Exposure to Triflusulfuron-methyl at levels which do not inhibit estradiol synthesis should not increase the incidence of Leydig cell tumours. The experimental results support a threshold for the Triflusulfuron-methyl-induced Leydig cell tumours in the rat lying within the range of 100 and 750 ppm.

In the mouse, Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was incorporated in the diet at 0, 10, 150, 2500 or 7000 ppm for 18 months. The NOAEL for Triflusulfuron-methyl was 150 ppm (20.9 mg/kg b.w in males and 27.7 mg/kg b.w in females), based on decrease in mean body weight gain and elevated liver weights and hepatic cytochrome P-450 content in both males and females fed 7000 ppm test article, as well as on the following microscopic findings: increased hepatic foci of cellular alteration and the presence of intra-hepatocellular erythrocytes in the mice fed 2500 and 7000 ppm. Slightly increased incidences of hepatocellular adenomas were also present in the 2500 and 7000 ppm male groups.
The pathogenesis and toxicological significance of the presence of erythrocytes in hepatocytes is unclear. As indicated in the dossier, a similar hepatic lesion was seen in mice from a 18 month feeding study with Bromacil. Atypical hepatocytes were also described in treated male mice from a chronic feeding study with doxylamine succinate, an antihistaminic compound. The lesion significance or aetiology was not determined.

To conclude, the target organs of Triflusulfuron-methyl in long term studies were the testis in rat and the liver in mouse. In the rat, the test compound might be considered a weak aromatase inhibitor, inducing a decrease in blood estradiol and a subsequent disruption of the hypothalamic-pituitary-testis axis, a well recognised mechanism of Leydig cell hyperplasia and adenoma formation in the rat by non-genotoxic compounds. In the mouse, Triflusulfuron-methyl slightly increased the incidence of hepatocellular adenomas in the high dose male groups.

4.10.5 Comparison with criteria

Rationale for classification as a Carcinogen:

The CLP criteria for classification as a category 2 Carcinogen (category 3 carcinogen according to Directive 67/548/EEC) are as follow :
“Substances are classified as a category 2 Carcinogen when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.”

In the mouse, Triflusulfuron-methyl slightly increased the incidence of hepatocellular adenomas at the higher dose. The increase in hepatocellular adenoma in the 2500 and 7000 ppm male mice groups was not considered relevant for human risk assessment for the following reasons: (i) the compound did not induce an increase in the formation of hepatic tumours in female mice at any dietary concentration, (ii) the occurrence of hepatic tumours was predominantly benign and the overall tumour incidence (of any type) was not increased in male or female treated mice when compared to the control, (iii) hepatic cell proliferation indices were similar between the control and the high dose group, (iv) Triflusulfuron-methyl was negative in a spectrum of short-term tests for mutagenicity and (v) it did not induce hepatic tumour formation in rats.
This does not fulfil the criteria for Cat 2 H351 classification.

In male rats, Triflusulfuron-methyl induced increased incidence of Leydig cell hyperplasia and adenomas at 750 or 1500 ppm. Mechanistic studies suggested that Triflusulfuron-methyl is a weak aromatase inhibitor, inducing a decrease in blood estradiol and a subsequent disruption of the hypothalamic-pituitary-testis axis, as well recognised mechanism of Leydig cell hyperplasia and adenoma formation in the rat by non-genotoxic compounds. This mechanism is considered relevant to humans. The notifier considers that the relevance of these tumours to human risk assessment is questionable mainly because (i) they are extremely rare in humans, (ii) the rat is more sensitive than human to disruption of the hypothalamus-pituitary-testis axis for physiological reasons (LH half-life, number of LH receptors on Leydig cells, qualitative and quantitative difference in hormonal stimulation effects) and (iii) of the results of some epidemiological studies. However, according to a specialized expert working group (see the Draft summary record of the meeting of Ispra, January 22_23 2004, ECBI/08/04 Rev 2, April 2004), substances causing Leydig cell tumours in rats by perturbating the HPT axis should be classified in Carcinogen Category 3, unless the mechanism can be proven not to be relevant for human Leydig cell carcinogenesis. Among the currently identified non-genotoxic mechanisms of rodent Leydig cell tumorigenesis (see review in Cook et al., *Critical Reviews in*

Toxicology, 1999, 29(2), 169-261), only dopamine and GnRH agonist mediated-effects are not considered relevant for humans. Therefore, as the mechanism of action of Triflusulfuron-methyl on Leydig cells was likely mediated by aromatase inhibition, the compound should be classified as a Cat 2 H351 classification.

4.10.6 Conclusions on classification and labelling

Based on the fact that evidence of carcinogenicity is restricted to a single experiment/specie, on the lack of genotoxicity potential and on the additional studies showing that disruption of the hypothalamic-pituitary-testis axis lead to the Leydig cell tumours, triflusulfuron-methyl can be considered as a non-genotoxic carcinogen and should not be classified as category 1B Carcinogen (category 2 carcinogen according to Directive 67/548/EEC).

In accordance with the criteria in Directive 67/548/EEC, classification in category 1 for carcinogenicity is not justified given that there is no evidence of Triflusulfuron-methyl having caused cancer in humans.

Based on the increased incidence of Leydig cell hyperplasia and adenomas at high doses in one specie which was considered as relevant to humans, a classification **Carc. Cat. 3; R40** is proposed.
Because evidence of carcinogenicity in rats is obtained from a single study, it is considered that there is a "limited evidence of carcinogenicity effects" which deserves a **classification Category 2– H351** according to CLP criteria.

4.11 Toxicity for reproduction

Table 18. Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Fertility			
Two generation feeding in the Sprague Dawley rats 0, 10, 100, 750 or 1500 ppm corresponding to 0, 0.588, 5.81, 44.0 and 89.5 mg/kg b.w for F0 males and 0, 0.764, 7.75, 58.0 and 115.0 mg/kg b.w. for F0 females 0, 0.785, 7.84, 59.6 and 123.0 for F1 males 0, 0.881, 8.96, 67.2 and 137.0 mg/kg b.w for F1 females OECD 416	No reproductive and developmental effects at 1500 ppm. NOAEL for reproductive effects ≥ 1500 ppm in M (89.5 – 123 mk/kg b.w/day) and F (115.0-137.0 mg/kg b.w/day) At doses ≥ 750 ppm: ↓ in body weight and nutritional parameters in parental rats and a slight ↓ of body weights in pups NOAEL for overall toxicity in adults and offspring = 100 ppm (5.81-7.75 mg/kg b.w. /day in males and 7.84-8.96 mg/kg b.w./day in females)	Purity: 95.6% "primary process"	Hurtt M.E., Kreckman K.H. (1993)
Developmental toxicity			
Teratogenicity oral (gavage) study in Sprague Dawley rats 0, 30, 120, 350 or 1000 mg/kg b.w/day OECD 414	↓body weight and food consumption at doses ≥ 350 mg/kg b.w NOAEL for maternal toxicity = 120 mg/kg b.w. ↓of the incidence of malformations at 1000 mg/kg and visceral and skeletal variations in foetuses at doses ≥ 350 mg/kg. NOAEL for developmental toxicity and teratogenicity = 120 mg/kg b.w.	Purity: 95.6% "primary process"	Mebus C.A. (1991), Mylchreest E. (2002)
Teratogenicity oral (gavage) study in (NZW)SPF rabbits 0, 15, 90, 270 or 800 mg/kg b.w/day OECD 414	↓body weight and nutritional changes at doses ≥ 90 mg/kg/day and ↑mortality and abortions in animals administered doses ≥ 270 mg/kg/day NOAEL for maternal toxicity = 15 mg/kg No teratogenic activity, absence of relevant adverse effects on the foetuses NOAEL for developmental toxicity ≥ 800 mg/kg	Purity: 95.6% "primary process"	Murray S.M. (1991)

4.11.1 Effects on fertility

4.11.1.1 *Non-human information*

Reference: Hurtt M.E., Kreckmann K.H. (1993)
GLP: Yes
Guidelines: OECD 416

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered to groups of 30 male and 30 female Sprague Dawley rats (F0, F1). The substance was incorporated in the diet at 0, 10, 100, 750 or 1500 ppm. The corresponding mean daily intake in function of body weight during the premating periods were: F0 males 0, 0.588, 5.81, 44.0, 89.5 mg/kg; F0 females 0, 0.764, 7.75, 58.0, 115.0 mg/kg; F1 males, 0, 0.785, 7.84, 59.6, 123.0 mg/kg; F1 females, 0, 0.881, 8.96, 67.2, 137.0 mg/kg. The F0 rats were bred within their treatment groups to produce F1 litters after 73 days on test. The F1 rats were bred within their respective treatment groups to produce F2 litters at least 105 days after weaning.
Parameters evaluated included body weight (weekly), body weight gain, food consumption (weekly), food efficiency, clinical signs (weekly), clinical pathology (daily), reproductive indices, litter and pup parameters, organ weights (testis), and gross (20 weanlings/sex/concentration and all parental rats) and microscopic (testis, epididymis, prostate, seminal vesicles, coagulating gland, pituitary, ovaries, uterus, vagina of control and high dose groups) pathology. Data were analysed by ANOVA, Dunnett's test, Kruskal-Wallis test and Fisher's exact test.

The stability and the homogeneity of the test compound in the diet was acceptable in the conditions of the assay. There were no test substance-related clinical signs or mortality observed during the course of the study.
The effects of the treatment on body weights and nutritional parameters of parental rats are shown in table 6.6.2 for the F0 generation and table 6.6.3 for the F1 generation. Changes occurred in the rats fed 750 and 1500 ppm of triflusulfuron-methyl and were observed mainly during the premating periods. The observed effects were moderate and generally less marked in parents of the F1. There were no biologically or statistically significant differences in mating indices, fertility indices, or gestation length in any of the F0 or F1 treatment groups. There were no test substance-related gross or microscopic observations at any dietary concentration. The observation of increased mean relative testes weights in 100, 750, and 1500 ppm F0, and 1500 ppm F1 males were considered reflective of lower body weights in these groups since there were neither associated with increases in absolute testes weights nor with any microscopic apparent lesions.

Minimal (< 10% compared to controls) decreases in mean pup weights were observed in F1 generation offspring of rats fed 750 and 1500 ppm. Female F2 pup weights were slightly decreased on Days 14 and 21 of lactation in the 750 and 1500 ppm groups; however, the reductions were not statistically significant. There were no biologically or statistically significant differences between control and treated groups for number of pups per litter or pup survival in the F1 or F2 litters. There were no significant differences in the incidence of any clinical observation in the F1 and F2 pups.

Table 4.11.1.1-1 - Two-generation reproduction study: Body weight/nutritional parameters in F0 parental rats

	10 ppm	100 ppm	750 ppm	1500 ppm
Males (premating; Day 0-70):				
Body weight	-	-	**↓(6.5)**	**↓(10)**
Body weight gain	-	-	**↓(13)**	**↓(23)**
Food consumption	-	-	-	**↓(6)**
Food efficiency	-	-	**↓(9.5)**	**↓(17)**
Females:				
Body weight				
Premating Day 70	-	-	-	-
Gestation Day 21	-	-	-	**↓(6)**
Lactation Day 21	-	-	**↓(7)**	**↓(16)**
Body weight gain				
Premating Day 0–70	-	-	**↓(14)**	**↓(18)**
Gestation Day 0–21	-	-	-	-
Lactation Day 0–21	-	-	-	-
Food consumption				
Premating Day 0–70	-	-	-	-
Gestation Day 0-14	-	-	**↓(9)**	-
Food efficiency[a]				
Premating Day 0-70	-	-	**↓(10)**	**↓(14)**
Gestation Day 0-14	-	-	-	-

↓: decrease, ↑: increase, -: no statistically significant change, ( ): % from control,

Table 4.11.1.1-2 -Two-generation reproduction study: Body weight/nutritional parameters in F1 parental rats

	0 ppm	10 ppm	100 ppm	750 ppm	1500 ppm
Males (premating; Day 0–105):					
Body weight	-	-	-	-	**↓(10)**
Body weight gain	-	-	-	-	**↓(10)**
Food consumption	-	-	-	-	-
Food efficiency	-	-	-	-	**↓(6)**
Females:					
Body weight					
Premating Day 105	-	-	-	**↓(7)**	**↓(10)**
Gestation Day 21	-	-	-	**↓(7)**	**↓(9)**
Lactation Day 21	-	-	-	**↓(7)**	**↓(8.5)**
Body weight gain	-	-	-		
Premating Day 0–105	-	-	-	-	**↓(11)**
Gestation Day 0–21	-	-	-	-	-
Lactation Day 0–21	-	-	-	-	-
Food consumption,	-	-	-		
Premating Day 0 - 105	-	-	-	**↓(5)**	**↓(7)**
Gestation Day 0 - 14	-	-	-	-	-
Food efficiency	-	-	-		
Premating Day 0-105	-	-	-	-	-
Gestation Day 0-14	-	-	-	-	-

↓: decrease, ↑: increase, -: no statistically significant change, ( ): % from control,

No reproductive and developmental adverse effects were observed with the highest (1500 ppm) dose of triflusulfuron-methyl, in a two generation study in rats. Then, the reproductive NOAEL for triflusulfuron-methyl was ≥ 1500 ppm (89.5-123.0 mg/kg b.w./day and 115.0-137.0 mg/kg b.w./day in males and females, respectively). The overall NOAEL (general toxicity) for the test compound in adults and offspring was 100 ppm (5.81-7.75 mg/kg b.w./day and 7.84-8.96 mg/kg b.w./day in males and females, respectively) based on decreases in body weight and nutritional parameters in parental rats and on slightly decreased body weights in pups at doses ≥ 750 ppm.

4.11.1.2 *Human information*

No data available

4.11.2 Developmental toxicity

4.11.2.1 *Non-human information*

- **Rat:**

Reference: Mebus C.A. (1991) and Mylchreest E. (2002)
GLP: Yes
Guidelines: OECD 414

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered by oral gavage to mated Sprague Dawley female rats (25/dose group) on gestation days 7-16 (the day mating was day 1). Gavage doses in methyl cellulose vehicle (0.5%) were 0, 30, 120, 350, or 1000 mg/kg/day. Parameters evaluated in dams were body weight, body weight gain (absolute and adjusted for the products of conception), food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, and skeletal malformations and variations. Statistical methods: depending on the analysed parameters, tests for linear trend were Cochran-Armitage, linear contrast of means and Jonckheere's tests and for comparison between groups, Fisher's exact and Mann-Withney tests were used.

Clinical signs: there were no statistically significant, test substance-related differences in the incidence of clinical observations detected during the study. Test substance-related mortality did not occur during the course of this study. A total of five rats died (3 rats in the 120 mg/kg/day group and 1 rat each in the 350 and 1000 mg/kg/day groups) as the result of gavage errors.

Dam body weight and food consumption: body weight loss, reduced body weight gain, and reduced food consumption were observed in the 350 and 1000 mg/kg/day groups, indicating some maternal toxicity. The effects on maternal body weight (table 6.6.4) were most pronounced during the first days of dosing (days 7-9) and contributed to a statistically significant compound-related reduction in the overall maternal weight gain (days 7-17) at 350 and 1000 mg/kg/day (81% and 70% of controls, respectively). There was a statistically significant compound-related reduction in mean maternal adjusted weight gain (adjusted to exclude the products of conception) to 70% of controls at 1000 mg/kg/day. An adjusted maternal weight gain at 350 mg/kg/day was reduced to 93% of controls, but was not statistically significant.

Table 4.11.2.1-1 - Developmental toxicity study in rats: Maternal body weight changes (g)

	0 mg/kg	30 mg/kg	120 mg/kg	350 mg/kg	1000 mg/kg
Days 1-7	**36.0**	**34.2**	**36.9**	**37.0**	**40.6**
Days 7-9	**2.3**	**5.5**	**4.1**	**-3.6***	**-11.5***
Days 9-11	**10.1**	**10.0**	**8.0**	**7.3**	**9.1**
Days 11-13	**10.7**	**11.3**	**10.5**	**10.7**	**12.7**
Days 13-15	**10.3**	**8.5**	**10.1**	**11.8**	**9.0**
Days 15-17	**18.1**	**10.5**	**15.6**	**15.5**	**16.7**
Days 7-17	***51.5***	***45.8***	***48.3***	***41.6****	***36.1****
Days 17-22	***82.3***	***83.3***	***90.3***	***81.5***	***83.5***
Days 7 – 22, Adjusted**	***32.0***	***36.1***	***37.6***	***29.7***	***22.7****

* Significant trend (Jonckheere's test, p ≤0.05). ** Maternal weight gains corrected for products of conceptions.

Statistically significant reductions (up to 28%) in food consumption were also present at various intervals in the 350 and 1000 mg/kg/day groups.

Reproductive performance: There were no adverse test substance-related effects on reproductive parameters.

Foetal development: No test substance-related effects on mean foetal weights were detected. No foetal abnormalities were observed at doses that were not associated with maternal toxicity (120 mg/kg/day and below). Malformations and variations were present at dose levels that produced maternal toxicity. The foetal effects are summarized in the table 4.11.2.1-2.

Table 4.11.2.1-2 - Developmental toxicity study in rats: Foetal effects

Parameters Observed	0 mg/kg	30 mg/kg	120 mg/kg	350 mg/kg	1000 mg/kg
Mean Foetal Weight (g)	**5.19**	**5.24**	**5.23**	**5.16**	**5.19**
Malformations - Mean % affected*	**0.7**	**0.0**	**0.4**	**0.3**	**1.4**
Total Variations due to Retarded Development Mean % affected*	**10.8**	**11.3**	**16.3**	**29.3****	**23.4****
Total number Foetuses with Variations Mean % affected	**10.8**	**11.7**	**16.6**	**29.8****	**23.8****

* Significant trend (Cochran-Armitage test) p ≤0.05. ** Significantly different from control by the Mann Whitney U criteria; p ≤0.05

At the maternally toxic dose of 1000 mg/kg/day, there was a slight but statistically significant increase in the number of malformed foetuses. Four foetuses from four litters had malformations and the majority of observed malformations occurred in one foetus. This foetus was from the dam most severely affected by compound administration (i.e., had the greatest weight gain reduction). The malformations in this one foetus included absent diaphragm, dextrocardia, exencephaly, gastroschisis, neural tube defect (spina bifida), fused rib, bent scapula, absent skull, and non-fused sternebra. In this foetus, the findings of exencephaly, neural tube defect (spina bifida), and absent skull were all likely related to the same underlying failure of the neural tube to close in regions of both the brain and spinal cord. The remaining malformations observed in three foetuses at 1000 mg/kg/day included micrognathia, microphthalmia, and absent mouth in one foetus, fused ribs in a second, and cleft palate and exencephaly in a third. There was no specific pattern of malformations at 1000 mg/kg/day; the four foetuses were affected at differing degrees of severity and generally at different anatomical sites. Malformations affected the diaphragm, the mouth, the heart, the neural tube (brain and/or spine), the abdominal wall, the eye, and various bones (ribs, jaw, palate, scapula, sternebra, and skull). The absence of a pattern for these malformations suggests these effects may be secondary to general maternal toxicity at 1000 mg/kg/day observed early in the dosing period and persisting throughout gestation. In a position paper the occurrence of malformations was compared to historical control data from 16 developmental toxicity studies conducted in the same laboratory. Some of the malformations have been observed at the same low incidences, others have not been observed in historical control foetuses (including all the malformations occurring in a control foetus of the current study). Irrespective of the type of malformation, the total number of affected foetuses varied from 1 foetus in 1 litter to 3 foetuses in 3 litters. The total number affected in the present study barely exceeds this incidence (4 foetuses in 4 litters).
At 350 and 1000 mg/kg/day, there was a statistically significant increase in the mean percent of foetuses affected per litter with "variations due to retarded development" (table 6.5.5). This increase was primarily

due to an increase in the occurrence of visceral variations at 1000 mg/kg/day and skeletal variations at 350 and 1000 mg/kg/day. The increased visceral variations resulted from a slight but significant increase in small renal papilla at 1000 mg/kg/day. The increased skeletal variations were due to a significant increase in partially ossified vertebra at 1000 mg/kg/day, and unossified skulls (hyoid) at 350 and 1000 mg/kg/day. As indicated in the position paper, the statistically significant variations due to retarded development in the 350 and 1000 mg/kg/day dose groups were within historical control ranges, with the exception of the incidences of partially ossified or unossified sternebra at 350 mg/kg/day (the increased incidences of these findings were however not dose-related). When considered with the facts that (i) the variations were observed only in the presence of some maternal toxicity, and (ii) the maternal toxicity was most evident for the intervals at the beginning of organogenesis, when many early embryonic organ systems are developing simultaneously and may be sensitive to poor maternal health, their direct relationship with the test compound exposure may be questionable.

Triflusulfuron-methyl administered to pregnant female rats at maternally toxic doses (decreased body weight and food consumption) induced small increases in the incidence of malformations at 1000 mg/kg and visceral and skeletal variations at doses ≥ 350 mg/kg. A direct effect of the compound in these foetal malformations and variations is questionable. The NOAEL for developmental toxicity and teratogenicity was 120 mg/kg b.w. as well as was the NOAEL for maternal toxicity.

- **Rabbit:**

Reference: Murray S.M. (1991)
GLP: Yes
Guidelines: OECD 414

Triflusulfuron-methyl (66037-24, primary process, purity 95.6%) was administered by oral gavage to time-mated Hra:(NZW)SPF female rabbits (20/dose group) on Gestation days 7–19 (the day of insemination equals day 0). Gavage doses in 0.5% methyl cellulose vehicle were 0, 15, 90, 270, or 800 mg/kg. [Doses were selected from the results pilot study: pregnant rabbits were dosed by gavage with dose levels of 0, 500, 1000, 2000 and 3000 mg/kg of body weight. Maternal toxicity was demonstrated as evidenced by death at 1000 mg/kg and above, and an increase in the incidence of abortions at 500 mg/kg and above. Based on these results, the maximum tolerated dose was judged to be between 500 and 1000 mg/kg]. Parameters evaluated in dams were body weight, body weight gain (absolute and adjusted for the products of conception), food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, and skeletal malformations and variations. Statistical methods: depending on the analysed parameters, tests for linear trend were Cochran-Armitage, linear contrast of means and Jonckheere's tests and for comparison between groups, Fisher's exact, ANOVA and Dunnett's and Mann-Withney tests were used.

Clinical symptoms and mortality: Compound-related mortality occurred in 2/20 and 9/20 rabbits in the 270 and 800 mg/kg/day groups, respectively. One rabbit from the 90 mg/kg/day group died as a result of injuries incurred during dosing. A significant increasing trend in the incidence of clinical observations occurred during the dosing period. This was primarily due to significantly increased reductions in stool quantity for the 270 and 800 mg/kg/day groups. Diarrhoea also occurred in the high dose group. Increases in signs associated with abortions were also present in these groups.

Body weight gain: During days 7-10, body weight loss occurred in animals administered doses ≥ 90 mg/kg/day, compared to a body weight gain of 46.5 grams in controls. By trend analysis, body weight gains in the 270 mg/kg/day group were significantly decreased for days 7-20 (excluding data from the 800 mg/kg/day group where only 3 dams delivered). A significant decrease (by trend analysis) in maternal food consumption was detected for days 16-20 in the 270 mg/kg/day group.

Reproductive performance: A significant increasing trend in the number of females that aborted was detected in the two highest dose groups. Eight of 16 dams in the 270 mg/kg/day group aborted and 12/20 dams in the 800 mg/kg/day group aborted. No other significant test substance-related trends for reproductive effects were detected.

Gross necropsy of does: All nine rabbits in the 800 mg/kg/day dose group that died before scheduled sacrifice had ulcerations of the gastric mucosa, lack of formed faeces in the large intestine, and gaseous distension in various parts of the digestive tract. Five additional animals that survived in this group had similar post-mortem findings. For the 270 mg/kg/day group, comparable findings were observed in five rabbits, but only one had ulcerations of the gastric mucosa. One rabbit in the 15 mg/kg/day group also had ulcerated gastric mucosa.

Observations on foetuses: Due to does mortality and/or abortions, 105[19], 106[17], 96[17], 54[8], and 16[3] foetuses[litters] were observed for developmental effects in the control, 15, 90, 270 and 800 mg/kg treated groups, respectively. No significant test substance-related effects were detected.

Triflusulfuron-methyl had no effect on the foetal development in the rabbit for doses up to 800 mg/kg that induced maternal toxicity. The NOAEL for maternal toxicity was 15 mg/kg/day based on body weight and nutritional changes at 90 mg/kg/day and above, and increased mortality and abortions in animals administered 270 mg/kg/day and above. The foetal NOAEL was 800 mg/kg/day, the highest dose tested, based on absence of foetal toxicity.

4.11.2.2 *Human information*

No data available

4.11.3 Other relevant information

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4.11.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of Triflusulfuron-methyl was evaluated in rats.
No adverse effects on reproduction were observed with the highest (1500 ppm) dose of Triflusulfuron-methyl, in a two generation study in rats. Then, the reproductive NOAEL for Triflusulfuron-methyl was ≥ 1500 ppm (89.5-123.0 mg/kg b.w./day and 115.0-137.0 mg/kg b.w./day in males and females, respectively). The overall NOAEL (general toxicity) for the test compound in adults and offspring was 100 ppm (5.81-7.75 mg/kg b.w./day and 7.84-8.96 mg/kg b.w./day in males and females, respectively) based on decreases in body weight and nutritional parameters in parental rats and on slightly decreased body weights in pups at doses ≥ 750 ppm.

Developmental toxicity studies were conducted with Triflusulfuron-methyl in rats and rabbits.
In the developmental study conducted in rabbits, the maternal NOAEL was 15 mg/kg based on compound-related body weight/nutritional effects at 90 mg/kg b.w./day and greater, and increased mortality and abortions in groups administered 270 mg/kg/day and above. The NOAEL for foetal toxicity in rabbits was 800 mg/kg/day, the highest dose tested, based on absence of foetal effects.
In rats, both the maternal and foetal NOAEL were 120 mg/kg/day. In maternal animals, this NOAEL was based on body weight effects, including body weight loss, in groups administered 350 mg/kg/day and above. These effects were most severe during gestation days 7-11, which coincide with the beginning of organogenesis. The foetal NOAEL was based on a slight increase in the mean percent of foetuses per litter with variations due to retarded development in groups administered 350 mg/kg/day and above. A slight increase in the number of malformed foetuses was also present at 1000 mg/kg/day. A direct effect of the

compound in these foetal malformations and variations is questionable since foetal effects were likely secondary to maternal toxicity noted in early gestation, were of low incidences and were in the historical control data limits.

4.11.5 Comparison with criteria

Reprotoxic substances can be toxic to the development of the unborn child or can cause impairment of fertility in male and female subjects.

Reprotoxic substances are divided into 2 groups;

- Effects on male or female fertility, including adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response.
- Developmental toxicity, including any effect interfering with normal development before and after birth.

1) Criteria for classification under Directive 67/548/EEC:

- Fertility:

The placing in category 1 is done on the basis of epidemiological data. Placing in categories 2 and 3 is done primarily on the basis of animal data. Data from *in vitro* studies are regarded as supportive evidence. When effects have been demonstrated only in high doses, classification in category 3 or even no classification will be warranted. Also when studies are performed in only 1 species, without other relevant supporting evidence, classification in category 3 may be appropriate.

- Developmental toxicity

For classification in category 2 there should be clear evidence of adverse effects in more species. The route of exposure is also important. Classification in category 3 or no category would be assigned when the only effects recorded are small changes in the incidences of spontaneous defects or small differences in postnatal developmental assessments.

2) Criteria in the CLP classification :

- Fertility and developmental toxicity

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

3) Comparison with criteria:

No adverse effects on reproduction were observed with the highest (1500 ppm) dose of Triflusulfuron-methyl, in a two generation study in rats. Classification is thus not necessary.

In the developmental study in rabbits, Triflusulfuron-methyl did not show any teratogenic potential at 800 mg/kg, the highest tested dose.

In the developmental study in rats, a slight increase in the mean percent of foetuses per litter with variations due to retarded development in groups administered 350 mg/kg/day and above. A slight increase in the number of malformed foetuses was also present at 1000 mg/kg/day. These effects of low incidences and in the historical control data limits were observed at a dose that also caused maternal toxicity and therefore probably due to the maternal toxicity. Classification is thus not necessary.

4.11.6 Conclusions on classification and labelling

Overall reproductive studies in rats and rabbits suggested that Triflusulfuron-methyl is not toxic for the reproduction and the development. In the rat, it was demonstrated that Triflusulfuron-methyl is a weak aromatase inhibitor leading to Leydig cell hyperplasia and adenoma formation. Thus, triflusulfuron-methyl could be an endocrine active substance. Nevertheless this point need to further be discussed when regulatory criteria identifying an endocrine disruptor substance will be defined.

Since no related effect to this mechanism of action has been observed in the reproductive and developmental *in vivo* toxicity studies, no classification is required.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 *Neurotoxicity*

Method	Results	Remarks	Reference
Acute oral neurotoxicity in Crl:CD BR VAF/Plus rats 0, 500, 1000 or 2000 mg/kg b.w US EPA 81-8	Lack of any specific evidence of neurotoxicity NOAEL for acute neurotoxicity > 2000 mg/kg b.w At doses ≥ 1000 mg/kg b.w and at least 2000 mg/kg b.w for F: ↓ body weight gain and food consumption NOAEL for systemic toxicity = 500 mg/kg b.w	Purity: 95.6% "primary process"	Foss J.A. (1994)
90-day feeding neurotoxicity in BR VAF/Plus rats 0, 100, 750, 1500 or 3000 ppm corresponding to 0, 6.1, 46.1, 92.7 and 186.2 mg/kg b.w/day in males and 0, 7.1, 51.6, 104.1 and 205.2 mg/kg b.w/day in females US EPA 82-7	No evidence of subchronic neurotoxicity NOAEL for subchronic neurotoxicity = 3000 ppm in M (186.2 mg/kg b.w/day) and F (205.2 mg/kg b.w/day) At doses 750 ppm in F: ↓ body weight and food consumption At 3000 ppm: ↓ body weight in M, ↓food consumption in M NOAEL for systemic toxicity = 1500 ppm in M (92.7 mg/kg b.w/day) and 100 ppm in F (7.1 mg/kg b.w/day)	Purity: 95.6% "primary process"	Foss J.A. (1994)

Acute (single dose gavage) and subchronic (90-day feeding) neurotoxicity studies were conducted in rats with Triflusulfuron-methyl. In both studies, no clinical or morphological evidence of neurotoxicity was present in male or female rats at any dose tested (up to 2000 mg/kg/day in the acute gavage study and up to 3000 ppm in the subchronic feeding study). In a 2-year feeding study in rats an increased incidence and/or severity of axonal degeneration of the sciatic nerve was observed in male and female rats fed 1500 ppm, the highest concentration tested. These findings were explained by an exacerbation, by some unknown indirect mechanism, of the spontaneous lesion seen commonly in the aging rat. The results of the acute and short-term neurotoxicity studies confirmed that interpretation and strongly suggested that Triflusulfuron-methyl is not a neurotoxicant.

4.12.1.2 *Immunotoxicity*

No data available

4.12.1.3 *Specific investigations: other studies*

No data available

4.12.1.4 *Human information*

No data available

4.12.2 Summary and discussion

The results of the acute and short-term neurotoxicity studies strongly suggested that Triflusulfuron-methyl is not a neurotoxicant.

4.12.3 Comparison with criteria

The results of the acute and short-term neurotoxicity studies confirmed that there have been no indications that triflusulfuron-methyl is selectively neurotoxic. No classification is proposed.

4.12.4 Conclusions on classification and labelling

No classification is required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for triflusulfuron-methyl is based on the Draft Assessment Report (EC, 2007), the Addendum to the Draft Assessment Report (EC, 2008) and the EFSA Scientific Report on the peer review of triflusulfuron (EFSA, 2010).

All the studies on the fate and behaviour in the environment and ecotoxicology of triflusulfuron-methyl were performed on GLP and according to EPA or OECD guidelines. Then, the reliability factor would be indicated in the summary only when different of 1.

5.1 Degradation

Table 19. Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD 301 D	Not readily biodegradable	None	Aldred D. (1992)

5.1.1 Stability

5.1.1.1 *Hydrolysis*

A hydrolysis study of triflusulfuron-methyl is available.

Hawkins D.R. et al. (1992a):
This study is performed according to EPA guidelines and is GLP.
Purified ^{14}C-ester carbonyl triflusulfuron-methyl (purity 95.8-98.9%) or ^{14}C-triazine triflusulfuron-methyl (purity > 97.0%) in acetonitrile was dissolved at about 1 mg/L in buffers at pH 5, 7 and 9 (solvent was < 1.1% in the final solutions). Solutions were kept at 25° C for 30 d. At each sampling time, radioactivity in solutions was measured by LSC and analysed by HPLC.
Hydrolysis of triflusulfuron-methyl involves cleavage of the sulfonylurea bridge to give IN-W6725 (methyl saccharin, 99.4%, 46.4% and 44.1% after 30 d at pH 5, 7 and 9, respectively) and IN-D8526 (triazine amine, 98.4%, 47.4% and 43.0% after 30 d at pH 5, 7 and 9, respectively).
DT_{50} is 3.7 d at pH 5, 32 d at pH 7 and 36 d at pH 9 (linear 1st order).

5.1.1.2 *Photolysis*

In water

Two studies of the photolysis of triflusulfuron-methyl in water are available.

Hawkins D.R. et al. (1992b):
This study is performed according to EPA guidelines and is GLP.
Purified ^{14}C-triazine or -ester carbonyl triflusulfuron-methyl in acetonitrile was dissolved at about 1 mg/L in sterile buffers at pH 5, 7 and 9 (solvent was < 1.1%). Solutions were exposed to Xenon arc light source (> 290 nm) for 15 d at 25°C (equivalent to 68-81 d of natural summer sunlight at latitude 52° N assuming that the average daily radiation intensity from the sun is about 75% of the peak intensity over a 12 h period). Radioactivity in solution was measured by LSC and analysed by HPLC and TLC. A preparative scale experiment was carried out for identification of photolysis products by MS. The dark controls were those from the hydrolysis study.
As compared to the dark conditions (hydrolysis), light slightly enhances degradation of triflusulfuron-methyl in aqueous solutions at pH 7 and 9 (in the light DT_{50} are 3.8 d at pH 5, 13.9 d at pH 7 and 24.6 d at pH 9) and a different degradation pathway is observed. At all pH, cleavage of the sulfonylurea bridge still occurs in the light and the metabolites IN-W6725 = methyl saccharin (max. 71.2% at pH 5, 18.1% at pH 7 and 18.9% at

pH 9) and IN-D8526 = triazine amine (max. 46.8% at pH 5, 18.2% at pH 7 and 12.1% at pH 9) are formed in significant amounts. However, light favours oxidation of triazine amine at pH 5 to give formyl N-desmethyl triazine amine = IN-E0Q47 = IN-JY947 (max. 20.1% at pH 5, < 1.5% at pH 7 and < 0.9% at pH 9) and N-demethylation of the AS (Active Substance) at pH 7 and 9 to give IN-66036 = N-desmethyl triflusulfuron-methyl (max. 4.0% at pH 5, 14.7% at pH 7 and 9.5% at pH 9). Formation in low amounts of IN-JL000 = triazine urea (< 1.3-3.8%) suggests that light could also favour another cleavage of the sulfonylurea bridge. An unknown photodegradation product (T9) derived from the triazine moiety is detected in significant amounts at all pH (max. 24.0% at pH 5, 13.5% at pH 7 and 16.2% at pH 9). Despite further investigations, the chemical structure of T9 has not been elucidated but it was shown to release triazine urea. IN-E7710 is formed in low amounts (< 6.9%) at all pH. With regard to the light intensity (15 d continuous artificial light would correspond to 68-81 d equivalent natural summer sunlight at latitude 52° N) photodegradation is expected to be not significant under real conditions at least in Northern Europe.

Singles S.K. (2001):
This study is performed according to EPA guidelines.
The quantum yield for direct photolysis of triflusulfuron-methyl was obtained by iterative calculations using the US EPA photolysis model GCSOLAR. When the calculated instantaneous photolysis rate at mid-day in summer at 50° latitude equaled the photolysis rate constant (half life 127 d natural sunlight equivalent at pH 7) observed in the light-irradiated experiment (Hawkins D.R., Kirkpatrick D., Dean G.M., Mellor S. , 1992, report HRC/DPT 218/91535 DuPont AMR-1629-90; summarised above) the calculations were concluded. The quantum efficiency that gave agreement between the model calculations and experiment was 0.0000685.
The quantum efficiency for photodegradation of triflusulfuron-methyl in water has been estimated to be 0.0000685 by iterative calculations using the US EPA model GCSOLAR and comparison between the model calculations at mid-day in summer at 50° latitude and the measured photodegradation at pH 7. Based on model calculation, photodegradation half lives at mid-day in summer are expected to decrease from 127 d to 112 d as latitude decreases from 50° to 30° and thus no significant photodegradation of triflusulfuron-methyl in water is expected even for Southern Europe.

On soil (informative data only)
A study of the photolysis of triflusulfuron-methyl on soil is available.

Hawkins D.R. et al. (1992):
This study is performed according to EPA guidelines and is GLP.
Purified ^{14}C-ester carbonyl or ^{14}C-triazine triflusulfuron-methyl (purity 95-97%) in acetonitrile was applied at 1 µg/cm^2 to thin layer (1 mm) of air dried Somersham soil. Soil layers (2.5 x 4.0 cm units) were kept at 25° C in darkness or continuously exposed to xenon arc light source (> 290 nm) using a Suntest apparatus for up to 15 d (equivalent to about 75 d of natural summer sunlight at latitude 52° N assuming that the average daily radiation intensity from the sun is about 75% of the peak intensity over a 12 h period). Volatiles were trapped. Soil samples were periodically removed and analysed for triflusulfuron-methyl and metabolites by HPLC and TLC as described in the aerobic studies (see DAR) except that acetonitrile : 0.1 M ammonium carbonate (9:1) was used as the first extraction solvent and extracts were concentrated to dryness and dissolved in 1 mL 0.1 M ammonium carbonate : acetonitrile (9:1) for analysis.
In the dark, triflusulfuron-methyl is rapidly degraded on dry soil (DT_{50} about 13 d) by cleavage of the sulfonylurea bridge to give IN-D8526 = triazine amine (max. 47.5% after 15 d) and IN-W6725 = methyl saccharin (max. 62.4% after 15 d). In the light (continuous artificial irradiation), degradation occurs at the same rate but a different pathway is observed. As compared to the dark conditions, the metabolites IN-D8526 and IN-W6725 are formed in lower amounts (11.8% and 11.7% respectively, after 15 d). Light favours N-demethylation of triflusulfuron-methyl to give IN-66036 = N-desmethyl triflusulfuron-methyl (max. 12.2% after 2 d, decline observed) which could be further degraded to IN-W6725 and IN-E7710 = N-desmethyl triazine amine (max. 6.9% after 15 d) by cleavage of the sulfonylurea bridge (IN-E7710 could be also formed by N-demethylation of IN-D8526). Light also favours another cleavage of the sulfonylurea bridge to give two triazine urea degradates IN-JL000 = triazine urea (max. 7.1% after 15 d) and IN-JM000 = N-desmethyl triazine urea (max. 13.5%, after 15 d) which could be derived from triflusulfuron-methyl and N-desmethyl triflusulfuron-methyl, respectively. These urea metabolites are derived from the triazine moiety

and the corresponding degradation products from the ester carbonyl moiety could be minor unknowns (< 6.8%). The urea metabolites could be further degraded to the corresponding triazine amine and N-desmethyl triazine amine. Small amounts of IN-M7222 = N,N-bis-desmethyl triazine amine (max. 4.2% at 15 d) are formed. Volatiles (< 10.2%) and bound residue (< 5.0%) are not significant. With regard to the light intensity (15 d continuous artificial light would correspond to about 75 d of natural summer sunlight at latitude 52° N), soil photolysis is not expected to play a significant role under real conditions as compared to biological degradation and even chemical degradation, at least in Northern Europe. Because latitude (30° - 50°) has been shown to have little effect on the rate of photodegradation in water, photodegradation on soil is expected to be not significant throughout Europe.

5.1.2 Biodegradation

5.1.2.1 *Biodegradation estimation*

No data.

5.1.2.2 *Screening tests*

A study on the ready biodegration of triflusulfuron-methyl is available.

Aldred D. (1992):

This study is a closed bottle test performed according to OECD 301 D guideline. This study is GLP.
Triflusulfuron-methyl (purity 91.2%) was dissolved at 2 mg/L in a mineral medium (pH 7.2) inoculated with a low level of micro-organisms obtained from a secondary effluent plant. Incubation was at 20°C for 28 d. Dissolved O_2 concentration was determined by the modified Winkler titrimetric method (combination of O_2 with manganous hydroxide, acidification in presence of iodide and titration of the released iodine). The oxygen depletion was used to calculate the percent biodegradation of the test substance against its theoretical oxygen demand. Sodium acetate was used as the reference standard (6 mg/L). Percents of biodegradation of triflusulfuron-methyl at day 5, day 15 and day 28 were 2.8%, 11.1% and 25%, respectively. For the reference standard, the corresponding figures were 71.2%, 74.7% and 100%.
Since the biodegradation of triflusulfuron-methyl after 28 days was determined to be 25%, triflusulfuron-methyl is not readily biodegradable.

5.1.2.3 *Simulation tests*

Water

In the two water sediment systems available (pHwater = 7.5 both) triflusulfuron-methyl degrades with a half-life of DT_{50} whole system = 22 – 40 d. In these systems, triflusulfuron-methyl partition to the sediment (max. 22% AR) and degrades by cleavage of the sulfonylurea urea bridge to methyl saccharin (IN-W6725, max. 38.4% AR (Applied Radioactivity) in water and 12% AR in sediment after 100 d) and triazineamine (IN-D8526, max. 23.2% AR in water and 18.9% AR sediment after 61 d) which subsequently degrades to *N*-desmethyl triazine amine (max. 10.7% AR in water after 61 d). In an alternative pathway triflusulfuron (JK-55517; max. 28.6% AR in water after 100 d and 19.7% AR in sediment after 61 d) is formed. A meeting of experts (PRAPeR meeting 47 at EFSA on May 2008) agreed that the half-life in water may only be considered a dissipation half-life and not a degradation half-life.

Soil The aerobic route of degradation study, the rate of degradation of triflusulfuron-methyl was investigated in four soils (pH 5.2-8.1; OC 0.72-1.96%; clay 5-13%; 40% MWHC (Maximum Water Holding Capacity)) under dark aerobic conditions at 20°C using triazine ^{14}C-labelled triflusulfuron-methyl. For one of the soils, incubation was carried out at a lower concentration of triflusulfuron-methyl (one tenth), 10°C and 21%

MWHC. Triflusulfuron-methyl exhibits low to moderate persistence in soil under dark aerobic conditions at 20°C (DT 50 lab aerobic = 5.3 – 15 d) or 25°C (DT 50 lab aerobic = 5.7 d [geometrical mean of the two labels]).

Under dark anaerobic conditions at 25°C, degradation of triflusulfuron-methyl is slower than under aerobic conditions (DT_{50} anaerobic = 21 d).

In the photolysis study, triflusulfuron-methyl was degraded at the same rate in the irradiated and the dark control (DT_{50} light = 11.6 d; DT_{50} dark = 12.6 d). The Rapporteur Member State considered that soil photolysis does not play a significant role in the environmental degradation of triflusulfuron-methyl.

5.1.3 Summary and discussion of degradation

Hydrolysis of triflusulfuron-methyl involves the cleavage of the sulfonylurea bridge to produce methyl saccharine (IN-W6725) and triazine amine (IN-D8526). Hydrolysis half-lives for triflusulfuron-methyl were DT_{50} = 3.7 d (pH 5), DT_{50} = 32 d (pH 7) and DT_{50} = 36 d (pH 9). According the available photolysis study, aqueous photolysis has no effect on the degradation of triflusulfuron-methyl at pH 5 and would increase the rate of degradation by a factor of 2.2 (pH 7) and 1.4 (pH 9) with respect to aqueous hydrolysis under dark conditions. Based on an aqueous photolysis study and model calculations photodegradation is not expected to play a significant role in the degradation of triflusulfuron-methyl in the environment and assessment of exposure to photolysis metabolites was not deemed necessary. This conclusion was confirmed by the meeting of experts (PRAPeR meeting 47 at EFSA on May 2008). Metabolites methyl saccharine (IN-W6725) and triazine amine (IN-D8526) are stable to hydrolysis and to aqueous photolysis. Triflusulfuron-methyl is not readily biodegradable according the available study.

Based on these available studies, we can conclude that triflusulfuron-methyl is not rapidly degradable in the environment according to the CLP Regulation.

In the two water sediment systems available (pHwater = 7.5 both) triflusulfuron-methyl degrades with a half-life of DT_{50} whole system = 22 – 40 d. In these systems, triflusulfuron-methyl partition to the sediment (max. 22% AR) and degrades by cleavage of the sulfonylurea urea bridge to methyl saccharin (IN-W6725, max. 38.4% AR in water and 12% AR in sediment after 100 d) and triazineamine (IN-D8526, max. 23.2% AR in water and 18.9% AR sediment after 61 d) which subsequently degrades to *N*-desmethyl triazine amine (max. 10.7% AR in water after 61 d). In an alternative pathway triflusulfuron (JK-55517; max. 28.6% AR in water after 100 d and 19.7% AR in sediment after 61 d) is formed. A meeting of experts (PRAPeR meeting 47 at EFSA on May 2008) agreed that the half-life in water may only be considered a dissipation half-life and not a degradation half-life.

Fate and behaviour on soil (for additional information only)

Triflusulfuron-methyl exhibits low to moderate persistence in soil under dark aerobic conditions at 20°C (DT 50 lab aerobic = 5.3 – 15 d) or 25°C (DT 50 lab aerobic = 5.7 d [geometrical mean of the two labels]).

Under dark anaerobic conditions at 25°C, degradation of triflusulfuron-methyl is slower than under aerobic conditions (DT_{50} anaerobic = 21 d).

In the photolysis study, triflusulfuron-methyl was degraded at the same rate in the irradiated and the dark control (DT_{50} light = 11.6 d; DT_{50} dark = 12.6 d).

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Hawkins D.R., Kirkpatrick D., Dean G.M., Mellor S. (1992c). Acceptable.
This study is performed according to EPA guidelines and is GLP.

Methods: Purified ^{14}C-ester carbonyl triflusulfuron-methyl in acetonitrile was dissolved at 4 concentrations (0.03-1.0 mg/L) in 0.005 M $CaCl_2$ (solvent was 0.1% in the final solutions). Solutions (20 mL) were added to 10 g samples of 5 sterile or non-sterile soils with 2 replicates (3 for the highest dose). Adsorption was allowed for 24 h (according to preliminary test) at 25°C. Following adsorption, RA (Radioactivity) in supernatant was measured by LSC. One replicate from each soil treated at the highest dose was used to assess degradation. Aqueous supernatant was removed, RA was measured by LSC and analysed by HPLC. The remaining soil with residual solution was extracted with acetonitrile: 0.1 M aqueous ammonium carbonate (3:1), extractable RA was measured by LSC and concentrated extracts were analysed by HPLC. Soil residue was combusted for mass balance. For the other 2 replicates, a portion of supernatant (10 mL) was removed (RA was measured by LSC) and replaced by fresh untreated solution to allow desorption for 24 h. After two desorption steps, the supernatant from each replicate was pooled for each soil type and aliquots were analysed by HPLC. The remaining soil was analysed as described for adsorption. Freundlich adsorption isotherms were calculated taking account of degradation observed at the highest dose.

Results: Recoveries were acceptable for adsorption and they could be slightly lower than 90 % for desorption. Significant degradation occurred and methyl saccharin was detected in aqueous supernatants and soil extracts. Non extractable RA was in the range 1.6-50 % for adsorption, 2.3-43.8 % for adsorption on sterile soils and 3.3-30.2 % for desorption. Concentrations of the AS in water and soil phases were calculated using the total RA in each phase and the corresponding HPLC analysis. For soil, non extractable RA was assumed to result from non exhaustive extraction and thus total RA was determined by difference between the applied RA and total RA in water. This assumption is acceptable with regard to the studies on degradation of triflusulfuron-methyl and methyl saccharin in soil. Because soils could contain significant volume of residual solution, HPLC chromatograms of soil extracts could be not representative of the composition of the adsorbed RA. However, because methyl saccharin is poorly adsorbed on soil, amounts of adsorbed AS are likely to be higher than predicted by soil extract analysis and thus adsorption could be underestimated. Accordingly, results can be used by default. Values for the Freundlich adsorption parameters are shown in Table 20.

Table 20. Adsorption of triflusulfuron-methyl on soils

	OC (%)	pH	Kf	Kfoc	1/n
Somersham	1.45	7.3	0.36	25	0.90
Hanford	0.38	6.2	0.50	132*	0.94*
Fargo	2.70	7.4	1.28	47	0.92
Portneuf	0.79	8.2	0.41	52	0.94
Speyer 2.2	1.93	5.9	0.67	35	0.92
Mean / median				**40 / 41**	**0.92 / 0.92**

* Values from this soil are not included in the mean and median. The soil is considered not representative of agricultural soils due to its low organic carbon content.

From the available studies (see DAR), triflusulfuron-methyl may be considered to exhibit high to very high mobility (Kfoc = 25 – 52 mL/g).

5.2.2 Volatilisation

Based on the low vapour pressure (1.01 x 10^{-5} Pa at 20°C), triflusulfuron-methyl is not considered as a volatile substance.

5.2.3 Distribution modelling

Not relevant for this report.

5.3 Aquatic Bioaccumulation

Based on its log Kow values of 2.3 (pH 5 at 25°C), 0.96 (pH 7 at 25°C) and -0.066 (pH 9 at 25°C), no concern over any potential for bioaccumulation could be concluded for triflusulfuron-methyl.

5.3.1 Aquatic bioaccumulation

Measured data on bioaccumulation of triflusulfuron-methyl are not available. Triflusulfuron-methyl has high water solubility and a low log Kow (0.96 at pH 7), it is therefore not predicted to bioconcentrate in aquatic organisms.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on a log Kow value of 0.96 (pH 7 at 25°C) for triflusulfuron-methyl, the cut-off value of log Kow ≥ 4 (as the experimental BCF measure is not available) set out in the CLP Regulation is not exceeded.

5.4 Aquatic toxicity

Only validated ecotoxicity tests accepted for risk assessment from Draft Assessment Reports were used.

All the aquatic toxicity studies of triflusulfuron-methyl were performed on GLP and according to EPA or OECD guidelines. Then, the reliability factor would be indicated in the summary only when different of 1. The reliability factors of the aquatic toxicity studies are reported in the Table 21, which summarised the available data on the toxicity for aquatic organisms. Algae and aquatic plants are the most sensitive species (see section "5.4.3. Algae and aquatic plants").

5.4.1 Fish

5.4.1.1 *Short-term toxicity to fish*

Three short-term toxicity studies to fish are available for triflusulfuron-methyl.

Baer K.N. (1991a):

This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Oncorhynchus mykiss* (rainbow trout).
The acute toxicity of triflusulfuron-methyl (technical substance, purity 95.6% w/w) was assessed in unfed juvenile rainbow trout exposed for 4 days under static conditions. Fish ranged from 3.3 to 4.3 cm in standard length (mean 3.7 cm) at the start of the study. Test solutions were maintained between 10.6 and 12.4°C. Exposure was performed in 20 L containers containing a pH (8.0) adjusted control and test water (respectively 0 and 130, 216, 360, 600, or 1000 mg/L, nominal). Ten fish were allocated randomly per container (loading rate: 0.45 g/L). Mortality and abnormal responses of fish were recorded at 24 h intervals throughout the exposure period.

Mean measured concentrations of triflusulfuron-methyl were 120, 210, 320, 660, and 1000 mg a.s./L. The LC50 was based on mean measured concentrations of triflusulfuron-methyl in the test media.
One of the surviving fish in the water control and one in the 600 mg/L group were observed to be upside down at the bottom of the test chamber after 24 h. The No Observable Effect measured Concentration was 210 mg/L.
LC50 – 96 h =730 mg a.s./L.

Baer K.N. (1991b):

This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Lepomis macrochirus* (bluegill sunfish).
The acute toxicity of triflusulfuron-methyl (technical substance, purity 95.6% w/w) was assessed in bluegill sunfish exposed for 4 days under static, unaerated conditions. Fish ranged from 2.3 to 3.6 cm in standard length (mean 2.6 cm) at the start of the study. Test solutions were maintained between 22.5 and 22.7°C. Exposure was performed in 15 L containers containing a water control, a pH (8.0) adjusted control and test water (respectively 130, 216, 360, 600, or 1000 mg/L, nominal). Ten fish were allocated randomly per container (loading rate: 0.31 g/L). Mortality and abnormal responses of fish were recorded at 24 h intervals throughout the exposure period.
The dissolved oxygen was above the theoretical saturation value at 0 and 24 h in all test conditions. This high dissolved oxygen was not biologically significant based on the lack of mortality at 24 h.
Mean measured concentrations of triflusulfuron-methyl were 120, 180, 370, 590, and 1100 mg a.s./L. The LC50 was based on mean measured concentrations of triflusulfuron-methyl in the test media.
No mortality and no abnormal behaviour were recorded in control fish. The highest test concentration causing no mortality was 370 mg/L. Two surviving fish at 600 mg/L and one surviving fish at 1100 mg/L exhibited dark coloration and were at the surface.
LC50 – 96 h = 760 mg a.s./L.

Baer K.N. (1993a):

This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Cyprinus carpio* (carp).
The acute toxicity of triflusulfuron-methyl (technical substance, purity 95.6% w/w, batch 66037-24) was assessed in unfed juvenile carp exposed for 4 days under static, unaerated conditions. Fish ranged from 2.3 to 2.8 cm in standard length (mean 2.4 cm) at the start of the study. Test solutions were maintained between 21.6 and 22.1°C. Exposure was performed in 20 L containers containing a water control, a pH (8.0) adjusted control and test water (respectively 130, 216, 360, 600, or 1000 mg/L, nominal). Ten fish were allocated randomly per container (loading rate: 0.23 g/L). Mortality and abnormal responses of fish were recorded at 24 h intervals throughout the exposure period.
Mean measured concentrations of triflusulfuron-methyl were 100, 210, 350, 550, and 830 mg a.s./L. The LC50 was based on mean measured concentrations of triflusulfuron-methyl in the test media.
There was no mortality observed. Some fish in the 830 mg a.s./L group were observed at the surface at 24, 48, and 96 hours. Because of the absence of mortality over the range of concentrations tested, no concentration-effect relationship, and therefore no LC50, could be established.
LC50 – 96 h > 830 mg a.s./L.

5.4.1.2 *Long-term toxicity to fish*

Two long-term toxicity studies to fish are available for triflusulfuron-methyl.

Baer K.N. (1992):

This test was GLP and performed according to OECD guideline no 204 (1984). The tested species was *Oncorhynchus mykiss* (rainbow trout).
The effects of triflusulfuron-methyl, technical substance (purity 95.6% w/w) on survival and growth of fingerling rainbow trout was assessed in an unaerated flow-through system over a 21-day exposure period. A dilution water control, and nominal test substance concentrations 6.0, 12, 25, 50, 100, and 200 mg/L were used during the study. Test solutions were delivered intermittently (about every 21 min.) to replicate 7-liter glass exposure chambers. The volume of each replicate was exchanged five times daily. A total of 10 embryos were exposed per concentration (5 embryos per replicate, 2 replicates per concentration) at test start. Mortality and abnormalities were recorded daily throughout the study and standard length and blotted wet weight of surviving fingerlings were calculated at the end of the test.
Analytical verification of triflusulfuron-methyl concentrations was made on test solutions sampled on Day 0, once weekly, and at test end (Day 21). Mean, measured concentrations of triflusulfuron-methyl were 0, 5.2, 12, 28, 56, 110, and 210 mg/L.
No mortality and no intoxication symptoms were observed in fish exposed to triflusulfuron-methyl over the exposure period. There was a statistically significant downward linear trend in fish length and weight with increasing test concentration. Due to the variability of these parameters, within each concentration, no single concentration was statistically significantly different from the control.
NOEC 21-d = 210 mg a.s./L.

Boeri R.L. et al. (1996):

This test was GLP and performed according to OECD guideline no 210 (1992). The tested species was *Oncorhynchus mykiss* (rainbow trout).
The effects of triflusulfuron-methyl, technical substance (purity 95.72% w/w) on the early life stage of rainbow trout was assessed in an unaerated, flow-through system for 97 days (61 days post-hatch). A dilution water control, and nominal test substance concentrations of 4.5, 8.8, 18, 35, 70, and 140 mg/L were used during the study. A total of 40 embryos were exposed per concentration (20 embryos per replicate, 2 replicates per concentration). Test solutions were maintained between 9.3 and 11.0°C.
Embryos and alevins were held in relative darkness until 1 week after hatching, and then held under a photoperiod of 16 hours light and 8 hours darkness for the remainder of the study. On Day 55, after swim-up had begun in the controls, the fingerlings were thinned to a total of 30 fish per concentration Following swim up, fish were fed commercial dry starter chow and live, newly hatched *Artermia salina* nauplii, *ad libitum,* 3 times per day except during the final 23 hours of the test. Daily observations were made for assessment of number of dead eggs, first and last day of hatching, first day of swim-up, survival and abnormalities from hatching to thinning, and survival and abnormalities from thinning to test end. Standard length and blotted wet weight of surviving fingerlings were determined at test end.
Analytical verification of triflusulfuron-methyl concentrations was made on test solutions sampled on Day 0, once weekly, and at test end (Day 90). Mean measured concentrations of triflusulfuron-methyl were 0, 2.41, 6.03, 11.8, 29.4, 57.7, and 136 mg/L.
Survival in the control averaged 73.8% at hatch, 72.5% at thinning, and 100% (after thinning) at the end of the study. The survival of test organisms at thinning was significantly decreased when compared to the controls at 136 mg/L. The time to hatch (start and end), time to swim up, and survival of test organism were not significantly different from the control at any concentration. In surviving fish exposed to triflusulfuron-methyl at 136 mg/L, the mean total length (43.1 cm) and mean wet weight (0.94 g) were significantly decreased when compared to control (48.3 cm and 1.21 g, respectively).
The 97-day NOEC of triflusulfuron-methyl was 57.7 mg/L, based on mean measured concentrations of triflusulfuron-methyl and survival of test organisms at thinning and total length and wet weight of surviving fish after 97 days of exposure.
NOEC 97-d = 57.7 mg a.s./L.

5.4.2 Aquatic invertebrates

5.4.2.1 *Short-term toxicity to aquatic invertebrates*

A single short-term toxicity study to aquatic invertebrates is available for triflusulfuron-methyl.

Baer K.N. (1991c):

This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Daphnia magna*.
The acute toxicity of triflusulfuron-methyl (technical substance, purity 95.6% w/w) was assessed in neonate (less than 24 h old) waterfleas exposed for 2 days under unaerated static conditions. Exposure was performed in 250 mL beakers containing 200 mL of test water with 0 (water control or pH adjusted control) or 130, 216, 360, 600, or 1000 mg/L (nominal) test substance. Dilution water originated from laboratory well and flowed through aquaria containing fathead minnows, prior to use in the daphnid test. Each replicate (four per concentration) contained 5 daphnids added randomly. Test solutions were held between 20.2°C to 20.4°C. Oxygen values were close to 100% saturation. Immobilisation of daphnids was recorded at 24h intervals throughout the exposure period.
Mean measured concentrations of the active substance were 130, 170, 310, 490, and 960 mg a.s./L.
No effects on mobility were recorded at any of the concentrations tested. Because of the absence of mortality over the range of concentrations tested, no concentration-effect relationship, and therefore no LC50, could be established.
EC50 48 h > 960 mg a.s./L.

Comments (RMS): the origin of dilution water used is questionable. The notifier stated that dilution water used for some early daphnid studies was conditioned prior to use to ensure adequate growth and survival of daphnid cultures. Considering that this dilution water fulfill all validity criteria, including good analytical recovery, it may be concluded that the dilution water did not affect the outcome of the study. The study may be considered as acceptable.

5.4.2.2 *Long-term toxicity to aquatic invertebrates*

A single long-term toxicity study to aquatic invertebrates is available for triflusulfuron-methyl.

Baer K.N. (1993b):

This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Daphnia magna*.
Effects of chronic exposure to triflusulfuron-methyl (technical substance, purity = 95.6% w/w) on *Daphnia magna* neonates was determined under semi-static conditions with test solution renewal 3 times a week for 21 d. Effects on survival and growth were assessed in tests vessels containing 200 mL test water with 0 (control), 0.33, 0.82, 2.0, 5.1, 13, 32, 80, 200, or 500 mg test substance/L. Each replicate (ten per concentration) contained four daphnids randomly assigned. Observations were made daily.
Mean measured concentrations of triflusulfuron-methyl were 0.28, 0.68, 1.7, 4.6, 11, 25, 67, 160, and 300 mg/L.
The first day of reproduction and the total number of young produced in 21 days were statistically different from controls at 11 mg/L and above. The variation at 11 mg/L was not considered biologically significant based on the lack of statistically significant effects in the total number of live young produced per surviving adult in 21 days. The total number of live young produced per surviving adult in 21 days was significantly reduced at 25 mg/L and above. There was no statistically significant effect on adult length at any test concentration where daphnids survived to day 21. The number of adults surviving to day 21 was significantly decreased at 160 mg/L and above.

NOEC 21-d = 11 mg a.s./L.

5.4.3 Algae and aquatic plants

Three toxicity studies to algae are available for triflusulfuron-methyl.

Douglas, M.T., Halls, R.W.S. (1991):

This test was GLP and performed according to OECD guideline no 201 (1984). The tested species was *Pseudokirchneriella subcapitata*.
The toxicity of triflusulfuron-methyl (technical substance, purity >97%, batch AGO216-11) to the green algae species Pseudokirchneriella subcapitata was determined under static conditions, continuous illumination over an exposure period of 120 h. The test was conducted in 250 mL flasks filled with 100 mL test water, containing 0 (control or solvent -acetone- control), 0.125, 0.250, 0.5, 1.0 and 2.0 mg test substance/L. The cell density was 9.69 x 10^4 cells/mL at the start of the test. Each concentration was repeated three times. Growth was monitored daily.
To assess recovery after the initial 120 hours exposure period, algae from the 2.0 mg/L concentration were placed in nutrient medium without triflusulfuron-methyl. Cell growth (absorbance at 665 nm) was assessed for up to 7 days following transfer to triflusulfuron-methyl-free medium.
Results based on nominal concentrations:
ErC50 24-48 h = 1.0 mg/L

EbC50 72 h = 0.50 mg/L; EbC50 120 h = 0.62 mg/L;

NOEC = 0.125 mg/L

Regrowth occurred in both the control and test culture (2.0 mg/L) within 7 days
Comments (RMS): analytical measurements were not performed. The results of this study should be considered with caution (reliability 2) and it is recommended to rely on the next study.

Hughes, J.S., Williams, T.L. (1993a):

This test was GLP and performed according to US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982). The tested species was *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*).
The toxicity of triflusulfuron-methyl (technical substance, purity 98.7%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions, continuous illumination over an exposure period of 120 h. Test treatments were control (medium only), solvent control (0.1 mL N,N-dimethylformamide (DMF)/L), and 4.50, 9.00, 18.0, 36.0, 71.9 µg triflusulfuron-methyl/L. Each treatment and control was replicated 3 times. The test concentrations were measured at test initiation and at the end of the study. The cell density was 3000 cells/mL at the start of the test. Each concentration was repeated three times. Treatments were incubated at 24±2°C for 120 hours and cell counts were made on days 3, 4, and 5.
Results based on nominal concentrations:
EbC50 120 h = 0.046 mg triflusulfuron-methyl/L [95% confidence limits 0.038 – 0.057 mg/L]

NOEC = 0.036 mg triflusulfuron-methyl/L

Comments (RMS): The cell density at the beginning of the test was 3000 cells/mL (consistent with the US-EPA test guideline used to conduct the test) (OECD recommendation: 10^4 cells/mL). Continuous illumination of 4306 ± 646 lumens/m^2 (300 µE/m^2s) is higher than the recommended value of 120 µE/m^2s. Cell concentration increase over 3 days was satisfactory in the controls. The study is acceptable.

Sloman, T.L. (1999a):

This test was GLP and performed according to US-EPA 850.5400 (1996). The tested species was *Anabaena flos-aquae*.
The toxicity of triflusulfuron-methyl (purity = 98.7%) to the blue-green alga *Anabaena flos-aquae* was determined under static conditions and continuous illumination over an exposure period of 96 h. The test was conducted in 250 mL flasks filled with 50 mL test water, containing 0 (control), 1, 2, 3, 4, and 5 mg test substance/L. Algae from a logarithmically growing stock culture were inoculated to achieve the cell density of 10^4 cells/mL at the start of the test. Each concentration was replicated three times. Cell density was measured every 24 h until the end of the test.
To assess recovery after the initial 96 hours exposure period, algae from the 2, 3, 4, and 5 mg/L concentrations were placed in nutrient medium without triflusulfuron-methyl. Cell counts were made approximately 72 and 144 h from recovery test initiation.
Results based on nominal concentrations:
Cell density:

96-hour EC50 = 1.46 mg/L
96-hour NOEC = 1 mg/L

Area under the growth curve:

96-hour EbC50 = 1.31 mg/L
96-hour NOEC = < 1 mg/L

Growth rate:

96-hour ErC50 = 2.80 mg/L
96-hour NOEC = 1 mg/L

Cell growth resumed within 6 days from recovery test initiation at concentration less than or equal to 5 mg/L.

Two toxicity studies to aquatic plants are available for triflusulfuron-methyl.

Sloman, T.L. (1999b):

This test was GLP and performed according to ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G3 1415-91 (1991). The tested species was *Lemna gibba*.
The effects of technical triflusulfuron-methyl (purity = 98.7%) on the growth and reproduction of the duckweed *Lemna gibba* were determined without test medium renewal over a 14 days period. Plants were exposed to the test substance in 250 mL flasks filled with 100 mL of test water, that contained 0 (control), 1, 1.5, 2, 3, and 4 µg triflusulfuron-methyl/L. A total of 15 fronds (5 plants each with 3 fronds per plant) were allocated per flask. Each concentration and the control were tested as 4 replicates. Frond counts were made on days 0, 2, 4, 7, 9, 12, and 14. Biomass was determined at the end of the test.
The ability of the organisms to recover was assessed for each treatment with 50% or greater growth inhibition based on healthy frond count relative to the blank control.
Initial, measured concentrations were 0.78, 1.1, 1.5, 2.2, and 2.9 µg triflusulfuron-methyl/L. Measured concentrations after 14 d were 0.8, 1.1, 1.5, 2.5, and 3.1 µg triflusulfuron-methyl/L. Based on nominal concentrations:
ErC50 (14 days) = 3.5 µg triflusulfuron-methyl/L, 95% CI = [3.4-3.5] µg/L. (healthy frond count)
NOErC (14 days) = 1.5 µg triflusulfuron-methyl/L
EbC50 (14 days) = 4.4 µg triflusulfuron-methyl/L, 95% CI = [4.0-5.1] µg/L.
NOEbC (14 days) = 2.0 µg triflusulfuron-methyl/L
The effects on growth and reproduction of *Lemna gibba* were found to be phytostatic, *i.e.* growth resumed, at concentration less than or equal to 4 µg/L within 14 days.

Hughes J.S., Williams T.L. (1993b):

This test was GLP and performed according to US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982). The tested species was *Lemna gibba*.
The effects of triflusulfuron-methyl (technical substance, purity = 98.7%) on the growth of the duckweed *Lemna gibba* were determined without test medium renewal over a 14 days period. Plants were exposed to the active substance at 0 (control, DMF control), 0.635, 1.27, 2.53, 5.05, and 10.1 µg triflusulfuron-methyl/L. A total of 12 fronds (3 plants each with 4 fronds per plant) were allocated per flask (temperature: 25 ± 2°C;

mean light intensity: 4198-5813 lux or 293 – 405 μE/m^2s; photoperiod: 24 h). Each concentration and the control were repeated three times. Effects on growth rate were assessed through the number of fronds measured on days 2, 4, 7, 9, 11, and 14.
The measured concentration values yielded from 91% to 148% of the nominal concentration on day 0 and from 80% to 151 % on day 14. The 3 highest concentrations had appreciable inhibitory effects upon the population growth of *L. gibba*. Effects on frond shape were noted at concentrations of 2.53 μg/L and higher, and fronds appeared smaller at the 1.27 μg/L concentration, relative to the 0.635 μg/L concentration and the controls. Based on nominal concentrations:
EC50 (14-d) = 2.82 μg triflusulfuron-methyl/L, 95% CI =[2.34-3.39]μg/L
NOEC (14-d) = 1.27 μg triflusulfuron-methyl/L

5.4.4 Other aquatic organisms (including sediment)

No data.

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

Data are summarised in Table 21 below.

Table 21. Summary of acute and long term toxicity of triflusulfuron-methyl to the most sensitive species within different groups of aquatic organisms

Organism	Species	Test conditions	LC_{50}/ EC_{50} (mg/L)	NOEC (mg/L)	GLP (Y/N)	Reliability
Fish	*Oncorhynchus mykiss* (Rainbow trout)	96 h static	760 (mean measured)	210 (mean measured)	Y	1
	Oncorhynchus mykiss (Rainbow trout)	Early life stage, flow-through, 97 days	-	57.7 (mean measured)	Y	1
Invertebrates	*Daphnia magna* (waterflea)	48 h, static	> 960 (mean measured)	960 (mean measured)	Y	1
	Daphnia magna (waterflea)	Growth and reproduction, semi-static, 21 days	-	11 (mean measured)	Y	1
Algae	*Pseudokirchneriella subcapitata*	Static Biomass (120 h): Growth rate:	 0.62 (nominal) 1.0 (nominal)	0.125 (nominal) - -	Y	2
	Pseudokirchneriella subcapitata	Static, 120 h Biomass: Growth rate:	 0.0463 (nominal) -	 0.036 (nominal) -	Y	1
	Anabaena flos-aquae	Static, 96 h Biomass: Growth rate:	 1.31 (nominal) 2.80 (nominal)	 < 1 (nominal) 1 (nominal)	Y	1
Aquatic plants	*Lemna gibba*	Static, 14 days Healthy frond count (ErC50) Biomass	 0.0035 (nominal) 0.0044 (nominal)	 0.0015 (nominal) 0.002 (nominal)	Y	1
	Lemna gibba	Static, 14 days	0.00282 (nominal)	0.00127 (nominal)	Y	1

In toxicity studies for algae and aquatic plants EC50s at concentrations ≤ 1 mg/L were observed. In addition, triflusulfuron-methyl is not readily biodegradable although it is unlikely for the substance to bioaccumulate in aquatic organisms (log Kow < 3). As a consequence and according to the CLP Regulation, due to its acute effect on algae/aquatic plants at a concentration ≤ 1 mg/L and due to its low degradability, triflusulfuron-methyl should be classified as R50-53 (Aquatic Acute 1 – Aquatic Chronic 1).

Based on the toxicity data for *Lemna gibba* (ErC50 = 0.0035 mg/L) an M-factor of 100 is proposed. The same approach was applied to determine specific concentration limits according to Directive 67/548/EEC:

Concentration Classification

C≥0.25% N ; R50-53

0.025%≤C<0.25% N; R51-53

0.0025%≤C<0.025% R52-53

where C is the concentration of triflusulfuron-methyl in the preparation (expressed as weight/weight percentage).

Here is the classification proposal for chronic according the 2nd ATP to the regulation (EC) 1272/2008. The lowest chronic toxicity value was the NOEC = **0.00127** mg/L (Hughes J.S., Williams T.L. (1993b)), determined with *Lemna Gibba*. As the NOEC-value is between 0.001 and 0.01 mg/L and Triflusulufron-methyl does not fulfill the criteria for rapid degradation, classification as **Aquatic Chronic 1 H410 'Very toxic to aquatic life with long lasting effects'** with a **M-factor of 10** according to Regulation EC 1272/2008 will be .proposed

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

Proposed classification based on Directive 67/548/EEC criteria:

N; R50-53

Proposed specific concentration limits (if any):

Proposed classification of mixtures		
N; R50-53	N; R51-53	R52-53
C ≥ 0.25%	0.25% > C ≥ 0.025%	0.025% > C ≥ 0.0025%

The concentration limits are expressed as weight/weight percentage.

Proposed classification based on CLP criteria:

Aquatic Acute 1 – H400

Aquatic Chronic 1 – H410

M-factor (acute): 100

M-factor (chronic): 10

6 OTHER INFORMATION

7 REFERENCES

Author(s)	Year	Title. Company (insert name) report no. . Source (where different) Published or not
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Hammond	1999	*Surface tension of triflusulfuron-methyl* *DuPont Stine Research Center* *DuPont-2280* GLP: Yes Published: No
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Huntley,	2001	Determination of density for triflusulfuron-methyl (DPX-66037) ABC Laboratories, Inc. (Missouri) DuPont-6280 GLP: Yes Published: No
Moore,	2002	Determination of the melting point/melting range for triflusulfuron-methyl (DPX-66037) technical DuPont Stine-Haskell Research Center DuPont-5775 GLP: Yes Published: No
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Moore,	1999	UV/visible absorption of triflusulfuron-methyl DuPont Experimental Station DuPont-2560 GLP: Yes Published: No
Moore, L.A., Schmuckler, M.E.	1997	The solubility of DPX-66037 in water DuPont Experimental Station AMR 4571-97 GLP: Yes Published: No

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Rhodes, B.C., Cooke, L.A.	1992	Solubility of DPX-66037 in pH 3, 5, 7 and 9 buffers and organic solvents DuPont Experimental Station AMR 1981-91 GLP: Yes Published: No
Rhodes, and Cooke,	1992	Determination of the octanol/water partition coefficient (K_{ow}) for DPX-66037 DuPont Experimental Station AMR 1984-91 GLP: Yes Published: No
Hawkins, D.R., Mayo, B.C., Pollard, A.D., Donschak, W.W.	1992	The metabolism of 14C-DPX-66037 in rats Huntingdon Research Centre AMR 1638-90 GLP: Yes Published: No
Clouzeau, J.	*1992*	*DPX-66037: Acute oral toxicity in rats* *Centre International de Toxicologie (CIT)* *8727 TAR* *GLP: Yes* *Published: No*
Sarver, J.W.	*1991 a*	*Acute oral toxicity study with DPX-66037-24 in male and female rabbits* *DuPont Haskell Laboratory* *HLR 7-91* *GLP: Yes* *Published: No*
Sarver, J.W.	*1991 b*	*Acute oral toxicity study with DPX-66037-24 in male and female rats* *DuPont Haskell Laboratory* *HLR 212-91* *GLP: Yes* *Published: No*
Clouzeau, J.	*1992*	*DPX-66037: Acute dermal toxicity in rats* *Centre International de Toxicologie (CIT)* *8728 TAR* *GLP: Yes* *Published: No*
Sarver, J.W.	*1991*	*Acute dermal toxicity study of DPX-66037-24 in rabbits* *DuPont Haskell Laboratory* *HLR 36-91* *GLP: Yes* *Published: No*
Panepinto, A.S.	*1991*	*Acute inhalation toxicity study with DPX-66037-28 in rats* *DuPont Haskell Laboratory* *HLR 553-91* *GLP: Yes* *Published: No*

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Atkinson, J.E.	*1991*	*A subchronic (3-month) toxicity study of DPX-66037-24 in the dog via dietary administration* *Bio/Dynamics, Inc.* *HLO 783-91* *GLP: Yes* *Published: No*
Biegel, L.B.	*1992*	*Subchronic oral toxicity: 90-day study with DPX-66037-59; feeding study in rats* *DuPont Haskell Laboratory* *HLR 528-92* *GLP: Yes* *Published: No*

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Biegel, L.B., Frame, S.R.	*1995*	*Response to the MAFF subcommittee on pesticides questions regarding triflusulfuron-methyl induction of testicular effects* *DuPont Haskell Laboratory* *TRIF/TOX 2* *GLP: Not given* *Published: No*
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Bentley, K.S.	*1991 b*	*In vitro evaluation of DPX-66037-24 for chromosome aberrations in human lymphocytes* *DuPont Haskell Laboratory* *HLR 775-91, Revision No. 1* *GLP: Yes* *Published: No*
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8 ANNEXES

See appendix 1 for confidential data.