Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

mesosulfuron-methyl (ISO); methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-*p*-toluate

EC Number: -
CAS Number: 208465-21-8

CLH-O-0000001412-86-131/F

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

Adopted

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Mesosulfuron-methyl

EC Number: **Not allocated**

CAS Number: **208465-21-8**

Index Number: **Not allocated**

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Version number: 2 **Date:** **November 2015**

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PART A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Mesosulfuron is included in the Annex I of Directive 91/414/EEC (CAS number 400852-66-6) and subsequently in Pesticide Regulation 540/2011.

However, as the active form of the substance is Mesosulfuron-methyl, all data belong to the methyl ester Mesosulfuron-methyl if not specified otherwise and the proposed classification is for Mesosulfuron-methyl.

Table 1.1-1: Substance identity

	Parent compound	**Active form** **All data belong to the Methyl ester Mesosulfuron-methyl if not specified otherwise**	**Mesosulfuron-methyl-sodium**
Substance name:	Common name: Mesosulfuron (parent compound of the active form: Mesosulfuron-methyl) Mesosulfuron (ISO 1750 (published)) IUPAC name: 2-[(4,6-dimethoxy pyrimidin-2-ylcarbamoyl) sulfamoyl]-α-methane sulfonamido-p-toluic acid (parent compound) CAS name: benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl] (parent compound)	Common name: Mesosulfuron-methyl IUPAC name: methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate CAS name: benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl]-, methyl ester	Common name: Mesosulfuron-methyl-sodium IUPAC name: methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate, sodium salt CAS name: benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl], methyl ester, monosodium salt
EC number:	not allocated	not allocated	not allocated
CAS number:	400852-66-6	208465-21-8	208465-19-4

Annex VI Index number:	not allocated	not allocated	not allocated
Degree of purity:	-	≥ 955 g/kg	-
Impurities:	The identity of the impurities is confidential		

1.2 Harmonised classification and labelling proposal

Table 1.2-1: The current Annex VI entry and the proposed harmonised classification

Mesosulfuron-methyl:

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not listed
Current proposal for consideration by RAC	Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Acute M-factor of 100 Chronic M-factor of 100

1.3 Proposed harmonised classification and labelling based on CLP Regulation criteria

Table 1.3-1: 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 applicable
2.3.	Flammable aerosols	None		None	Not applicable
2.4.	Oxidising gases	None		None	Not applicable
2.5.	Gases under pressure	None		None	Not applicable
2.6.	Flammable liquids	None		None	Not applicable
2.7.	Flammable solids	None		None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None		None	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	None		None	Not applicable
2.10.	Pyrophoric solids	None		None	Not applicable
2.11.	Self-heating substances and mixtures	None		None	Not applicable
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not applicable
2.13.	Oxidising liquids	None		None	Not applicable
2.14.	Oxidising solids	None		None	Conclusive but not sufficient for classification

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

Table 1.3-1: Proposed classification according to the CLP Regulation continued

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification 1)	Reason for no classification 2)
2.15.	Organic peroxides	None		None	Not applicable
2.16.	Substance and mixtures corrosive to metals	None		None	Not applicable
3.1.	Acute toxicity - oral	None		None	Conclusive but not sufficient for classification
	Acute toxicity - dermal	None		None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	None		None	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None		None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None		None	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Data lacking
3.4.	Skin sensitisation	None		None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None		None	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	None		None	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None		None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None		None	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None		None	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	None		None	Not applicable
4.1.	Hazardous to the aquatic environment	Aquatic acute 1; H400 Aquatic chronic 1; H410	Acute M factor: 100 Chronic M factor: 100	None	Conclusive

5.1.	Hazardous to the ozone layer	None		None	Data lacking

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

Labelling:

Pictogram:	GHS09
Signal word:	Warning
Hazard statements:	H410: Very toxic to aquatic life with long lasting effects
Precautionary statements:	P273: Avoid release to the environment. P501: Dispose of contents/container in accordance with local regulation. Precautionary statements are not included in Annex VI

Proposed notes assigned to an entry: None proposed

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Mesosulfuron-methyl is a pesticide herbicide that has been reviewed as a new active substance, in the context of the work of Directive 91/414/EEC. The overall conclusion from the evaluation is that it may be expected that plant protection products containing Mesosulfuron will fulfil the safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC and Mesosulfuron was approved in accordance with Directive 91/414/EEC (Directive 2003/119/EC) and subsequently in Regulation 540/2011.

Within the framework of that decision, the French and the German authorities proposed in the draft assessment report the following classification for the technical active ingredient Mesosulfuron-methyl (carried out according to 67/548/EEC):

Hazard symbol:	N	Dangerous for the environment
Risk phrases:	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases:	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/Safety data sheets

At the time of submission, there are no registrations for this substance under REACH or under the Biocidal Products Regulation.

2.2 Short summary of the scientific justification for the CLH proposal

The lowest EC50 value obtained for Mesosulfuron-methyl is 0.00129 mg a.s./L for aquatic plants, that therefore fulfils the criteria for classification as Aquatic Acute Cat. 1 (L(E)C50 ≤ 1 mg/L) and the acute M-factor is 100 (EC_{50}value falls within the >0.001 to ≤0.01 mg/L). The lowest NOEC value obtained for Mesosulfuron-methyl is 0.000388 mg a.s./L for aquatic plants, that therefore fulfils the criteria for classification as Aquatic Chronic Cat. 1 (chronic NOEC ≤ 0.1 mg/L) and chronic M-factor is 100 (The substance is not rapidly degradable and the NOEC value falls within the >0.0001 to ≤0.001 mg/L band).

2.3 Current harmonised classification and labelling

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

Not currently listed on Annex VI of the CLP Regulation.

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

Not currently listed on Annex VI of the CLP Regulation.

2.4 Current self-classification and labelling

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

Aquatic Acute 1; H400,

Aquatic Chronic 1; H410.

2.4.2 Current self-classification and labelling based on DSD criteria

N; R50/53

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Mesosulfuron has also been evaluated as a new pesticide active substance, for use as an herbicide, in the context of Directive 91/414/EEC concerning the placing of plant protection products on the market.

In accordance with Article 36 (2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, Mesosulfuron is therefore subject to harmonised classification and labelling and should be considered for it. As the substance is not listed on Annex VI of CLP this proposal covers all hazard classes.

The proposal is based mainly on the information presented in the renewal assessment report submitted by the Rapporteur Member State, France, in the context of the Approval Renewal of the active substance under Pesticide Regulations EC 1107/2009 and EC 844/2012. The Renewal assessment report is available on EFSA website.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

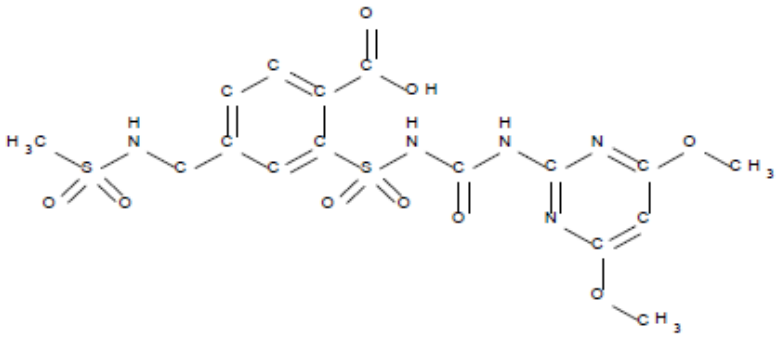
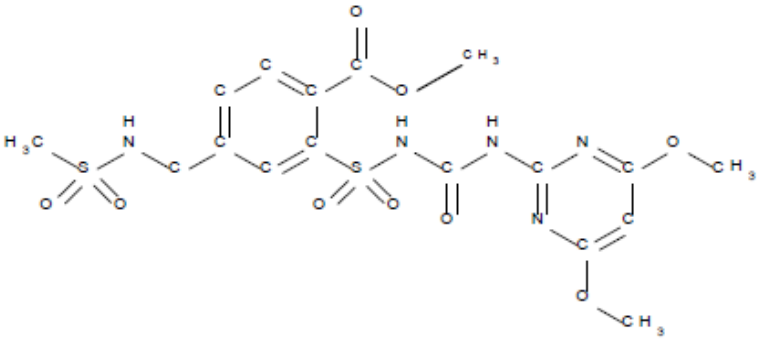
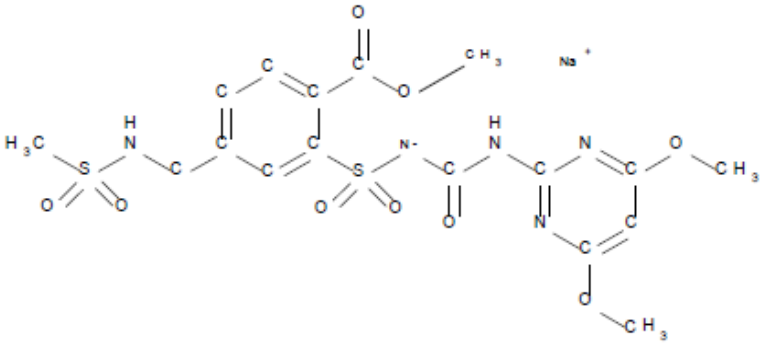
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1.1-1: Substance identity

	Parent compound	Active form All data belong to the methyl ester, Mesosulfuron-methyl, if not specified otherwise	Mesosulfuron methyl sodium
EC number:			
EC name:	Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl] -	Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]-, methyl ester	Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]-, methyl ester, sodium salt (1:1)
CAS number:	400852-66-6	208465-21-8	208465-19-4
CAS name:	benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl]	benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl]-, methyl ester	benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl], methyl ester, monosodium salt
IUPAC name:	2-[(4,6-dimethoxy pyrimidin-2-ylcarbamoyl) sulfamoyl]-α-methane sulfonamido-p-toluic acid	methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate	methyl 2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-α-(methanesulfonamido)-p-toluate, sodium salt
CLP Annex VI Index number:	not allocated	not allocated	not allocated
Molecular formula:	$C_{16}H_{19}N_5O_9S_2$	$C_{17}H_{21}N_5O_9S_2$	$C_{17}H_{20}N_5NaO_9S_2$
Molecular weight range:	489.48 g/mol	503.51 g/mol	525.50 g/mol

Chemical name	**Structural formula**

Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]-	
Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]-, methyl ester	
Benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]-, methyl ester, sodium salt (1:1)	

1.2 Composition of the substance

Table 1.2-1: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
benzoic acid, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-4-[[(methylsulfonyl) amino]methyl]-, methyl ester (Mesosulfuron-methyl)	≥ 955 g/kg	≥ 955 g/kg	

Current Annex VI entry: Not listed

Table 1.2-2: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
All impurities are confidential	The identity of the impurities and their maximum contents are confidential		

Current Annex VI entry: Not listed

The impurities were thoroughly evaluated during the review under Directive 91/414/EEC and do not additionally impact on the classification proposed in this dossier. All impurities are confidential business information.

Table 1.2-3: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives are present in the active substance as manufactured.				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

In the original Draft Assessment Report, the minimum purity was 930 g/kg. However, French authorities reviewed the specification and agreed the minimum purity is 955 g/kg. This minimum purity of 955 g/kg has been notified to all Member States and is presented in the Application for Approval Renewal for Mesosulfuron.

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Light yellow crystalline (powder) with a weakly pungent odour	M-198767-01-1, 2000	Visual inspection US EPA OPPTS 830.6303 Mesosulfuron-methyl; pure (98.7% w/w)
	Crème coloured (Munsell colour 5) fine powder with a weakly pungent odour	M-198490-01-1, 2000	Visual inspection US EPA OPPTS 830.6303 Mesosulfuron-methyl; technical (95.7% w/w)
Melting/freezing point	Mean: 195.4°C followed by a decomposition of the test item	M-198734-01-1, 2000	Measured EU 92/69 A.1 Mesosulfuron-methyl; pure (98.7 % w/w)
	189-192°C	M-198629-01-1, 2000	Measured EU 92/69 A.1 Mesosulfuron-methyl; technical (95.7 % w/w)
Boiling point	The test item decomposes before reaching the boiling point. The test item therefore has no boiling point at atmospheric pressure. There is an exothermal decomposition in the temperature range 195 - 360°C, with a decomposition energy of 696 J/g.	M-198734-01-1, 2000	Measured EU 92/69 A.1 Mesosulfuron-methyl; pure (98.7 % w/w)
	There is an exothermal decomposition in the temperature range 190°C up to 360°C, with a decomposition energy of 846 J/g.	M-198629-01-1, 2000	Measured EU 92/69 A.1 Mesosulfuron-methyl; technical (95.7 % w/w)
Relative density	D^R_4 = 1.48	M-198737-01-1, 2000	Measured EU 92/69 A.3 OECD 109 Mesosulfuron-methyl; pure (98.7 % w/w)
	D^R_4 = 1. 53	M-198633-01-1, 2000	Measured EU 92/69 A.3 OECD 109 Mesosulfuron-methyl; technical (95.7 % w/w)

Table 1.3-1: Summary of physico - chemical properties continued

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	$3.5 * 10^{-12}$ Pa at 20°C; $1.1 * 10^{-11}$ Pa at 25°C; $3.3 * 10^{-11}$ Pa at 30°C.	M-141806-01-1, 1996	Measured EEC A.4 OECD 104 Mesosulfuron-methyl; pure (98.1 % w/w)
Surface tension	61.9 mN/m at 20 °C (19.27 mg/L in distilled water).	M-198739-01-1, 2000	Measured 84/449/EWG, A.5. Mesosulfuron-methyl; pure (98.7% w/w)
Explosive properties	No danger of explosion according to the explosive properties in the sense of Guideline test A.14.	M-198629-01-1, 2000	Measured EU 92/69 A.14 Mesosulfuron-methyl; technical (95.7% w/w)
Self-ignition temperature	Upon increasing the temperature in a test oven, a slight endothermal effect is observed at about 150°C, probably due to melting. No self-ignition occurs up to 401°C.	M-142717-01-1, 1997	Measured EU 92/69 A.16 Mesosulfuron-methyl; technical (95.7% w/w)
Water solubility	Solubility in water determined at 20°C (mg/L): 2.15 (pH 4) 7.24 (pH 5) 483 (pH 7) 15390 (pH 9) 13800 (pH 10) In deionised water without buffer, the test substance was soluble to 21.4 mg/L at a pH of 5.66.	M-141705-01-1, 1996	Measured EEC A.6 OECD 105 Mesosulfuron-methyl; pure (98.1% w/w)
Partition coefficient n-octanol/water	log P_{OW} at 25°C: 1.90 (pH 4) 1.39 (pH 5) -0.48 (pH 7) -2.06 (pH 9) -2.10 (pH 10)	M-142043-02-1, 1996, 1997 (amendment)	Measured EEC A.8 OECD 107 Mesosulfuron-methyl; pure (98.1% w/w)
Flash point	Not required, as melting point > 40°C		-

Table 1.3-1: Summary of physico - chemical properties continued

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	The test substance could be ignited, but the flame went out without propagation within 10 - 60 seconds. The test item is considered to be not flammable in the sense of Guideline test A.10.	M-142716-01-1, 1997	Measured EU 92/69 A.10 Mesosulfuron-methyl; technical (94.6% w/w)
Oxidising properties	No oxidizing properties in the sense of Guideline test A.17.	M-198635-01-1, 2000	Measured 84/449/EWG, A.17 Mesosulfuron-methyl; technical (95.7% w/w)
Granulometry	Not available.		
Stability in organic solvents and identity of relevant degradation products	Not available.		
Dissociation constant	At 20°C, the pKa value was found to be 4.35 ± 0.04.	M-143499-02-1, 1996 M-141806-01-1,1997 (Amendment)	Measured OECD 112 Mesosulfuron-methyl; pure (98.1% w/w)
Viscosity	Not applicable		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Mesosulfuron-methyl is used as a sulfonylurea herbicide for post-emergence use in cereals (soft and durum wheat, triticale).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of Mesosulfuron-methyl were assessed in the Draft Assessment Report prepared in the context of the inclusion of Mesosulfuron in Annex I of Council Directive 91/414/EEC

(Draft Assessment Report, December 2001, volume 3, B2 and subsequent addenda, RMS France) concerning the placing of plant protection products on the market.

Mesosulfuron-methyl is not flammable and not self-ignitable. The substance has no oxidizing or explosive properties. Therefore, no classification for physico-chemical properties is proposed.

Table 3-1: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Based on data mentioned in the Table 1.1-1, no classification is proposed for Mesosulfuron regarding physico-chemical hazards according to CLP criteria.			

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of Mesosulfuron-methyl were assessed in the Draft Assessment Report and the Addendum to the Draft Assessment Report prepared in the context of the inclusion of Mesosulfuron in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2001 and subsequent addenda, RMS France) concerning the placing of plant protection products on the market.

The results included in this proposal are taken from the DAR (and its addenda and assessment reports when these contain updated information) and the DRAR (draft Renewal Assessment Report) prepared in 2015 by France in the context of the approval renewal of the active substance under Regulation (EC) No1107/2009.

For several toxicological studies, the synonym AE F130060 is used for Mesosulfuron-methyl.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The absorption, distribution, metabolism and excretion, including plasma and blood pharmacokinetics, of Mesosulfuron-methyl (AE F130060) was investigated in the Wistar rat following single oral gavage (10 or 1 000 mg/kg bw) and repeated oral administration (up to 7 daily oral doses of 250 mg/kg bw/d).

Following a single oral low dose (10 mg/kg bw), Mesosulfuron-methyl (AE F130060) is moderately absorbed with a 23% absorption rate, 13% of the dose being recovered in the urine, 8% in the bile and < 2% in cage wash and carcass; following a single oral high dose (1000 mg/kg bw) absorption is low (ca.3%), indicating a non-linear absorption profile. Mesosulfuron-methyl (AE F130060) is rapidly excreted (ca. 95% found in the 0-24 h excreta), mainly in the faeces, with unchanged Mesosulfuron-methyl (AE F130060) being the main excretion product (>68% and >81% for the low and the high dose, respectively). There were no major sex-specific differences in the route of excretion and no exhalation of radiolabelled carbon dioxide during the first 24 hours post-dosing of 1000 mg/kg bw/d. Repeated dosing at 250 mg/kg bw for 7 days had no significant effect on the excretion profile and more than 93% of the totally administered radioactivity were found in rat faeces.

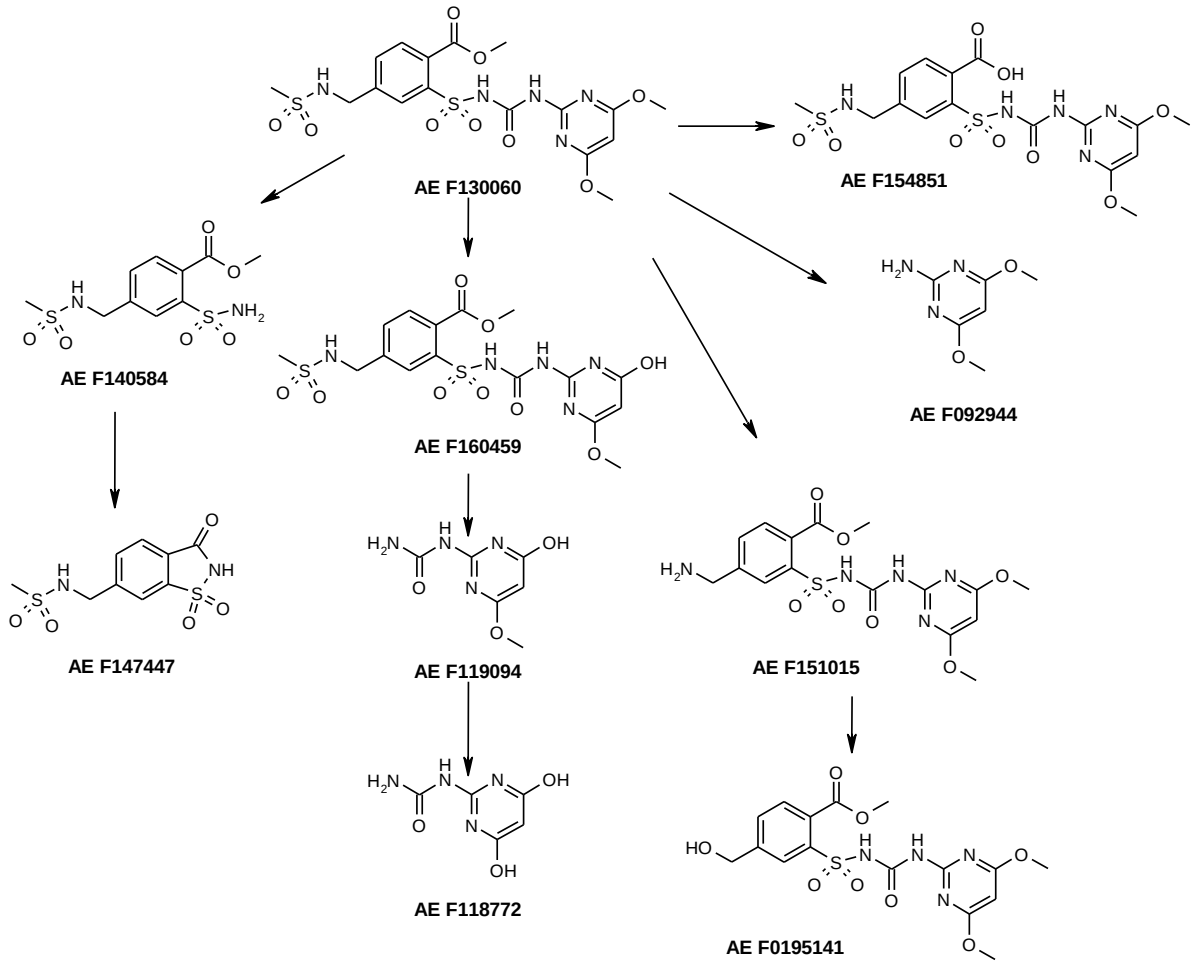
Pharmacokinetics studies indicated rapid absorption with maximum concentration of radioactivity in whole blood and plasma (Cmax) at 2-4 hour post-dosing for males and females respectively in the

10 mg/kg dose group, and 4 hours after dosing for the 1000 mg/kg dose group. Monophasic plasma elimination half-lives ($T_{1/2}$) of ~11 hours for males and ~8 hours for females reflected the rapid clearance of the compound in both sexes. Tissue residues were generally low in rats 72 hours post-dosing with several tissues containing residues below the limit of quantification at both dose levels. Following a single oral dose of 10 mg/kg body weight, only traces of radioactivity were detected in the organs and tissues. In the males, only liver (mean 0.17 µg equivalents/g) and plasma (mean 0.30 µg equivalents/g) showed radioactivity. In the females, the mean values in organs and tissues were below the limit of quantification. At the high-dose level (1000 mg/kg body weight) all tissue residue levels were below 0.04 µg equivalents/g tissue. Repeated dosing (7 x 250 mg/kg bw/d) resulted in an early steady state of residues in organs and tissues with rapid biphasic elimination.

The metabolism of Mesosulfuron-methyl (AE F130060) showed that at both dose rates the main excretion product was unchanged Mesosulfuron-methyl (AE F130060) excreted mainly in the faeces. The major metabolic pathway was the breakdown of the sulfonylurea-bridge leading to 2-amino-4,6-dihydroxypyrimidine (AE F092944) and methyl-4-methanesulfonamidomethyl-2-sulfamoyl-benzoate (AE F140584) which cyclized to (6-methane-sulfonamidomethyl-1,2-benzisothiazol-3(2H)-on-1,1-dioxid (AE F147447). Minor metabolic reactions observed were the O-demethylation at the pyrimidine moiety leading to methyl 2-[3-(4-hydroxy-6-methoxypyrimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate (AE F160459) and the cleavage of the methanesulfonamidomethyl side chain leading to methyl-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-aminomethylbenzoate (AE F151015) and 2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethyl-benzoic acid (AE 0195141). A breakdown of the sulfonylurea-bridge of AE F160459 leads to AE F119094 which is further metabolised by O-demethylation to AE F118772. The formation of the benzoic acid metabolite AE F154851 due to hydrolysis of the methyl ester by esterases was detected to be a minor metabolic reaction in rats.

The metabolic profile of mesosulfuron-methyl in the rat is shown in Figure 1.

Figure 1: Metabolic profile of mesosulfuron-methyl in rats



4.1.2 Human information

No data available

4.1.3 Summary and discussion on toxicokinetics

In the rat, Mesosulfuron-methyl (AE F130060) is moderately to poorly absorbed and rapidly excreted within the first 24 hours following oral administration. There is no significant sex-related differences regarding absorption, distribution, route of excretion, or in the excretion profile of the phenyl or in the pyrimidyl-labelled test substance. There was no evidence for elimination via expired volatiles. The metabolism of ^{14}C- is low without sex-related or dose-related differences in the metabolite pattern; excretion is primarily as unchanged parent which is mainly found in the faeces with small amounts of one of the sulfonyl-urea-bridge breakdown products (AE F 140584).

4.2 Acute toxicity

Table 4.2-1: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral Groups of 10 (5/sex) Wistar rats, 7-8w old Mesosulfuron-methyl (AE F130060) technical (purity: 95.6% suspended in deionised water) Dose levels: 2000 or 5000 mg/kg bw OECD 401, gavage; 1987	LD_{50} > 5000 mg/kg bw for both sexes	No deaths occurred. For both sexes, clinical signs were observed during 10 min and persisted in some cases up to 6h after administration and comprised decreased spontaneous activity, squatting posture, stilted gait and also irregular respiration, uncoordinated gait and increased fright reaction. There was no impairment of body weight gains, nor gross pathological changes at necropsy that were attributed to administration of the test material.	M-140405-01-1; 1996 (Ehling G., 1996a)

Inhalation One group of 10 (5/sex) Sprague-Dawley rats, 8-10 w old Fine dust aerosol of Mesosulfuron-methyl (AE F130060) technical (94.6% purity) Dose level: 1.33 mg/L air (gravimetrical analysis highest achievable; concentration); 4-h (nose only) exposure OECD 403; 1981	LD_{50} > 1.33 mg/L for both sexes.	Mean analytical exposure concentration determined to be 1.33 mg/L with a nominal concentration of 0.76-1.09 mg/L; it was considered to be the maximum attainable concentration with the test substance. The mass median aerodynamic diameter (MMAD) was 3.57-3.62 µm with a geometric standard deviation (GSD) of 1.85-1.97; 2.4% of the particles were < 1µm in size, and 56.6% < 4 µm. No deaths occurred during the 14-d study period. During exposure, rats exhibited irregular respiration; no clinical signs of toxicity nor body weight impairment were recorded until study termination. Gross pathological examination did not reveal any macroscopic change.	M-186735-02-1; 1999 (Hofmann Th.,1999)
Dermal One group of 10 (5/sex) Wistar rats, 7-8 w old Mesosulfuron-methyl (AE F130060) technical (purity: 95.6% moistened with deionised water) Limit Dose: 5000 mg/kg bw; 24-h (occlusive) exposure OECD 402; 1987	LD_{50} > 5000 mg/kg bw for both sexes.	No deaths occurred and there were no clinical signs of toxicity, nor impairment of the bw gains, nor gross pathological changes at necropsy.	M-140406-01-1; 1996 (Ehling G., 1996b)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Groups of 10 Wistar rats (5/sex), 7-8 weeks old, were given, by oral gavage, a single dose of 2000 or 5000 mg/kg bw of AE F130060 (95.6% purity) suspended in deionised water (dose volume of 20 ml/kg bw). Overt signs of toxicity and mortality were recorded daily for the 14 day test period. Individual body weights were recorded prior to dosing, on day 7 post dosing and at termination (day 14), prior to sacrifice and necropsy, which was performed on all animals with gross examination of abdominal and thoracic cavities. No deaths occurred during the study period. For both sexes, clinical signs were observed from 10 min and persisted in some cases up to 6h after administration, comprised decreased spontaneous activity, squatting posture, stilted gait and also irregular respiration, uncoordinated gait and increased fright reaction. There was no impairment of body weight gains and no adverse gross pathological changes at necropsy where observed which were attributed to administration of the test material. The oral LD_{50} of AE F130060 was > 5000 mg/kg bw for both sexes of Wistar rats.

4.2.1.2 Acute toxicity: inhalation

Ten (5/sex) 8-10 week old Sprague-Dawley rats [Harlan Winkelmann strain] were exposed "nose-only" for 4 h to a fine dust aerosol of AE F130060 (94.6% purity) which was generated at the highest technically administrable concentration of 1.33 mg/l air (agglomeration of particles was prevented by earthing the exposure chamber). Gravimetric analysis of the dust concentration in the breathing zone was performed at regular intervals; chemical analytical determination of the active substance was performed using HPLC and aerosol particle size distribution was carried out using a cascade impactor. Clinical observations were conducted on all rats at hourly intervals during the exposure, 1 hour after termination of exposure and once daily thereafter until study termination on day15. Individual body weights were determined prior to exposure and on day 7 & day 14 after the exposure period. Necropsies were performed on all rats at termination for macroscopic examination.

The mean analytical exposure concentration of AE F130060 technical was determined to be 1.33 mg/L with a nominal concentration of 0.76-1.09 mg/L; this was considered to be the maximum attainable concentration with the test substance. The MMAD was 3.57-3.62 μm with a gsd of 1.85-1.97; 2.4% of the particles were < 1μm in size, and 56.6% < 4 μm.

No deaths occurred during the study period. During exposure, rats exhibited irregular respiration. No clinical signs of toxicity or body weight impairment were recorded up to study termination. Gross pathological examination did not reveal any macroscopic change. The LC_{50} was > 1.33 mg/L (analytical) for both sexes.

4.2.1.3 Acute toxicity: dermal

Ten (5/sex) 7-8 week old Wistar rats received a topical application of 5000 mg/kg bw of AE F 130060 (95.6% purity; moistened with deionised water) to the shaved dorsal skin, under occlusive bandage for a 24-hour exposure period, after which the skin was washed with warm water. Clinical observation (local signs and systemic toxicity) were performed during the 14 day study period; individual body weights were recorded on the day of dosing, at day 7 and at termination; necropsies were performed on all rats. No deaths occurred and there were no clinical signs of toxicity observed. Body weight gain was not and there were no gross pathological changes observed at necropsy. The dermal LD50 was > 5000 mg/kg bw for both sexes.

4.2.1.4 Acute toxicity: other routes

No information provided

4.2.2 Human information

No information is available.

4.2.3 Summary and discussion of acute toxicity

Mesosulfuron-methyl (AE F130060) exhibited low acute toxicity to rats via all routes of exposure (oral, percutaneous administration, inhalation exposure). The rat acute oral and dermal LD_{50} were

>5 000 mg/kg body weight. The rat acute inhalation LC_{50} (4-hour) was > 1.33 mg/L air, which was the highest achievable concentration.

4.2.4 Comparison with criteria

Mesosulfuron-methyl does not meet the criteria for classification for acute oral or dermal toxicity according to CLP regulations.

For inhalation toxicity, the LC_{50} of 1.33 mg/L is lower than the cut-off. However classification is not considered to be warranted as this was the maximum attainable concentration and due to the low toxicity observed in this study (no mortality or body weight effect; transient clinical signs during exposure only; no macroscopic changes). Therefore, it is proposed not to classify Mesosulfuron-methyl for acute inhalation toxicity.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity via oral, inhalation or dermal routes is warranted based on results of the acute toxicity studies and the CLP criteria.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

One oral, one dermal and one inhalation acute toxicity study, all in rats, are were summarised in the CLH report.

After oral exposure of 10 Wistar rats (5 / sex) to 2,000 and 5,000 mg/kg bw, no deaths occurred and no effects on weight gain or gross pathological findings were seen. For both sexes, clinical signs were observed from 10 min and persisted in some cases up to 6 h after administration and comprised of decreased spontaneous activity, squatting posture, stilted gait and also irregular respiration, uncoordinated gait and increased fright reaction. The oral LD_{50} was > 5,000 mg/kg bw for both sexes.

No deaths also occurred after dermal exposure to 5,000 mg/kg bw (Wistar rat, 5 / sex, occlusive) and there were no effects on weight gain or gross pathological findings and there were no clinical signs of toxicity. The dermal LD_{50} was > 5,000 mg/kg bw for both sexes.

In the acute inhalation toxicity study, no deaths occurred among 10 Sprague-Dawley rats (5 / sex) exposed for 4 h to the highest technical achievable concentration of 1.33 mg/L (nominal concentration of 0.76 – 1.09 mg/L). During exposure, rats exhibited irregular respiration. No clinical signs of toxicity or body weight impairment were recorded up to the study termination. Gross pathological examination did not reveal any macroscopic changes. The LC_{50} was > 1.33 mg/L for both sexes.

The Dossier Submitter (DS) therefore proposed no classification for acute toxicity.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

The substance induced no mortalities after oral or dermal exposure at or above the doses defining the categories for classification or inhalation exposure at concentrations at the highest technically achievable concentration. RAC thus agrees with the DS that **no classification for acute toxicity is warranted for any of the exposure routes**.

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

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

There was no evidence from available single exposure studies of any specific toxic effects on any target organ or tissue. Clinical signs of toxicity were observed after single exposures to Mesosulfuron-methyl but were transient in nature and are considered to be unspecific signs of general acute toxicity (refer to section 4.2). There are no specific reports for human data to provide additional information on this end point. Classification as STOT SE under Regulation CLP is not warranted.

4.3.2 Comparison with criteria

Substances that have produced significant non-lethal toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following single exposure are classified as STOT SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a constant and identifiable effect.

Classification in STOT SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract infection.

The signs that were apparent after single oral and inhalation exposure (no adverse effects were observed after dermal exposure) to Mesosulfuron-methyl were indicative of non-specific, general acute toxicity. As there was no clear evidence of specific effects on a target organ or tissue that were independent of mortalities, no definitive signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) under CLP is required.

4.3.3 Conclusions on classification and labelling

Based on the results of the studies performed, no classification as STOT SE is proposed.

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

Summary of the Dossier Submitter's proposal

No toxicity to a specific organ was observed in acute oral, inhalation or dermal toxicity studies in animals. Clinical signs of toxicity were observed after single oral exposure to mesosulfuron-methyl, but these were transient in nature and considered to be unspecific signs of general acute toxicity (see section on acute toxicity). Additionally, no acute organ toxicity was observed in short-term nor long-term studies.

The Dossier Submitter (DS) therefore proposed no classification for specific target organ toxicity after repeated exposure (STOT-SE).

Comments received during public consultation

In the only comment received addressing this endpoint, one Member State supported the proposal for no classification.

Assessment and comparison with the classification criteria

No effects that could lead to classification as STOT SE were reported. RAC thus agrees with the DS that **no classification for STOT SE is warranted**.

4.4 Irritation

4.4.1 Skin irritation

Table 4.4.1-1: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
3 female New Zealand White rabbits (3-5 months old) 500 mg Mesosulfuron-methyl (AE F130060) technical (purity: 95.6% moistened with deionised water); 4-h exposure; applied using a 2.5 cm^2 cellulose patch under semi-occlusive bandage OECD 404; 1992	**Non irritating**	No deaths and no signs of toxicity occurred during the 3-d observation period. The mean Draize scores for erythema and oedema were 0.0 at all intervals (0.5, 1, 24, 48 and 72 hours).	M-140524-01-1; 1996 (Hammerl R., 1996 d)

4.4.1.1 Non-human information

500 mg of AE F130060 (95.6% purity; moistened with deionised water) was applied to a 2.5 cm^2 cellulose patch which was fixed to the shaved dorsal skin of 3 female New Zealand White rabbits under semi-occlusive bandage for a 4 hour exposure period, after which test sites were cleaned with warm tap water. The applications sites were examined at 0.5; 1; 24; 48 and 72 hours post patch removal and scored for erythema and oedema using the Draize scale. No deaths or overt signs of toxicity occurred during the 3 day observation period. The mean Draize scores for erythema and edema were 0.0 at all intervals. The overall mean erythema and oedema scores from the 1-, 24-, 48- and 72-hour observations were 0.0. AE F130060 should be classified as non-irritating to rabbit skin.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

No signs of erythema, oedema or any other signs of skin irritation were observed in a rabbit skin irritation study. There is no data in humans to suggest that Mesosulfuron-methyl has a potential to cause skin irritation.

4.4.1.4 Comparison with criteria

Mesosulfuron-methyl did not cause skin irritation in a rabbit study and no human data is available to suggest that Mesosulfuron-methyl has a potential to cause skin irritation. Therefore, Mesosulfuron-methyl does not meet the criteria for classification according to CLP regulation.

4.4.1.5 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of mesosulfuron-methyl was tested in a standard guideline compliant study (OECD TG 404) in rabbits using the Draize scores for erythema and oedema formation. The overall mean erythema and oedema scores from the 1-, 24-, 48-, and 72-h observations were in all cases 0. No signs of toxicity occurred during the 3-day observation period. Furthermore, there were no human data demonstrating skin irritation potential.

The DS proposed no classification for this endpoint.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

In the reported skin irritation study, there were no indications of irritation or corrosion. RAC thus agrees with the DS that **no classification for skin corrosion/irritation is warranted**.

4.4.2 Eye irritation

Table 4.4.2-1: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
3 female New Zealand White rabbits (3-5 months old) Mesosulfuron-methyl (AE F130060) technical (purity: 95.6%); 0.1 g moistened with deionised water; eyes rinsed after 24 hr. examination point 1, 24, 48, 72 hr after exposure OECD 405; 1987	Slightly irritating	1 h up to 24h following instillation of the test substance, all 3 rabbits exhibited evident hyperaemia of the blood vessels up to a diffuse, deeper crimson reddening; 1h after application, swelling with partial everision of the lids and a clear colourless discharge were observed. All signs of irritation had disappeared after 2 days. No overt signs of toxicity occurred during the study period. Ocular scores (mean after 24, 78 and 72 hours) for all three tested rabbits: Conjuctival redness: 0.00, 0.66 and 0.33 Conjunctival chemosis: 0, 0 and 0 Corneal opacity: 0, 0, 0 Iris: 0, 0, 0 No other ocular findings which would trigger classification.	M-140517-01-1; 1996 (Hammerl R., 1996 c)

4.4.2.1 Non-human information

AE F130060 (95.6% purity; 100 mg moistened with deionised water) was instilled into the conjunctival sac of the left eye of each of 3 female New Zealand White rabbits, with the untreated eye serving as control in each case. After a 24-hour exposure period, treated eyes were rinsed with isotonic saline and examined after 1; 24; 48; 72 hours post exposure (corneal lesions were examined with the aid of UV light and fluorescein solution at 24 and 72 hours). At 1 hour and up to 24 hours post-instillation, evidence of hyperaemia of the blood vessels up to a diffuse, deeper crimson reddening was observed in all 3 rabbits; at 1 hour post-instillation swelling with partial everision of the lids and a clear colourless discharge were observed. All signs of irritation had disappeared after 2 days. No overt signs of clinical toxicity occurred during the study period. The overall mean scores from the 24-, 48- and 72-hour observations were 0, 0 and 0.33 for corneal opacity, iris and conjunctival redness, respectively; according the current EU guideline, AE F130060 should be classified as non-irritating to the rabbit eye.

4.4.2.2 Human information

No information available.

4.4.2.3 Summary and discussion of eye irritation

Mesosulfuron-methyl was slightly irritating to the rabbit eye. No human data is available.

4.4.2.4 Comparison with criteria

The slight ocular irritation seen in the available *in vivo* eye irritation study does not meet CLP classification criteria.

4.4.2.5 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of mesosulfuron-methyl was tested in a standard guideline compliant study (OECD TG 405). Mesosulforon-methyl was applied to the eye of three female rabbits. After 24-h exposure period, treated eyes were rinsed and examined after 1-, 24-, 48- and 72-h post exposure. At 1 h up to 24 h post installation, evidence of hyperaemia of the blood vessels up to a diffuse deeper crimson reddening was observed. Furthermore, at 1 h post-installation swelling with partial eversion of the lids and a clear colourless discharge were observed. The irritation signs disappeared after two days and no signs of other signs of clinical toxicity occurred during the study.

According to the DS the overall mean scores for the 24-, 48-, and 72-h observations were 0, 0 and 0.33 for corneal opacity, iris and conjunctival redness, respectively. The DS concluded that mesosulfuron-methyl was slightly irritating to the rabbit eye. However, these slight effects seen in the *in vivo* eye irritation study did not meet the CLP classification criteria.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

According to the CLP criteria, a substance needs to be classified as "Irritating to eyes" (Category 2) if a substance produces, in at least 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 (chemosis) ≥ 2, calculated as mean scores, following grading at 24, 48 and 72 h after installation of the test material and which fully reverses after 21 days.

The overall mean scores from the 24-, 48- and 72-h observations were 0, 0 and 0.33 for corneal opacity, iris and conjunctival redness, respectively. Thus, the criteria for

classification were not met. RAC thus agrees with the DS that **no classification for eye corrosion/irritation is warranted**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available. In the acute toxicology study via the inhalation route (section 4.2), no specific signs of respiratory tract irritation was observed and the clinical signs of toxicity observed were considered as common observations during acute inhalation studies and do not indicate a potential for Mesosulfuron-methyl to cause respiratory tract irritation.

4.4.3.2 Human information

No information available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No studies which specifically investigate respiratory tract irritation are available. However, the available acute inhalation study does not suggest that Mesosulfuron-methyl is a respiratory tract irritant. No human data is available.

4.4.3.4 Comparison with criteria

CLP criteria for classification are not met.

4.4.3.5 Conclusions on classification and labelling

No classification is proposed for this endpoint.

4.5 Corrosivity

Table 4.5-1: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
In vivo rabbit skin irritation study OECD 404; 1992	Not corrosive.	No skin irritation was observed.	M-140524-01-1; 1996 (Hammerl R.; 1996d)
In vivo rabbit eye irritation study OECD 405; 1987	Not corrosive.	Slight irritation only. No evidence of corrosivity.	M-140517-01-1; 1996 (Hammerl R.; 1996c)

See section 4.4 for further study details.

4.5.1.1 Non-human information

Mesosulfuron-methyl showed no potential to cause corrosivity in the available *in vivo* rabbit skin and eye irritation studies (see section 4.4 for full study details).

4.5.1.2 Human information

No information available.

4.5.1.3 Summary and discussion of corrosivity

Mesosulfuron-methyl showed no potential to cause corrosivity in the available in vivo rabbit skin and eye irritation studies. No human data available.

4.5.1.4 Comparison with criteria

Mesosulfuron-methyl does not meet the criteria to be classified as corrosive to the skin or eyes.

4.5.1.5 Conclusions on classification and labelling

No classification is proposed for this endpoint.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 4.6.1-1: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Magnusson & Kligman method / 20 treated and 10 control female White guinea pigs Mesosulfuron-methyl (AE F130060) technical (purity: 94.6% diluted) Induction Intradermal injections: 5 % w/v test substance in deionised water and in 50% FCA Topical induction: 25% w/w in deionised water Challenge Topical induction: 25% w/w in deionised water OECD 406; 1992	**Non sensitizing:** 0% incidence of animals in the test group exhibiting sensitization reactions at challenge.	No clinical signs of toxicity nor body weight impairment were observed throughout the study period. No signs of irritation were observed after challenge with test substance (25%). Positive response in 50% of animals was observed in a concurrently performed control assay with benzocaine.	M-148033-01-1; 1998 (Hammerl R., 1998d)

4.6.1.1 Non-human information

In a Magnusson and Kligman guinea pig maximization test, 20 female Pirbright White guinea pigs were treated with intradermal injections of 0.1 ml AE F 130060 (94.6% purity) at 5% in deionized water and 50% Freuds Complete Adjuvant (FCA), by topical induction (0.5 ml) at 25% in deionized water, and

challenged with 25% in deionized water. Concentrations for induction and challenge were determined in pre-screening range finding studies. A further 10 animals were used as a control group.

No clinical signs of toxicity or evidence of body weight gain impairment were observed throughout the study period. Skin reactions were observed at FCA alone and FCA + test material injection sites (severe erythema and oedema, but also indurations, encrustations and necrosis) but not at the test material alone intradermal injection sites. No signs of irritation were observed after challenge with test substance (25%). Positive response in 50% (5/10) of animals was observed in a concurrently performed control assay with benzocaine. Based on the 0% incidence of animals in the test group exhibiting sensitization reactions at challenge, AE F 130060 technical can be classified as a non-sensitizer according to EC criteria, which specify that at least 30% of the test animals show a sensitization response in the Magnusson and Kligman assay to be considered a sensitizer.

4.6.1.2 Human information

No dermal allergic reactions have been detected during the handling of Mesosulfuron-methyl to date.

4.6.1.3 Summary and discussion of skin sensitisation

An *in vivo* Magnusson & Kligman skin sensitisation study in the guinea pig was negative for skin sensitisation. No dermal allergic reactions have been detected during the handling of Mesosulfuron-methyl to date.

4.6.1.4 Comparison with criteria

Mesosulfuron-methyl does not meet the criteria to be classified as a skin sensitiser.

4.6.1.5 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of skin sensitisation
Summary of the Dossier Submitter's proposal An *in vivo* Magnusson & Kligman (M&K) skin sensitisation study in guinea pigs, conducted according to OECD TG 406, was negative for skin sensitisation. There were no reports that mesosulfuron-methyl caused dermal allergic reactions in humans after dermal contact. The DS concluded that mesosulfuron-methyl did not meet the CLP criteria for classification as a skin sensitiser.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

In a M&K test, 20 female guinea pigs were treated with intradermal injections of 0.1 mL mesosulfuron-methyl at 5% in deionized water and 50% Freund's Complete Adjuvant (FCA), by topical induction (0.5 mL) at 25% in deionised water, and challenged with 25% in deionised water. No signs of toxicity were observed throughout the study and no sensitisation symptoms were observed.

According to the DS, the concentrations for induction and challenge were determined in pre-screening range finding studies. As stated in the OECD TG 406, in the guinea pig maximization test method, "the concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose." Based on the presented data it cannot be determined whether appropriate dosages were applied.

RAC takes into consideration that no explicit evaluation of the applied dose could be made since no details of the dose range finding tests were provided.

In conclusion, since no signs of sensitisation were detected in the M&K test, the criteria for classification were not met and **no classification for skin sensitisation is warranted**.

Supplemental information - In depth analyses by RAC

ECHA was informed by the European Food Safety Authority (EFSA), that in the course of the reviewing process, ESFA could not conclude on the skin sensitisation potential of mesosulfuron-methyl on the following basis:

Two M&K tests, one of them presented in the CLH dossier and the second one submitted after EFSA's request, were carried out. Both tests were negative.

In the study *confidential* (1998) and also in the second test (*confidential*, 2003), which was submitted after a request, the intra-dermal induction concentration of 5% w/v, which caused slight to well defined erythema and light oedema was used. The topical induction/challenge of 25% w/w (*confidential*, 1998) and 50% (*confidential*, 2003) challenge concentration were considered, however, too low as no irritation was obtained in the preliminary irritation tests.

Furthermore, a QSAR analysis (DEREK Nexus 4.0.5, Nexus 1.7.5) revealed an equivocal alert for skin sensitisation potential in mammals (Alert 437, activated N-heterocycle).

EFSA concluded that, although no positive response was obtained in the two M&K tests, since the two assays were not conducted at concentrations of the active substance as

recommended by OECD guideline and taking into account the results of the QSAR analysis, no clear conclusion could be drawn on the sensitising potential of mesosulfuron-methyl.

In the present CLH report, the study of Hammerl *et al.* (1998) is presented and it is mentioned that the concentration for induction (induction: intradermal injection, 5% w/v and topical induction, 25% w/v, challenge: topical induction, 25% w/v) and challenge were determined in pre-screening studies. No further details regarding range finding studies were given.

According to the OECD TG 406, in the guinea pig maximization test method, "the concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose.

Based on EFSA's information, the concentrations used in the range-finding studies did not induce any irritation signals, thus the applied doses were considered as too low.

Although there were no positive responses in M&K tests, RAC takes into account that there are still uncertainties on the sensitising properties of mesosulfuron-methyl since the dosage applied deviated from the instructions of the guideline (OECD TG 406).

4.6.2 Respiratory sensitisation

Table 4.6.2-1: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data available			

4.6.2.1 Non-human information

There are no studies available that specifically investigate respiratory sensitisation.

4.6.2.2 Human information

No information available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No human or animal data are available.

4.6.2.4 Comparison with criteria

No comparison with criteria can be made as appropriate data is not available.

4.6.3 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No studies were available which specifically investigated respiratory sensitisation and no human data were present. Thus, no comparison with the criteria could be made. The DS therefore proposed no classification for this endpoint.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

RAC concludes that no comparison with the classification criteria can be made since there were no human or animal data available and therefore **no classification for respiratory sensitisation** is warranted.

4.7 Repeated dose toxicity

Mesosulfuron-methyl has been studied extensively in standard GLP/OECD- compliant studies involving repeated oral treatments of rats and mice for 90 days, and for up to 1 year in dogs. Due to low intrinsic toxicity observed in studies reported elsewhere in this document, repeat dose studies via the dermal and inhalation routes have been deemed unnecessary (see sections 4.7.1.2 and 4.7.1.3 for full justifications.

Table 4.7-1: Summary table of repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral 90-day study in rat, dietary administration + 4 weeks recovery Groups of 20 (10/sex) Wistar rats Mesosulfuron-methyl (AE F130060) technical (purity: 96.0%) Dose levels: 0; 240; 1200; 6 000 and 12 000 ppm OECD 408; 1981	NOAEL =12 000 ppm, the highest concentration tested, which was equivalent to an average daily intake of: • 907.5 mg/kg bw/d in males. • 976.5 mg/kg bw/d in females.	Minor changes in some haematological and biochemical parameters considered as non-adverse effects of test substance.	M-187497-01-1; 1999 (Hammerl R.; 1999c)
Oral 90-day study in mice, dietary administration Groups of 20 (10/sex) CD-1 mice Mesosulfuron-methyl (AE F130060) technical (purity: 96.0%) Dose level: 0; 140; 1 000 and 7000 ppm OECD 408; 1981	NOAEL = 7000ppm, the highest concentration tested, which was equivalent to approximately: • 1238 mg/kg bw/d in males. • 1603 mg/kg bw/d in females.	Statistically significant decreased leukocyte counts (in males at 1000 ppm and 2 sexes at 7000 ppm) exhibited some dose-dependency, so that a possible treatment effect was suggested. However, this was disregarded since no histopathological or clinical corroborates were seen. Moreover, mouse leukocyte counts were decreased in the 90-day study and increased in the 18-month study, so that they were not considered at toxicologically relevant. The slight reduction of liver weights (absolute and relative) in the top dose males only was also an isolated finding without histopathological corroborate.	M-194489-01-1; 1999 (Hammerl,R.,1999b)
28-day study in dog, with dietary administration Groups of 4 (2/sex) Beagle dogs (6 months old) Mesosulfuron-methyl (AE F130060) technical (purity: 94.4%) Dose levels: 0; 400; 2000; 10 000 and 20 000 ppm OECD 409; 1981	The NOAEL for this study was 20000 ppm, the highest concentration tested, which was equivalent to an average daily intake of: • 775.9 mg/kg bw/d in males • 804.5 mg/kg bw/d in females	No adverse effects were seen in this study.	M-142958-01-1; 1997 (Stammberger,I.,1997a)

Table 4.7-1: Summary table of repeated dose toxicity studies continued

Method	Results	Remarks	Reference
Oral 90-day study in dog, with dietary administration Groups of 8 (4/sex) Beagle dogs (6 months old) Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) Dose levels: 0; 2000; 10 000 and 20 000 ppm OECD 409; 1981	NOAEL = 20 000 ppm, the highest concentration tested, which was equivalent to an average daily intake of: • 648 mg/kg bw/d in males. • 734 mg/kg bw/d in females.	The minor changes in some biochemical parameters considered as non-adverse effects of test substance.	M-198012-02-1; 2000 (Stammberger,I.,2000a)
Oral 1-year study in dog, with dietary administration Groups of 12 (6/sex) Beagle dogs (6-7 months old) Mesosulfuron-methyl (AE F130060) technical (purity: 95.3 – 95.7%) Dose levels: 0; 400; 4000 and 16000 ppm OECD 452; 1981	NOAEL= 16 000 ppm, which was equivalent to an average daily intake of: • 574 mg/kg bw/d in males. • 646 mg/kg bw/d in females. Local NOAEL = 4000 ppm which was equivalent to an average daily intake of: • 155 mg/kg bw/d in males.	No adverse treatment related effects were seen in the general health status, body weight gain or food consumption, haematological and biochemical parameters up to the highest dose tested. There were no substance-related changes on organ weights or any other systemic macroscopic and microscopic findings after terminal kill. Local findings in the stomach mucosa were observed in 3/6 males at 16 000 ppm.	M-198511-01-1; 2000 (Mallyon,B.,2000a)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rodent Studies

There are two studies in rodents; 1 in rats and 1 in mice, both for 90 day duration.

Groups of 20 (10/sex) Wistar rats (Hoe:Wisk strain; 4-6 w old) were administered dietary concentrations of 0; 240; 1200; 6 000 and 12 000 ppm of AE F130060 technical (96.0% purity) daily for 13 consecutive weeks; in the control and top dose groups, 10 additional rats/sex were kept on control diet for a further 4 weeks to examine the reversibility of potential effects (M-187497-01-1; 1999). The animals were observed twice daily for signs of toxicity, morbidity and mortality. Detailed clinical observations, including examination of eyes, teeth, oral mucosa and neurological status, individual body weights and food consumption were recorded weekly. Ophthalmological examinations were performed on all animals

at initiation and at termination of the study period. At termination or/and at end of recovery period, 10 rats/sex/group were submitted to haematological determinations and clinical chemistry determinations. Urinalysis determinations were performed on all rats. Gross-pathological examination were performed and selected organs were weighed (heart, liver, lung, kidney, spleen, brain, pituitary, thyroid gland/parathyroids, adrenals, testes, ovaries); other organs and tissues being preserved in formaldehyde. Extensive histopathological examinations were performed on all tissues from all rats of the control and top dose groups but without those of the recovery groups (heart, liver, kidney, lung, brain, pituitary, thyroid, adrenals, spleen, testes, ovaries, bone marrow).

Food consumption values for treated males and females were comparable in all groups. No deaths occurred, no signs of toxicity and no ophthalmological changes were observed during the 13-week study period. Body weight and body weight gains were not affected by treatment. There were no statistically significant changes in any of the haematological (see table 4.7.1.1-1), clinical chemistry or urinary parameters for either sex at any treatment level. There was no indication of treatment-related ocular abnormalities at the termination of the study. There were no macroscopic, nor histopathological findings that could be attributed to the test material; organ weights were not affected by the treatment

Statistically significant changes in some haematological parameters were seen in the high dose group (see table 4.7.1.1-1): there was a slight decreased prothrombin time in males (but in the recovery group, values were increased in males and decreased in females) and a slight increased red blood cell count and Hct in females; this changes were not dose related and within the historical control range. In addition, changes seen in the leukocyte counts should be considered as incidental findings since the high dose group males exhibited increased values after 13 w in the main group and decreased values after 17 weeks in the recovery group.

Table 4.7.1.1-1: Selected haematological parameters after 13 weeks

		Main groups					Recovery groups	
Sex	**Parameter**	**0 ppm**	**240 ppm**	**1200 ppm**	**6000 ppm**	**12000 ppm**	**0 ppm**	**12 000 ppm**
Males	Red blood cells (10^{12}/L)	7.65	7.30	7.57	7.42	7.79	7.01	7.74
	Haemoglobin (g/L)	138	137	139	136	145	139	140
	Haematocrit	0.42	0.41	0.42	0.41	0.44	0.43	0.43
	Prothrombin time	8.72	9.06	8.42	8.63	8.34*	8.64	9.08*
	Leucocytes (10^9/L)	3.3	2.6	3.2	4.0	5.0*	3.1	2.5*
Females	Red blood cells (10^{12}/L)	6.83	6.86	6.86	6.94	7.31*	7.14	7.36
	Haemoglobin (g/L)	134	134	131	134	139	140	138
	Haematocrit	0.40	0.40	0.40	0.40	0.42*	0.42	0.42
	Prothrombin time	7.83	8.10	8.04	7.67	7.94	8.01	7.64*
	Leucocytes (10^9/L)	2.2	2.2	2.7	2.8	2.6	2.5	2.7

*Significantly different from control. Method of statistics: Wilcoxon test. Two-tailed test for the highest dose and one-tailed tests for lower doses, if all higher doses are significantly different from control ($p < 0.05$).

Statistically significant changes in some biochemical parameters were seen mainly in the males of the mid- and top dose groups (see table 4.7.1.1-2): all values were within the historical control range except

for glucose in males of the 6000 ppm group and of the 12 000 ppm recovery group (serum glucose values were generally high in all dose groups). All these changes were of doubtful biological significance since there were either seen in 1 sex, or exhibited no dose dependency, or were within the biological historical changes, or showed no consistent pattern when comparing main high dose group and high dose recovery group. Furthermore, no histopathological changes were observed to corroborate these findings.

Table 4.7.1.1-2: Selected biochemical parameters after 13 weeks

		Main group					Recovery group	
Dose (ppm)		**0**	**240**	**1200**	**6000**	**12000**	**0**	**12000**
Sex	**Parameter**							
Males	Calcium (mmol/L)	2.35	2.36	2.36	2.40*	2.42*	2.37	2.38
	Chloride (mmol/L)	104	105	103*	101*	100*	104	102
	Inorg. phosph.(mmol/L)	1.84	1.56	1.80	2.36*	2.53*	1.91	1.86
	Bilirubin total (µmol/L)	3.7	3.9	4.3*	4.5*	5.0*	4.0	3.8
	Serum glucose (mmol/L)	13.06	13.26	15.89*	18.36*	15.89*	13.24	18.09*
	Total Lipids (g/L)	4.24	4.06	4.19	3.61*	3.58*	4.47	4.25
	Albumin (g/L)	29.2	30.1	29.5	29.3	30.3*	29.6	28.5*
	ASAT (U/L)	73	71	87	74	66*	124	117
Females	Sodium (mmol/L)	142	141	141*	140*	140*	143	144
	Chloride (mmol/L)	105	105	104	104*	102*	103	105*
	Inorg. phosph. (mmol/L)	1.68	1.78	1.76	1.78	1.94	1.61	2.75*
	Uric acid (µmol/L)	118	81*	79*	74*	79*	81	151*
	Triglycerids (g/L)	0.84	0.59*	0.54*	0.71*	0.52*	0.78	0.74
	Total lipids (g/L)	3.77	3.11*	3.00*	3.11*	3.12*	3.74	3.70

* Significantly different from control ; Method of statistics: Wilcoxon test. Two-tailed test for the highest doses and one-tailed tests for lower doses, if all higher doses are significantly different from control ($p < 0.05$).

The minor changes in some haematological and biochemical parameters should be considered as non-adverse effects of test substance. The NOAEL for this study was 12 000 ppm, the highest concentration tested, which was equivalent to an average daily intake of 907.5 and 976.5 mg/kg bw/d in males and females, respectively.

In a dietary study in mice (M-194489-01-1; 1999) groups of 20 (10/sex) CD-1 mice were administered daily dietary concentrations of 0; 140; 1 000 and 7000 ppm of AE F130060 technical (96.0% purity) for 13 consecutive weeks. Clinical observations for mortality and signs of toxicity were performed at least twice daily; detailed physical examinations for neurological disturbances, impairment of dental growth and changes in oral mucosa were performed weekly. Ophthalmological examinations using slit lamp were carried out on all mice from the control and top dose groups, pre-test and study termination: body weight and food consumption were determined weekly. At study end, haematological and clinical chemistry parameters were measured on the last 5 mice/sex/group. After 90 days of treatment, all mice were sacrificed, selected organs were weighed and some were preserved. Complete gross post-mortem and extensive histopathological examinations were conducted on all animals.

No deaths occurred and no treatment related clinical signs of toxicity or ophthalmological abnormalities were observed during the 90 day study period. Body weights and body weight gain were comparable in

all groups; no dose-related effect on food consumption was seen. Absolute and relative liver weights were statistically significantly decreased in males from the top dose group (-12% and -9% compared to control group respectively, see table 4.7.1.1-3) without histo-pathological corroborate. There were no gross or microscopic findings attributed to treatment.

Haematological parameters (see table 4.7.1.1-3) did not reveal any treatment related effects except statistically significant decreased leukocyte counts in the males of the mid group and in both sexes of the top dose group. Increased levels of total bilirubin in males of the mid- and top dose groups; this change which was within historical control values and was seen in only one sex, could be considered as not treatment related. Other changes observed were statistically significant increase of total protein in males of the top dose group and statistically significant increase of potassium in females of the top dose group.

Table 4.7.1.1-3: Group mean changes in selected haematological/biochemical parameters and liver weights

Dose level	**0**		**140 ppm**		**1000 ppm**		**7000 ppm**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Leukocytes (10^9/L)	3.5	3.2	3.3	2.5	2.3*	2.6	1.9*	2.3*
Bilirubin (µmol/L)	6.2	8.3	6.6	8.4	7.4*	7.8	8.3*	8.3
Protein (g/L)	51		47		51		53*	
Potassium (mmol/L)		4.14		4.96		4.48		4.70*
Liver Weight								
Absolute	1.88	1.4	1.76	1.36	1.75	1.4	1.65*	1.46
Relative (%)	5.05	4.98	4.92	4.90	4.71	4.98	4.58*	5.11

* significance level : $p \leq 0.05$ (Wilcoxon test).

The slight but statistically significant decrease in leukocyte counts (see table 4.7.1.1-3) in males at 1000 ppm and in both sexes at 7000 ppm exhibited some dose-dependency, so that a possible treatment effect was suggested. However, this was disregarded since no histo-pathological or clinical corroborates were seen. Moreover, mouse leukocyte counts were decreased in the 90 d study and increased in the 18 months study, so that they were not considered toxicologically relevant. The slight reduction of liver weights (absolute and relative) in the top dose males only was also an isolated finding without histo-pathological corroborate. The NOAEL was 7000 ppm, the highest concentration tested, which was equivalent to approximately 1238 and 1603 mg/kg bw/d in males and females, respectively.

Dog Studies

There are three studies in dogs for 28 day, 90 day and 1 year duration, respectively.

In a 28-day study in dogs (M-142958-01-1; 1997), groups of 4 (2/sex) Beagle dogs were administered dietary concentrations of 0; 400; 2000; 10 000 and 20 000 ppm of AE F130060 (94.4% purity) for 28 consecutive days. Clinical observations and food consumption were recorded daily; body weights were determined weekly. Examination of the neurological status and ophthalmological examinations were conducted pre-test and at termination. Haematological parameters, clinical chemistry parameters and urinalysis were performed on all animals pre-test, at week 1 and terminally at week 5. At termination, all dogs were sacrificed, selected organs were weighed and histopathological examinations were conducted on selected organs from the control and the top dose groups.

No deaths occurred and no treatment-related clinical signs of toxicity were observed during the 28 day study period. No statistically significant changes were observed in food consumption and body weight gains, which were generally comparable for both sexes at all dietary concentrations to those of control animals at most measurement intervals. No changes with the initial status when testing reflexes of excitability, postural reactions and hearing tests were recorded, or in ophthalmological investigations. There was no treatment-related effect on the haematological and urinalysis parameters; no substance related significant changes in clinical chemical parameters were recorded. There was no treatment related effect on organ weight. Not dose-related increase of liver weight was observed in males and females: relative liver weight changes are -4%, +14%, +10% and +17% in males, +19%, +9%, +7% and +24% in females at 400, 2000, 10000 and 20000 ppm respectively compared to controls. Absolute liver weight changes are +6%, +20%, +26% and +22% in males, +2%, +4%, +4% and +16% in females at 400, 2000, 10000 and 20000 ppm respectively compared to controls. No statistical analysis was conducted due to the small number of dogs used in this study (2 dogs per sex per group). In the absence of any liver histopathological findings and hepatic clinical chemistry parameter changes, these findings are not considered adverse. There were no gross pathological or histopathological changes that were attributed to treatment with AE F130060 in any of the tissues evaluated. The NOAEL for this study was 20000 ppm, the highest concentration tested, which was equivalent to an average daily intake of 775.9 and 804.5 mg/kg bw/d in males and females, respectively.

In a 90 day dietary study in dogs (M-198012-02-1; 2000) groups of 8 (4/sex) Beagle dogs were administered dietary concentrations of 0; 2000; 10 000 and 20 000 ppm of AE F130060 (94.6% purity) for 13 consecutive weeks; in the control and top dose groups, 4 additional dogs/sex were kept on control diet for a further 6 weeks to examine the reversibility of potential effects (recovery groups). Clinical observations and food consumption were recorded daily; body weights were determined weekly. Examination of the neurological status and ophthalmological examinations were conducted pre-test and at termination. Haematological parameters, clinical chemistry and urinalysis were performed on all animals pre-test and terminally and on animals from control and top dose groups at week 6. At termination (week 13 in the main groups and week 18 in the recovery control and top dose groups), all dogs were sacrificed, selected organs were weighed and histopathological examinations were conducted on a large number of organs.

No deaths occurred and no treatment related clinical signs of toxicity, no changes when testing reflexes of excitability, postural reactions and hearing, nor ophthalmological abnormalities were observed during the 90 day study period. Body weight and body weight gain was comparable in all groups; no dose-related effect on food consumption was seen. Haematological parameters were not affected by treatment. Examination of clinical chemistry parameters (see table 4.7.1.1-4) revealed some statistically significant changes but none of these changes exhibited a dose response pattern and most of them were either seen in only 1 sex or were within historical ranges. There were no macroscopic or histo-pathological findings that could be attributed to the test material.

Although effects on organ weights were observed in this study, they were not considered treatment-related (see table 4.7.1.1-4).

As highlighted by the study author, statistically significant changes in testes, ovary and uterus were due to individual status of sexual cycle and/or maturity (e.g. one control dog with underdeveloped testes or 3 control and one high dose females already being in the estrous cycle). No dose-relation was observed (except for testes weight). Furthermore, weights of testes, ovaries, epididymides and uterus were inside the available historical control data provided in an amendment to the study report (see table 4.7.1.1-4).

No histopathological effects were noted in these organs. It is also to be noted that no changes were observed in the recovery groups. Hence these findings are considered incidental and not related to the test compound.

Relative pituitary and adrenals weights were decreased in males and increased in females, reaching sometimes statistical significance. No dose-relation was observed and no histopathological findings were noted in these organs. No effect was noted in the recovery groups. Therefore, these finding are not considered to be adverse.

It should be noted that statistical analyses have to be considered with caution, since they were performed on 4 animals per sex per group only.

Table 4.7.1.1-4: Group mean values for selected biochemical parameters at terminal sacrifice and organ weights

Dose level	**0 ppm**		**2000 ppm**		**10000 ppm**		**20000 ppm**		**Historical control data****
Sex	M	F	M	F	M	F	M	F	
Clinico chemical parameters									
Creatinin (µmol/L)	61	62	65	63	66	60	65*	64	
Urea (mmol/L)	3.25	3.45	3.22	3.35	3.68	3.92	3.22*	3.73	
Cholesterol (mmol/L)	2.83	2.90	2.90	2.82	2.80	2.93	2.57	2.87*	
Triglycerides (g/L)	0.36	0.32	0.40	0.32	0.35	0.37	0.24*	0.34	
Total lipids (g/L)	5.07	5.22	5.42	4.74	5.04	5.28	4.72	5.24*	
Protein (g/L)	54	53	52	52	53	52	54	56*	
ALAT (U/L)	24	19	27	27	27	23	28*	22	
ASAT (U/L)	15	12	15	14*	20*	15*	16*	15*	
CK (U/L)	57	43	60	40	159	55*	49*	59*	
α2 globulin (U)	0.077	0.078	0.081	0.075	0.079	0.078*	0.080	0.081*	
β2 globulin (U)	0.039	0.037	0.036	0.034	0.039	0.036	0.038	0.038*	
Organ Weights									
Absolute (g)									
Testes	11.86		13.24		14.25		15.46*		7.85-16.02
Epididymides	2.46		3.0		2.79		3.48*		1.89-3.51
Ovary		1.57		0.83*		0.74*		0.92*	0.14-3.25
Uterus		15.32		5.65*		4.40*		6.40*	1.50-20.17
Relative W (%)									
Adrenals	0.1263	0.1047	0.0972	0.1450	0.1198	0.1158	0.0985*	0.1132	
Pituitary	0.0083	0.0061	0.0071	0.0084	0.0059*	0.0073	0.0063*	0.0077*	

* : Two-tailed Wilcoxon (based on comparison with control and preliminary value at start of the study; $p < 0.05$).

**: studies from Jan 1998 to August 1998 in beagle dogs from Marshall Farms (US), n=22

The minor changes in some biochemical parameters should be considered as non-adverse effects of test substance. The NOAEL for this study was 20 000 ppm, the highest concentration tested, which was equivalent to an average daily intake of 648 and 734 mg/kg bw/d in males and females, respectively.

In a 1 year dog study (M-198511-01-1; 2000) groups of 12 (6/sex) Beagle dogs were administered dietary concentrations of 0; 400; 4000 and 16000 ppm of AE F130060 (purity 95.3 – 95.7%) for 52 consecutive

weeks. Clinical observations and food consumption were recorded daily; body weights were determined weekly. Ophthalmological examinations were conducted pre-test on all dogs and at termination on control and top dose groups. Haematological parameters, clinical chemistry parameters and urinalysis were performed on all animals pre-test and 3, 6 and 12 months of study period. At termination, all dogs were sacrificed, selected organs were weighed and extensive histopathological examinations were conducted on all organs and tissues.

No excess of mortality was recorded. No clinical signs of toxicity, no ophthalmological abnormalities, no effects on body weight or body weight gain impairment were observed during the 1 year study period. There was no effect on food consumption and food conversion. No treatment related effects on haematological, clinical chemical or urinalysis parameters were recorded (most of the statistically significant changes were seen mostly in the top dose group and were limited to 1 sex, not dose-related and within historical range (see table 4.7.1.1-5); in addition, no histopathological corroborative effects were recorded).

Table 4.7.1.1-5: Statistically significant changes in haematology and clinical chemistry

Parameter	Period	Dose level			
		0 ppm	400 ppm	4000 ppm	16000 ppm
Males					
Reticulocytes (10^9/L)	3 months	75	55	58	36**
	6 months	52	51	69	45
	12 months	64	47	64	43
White blood cells (10^9/L)	3 months	9.5	10.4	9.8	11.6*
	6 months	8.9	10.4	9.8	11.4*
	12 months	9.6	10.9	9.2	11.4
Neutrophils (10^9/L)	3 months	5.9	6.8	6.1	7.5*
	6 months	5.6	6.8	6.4	7.4*
	12 months	6.2	7.3	6.1	7.6
AST (U/L)	3 months	35	39	40	38
	6 months	41	44	54*	54*
	12 months	49	48	49	48
CPK (U/L)	3 months	190	236	253*	249*
	6 months	190	228	256*	256*
	12 months	239	256	312	270
Females					
Reticulocytes (10^9/L)	3 months	51	42	57	39
	6 months	69	50	71	66
	12 months	73	46*	50	46*
White blood cells (10^9/L)	3 months	10.6	12.2	10.3	13.0
	6 months	10.3	9.9	10.1	9.7
	12 months	9.0	11.3	9.9	11.4
Neutrophils (10^9/L)	3 months	6.9	7.7	6.3	8.2
	6 months	7.0	6.1	6.2	5.9
	12 months	5.8	7.0	6.0	7.2
Potassium (mmol/L)	3 months	4.16	3.89*	4.07	3.84*

	6 months	4.20	3.97	4.11	3.93
	12 months	4.55	4.08*	4.12	3.93**
AST (U/L)	3 months	37	37	46	39
	6 months	38	38	42	44
	12 months	48	42	51	51
CPK (U/L)	3 months	227	215	354*	248
	6 months	199	215	264	230
	12 months	350	197**	222*	241*

2 tailed Dunnett test; significant * $p < 0.05$; ** $p < 0.01$

There were no macroscopic findings that could be attributed to the test material; organ weights were not affected by the treatment. At histopathological examination, 3/6 males from the top dose group exhibited minimal to slight increased foveolar mucous secretion in the cardiac and fundic sections of the stomach (see table 7.4.1.1-6), which was accompanied by a chronic superficial gastritis in 1 dog. From its nature, this finding was considered to be due to local irritation in the stomach following treatment with a very high concentration of test substance in the diet. No similar finding was observed in the females.

Table 4.7.1.1-6: Histopathological changes in the stomach of males

Dose level	0 ppm	400 ppm	4000 ppm	16000 ppm
Cardiac section				
Increased foveolar mucous secretion				
-minimal	0/6	0/6	0/6	1/6
-slight	0/6	0/6	0/6	2/6
CHRONIC SUPERFICIAL GASTRITIS				
-minimal	0/6	0/6	0/6	1/6
FOCAL MUCOSAL CONGESTION				
-present	1/6	0/6	0/6	0/6
Fundic section				
Increased foveolar mucous secretion				
-slight	0/6	0/6	0/6	3/6
Chronic superficial gastritis				
-slight	0/6	0/6	0/6	1/6
Pyloric antrum				
Chronic gastritis				
-moderate	0/6	0/6	0/6	1/6

No adverse treatment related effects were seen in the general health status, body weight gain or food consumption, haematological and biochemical parameters up to the highest dose tested. There were no substance-related changes on organ weights or any other systemic macroscopic and microscopic findings after terminal kill. The local findings in the stomach mucosa of 3/6 males at 16 000 ppm were not considered as an adverse effect and were of questionable significance with regard to treatment-relatedness in the absence of this findings in the females. A “local NOAEL” may be set at 4000 ppm, i.e. 155 mg/kg bw/day.The no observed adverse effect level (NOAEL) in this study as 16 000 ppm i.e. 574 mg/kg bw/day for males and 646 mg/kg bw/day for females.

4.7.1.2 Repeated dose toxicity: inhalation

No compound-specific toxic effects were seen after acute inhalation testing of technical Mesosulfuron-methyl (AE F130060) under experimental (respirable) conditions as a fine, respirable dust. Hence, a study with repeated inhalation exposure is not considered necessary as it would not yield significant new toxicological information. In addition, based on the physico-chemical properties of technical Mesosulfuron-methyl, relevant inhalation exposure to the active substance does not occur during production. A health risk from repeated inhalation exposure to humans can therefore be excluded.

4.7.1.3 Repeated dose toxicity: dermal

Based on the very low acute dermal and oral toxicity of technical Mesosulfuron-methyl (AE F130060) and relatively low dermal absorption figures of the oil flowable product, which is similar to oral absorption, a potential human health risk resulting from repeated dermal exposure can be excluded.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

No further relevant available.

4.7.1.7 Summary and discussion of repeated dose toxicity

No adverse effects or signs of serious damage (‘clear functional disturbance or morphological change which has toxicological significance’) were seen at any dose levels up to the highest tested dose of 1000 mg/kg bw/d of Mesosulfuron-methyl (AE F130060) in rats, mice and dogs. In all of the studies the NOAEL was the highest dose level given. This can be explained by the low toxicity of the test substance in combination with a non-linear resorption from the gastrointestinal tract, *i.e.*, up to 23 % after oral low doses of 10 mg/kg bw and only 2 % or slightly above at the limit dose of 1 000 mg/kg bw after oral exposure.

Slight changes of some biochemical parameters (bilirubin, lipids, calcium, inorganic phosphate) were seen in the sub-chronic study in rats. Slightly reduced values for leucocytes were observed in male mice after 90 days. These findings occurred in the mid and high dose groups without showing a clear dose-related pattern and were not consistently seen in both sexes and were therefore in the absence of any histopathological findings, considered as non-adverse effects. Slightly reduced values for leucocytes in male mice after 90 days were not reproduced with the same strain in the respective oncogenicity study, which to the contrary showed slightly increased leucocytes in the top dose group only after 18 months. This minimal shift of leucocyte counts within the biological range over the time period may reflect the bioavailability of the Mesosulfuron-methyl (AE F130060) in particular in male blood (as indicated by

the pharmacokinetic investigations). However, in the absence of any clinical and histo-pathological correlate, it was not considered to be toxicologically relevant.

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

Not required.

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

Not required.

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

Not required.

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

There was no clear evidence of any specific toxic effects on a target organ or tissue from repeated exposure in experimental studies.

The standard animal studies in rats, mice and dogs include haematological, clinic-chemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Slight changes of some biochemical parameters (bilirubin, lipids, calcium, inorganic phosphate) were seen in the sub-chronic study in rats. Slightly reduced values for leucocytes were observed in male mice after 90 days. However, in the absence of any clinical and histo-pathological correlate, it was not considered to be toxicologically relevant. Other long-term exposure studies, such as carcinogenicity or reproductive toxicity studies, also do not provide evidence of specific target organ toxicity that could lead to classification.

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

Substances are classified for repeated dose toxicity when serious damage (‘clear functional disturbance or morphological change which has toxicological significance’) is seen following repeated or prolonged exposure below guidance values provided in the classification criteria.

Mesosulfuron-methyl shows low toxicity after repeat exposure to the mouse, rat and dog. No target organs were identified at dose levels up to the limit dose. Criteria for classification as STOT RE are not met.

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

Based on the results of the studies performed, no classification as STOT RE is proposed.

4.9 Germ cell mutagenicity (Mutagenicity)

The genotoxic potential of Mesosulfuron-methyl has been investigated in several *in vitro* studies and in an *in vivo* micronucleus test.

Table 4.9-1: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
In vitro			
Bacterial gene Mutation - Bacterial reverse mutation test (Ames test) Test strains: *Salmonella typhimurium* TA98; TA100; TA1535, TA1537 and WP2 uvrA of E. *coli*. Mesosulfuron-methyl (AE F130060) technical (purity: 95.6%) Dose levels: 4; 20; 100; 500; 2500 and 5000 µg/plate (1st experiment) ; 0.16; 0.8; 4; 20; 100 and 500 µg/plate (2nd experiment), with and without S-9 metabolic activation for 3 replicates per dose. Appropriate negative and positive controls used. OECD 471 and 472; 1983	Negative	Mesosulfuron-methyl was negative for mutagenicity in 6 tester strains of bacteria at concentrations up to and including 5,000 µg/plate in the presence and absence of S-9 metabolic activation.	M-140530-01-1, 1996 (Muller W. 1996i)
Clastogenicity - Mammalian chromosome aberration test Test system: Mesosulfuron-methyl was tested in an in vitro chromosome aberration test in V79 cells of Chinese hamster lung. Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in distilled water Dose levels: 0; 250; 790 and 2500 µg/mL for a 20h harvest interval and 1 selected dose 2500 µg/mL for a 28h harvest interval With and without S-9 metabolic activation OECD 473; 1983	Negative	The test material was negative in the in vitro chromosome aberration assay in V 79 Chinese hamster lung cells, when tested with and without metabolic activation.	M-147927-01-1; 1998 (Muller W. 1998b)
Gene mutation - Mammalian cell gene mutation (HGPRT test) Test system: potential of test substance to induce mutations at the HGPRT locus in V79 Chinese hamster lung fibroblasts in vitro. Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in deionised water Dose levels: 0; 25; 79; 250; 790 and 2500 µg/ml With and without S-9 metabolic activation OECD 476; 1984	Negative	No relevant reproducible enhancement of the mutation rate over the range of control values were induced by Mesosulfuron-methyl at the HGPRT locus in V79 Chinese hamster lung fibroblasts with and without metabolic activation.	M-147480-01-1; 1998 (Muller W. 1998d)
DNA damage and repair - UDS-test Freshly prepared Sprague Dawley primary rat hepatocytes Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in double deionised water Dose levels: 0; 1; 3; 10; 100; 300 and 2500 µg/mL OECD 482; 1986	Negative	No induction of DNA damage and repair was induced by test substance in primary rat hepatocytes in vitro.	M-148054-01-1; 1998 (Muller W. 1998c)
In vivo			

Method	Results	Remarks	Reference
Micronuclei in Mouse Bone Marrow Dose levels: 30 (15/sex) Hsd:Win:NMRI mice (7-8 w old) - single oral dose (gavage) Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in deionised water Dose levels: 200; 1000 and 2000 mg/kg bw OECD 474; 1988	Negative	Test material was negative for causing cytogenetic damage as measured by micronucleus induction in NMRI mice.	M-147538-01-1; 1998 (Muller W. 1998e)

4.9.1 Non-human information

4.9.1.1 *In vitro* data

The genotoxic potential of Mesosulfuron-methyl has been investigated in several *in vitro* studies and gave negative results. In all tests with the exception of the Ames test (which was performed at a relatively early stage of development with a purity of 95.6 %) test material from the same batch (purity 94.6 %) as in most other toxicological studies was used.

The *in vitro* testing battery comprised investigations for gene mutation in bacterial and mammalian cells, examination of chromosomal aberration in Chinese Hamster cells and testing for unscheduled DNA-synthesis in primary rat hepatocytes.

AE F130060 technical (95.6% purity) was tested in a standard plate incorporation assay (Ames test) using 5 bacterial tester strains: TA98; TA100; TA1535 and TA1537 of *S. typhimurium* and WP2 *uvrA* of *E. coli* (M-140530-01-1, 1996). All 5 tester strains received either the test material in aqua bidest. at dose levels of 4; 20; 100; 500; 2500 and 5000 µg/plate (1st experiment) or 0.16; 0.8; 4; 20; 100 and 500 µg/plate (2nd experiment) or aqua bidest as negative solvent control or no treatment as untreated controls, with and without S-9 metabolic activation for 3 replicates per dose.

Parallel to the 2nd experiment, a precise toxicity testing was performed using 0.1 ml of the different concentrations of the test compound mixture with 0.1 ml of a 10^6 solution of an overnight culture of TA100 which was plated subsequently (3 plates/dose). Positive controls without metabolic activation included sodium azide (1 µg/plate for TA100 and TA 1535), 9-aminoacridine (50.0µg/plate for TA 1537), 2-nitrofluorene (2.5 µg/plate for TA 98) and MNNG (2.5 µg/plate for WP2uvrA E Coli); positive control with metabolic activation was 2-Aminoanthracene (0.5 µg/plate for TA98 and TA100; 1.0 µg/plate for TA1535 and TA 1537 and 10.0µg/plate for WP2uvrA E Coli). After 48 h of incubation at about 37°C, revertant colonies were counted using an automated counter (see table 4.9.1.1-1).

Visible precipitation of test substance was observed at 5000 µg/plate. In the 1st experiment, dose related toxicity was observed at concentrations ≥ 20 µg/plate in the absence of metabolic activation and at concentrations ≥ 100 µg/plate in the presence of metabolic activation in all Salmonella tester strains; no cytotoxicity was observed for the WP2uvrA *E. Coli* strain. Cytotoxic effects were reproduced with Samonella strains in the 2nd experiment. In the toxicity trial which was performed in parallel with the 2nd experiment with a dilution of TA 100 tester strain, toxicity was only observed in the absence of metabolic activation at concentrations ≥ 100 µg/plate (no cytotoxicity was found up to the highest concentration of 500 µg/plate in the presence of metabolic activation). Test substance did not induce increases in the

number of revertant colonies at any of the concentrations tested in presence or in absence of metabolic activation for any of the tester strains in both independent trials. The positive control materials elicited positive responses indicating that the test system was capable of detecting mutations and that the metabolic activation system was functioning properly (see table 4.9.1.1-1). AE F130060 was negative for mutagenicity in 6 tester strains of bacteria at concentrations up to and including 5,000 µg/plate in the presence and absence of S-9 metabolic activation.

Table 4.9.1.1-1 Mean number of revertant colonies in bacterial reverse mutation test

Mean number of revertant colonies (3 replicates)											
	µg/plate	TA100		TA1535		TA1537		TA98		E Coli	
		+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9
1st experiment											
AE F130060	0	134	135.3	10.3	9.3	9.3	8	23.7	24	34.7	27.3
	4	113.3	145	13	12.7	8.7	8	29.3	28.3	32.3	21.7
	20	94.7	55	8.3	3	3.3	5.7	26.3	14	27.3	24.7
	100	57.3	18.7	0.3	0.3	2	1	13	9	26.3	25.7
	500	6.7	2.7	0	0	0.3	0	0	1	30	16.3
	2500	1.3	0	1	1	0	0	0	0	28.7	23.7
	5000	0	0	0	0	0	0	0	0	28.3	17
2 AAT		1434		175		254		1836.7		456.7	
Na azide			689		357.7						
9 AAC							175.7				
2 NF									434.3		
MNNG											242.7
2nd experiment											
AE F130060	0	166	180	13.3	14.3	9.7	12.3	36.7	35.3	38.7	28.3
	0.16	172.7	140.7	16.7	13.3	6.7	11	24.7	24		
	0.8	173	141.3	16	11	8.7	11.7	23	22.3		
	4	159	119	11.7	13.7	11	9	21.3	21.3	28.7	23.3
	20	119	61	11.7	7.7	10	9	19	12.7	31.3	26.3
	100	48.7	27	8.3	5.7	5.3	3.7	11.7	7	33.2	21.3
	500	2.3	1	4.3	1.7	3.3	1.7	2	2	29	22.7
	2500									31.7	22.3
	5000									28.7	28.7
2 AAT		1644.7		150		321		1995		389	
Na azide			743.3		382.7						
9 AAC							191.7				
2 NF									587.3		
MNNG											268

2AAT = 2 Aminoanthracene
9 AAC = 9 Aminoacridine
2 NF = 2 Nitrofluorene
MNNG = N-Methyl-N-nitro-N-nitrosoguanidine

AE F130060 technical (94.6% purity) was tested in an *in vitro* chromosome aberration test in V79 cells of Chinese hamster lung with and without S-9 metabolic activation in accordance with OECD 473

guidelines (M-147927-01-1; 1998). The highest practicable concentration of AE F130060 in culture medium was determined in range finding studies to be 2500 µg/mL, therefore concentrations of 50; 100; 250; 500; 750; 1000; 2000 and 2500 µg/mL were used for the preliminary toxicity study: precipitation was observed microscopically for the concentrations ≥ 100 µg/mL.

Two independent chromosome aberration assays were performed with and without metabolic activation, including 3 selected dose levels of test material in distilled water (0; 250; 790 and 2500 µg/mL) for a 20h harvest interval and 1 selected dose (2500 µg/mL) for a 28h harvest interval. All cultures were exposed to 0.1 µg/ml Colcemid for 2 h prior to the harvests. Positive controls were used only for the 20h preparation time (0.5µg/mL EMS in the absence of metabolic activation and 3.5 µg/mL cyclophosphamide and the presence of metabolic activation). Chromosome aberrations were scored on 25-100 metaphases from each of 2 replicate cultures at each dose level of test material and from the corresponding untreated, solvent and positive controls, from each harvest in the activated and non-activated systems. The total number of aberrations and the percentage of cells with one or more aberrations which were classified as structural aberrations or chromosomal disintegration were calculated for each dose level.

Test substance was not toxic to the V79 lung cells in the absence or in the presence of metabolic activation, as shown by the mitotic index (see table 4.9.1.1-2). The highest dose level selected for the mutagenic assay represented the solubility limit of the test material in the culture medium; microscopic examination revealed precipitation of test substance for concentrations ≥ 100 µg/mL. No reproducible statistically significant increases in the number of cells with aberrations over the controls were found at any dose or time interval in the presence and in the absence of metabolic activation: the statistically significant increase of the number of aberrations (including gaps) which was recorded in the 1st experiment without metabolic activation at the 28h harvest time was not reproduced in the 2nd experiment. There was no significant increase in the number of polyploid cells. The concurrent positive control materials elicited positive responses indicating that the test system was valid and that the metabolic activation system was functioning properly (see table 4.9.1.1-3).

The test material was negative in the *in vitro* chromosome aberration assay in V 79 Chinese hamster lung cells, when tested with and without metabolic activation.

Table 4.9.1.1-2: Mitotic index (determined in 1000 cells)

Test group		1st experiment				2nd experiment			
		Without S9 mix		With S9 mix		Without S9 mix		With S9 mix	
	Dose (µg/mL)	Mean MI	Relative MI (%)	Mean MI	Relative MI (%)	Mean MI	Relative MI (%)	Mean MI	Relative MI (%)
20 h harvest									
Control	0.0	4.8	100	4.2	100	9.0	100	6.0	100
AE F130060	250	6.9	143.8	5.0	119	5.1	56.7	5.0	83.3
	790	6.2	129.2	5.2	123.8	6.3	70	5.7	95
	2500	7.1	148	5.1	121.4	5.3	58.9	6.2	103.3
EMS		5.1	106.3			7.2	80.0		
CPA				5.1	121.4			4.6	76.7
28h harvest									
Control	0.0	6.5	100	5.4	100	10.9	100	6.8	100
AE F130060	2500	10.4	160	7.4	137	8.9	81.7	5.2	76.5

MI : mitotic index
CPA = cyclophosphamide
EMS = ethyl methane sulfonate

Table 4.9.1.1-3. Percentage of cells with aberrations (28 h harvest; 100 metaphases analysed)

Dose (µg/mL)	Culture ± S9-mix	No. of phases with aberrations		No. of aberrations		g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
		incl.	excl.	incl.	excl.													
		Gaps		Gaps														
1st experiment																		
0.0	-	2	1	2	1	1			1									7.5
0.0	-	3	2	3	2	1								1	1			5.5
Total		5	3	5	3	2			1					1	1			
2500.0	-	6	5	9	7	2		2			1		2		1	1²		9.3
2500.0	-	4	2	5	2	3							1		1			11.5
Total		10	7	14*	9	5		2			1		3		2	1		
0.0	+	1	1	1	1						1							6.8
0.0	+	5	4	5	4	1		2	1								1 di	4.0
Total		6	5	6	5	1		2	1		1						1	
2500.0	+	3	0	3	0	3												8.3
2500.0	+	6	4	7	5	2					2		2				1 di	6.5
Total		9	4	10	5	5					2		2				1	
2nd experiment																		
0.0	+	3	2	3	2	1			1						1			11.2
0.0	+	5	4	6	5	1		1			1				2		1 im	10.5
Total		8	6	9	7	2		1	1		1				3		1	
2500.0	+	1	1	1	1						1							10.3
2500.0	+	4	4	4	4								2		2			7.4
Total		5	5	5	5						1		2		2			
0.0	+	2	2	4	4			1			2						1 ri	6.0
0.0	+	3	3	6	6			1	1		3		1					7.5
Total		5	5	10	10			2	1		5		1				1	
2500.0	+	1	1	1	1						1							5.7
2500.0	+	5	3	5	3	2		1	1		1							4.7
Total		6	4	6	4	2		1	1		2							

S = solvent control (DMSO)

MI: mitotic index

* = $p \leq 0.05$

1² = two chromosomes of the metaphase were disintegrated

g = gap ; ig = isogap ; b = break ; ib = isobreak ; f = fragment ; if = isofragment ; d = deletion ; id = isodeletion ; ma : multiple aberrations ; ex : exchange ; cd : chromosome disintegration

In a study conducted to OECD 476 guidelines (M-147480-01-1; 1998), AE F130060 technical (94.6% purity) in deionised water was tested for its potential to induce mutations at the HGPRT locus in V79 Chinese hamster lung fibroblasts *in vitro*. Results from dose-range finding study showed that AE F130060 was not toxic both in the presence and in the absence of S9-mix at concentrations up to 2500 µg/ml and that precipitation occurred in the culture medium for doses ≥ 100 µg/mL.

Two independent trials were conducted with 0; 25; 79; 250; 790 and 2500 µg/ml AE F130060, with and without metabolic activation; 7.7 µg/ml 7,12-dimethylbenzanthracene (DMBA) in DMSO with metabolic activation and 1.0 µg/ml ethylmethanesulfonate (EMS) in culture medium without metabolic activation were used as positive controls. Approximately 6 x 10^5 – 1 x 10^6 cells/well were incubated at 37°C for 4 hours treatment by test material; only colonies with more than 50 cells were counted. Plating efficiency was determined on wells seeded with 4500 cells.

Test substance was not toxic to the V79 lung cells in the absence or in the presence of metabolic activation, but a slight decrease of the survival rate (68.6% of the controls) occurred at the highest dose level of 2500 µg/mL in presence of metabolic activation (see table 4.9.1.1-4).

In presence of metabolic activation, a slight but statistically significant increase in the mutant frequency was found at the lowest concentration of test substance (25 µg/mL) in the 1st experiment, but not in the 2nd experiment. The mutation frequencies in controls of the 2nd experiment without metabolic activation was relatively high compared with those of the 1st experiment (and those of historical controls as stressed in the laboratory report). Taking into account values found at the different dose levels, these no-reproducible findings are to be considered as not biologically relevant. The positive controls elicited the expected positive responses, indicating that the test system was valid (see table 4.9.1.2-4).

No relevant reproducible enhancement of the mutation rate over the range of control values were induced by AE F130060 at the HGPRT locus in V79 Chinese hamster lung fibroblasts with and without metabolic activation.

Table 4.9.1.1.-4.: Results of the two independent experiments

Test group	Dose (µg/mL)	1st experiment		2nd experiment	
		Without S9-mix	With S9-mix	Without S9-mix	With S9-mix
Relative survival (%)					
Neg. control	0	119.1	112.1	96.6	122.6
Solv. control	0	100	100	100	100
AE F130060	25	105.4	98.1	98.1	113.4
	79	97.7	101.8	107.7	106.3
	250¤	100.4	96.9	103.4	92.2
	790¤	96.7	95	104.1	93.2
	2500¤	96.9	68.6	92.4	89.7
EMS	1000	81.6	64.7		
DMBA	7.7			73.2	83.8
Mutation frequency					
Neg. control	0	13.4	8.1	96.6	42.7
Solv. control	0	10.8	8.5	80.0	28.9
AE F130060	25	6.9	**22.0***	41.5	16.5
	79	3.7	18.1	78.6	21.5
	250♣	1.4	4.1	45.7	25.5
	790♣	22.2	9.3	74.5	33.1
	2500♣	5.4	8.9	50.9	17.3
EMS	1000	597.8*		852.4*	
DMBA	7.7		44.8*		114.3*

EMS = ethyl methane sulfonate
DMBA = 9,10-dimethyl-1,2-benzanthracene
Mutation frequency (mutant colonies per 1 million cells): mean values /cells surviving
* Statistical significance ($p<0.05$) Mann-Whitney-U-test; solvent control = double-distilled water
♣precipitation of test compound

In a study conducted to OECD 482 guidelines (M-148054-01-1; 1998) duplicate cultures of freshly prepared Sprague Dawley primary rat hepatocytes were exposed to 0; 1; 3; 10; 100; 300 and 2500 µg/mL AE F130060 technical (94.6% purity) suspended in double deionised water for 16-20 hours at 37°C and to 1 µg/ml 2-Acetylaminofluorene (2-AAF) in DMSO as positive control. A preliminary solubility test was carried out to determine to appropriate dose levels and cytotoxicity tests were performed in parallel to each of 2 independent UDS experiments. Taking into account the highest practicable concentration of test substance in double distilled water (250 mg/ml) and the highest concentration at which no visible precipitation in culture medium (30 µg/mL), the main experiments were carried out using a maximum concentration of 2500 µg/mL.

In two independent experiments, no relevant reproducible increase in the incorporation of ^{3}H thymidine over the range of control values was found with test substance; the positive control elicited the expected positive response, indicating that the test system was valid (see table 4.9.1.2-5).

Table 4.9.1.1-5: Results of UDS asssay

Test group		1st experiment	2nd experiment

	Dose (µg/mL)	Cytotoxicity	Net grains/nucleus (mean ± SD)	% cells in repair*	Cytotoxicity	Net grains/nucleus (mean ± SD	% cells in repair*
Neg. Controls	0	(a)	1.7 ± 1.9	3	(a)	1.5 ± 1.8	2
Solvent controls	0	100.0	2.2 ± 1.7	2	100.0	1.4 ± 1.8	2
AE F130060	1	122.4	1.7 ± 1.6	2	90.6	1.7 ± 1.9	1
	3	111.8	2.5 ± 1.5	2	103.4	1.9 ± 1.8	1
	10	124.9	2.6 ± 1.7	4	113.5	1.5 ± 1.8	1
	30	124.4	2.2 ± 1.8	3	115.4	1.6 ± 2.2	3
	100	128.9	2.1 ± 1.9	5	111.5	1.8 ± 1.9	5
	300	136.0	1.8 ± 1.8	4	97.4	1.4 ± 1.6	1
	1000	124.3	1.7 ± 1.9	2	90.1	1.4 ± 1.8	2
	2500	151.0	1.3 ± 2.0	3	79.2	1.4 ± 1.8	2
2-AAF	1		36.2 ± 5.8	100		35.3 ± 6.7	100

2-AAF = 2-acetamidofluorene

* net grains > 5

(a): No cytotoxicity was not evaluated in the negative control (*i.e.* cells from animals which did not received neither the vehicle nor the test substance

No induction of DNA damage and repair was induced by test substance in primary rat hepatocytes *in vitro*.

4.9.1.2 *In vivo* data

The test program for possible genotoxic properties of the technical Mesosulfuron-methyl in several *in vitro* test systems was complemented by a mouse micronucleus assay as an indirect investigation on the end-point chromosomal aberration *in vivo* and gave negative results.

As no positive results were seen during *in vitro* testing for DNA damage and repair in the UDS test and no oncogenic response was seen in long-term studies in 2 species, *in vivo* testing for unscheduled DNA synthesis or mouse spot test was not considered necessary.

An *in vivo* mouse micronucleus assay, in accordance to OECD 474 guidelines was conducted on groups of 30 (15/sex) Hsd:Win:NMRI mice which were given a single oral dose (gavage) of 200; 1000 and 2000 mg/kg bw AE F130060 technical (94.6% purity) suspended in deionised water, along with concurrent vehicle controls (deionised water) and positive controls (5/sex) which were given an 50 mg/kg bw cyclophosphamide (M-147538-01-1; 1998). Dose levels were selected based on results from a range-finding test. Animals were individually examination for clinical signs of toxicity and mortality at regular intervals after dosing. 10 mice /group (5/sex) were sacrificed 24-; 48- and 72-hours following dosing for collection of bone marrow cells (positive control animals were sacrificed at 24 hours following dosing). For each animal, 1,000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei and the ratio of polychromatic to normochromatic erythrocytes (NCE) was calculated from 1,000 erythrocytes per animal; the incidence of micronucleated normochromatic erythrocytes was also recorded.

No deaths occurred, or clinical signs of toxicity, nor macroscopic findings were recorded in any dose group for either sex in the range finding test. The incidence of normochromatic and polychromatic

erythrocytes and of micronucleated normochromatic erythrocytes did not differ from controls; the positive control elicited the expected positive response. No treatment related effect was observed on the micronucleated PCE and for the percent of PCE at any harvest time in either sex; the ratio of PCE to normocytes differed from control values in 3 mice (in 1 from each the control group, the 200 mg/kg bw group and the 1000 mg/kg bw group) which reflect the normal biological variation (see table 4.9.1.2-1).

Table 4.9.1.2-1: Summary results (mean values ± SD for 10 mice / dose level)

		Erythrocytes with micronuclei					
		% PCE	% NCE	% PCE	% NCE	% PCE	% NCE
Sampling time		12h		24h		48h	
Test group	**Dose (mg/kg bw)**						
Controls	0	0.1 ± 0.07	0.1 ± 0.13	0.1 ± 0.10	0.4 ± 0.05	0.1 ± 0.09	0.6 ± 0.05
AE F130060	200	0.2 ± 0.15	0.1 ± 0.05	0.2 ± 0.12	0.5 ± 0.08	0.1 ± 0.09	0.4 ± 0.05
	1000	0.1 ± 0.11	0.1 ± 0.10	0.3 ± 0.20	0.8 ± 0.06	0.1 ± 0.11	0.6 ± 0.05
	2000	0.2 ± 0.15	0.1 ± 0.05	0.1 ± 0.10	0.5 ± 0.05	0.1 ± 0.10	1.3 ± 0.11
CPA	50			2.3 ± 0.57*	1.4 ± 0.07		

PCE = polychromatic erythrocytes
NCE = normochromatic erythrocytes
CPA = Cyclophosphamide = Endoxan®
* significantly different from controls ($p < 0.05$); Wilcoxon test

Test material was negative for causing cytogenetic damage as measured by micronucleus induction in NMRI mice.

As results from all of the genotoxicity studies conducted were negative, i.e., no positive results were seen during *in vitro* and *in vivo* testing in somatic cells, and as there is no evidence for heritable toxicological effects presented in the multigeneration study and no oncogenic response was seen in long-term studies in two species, an *in vivo* study in germ cells was not triggered under the EC Commission Directive 94/79/EC of 21 December 1994.

4.9.2 Human information

No information available.

4.9.3 Other relevant information

No further relevant information available.

4.9.4 Summary and discussion of mutagenicity

Mesosulfuron-methyl has been demonstrated to be negative for genotoxicity in a complete package of *in vitro* studies and in an *in vivo* micronucleus assays for genotoxicity. The *in vitro* testing battery comprised investigations for gene mutation in bacterial and mammalian cells, examination of chromosomal aberration in Chinese Hamster cells and testing for unscheduled DNA-synthesis in primary rat hepatocytes. Furthermore, a mouse micronucleus assay on chromosomal aberration *in vivo* was

performed. Since all 5 tests were negative and no evidence for carcinogenic properties was seen in life-time experiments in two species, further testing, e. g. tests using germ cells was not triggered

No human data is available.

4.9.5 Comparison with criteria

The available data base indicates that Mesosulfuron-methyl is not genotoxic.

4.9.6 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The *in vitro* test battery compromised of the following tests: bacterial gene mutation assay (according to OECD TG 471 and 472), mammalian chromosome aberration test (according to OECD TG 473), mammalian cell gene mutation (HPGRT test, according to OECD TG 476) and DNA damage and repair (UDS test, according to OECD TG 482). Furthermore, an *in vivo* mouse micronucleus assay in bone marrow according to OECD TG 474 was carried out.

None of the five tests, which were all considered as reliable showed any indication of germ cell mutagenicity due to the test substance.

The DS concluded that mesosulfuron-methyl is not genotoxic and proposed no classification for this endpoint.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments were received during public consultation.

Assessment and comparison with the classification criteria

No germ cell mutagenicity potential was identified in an acceptable battery of tests conducted in accordance with applicable TG.

RAC agrees that **no classification for germ cell mutagenicity** is warranted.

4.10 Carcinogenicity

Table 4.10-1: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Rat combined dietary chronic and oncogenicity study Wistar [Hoe:WISKf(SPF71)] (5-6 w of age at initiation) Interim toxicity: 52 weeks; 10/sex/dose Chronic toxicity study : 104 weeks; 20/sex/dose Carcinogenicity study: 104 weeks; 50/sex/dose Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) Dose levels: 0; 160; 1600 and 16000 ppm OECD 453; 1981	NOAEL = 16000 ppm, highest concentration tested, which was equivalent (oncogenicity group) to approximately: • 764 mg/kg bw/d in the males. • 952 mg/kg bw/d in the females.	No evidence of toxicity or oncogenicity.	M-198434-01-1; 2000 (Seeberger A.,2000a)
Mouse dietary oncogenicity / toxicity study CD-1 mice (Crl:CD-1® (ICR)BR strain) (5-6 w of age at initiation) Interim toxicity: 52 weeks; 10/sex/dose Carcinogenicity study: 78 weeks; 50/sex/dose Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) Dose levels: 0; 80; 800 and 8000 ppm in the diet OECD 451; 1981	NOAEL = 800 ppm based on lower body weight gains in females at the high dose level. This is equivalent to a mean achieved intake of • 103 mg/kg bw/d in the males. • 130 mg/kg bw/d in the females.	Slight reductions in bw gain seen in females of the top dose group (8000 ppm). Transient and sporadic changes in haematological parameters without histolopathological corroborate. The statistically significant increased leukocyte counts, seen in males at 800 ppm and in 2 sexes at 8000ppm, also exhibited some dose dependency suggesting a treatment related effect ; these changes were slight and not corroborated by any clinical or histopathological change so that they were not considered as toxicologically relevant. Moreover, mouse leukocyte counts were decreased in the 90 d study and increased in the 18 months study, so that they were not considered at toxicologically relevant No carcinogenic effect and no - neoplastic lesions.	M-198596-01-1; 2000 (Seeberger, 2000b)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In a rat combined chronic toxicity and carcinogenicity study, groups of 160 (80/sex) Wistar [Hoe:WISKf(SPF71)] rats were administered 0; 160; 1600 and 16000 ppm of AE F130060 (94.6 % purity) in the diet for 52 consecutive weeks (10/sex/dose : interim toxicity study) and for 104 weeks (20/sex/dose for chronic toxicity study and 50/sex/dose for carcinogenicity study) (M-198434-01-1; 2000). All animals were observed twice daily for signs of overt-toxicity, behaviour, general health condition and mortality; examinations for neurological disturbances, impairment of dental growth and eye changes were performed monthly; ophthalmological investigations were done on all animals at start of study and on all control and top dose rats at study termination (i.e. at 12 months for the interim group and 24 months for the chronic toxicity and carcinogenicity groups). Individual body weights and food consumption were recorded weekly. Haematological parameters clinical chemistry parameters and urinalysis were determined on all or part of the animals at various sampling intervals depending on the study groups (see table 4.10.1.1-1). At termination, all surviving animals were sacrificed for gross necropsy and selected organs were weighed; gross necropsy was also performed on unscheduled deaths. Extensive histopathological examinations were performed on all tissues from the control and high dose groups, on all gross lesions, and on the kidney, the liver, and the lung from all low and mid dose groups at terminal sacrifice, as well as on a full spectrum for tissues from unscheduled dead rats.

Table 4.10.1.1-1: Laboratory investigations in study groups

Study group	Urinalysis	Haematology	Clinical chemistry
12 months interim toxicity	All rats (on w-52 – 53)		
24 months chronic toxicity	First 10 rats/sex on w-14, -27, -51, -79 and -104	First 10 rats/sex on w-14, -27, -51, -79 and –104 Moribund rats : at killing	Last 10 rats/sex on w-15, -28, -52, -80, and -105
24 months carcinogenicity		All surviving rats on w-106/107 Moribund rats : at killing	

Survival was not affected by the treatment (see table 4.10.1.1.-2); a total of 127 rats died or were killed in extremis; in the 12 months toxicity group, 1 top dose female rat died; in the 24 months chronic/carcinogenicity groups, the mortality rate was low and raised, as expected, during the 2nd year, but the cause of death did not distinguish treated rats from controls. Food consumption values for both male and female treated rats levels were generally comparable to that of controls. In both sexes, body weights and overall body weight gain of treated rats were generally comparable to that of controls in the interim toxicity, chronic toxicity and carcinogenicity groups.

Table 4.10.1.1-2: Summary of mortality and survival rates (intercurrent dead and sacrificed rats) in 24 months chronic / carcinogenicity groups.

	Controls		160 ppm		1600 ppm		16000 ppm	
Chronic toxicity	**20M**	**20F**	**20M**	**20F**	**20M**	**20F**	**20M**	**20F**
		20	20	20	20	20	20	20
W1-26	-	-	-	-	-	-	-	-
W27-52	-	-	-	-	-	-	1	-
W53-78	-	2	1	1	-	1	1	-

W79-105	3	5	1	4	2	7	1	4
Σ deaths	3	7	2	5	2	8	3	4
% deaths	*15*	*35*	*10*	*25*	*10*	*40*	*15*	*20*
Carcinogenicity	**50M**	**50F**	**50M**	**50F**	**50M**	**50F**	**50M**	**50F**
W1-26	-	-	-	-	-	-	-	-
W27-52	1	-	-	1	-	-	2	1
W53-78	2	2	2	2	1	7	-	3
W79-105	4	15	6	11	4	11	6	11
Σ deaths	7	17	8	14	5	18	8	15
% deaths	*14*	*34*	*16*	*28*	*10*	*36*	*16*	*30*

There were no overt signs of toxicity and no changes in general health and behaviour, in neurological condition, in teeth and oral mucosa, and in eyes which could be attributed to treatment; all observed clinical signs, palpable masses and/or ophthalmological lesions were considered as incidental changes which occurred at similar rates in all groups including controls (see table 4.10.1.1-3).

Table 4.10.1.1-3 Incidence of lens opacities (lens findings/n° of examined rats and percentage among groups)

	After 12 months		**After 24 months**			
	Interim toxicity study		**Chronic toxicity study**		**Carcinogenicity study**	
	M	F	M	F	M	F
Controls	0/10	0/10	9/20 *(45%)*	3/13 *(23%)*	25/43	13/34 *(38%)*
160 ppm	-	-	-	-	6/6	-
1600 ppm	-	-	2/2	1/1	2/2	2/2
16000 ppm	0/10	0/9	6/17 *(28%)*	5/16 *(31%)*	16/43	10/35 *(29%)*

Food consumption values for both male and female treated rats levels were generally comparable to that of controls. The mean daily intake of test material during the different study periods (w-1 to w-52 or w-1 to w-105-106) which was calculated on the basis of the mean weekly relative food consumption and nominal dose levels, is summarised in Table 4.10.1.1-4.

Table 4.10.1.1-4: Relative mean food consumption (g/kg bw/d) and mean daily intake of test material (mg/kg bw/d) during the study period

		Controls		160 ppm		1600 ppm		16000 ppm	
		M	F	M	F	M	F	M	F
Interim toxicity study									
Food consumption (d-d365)	g/kg bw/d	53.0	66.8	54.3	64.5	52.8	63.0	54.0	66.0
	% of controls			+2.4	-3.5	-0.5	-5.7	+1.9	-1.2
Mean substance intake	mg/kg bw/d			6.7	10.3	84.5	100.8	865.3	1056.0
Chronic toxicity study									
Food consumption (d-d729)	g/kg bw/d	46.3	61.1	47.2	59.8	47.3	60.2	48.4	59.5
	% of controls			+1.9	-2.0	+2.2	-1.4	+4.6	-2.5
Mean substance intake	mg/kg bw/d			7.6	9.6	75.7	96.4	774.3	952.6
Carcinogenicity study									
Food consumption (d-d729)	g/kg bw/d	46.6	60.5	46.6	58.7	46.1	59.2	47.8	59.5
	% of controls			+0.1	-3.0	-1.0	-2.2	+2.5	-1.7
Mean substance intake	mg/kg bw/d			7.5	9.4	73.8	94.7	764.0	952.3

No treatment related changes were noted for either sex in the urinalysis, haematological and clinical chemistry parameters (see tables 4.10.1.1.-5 and 6).

Table 4.10.1.1-5: Red blood parameters taken at different sampling time points during the 24-month treatment period

Parameter study	t	Males				Females			
		0 ppm	160 ppm	1 600 ppm	16 000 ppm	0 ppm	160 ppm	1 600 ppm	16 000 ppm
12 months interim toxicity group									
Erythrocytes (10^{12}/L)	I	8.61	8.52	8.61	8.77	7.08	7.04	7.23	7.18
Haemoglobin (g/L)	I	149	146	148	148	131	130	**138***	**136***
Haematocrit (U)	I	0.46	0.45	0.45	0.45	0.39	0.39	**0.42***	**0.40***
MCV (10^{-15}/L)	I	53	53	52	**51***	55	56	57	56
Reticulocytes (U)	I	0.024	0.026	0.025	0.027	0.029	0.028	0.028	0.029
24 months chronic toxicity group									
Erythrocytes (10^{12}/L)	1	8.60	8.72	8.46	8.78	7.97	7.90	8.02	7.99
	2	8.69	8.96	8.74	**8.98***	8.15	8.10	8.11	8.02
	3	8.97	9.16	9.06	9.02	7.86	7.97	8.10	7.84
	4	8.95	9.09	8.94	9.05	7.72	7.55	7.48	7.72
	F	8.43	8.43	8.20	8.09	6.85	7.03	7.40	7.07
Haemoglobin (g/L)	1	154	154	150	154	148	148	150	149
	2	149	150	148	151	142	145	141	139
	3	156	157	156	154	145	148	150	146
	4	163	165	161	162	148	148	149	151
	F	159	154	154	150	134	142	146	138
Haematocrit (U)	1	0.45	0.44	0.44	0.45	0.42	0.42	0.43	0.42
	2	0.47	0.48	0.47	**0.49***	0.46	0.46	0.46	0.45
	3	0.47	0.47	0.47	0.47	0.42	0.44	0.44	0.43
	4	0.47	0.48	0.47	0.47	0.43	0.43	0.42	0.43
	F	0.48	0.47	0.47	0.46	0.41	0.43	0.43	0.41
MCV(10^{-15}/L)	1	52	51	51	51	53	53	53	53
	2	54	53	54	55	56	57	57	56
	3	53	52	53	53	54	55	55	55
	4	53	52	53	52	55	57	57	56
	F	57	56	57	58	60	62	58	58
Reticulocytes (U)	1	0.023	0.025	0.022	0.021	0.018	0.020	0.019	0.022
	2	0.033	0.032	0.038	0.039	0.033	0.37	0.033	0.035
	3	0.029	0.028	0.026	0.028	0.023	0.024	0.027	0.027
	4	0.022	0.022	0.023	0.024	0.023	0.023	0.022	0.023
	F	0.029	0.028	0.026	0.030	0.031	0.046	0.034	0.032
Normoblasts	4	1	-	2	2	-	-	-	-

* Mean value of treated group statistically different from the control group

1, 2, 3, and 4 = 3, 6, 12, and 18 month values; I = final, 12 month values, F = final, 24 month values

Table 4.10.1.1-6: Serum parameters taken at different time points

Parameter study	V	Males				Females			
		0 ppm	160 ppm	1 600 ppm	16 000 ppm	0 ppm	160 ppm	1 600 ppm	16 000 ppm
12 months interim toxicity study									
Ca (mol/L)	I	2.40	**2.45***	**2.45***	**2.51***	2.42	2.49	2.41	2.50
Ph (mmol/L)	I	1.13	**1.45***	**1.69***	**1.68***	1.40	1.48	**1.68***	**1.80***
Urea (mmol/L)	I	6.71	6.14	**5.37***	**5.62***	6.54	5.65	5.32	5.90
ASAT (U/L)	I	47	46	**35***	**36***	68	56	54	67
24 months chronic toxicity study									
Sodium (mmol/L)	1	141	141	141	142	141	141	**144***	**142***
	2	148	150	149	150	148	148	**148***	**148***
	3	145	146	146	145	145	145	144	144
	4	144	144	144	145	143	143	144	143
	F	142	142	143	143	142	143	142	141
Chloride (mmol/L)	1	105	105	105	**106***	107	106	107	**108***
	2	107	108	107	108	105	106	**106***	**107***
	3	104	104	103	104	101	101	101	101
	4	104	104	104	**105***	101	102	103*	103*
	F	103	103	103	105	100	102	104	100
Phosphorus (mmol/L)	1	2.04	1.99	2.14	2.12	1.78	1.85	1.92	1.76
	2	2.08	2.19	2.23	2.22	1.64	1.51	1.52	1.65
	3	1.77	**2.20***	**2.24***	**2.00***	1.61	1.66	1.60	1.61
	4	1.61	1.57	1.57	1.63	1.46	1.48	1.41	1.46
	F	1.25	**1.45***	**1.54***	**1.55***	1.64	1.51	1.61	1.86
Bilirubin (µmol/L)	1	3.7	3.5	3.7	3.8	4.0	4.5	4.7	**5.4***
	2	4.5	4.1	4.3	4.1	4.6	4.7	4.2	4.8
	3	3.3	3.3	3.6	3.1	4.3	4.9	4.2	4.6
	4	4.4	4.2	4.6	4.5	4.6	4.8	4.6	5.0
	F	4.7	4.3	4.9	4.9	5.1	5.5	5.3	5.4
Glucose (mmol/L)	1	5.86	5.81	5.90	6.05	6.04	5.98	5.61	6.13
	2	6.39	6.18	6.19	6.62	6.19	6.67	5.84	5.94
	3	5.55	5.83	**6.15***	**6.12***	5.41	5.19	5.87	5.14
	4	5.40	5.30	6.20	6.00	5.11	6.00	5.30	5.60
	F	10.56	11.94	12.08	11.40	9.54	7.51	8.82	8.51
Creatinine (µmol/L)	1	41	41	42	43	39	39	42	42
	2	44	**48***	**49***	**49***	49	47	46	46
	3	43	**46***	**45***	**49***	41	43	42	**44***
	4	41	41	42	41	41	43	41	45
	F	58	46	47	44	47	49	48	46
Urea (mmol/L)	1	6.05	5.79	5.76	5.84	7.46	7.25	7.23	7.09
	2	5.83	5.77	6.00	6.00	7.46	7.66	8.13	**8.33***
	3	5.24	5.46	5.36	5.59	4.95	5.38	5.27	**5.84***
	4	5.37	5.12	5.28	5.07	6.03	6.46	6.12	6.91
	F	7.04	5.86	**4.93***	**4.56***	5.89	5.36	5.13	5.66
Protein (g/L)	1	64	63	64	64	65	66	69	**69***
	2	65	66	65	66	72	76	73	72
	3	69	68	70	68	75	76	74	76
	4	69	69	70	68	72	71	70	71
	F	60	58	62	60	63	64	59	64
Albumin (g/L)	1	34.5	33.2	34.1	34.2	38.1	39.1	**41.6***	**40.4***
	2	32.5	33.7	33.5	33.1	41.1	43.7	42.5	42.1
	3	36.1	35.7	36.7	36.7	43.0	44.8	43.2	43.9
	4	36.0	36.6	37.9	36.8	40.9	41.1	40.5	41.4
	F	28.8	27.6	29.8	29.1	31.6	32.6	29.8	33.0
ALAT (U/L)	1	40	42	36	39	36	33	43	34
	2	35	35	31	34	37	53	38	35
	3	44	46	37	39	39	39	38	35

Parameter study	V	Males				Females			
		0 ppm	160 ppm	1 600 ppm	16 000 ppm	0 ppm	160 ppm	1 600 ppm	16 000 ppm
	4	38	34	42	52	38	**32***	**27***	**29***
	F	40	46	44	36	30	30	24	33

* Mean value of group statistically different from the control group; 1, 2, 3, and 4 = 3, 6, 12, and 18 month values; I = final, 12 month values ; F = final, 24 month values

A few statistically significant changes were noted for various treated groups, in either absolute or relative (to bw) organ weights, mostly for one sex (higher absolute spleen weight and lower relative adrenals, lung, liver and brain weights in the 12 months interim toxicity study females; decreased relative liver weight in top dose males of the 24 months chronic toxicity and carcinogenicity studies). No dose response relationship was seen for these findings which were not correlated with clinical and histopathological changes (see table 4.10.1.1-7).

Table 4.10.1.1-7: Organ weight changes

12 months interim group						
Weight	**Organ**	**Sex**	**0 ppm**	**160 ppm**	**1600 ppm**	**16000 ppm**
Absolute (g)	Spleen	F	0.519	0.545	**0.613*** *(+18.1%)*	**0.616*** *(+18.7%)*
	Adrenal	F	0.0732	0.0678	0.0685	**0.0622*** *(-15.0%)*
Relative (g/kg bw)	Thyroid	M	0.0472	0.0437	**0.0383*** *(-18.9%)*	**0.0380*** *(-19.5%)*
	Lung	F	4.481	**3.940*** *(-12.1%)*	**4.017*** *(-10.4%)*	**3.923*** *(-12.5%)*
	Liver	F	33.193	33.638	33.436	**29.957*** *(-9.8%)*
	Adrenal	F	0.2729	**0.2284*** *(-16.3%)*	**0.2315*** *(-15.2%)*	**0.2139*** *(-21.6%)*
	brain	F	7.392	**6.939*** *(-5.7%)*	**6.887*** *(-6.8%)*	**6.886*** **(-6.9%)**
24 months chronic toxicity group						
Relative (g/kg bw)	Liver	M	31.517	31.497	30.925	**29.512*** *(-6.4%)*
	Lung	F	5.158	4.635	4.498	**4.449*** *(-13.8%)*
24 months carcinogenicity group						
Absolute (g)	Kidney	F	2.273	2.242	2.201	**2.091*** *(-8.0%)*
Relative (g/kg bw)	Heart	M	2.623	2.768	2.559	**2.774*** *(+5.8%)*
	liver	M	30.297	29.194	29.367	**28.856*** *(-4.8%)*

*: Significantly different from control ($p<0.05$)

There were no treatment related macroscopic findings of toxicological significance at any treatment level. All significant non neoplastic microscopic changes occurred with similar type, incidence and severity among all groups including controls and were primarily inflammatory, degenerative and/or hyperplastic changes which affected mainly the endocrine, the reproductive and large parenchymatous organs of the top dose groups (decreased incidence of liver glycogen deposits and clear cells altered foci in males, decreased incidence of cortical cystic/haemorrhagic degeneration of adrenals in females). The type, the incidence and the organ distribution of neoplastic changes and of primary neoplasms was similar among treated and control groups (see table 4.10.1.1-8).

Table 4.10.1.1-8. Summary of incidence of the most common neoplastic and neoplastic findings seen at terminal sacrifice in the 24 months carcinogenicity study groups (50 rats/sex/dose)

	Males				Females			
Test material (ppm)	**0**	**160**	**1600**	**16000**	**0**	**160**	**1600**	**16000**
Adrenal gland								
Focal fatty change	17	4	1	17	4	1	-	9
Degeneration:cystic/hemorrhagic	-	1	2	1	34	9	12	20
Focal hyperplasia	1	-	-	1	-	1	-	1
Focal hypertrophy	-	-	-	1	1	-	1	-
Kidney								
Nephropathy chronic progressive	12	17	26	18	6	4	3	2
Mineralisation: pelvic/caliceal	5	5	4	2	10	15	12	16
Tubular degeneration/regeneration	15	6	4	8	2	4	2	1
Pelvic dilatation	-	6	5	2	2	5	3	6
Transitional cell hyperplasia	1	-	-	1	4	3	3	3
Cortical cysts	-	2	2	2	-	-	1	-
Liposarcoma	0	0	1	1	0	0	0	0
Malignant mesenchymal	0	1	0	0	0	0	0	1
Liver								
Clear cell foci	22	12	25	12	3	6	8	4
Basophilic cell foci	2	5	7	7	3	8	7	7
Hypertrophy	-	1	-	-	-	-	-	1
Bile duct hyperplasia	21	15	14	16	9	18	12	13
Increased glycogen deposits	19	15	13	9	3	7	6	4
Focal necrosis	-	1	-	-	2	3	3	3
Hepatocellular Adenoma	0	0	0	0	0	1	0	0
Lung								
Alveolar haemorrhage	2	2	-	2	7	2	2	8
Congestion	4	3	2	3	2	7	5	3
Thyroid								
Follicular cell hyperplasia	10	4	-	6	1	1	2	-
Focal C cell hyperplasia	6	1	-	3	2	-	-	1
Diffuse C cell hyperplasia	2	-	-	4	nd	nd	nd	nd
Follicular cell adenoma	3	0	1	3	1	1	3	0
C cell adenoma	1	0	0	1	1	0	1	3
Follicular cell carcinoma	0	0	2	0	0	0	1	0
Spleen								
Increased haematopoiesis	3	2	1	8	14	8	6	11

No carcinogenic effect of test substance was demonstrated; the NOAEL in this combined chronic toxicity/carcinogenicity study was 16000 ppm and was equivalent to an average daily intake of 865 and 1056 mg/kg bw/d (chronic toxicity group) and 764 and 952 mg/kg bw (oncogenicity group) of test substance in the males and females, respectively.

In a mouse carcinogenicity study groups of 120 (60/sex) CD-1 mice (Crl:CD-1® (ICR)BR strain) were administered 0; 80; 800 and 8000 ppm of AE F130060 test substance technical (94.6 % purity) in the diet for 52 consecutive weeks (10/sex/dose for the interim toxicity group) and consecutive 78 weeks (50/sex/dose for the carcinogenicity study group). All animals were observed twice daily for signs of overt-toxicity, behaviour, general health condition and mortality; examinations for neurological disturbances, impairment of dental growth and eye changes as well as palpation of skin for nodules were performed monthly. Individual body weights and food consumption were recorded weekly. Haematological parameters were determined at week 52 (5 mice/sex/dose from interim toxicity group and 50 mice/sex/dose from the carcinogenicity group) and at weeks 79-80 (50 mice/sex/dose from the carcinogenicity study); clinical chemistry parameters were determined at week 52 on 5 mice/sex/dose from interim toxicity group. At termination, all surviving animals were sacrificed for gross necropsy and selected organs were weighed; gross necropsy was also performed on unscheduled deaths. Extensive histopathological examinations were carried out on all tissues from the control and high dose groups, on all gross lesions, and on the kidney, the liver, and the lung from all low and mid dose groups at terminal sacrifice, as well as on a full spectrum for tissues from unscheduled dead animals.

Survival was not affected by the treatment; in the 24 months carcinogenicity study, the mortality rate was low and rose, as expected, during the 2nd year (see table 4.10.1.1-9). The cause of death did not distinguish treated mice from controls.

Table 4.10.1.1-9: Summary of mortality and survival rates (intercurrent dead and sacrificed mice) in 80-w carcinogenicity study

	Controls		**80 ppm**		**800 ppm**		**8000 ppm**	
Carcinogenicity	**50M**	**50F**	**50M**	**50F**	**50M**	**50F**	**50M**	**50F**
W1-26	1	0	0	0	0	0	0	0
W27-52	2	4	1	1	0	1	1	2
W53-80	8	5	4	8	7	7	6	10
Σ deaths	11	9	5	9	7	8	7	12
% deaths	*18.3*	*15.0*	*8.3*	*15.0*	*11.7*	*13.3*	*11.7*	*20.0*

There were no overt signs of toxicity, and no changes in general health and behaviour, in neurological condition, in teeth and oral mucosa, and in eyes which could be attributed to treatment; all observed findings were considered as incidental changes which occurred at similar rates in all groups including controls. Palpation of skin for nodules and skin masses did not reveal significant treatment related changes.

Food consumption values for both male and female mice were generally comparable to that of controls (see table 4.10.1.1-10).

Table 4.10.1.1-10: Relative mean food consumption (g/kg bw/d), mean daily intake of test material (mg/kg bw/d) and mean bw gain during the study period

Carcinogenicity study	Controls		80 ppm		800 ppm		8000 ppm	
	M	F	M	F	M	F	M	F
Food consumption (d1-d547)								
g/kg bw/d	132.8.	168.8	131.8	173.26	128.5	162.2	133.7	169.5
% of controls			-0.7	+2.6	-3.3	-3.9	+0.6	+0.4
Mean substance intake (mg/kg bw/d)			**10.6**	**13.9**	**102.8**	**129.8**	**1069.4**	**1355.6**
Bw gain (g & difference with controls in %)								
Day –0	25.8	20.7	25.6 (-0.78)	19.9 (-3.86)	26.0 (+0.78)	19.7 (-4.83)	25.5 (-1.16)	21.3 (+2.90)
Day- 85	35.1	26.8	34.5 (-1.71)	26.7 (-0.37)	34.9 (-0.57)	26.9 (+0.37)	**34.0 (-3.13)***	**26.7 (-0.37)***
Day-176	39.1	28.9	**37.8 (-3.32)***	29.1 (+0.69)	**38.1 (-2.56)***	29.1 (+0.69)	**37.6 (-3.84)***	**28.6 (-1.04)***
Day-358	40.6	30.6	39.8 (-1.97)	30.4 (-0.65)	40.2 (-0.99)	30.3 (-0.98)	**39.2 (-3.45)**	**29.2 (-4.58)***
Day-547	41.5	32.1	40.4 (-2.66)	31.6 (-1.56)	41.1 (-0.97)	31.2 (–2.81)	**39.3 (-5.30)***	**31.0 (-3.43)***
Days 1-547	15.7	11.4	14.8 (-5.7 %)	11.7 (+2.6 %)	15.1 (-3.8 %)	11.5 (+0.9 %)	13.8 (-12.1%)	9.7 (-14.9 %)

*: Significantly different from control ($p<0.05$)

At 18 months, slight but statistically significant increases in mean RBC count and Hb and decrease in mean MCV were observed in the top dose males only. The statistically significantly increased WBC counts in top dose males and females and in mid-dose males at 18 months (but not at 12 months interim evaluation) could be treatment related as far as some dose dependency was observed in males; this change, which was slight and not corroborated by any clinical and histopathological changes, should be regarded of limited toxicological significance; Moreover, leukocytes counts were decreased in the mouse-90d toxicity study for dose levels ≥ 1000 ppm in males and 7000 ppm in females (i.e. ≥ 176 and 1603 mg/kg bw, respectively). All other haematological parameters did not differ significantly from controls or were inside the expected biological ranges and not considered treatment-related. At the 12 and 18 months examination of the differential blood counts, no differences were observed between the animals of the treatment groups and the control group (see table 4.10.1.1-11). No statistically significant or biologically relevant differences were observed in clinical chemistry examinations. At interim and terminal sacrifice the overall absolute mean organ weights of treated mice did not differ from those of controls (see table 4.10.1.1-12).

Table 4.10.1.1-11: Selected haematological parameters after 12 / 18 months

	Dose group	**0 ppm**		**80 ppm**		**800 ppm**		**8 000 ppm**	
	Time points	**12 months**	**18 months**	**12 months**	**18 months**	**12 months**	**18 months**	**12 months**	**18 months**
Males	Red blood cells (10^{12}/L)	7.80	8.75	8.18	8.74	7.89	8.69	7.96	**9.18** $^{+}$
	Haemoglobin (g/L)	123	138	127	138	121	137	124	**142** $^{+}$
	Haematocrit [U]	0.40	0.42	0.41	0.42	0.39	0.41	0.40	0.43
	MCV (10^{-15}/L)	51	48	50	48	50	48	50	**46** $^{-}$
	MCHC (g/L)	308	329	307	332	307	332	309	334
	Leucocytes (10^{9}/L)	2.8	3.5	2.8	3.5	1.9	**4.3** $^{+}$	2.5	**4.7** $^{+}$
Females	Red blood cells (10^{12}/L)	8.30	8.51	8.78	8.14	8.93	8.49	8.08	8.74
	Haemoglobin (g/L)	135	135	140	130	140	137	136	140
	Haematocrit [U]	0.42	0.41	0.44	0.40	0.45	0.41	0.45	0.41
	MCV (10^{-15}/L)	51	48	51	49	50	49	**58** $^{+}$	47
	MCHC (g/L)	318	331	314	327	313	334	**298**	338
	Leucocytes (10^{9}/L)	1.8	3.8	2.6	3.8	3.1	3.2	**6.1**	**5.9** $^{+}$

$^{+-}$ Significantly different from control (Wilcoxon test. Two-tailed test for the highest does and one-tailed tests for lower doses, if all higher doses are significantly different from control ;$p < 0.05$).

Table 4.10.1.1-12: Organ weights at final sacrifice (after 12-month treatment)

Organ Weight		**0 ppm**		**80 ppm**		**800 ppm**		**8000 ppm**	
		Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Heart	Absolute (g)	0.23	0.17	0.22	0.16	0.21*	0.16	0.21*	0.16
	Relative (g/kg bw)	5.57	5.18	5.54	5.14	5.2	5.0	5.33	5.05
Brain	Absolute (g)	0.49	0.50	0.50	0.49	0.50	0.50	0.50	0.50
	Relative (g/kg bw)	12.07	15.85	12.48	15.80	12.32	16.09	12.73*	16.38
Liver	Absolute (g)	1.96	1.54	1.85	1.50	1.86	1.53	1.87	1.42
	Relative (g/kg bw)	47.44	47.75	45.77	47.58	45.52	48.91	47.78	45.93
Kidney	Absolute (g)	0.69	0.43	0.70	0.43	0.69	0.41	0.68	0.40
	Relative (g/kg bw)	16.70	13.50	17.36	13.61	16.78	13.24	17.34	13.19

*: Significantly different from control ($p<0.05$)

There were no compound-related non-neoplastic findings in any dose group during histopathology at interim sacrifice.

At terminal sacrifice several non-neoplastic lesions achieved statistical significance in some occasions in high dose mice; in males (see table 4.10.1.1-13), a positive trend with significant p-value was observed for pancreatic lymphoid cell infiltration (but no dose dependent pattern was seen), in females, a negative trend was found for liver single cell necrosis (no dose dependent pattern) and a positive trend for liver diffuse fatty acid changes (occurring in lower incidence in the low and mid dose groups; incidence in top dose females lower than that of controls). In both sexes, lesions which were found in the liver, in the lung, in the duodenum, in the jejunum and in the ovaries, included amyloidosis, and were mainly age-related. Additionally, several lesions (splenic lymphoid hyperplasia in males, ovarian follicular cysts and thyroid follicular dilatation in females) occurred in lower incidence in high dosed mice than in controls. All these changes could be considered as incidental.

Table 4.10.1.1-13: Incidence of non-neoplastic lesions in selected organs: final sacrifice (50 animal/sex/dose group)

	Males				Females			
	Dose levels (ppm)				Dose levels (ppm)			
Organ	**0**	**80**	**800**	**8 000**	**0**	**80**	**800**	**8 000**
Brain								
Gliosis	17	1	4	25	20	4	2	25
Kidneys								
Infiltration: lymphoid cell	42	36	40	39	27	30	35	27
Hyperplasia: tubular; basophilic	30	31	37	35	7	13	11	12
Cysts: tubular	11	11	11	8	10	7	8	11
Casts: tubular	11	20	16	19	12	13	14	16
Nephropathy: chronic progressive	2	5	4	5	7	7	6	11
Amyloidosis	6	5	5	9	8	10	6	4
Vacuolation: tubular cell	1	2	2	2	0	0	2	0
Liver								
Infiltration: lymphoid cell	26	31	29	29	28	33	35	32
Increased hepatocytic glycogen deposits	1	4	4	4	0	0	0	0
Fatty change diffuse	19	5	11	16	42	14	11	36
Amyloidosis	5	4	5	9	8	10	4	3
Necrosis: single cell	2	3	5	4	6	3	6	1
Necrosis: focal	0	4	0	2	7	5	4	7
Lung								
Infiltration: lymphoid cell	3	8	7	7	5	14	9	10
Alveolar hystiocytosis	2	2	8	4	6	3	12	3
Amyloidosis	2	1	2	3	4	2	1	0
Congestion	7	3	4	3	6	3	2	4
Haemorrhage	9	4	7	7	10	4	6	7
Pancreas								
Infiltration: lymphoid cells	4	0	0	7	7	0	0	5
Hyperplasia: Islet cells	7	10	9	5	4	7	8	9
Adrenal cortex								
Amyloidosis	8	1	2	8	8	0	1	3
Hyperplasia: A cells	12	2	2	16	42	7	7	46

A number of neoplastic lesions were diagnosed but did not differ significantly between treated and control groups. Test substance did not exhibit any carcinogenic potential in mice (see table 4.10.1.1-14).

Table 4.10.1.1-14 Neoplastic lesions (incl. deaths, final sacrifice)

	Males				Females			
	0	**80**	**800**	**8 000**	**0**	**80**	**800**	**8 000**
No. examined	50	50	49	50	49	50	50	50
No. animals with neoplasm	15	13	8	16	26	18	15	21
No. animals with more than 1 primary neoplasm	1	0	1	2	7	2	2	2
No. animals with metastases	0	0	0	0	2	2	1	0
No. animals with								
Benign T.	11	6	3	13	12	7	6	8
Unclassified T.	0	0	0	0	0	0	0	0
Malign T.	4	7	6	5	21	12	11	14
Lung								
Adenoma: bronchioalveolar	5	4	1	3	4	2	1	3
Adenocarcinoma: bronchioalveolar	3	2	3	3	1	2	1	1
Liver								
Haemangioma	0	0	0	1	0	0	0	1
Adenoma: hepatocellular	3	0	1	3	1	1	1	1
Carcinoma: hepatocellular	0	3	1	1	1	0	0	0

Dietary administration of up to and including 8 000 ppm of AEF 130060 to mice for up to 18 months was not tumorigenic and did not cause non-neoplastic lesions.

The dose level of 800 ppm is considered to be the 'no observed adverse effect level' (NOAEL) in this study, based on lower body weight gains in females at the high dose level. This is equivalent to a mean achieved intake of 103 and 130 mg test substance/kg body weight/day in males and females, respectively.

4.10.1.2 Carcinogenicity: inhalation

No information available.

4.10.1.3 Carcinogenicity: dermal

No information available.

4.10.2 Human information

No information available.

4.10.3 Other relevant information

The lack of oncogenic potential of Mesosulfuron-methyl (AE F130060) was supported by the absence of genotoxic activity, as determined by results from a battery of 4 *in vitro* and 1 *in vivo* genetic toxicity tests.

4.10.4 Summary and discussion of carcinogenicity

In the combined chronic toxicity and oncogenicity study in rats, continuous dietary treatment for 106 weeks with dose levels of up to 16 000 ppm, which approximated to the international regulatory limit dose of 1000 mg/kg bw/day, did not produce any evidence of toxicity or oncogenicity during their natural lifespan. Similarly in mice, dietary treatment with up to 8 000 ppm (ca. 1000 mg/kg bw/d) for 80 consecutive weeks provoked no evidence of oncogenic activity.

Taking into account the studies available, there is no indication that Mesosulfuron-methyl is carcinogenic.

4.10.5 Comparison with criteria

Taking into account the studies available in rats and mice, classification for carcinogenicity is not justified.

4.10.6 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Carcinogenicity was investigated in two GLP carcinogenicity studies, one dietary combined chronic and oncogenicity study in the rat conducted in accordance with OECD TG 453 and one dietary mouse oncogenicity study conducted according to OECD TG 451.

Rat

In the rat study, continuous dietary treatment for 106 weeks with dose levels of up to 16,000 ppm, which approximated to the international regulatory limit dose of 1,000 mg/kg bw/d did not produce any evidence of toxicity or oncogenicity during the natural life span of the animals.

A few statistically significant changes in absolute and/or relative organ weights were noted mostly for one sex, with relatively strong effects on adrenal and lung weights.

At the interim sacrifice, the relative lung weight was statistically significantly decreased in all dosed females, ranging between 10.4% to 12.5%, but not in a dose-manner. At the end of the study, a statistically significant decrease in lung weight was only seen in high dose females (-13.8%).

Absolute and relative adrenal weight loss was observed at the interim sacrifice only in females. Statistical significance was reached at the highest dose level for the absolute

weight and at all doses for the relative weight. The decreases ranged between 15% and 21.6%; no dose-response relationship was observed.

As there was no clear dose-response relationship and no corroborating histopathological findings the organ weight effects were considered not adverse.

Mouse

Dietary treatment with up to 8,000 ppm (ca. 1,000 mg/kg bw/d) for 80 weeks did not produce any evidence for an oncogenic potential. Survival rates were equal in all groups, only body weight gain was slightly reduced in the high dose females. As a result resulting the NOAEL was set at 103 and 130 mg/kg bw/d in males and females, respectively.

Slight but statistically significant increases in the mean RBC count and Hb, and a decrease in mean MCV, were observed in high dose males only. A statistically significant increase in WBC counts in high dose males and females and in mid dose males at 18 months (not seen at 12 months) could be treatment related, since some dose dependence was seen in males. However, this change was slight and not corroborated by any clinical or histopathological changes and was therefore regarded as of limited toxicological significance. Moreover, it should be noted that leuckocyte counts were decreased in the mouse 90-day toxicity study for dose levels ≥ 176 mg/kg bw/d in males and at 1,603 mg/kg bw/d in females.

No other relevant toxicological findings were reported and there was no evidence for an oncogenic potential of mesosulfuron-methyl.

The available studies in mouse and rat gave no indication that mesosulfuron-methyl is carcinogenic.

The lack of oncogenic potential of mesosulfuron-methyl is supported by the absence of genotoxic activity, as determined by results from a battery of 4 *in vitro* and 1 *in vivo* genotoxicity tests.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

In TG compliant studies in 2 species no evidence of carcinogenicity or pre-neoplastic lesions was observed. Based on the available data, RAC agrees with the DS that **no classification for carcinogenicity** is warranted.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Table 4.11.1-1: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat two-generation feeding reproduction toxicity Groups of 50 (25/sex) Sprague Dawley rats (Hsd:SD strain; 6 - 8 w old at start of study) Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) Dose levels: 0; 160; 1600 and 16000 ppm in the diet OECD 416;1981	The NOAEL for reproductive toxicity (parental, reproductive and development) was 16000 ppm equivalent to an average daily intake of test substance of: • 1175 mg/kg bw/d in the males. • 1388 mg/kg bw/d in the females.	No adverse effects were indicated from the evaluation of parental or neonatal parameters and no treatment related effects on reproductive performance were noted at dietary levels up to and including 16000 ppm.	M-198366-01-1; 2000 (Horstmann G., 2000a)

4.11.1.1 Non-human information

In a 2-generation study in rats, groups of 50 (25/sex) Sprague Dawley rats were administered dietary concentrations 0; 160, 1600 and 16000 ppm of AE F130060 (94.6% purity) through 2 generations; P1 and F1 generation animals (25/sex/generation) were treated over a 10-week pre-mating period or following weaning, throughout mating and gestation with 2 litters including a 2-3 week rest period between the 2 cohabitation periods, until sacrifice after 3w of the final lactation period; duration of treatment was approximately 26-28 weeks and 26-31 weeks in P and F1 generation rats, respectively. All females were allowed to deliver their litters and rear the pups for 21 days.

The same mating procedure was applied for producing litters in each generation; after 10-weeks premating treatment period, a female was co-housed with a male of the same treatment group for 14 days or beyond that until 3 oestrus cycles had elapsed. On day of evidence of mating (microscopic observation of sperm in the vaginal smear) which was defined as day-0 of gestation, female returned to her individual cage.

Parental animals (P1 & F1) were observed twice daily for mortality and signs of toxicity (behaviour and general condition); food consumption was monitored daily and recorded weekly, except during the cohabitation period; determination of body weights were performed weekly and on day18 of the 2nd gestation period for F1 dams, and on day 4 of lactation period for all dams.

In P and F1 parental generation, males were killed when most of the inseminated females had delivered (when most of their female partners had reared the second litter F1b and F2b, respectively) and dams were sacrificed within 2 days after weaning of the last litter (dams without litter were sacrificed approximately 6 weeks after completion of the mating period). All parental rats were given a gross post-mortem examination; reproductive tissues and selected organs were collected, weighed and preserved for microscopic examination which was performed on all P and F1 adults from the control and high dose groups (no histological examination was carried out for the low and mid dose groups, since no treatment related histological changes were seen in the high dose group) as well as on all inter-currently dead rats and on all organs and tissues exhibiting gross macroscopic changes. Uterine implantation scars were counted in females failing to deliver for either pairing. Primordial follicles in the ovaries from 10 control and high dose F1 selected females were counted.

Sperm assessments were carried out on semen samples collected at necropsy in all surviving P and F1 males, testicular spermatids and caudal spermatozoa were counted using deep frozen left testes and caudal epididymides, respectively.

Litter size, number of live and dead pups were recorded as soon as possible after delivery along with pups body weight, sex distribution, external abnormalities; during lactation, sex was verified on days 4; 7; 12 and 21; individual pup body weights were recorded on postnatal days 4 and 7 and once weekly thereafter. On lactation day 4, all F1a litters with more than 8 pups were reduced (random selection) to equalize sex distribution (4/sex) when possible (litters with fewer than 8 pups were not adjusted for sex distribution). Sexual development was evaluated by determining the balano-preputial separation in the males and the vaginal opening in the females.

All F1a, F1b (except those selected to become the F1 adult generation), F2a and F2b pups were killed on day 21 of lactation and given a gross post-mortem examination; reproductive and selected organs were preserved. Microscopic examination and organ weights were carried out on 1 randomly selected pup/sex/litter (the histological examination performed on the selected organs of the F2b pups from the control and high dose groups, as well as on all organs/tissues exhibiting macroscopic changes of all groups); since no treatment related histological changes were found in the high dose group, the low and mid dose F2 pups and all F1 pups were not examined histologically.

There was no effect of treatment on mortality of the parental P1, F1 generations. There were no clinical findings that could be attributed to treatment in any of the P1 and F1 parental animals.

Mean weekly food consumption for males and females of both parental generations was either comparable to controls or slightly higher than controls (high dose P males only) or exhibited slight variations which were not directly related to the body weight gain during the various phases of the study (see table 4.11.1.1-1).

Table 4.11.1.1-1: Mean substance intake (mg/kg bw)

	P generation			F1 generation		
	160 ppm	1600 ppm	16000 ppm	160 ppm	1600 ppm	16000 ppm
Males						
Premating period litter a						
w-1	15.5	150.0	1462.0	31.8	288.1	2881.7
w-10	9.4	61.2	945.7	10.8	105.9	1073.7
w 1-10	11.7	115.3	1175.2	16.9	163.3	1664.9
Pre-mating period litter b						
w-1	8.4	82.7	848.1	8.7	85.4	876.6
w-2	8.1	80.8	840.4			
post-mating period litter a						
w-1	9.0	92.5	964.7	10.3	101.1	1053.4
w-6	8.9	87.8	899.7	8.9	95.0	950.5
w 1-6	8.8	87.7	905.4	9.4	95.9	978.1
Post-mating period litter b						
w-1	8.6	86.6	894.0	9.8	96.6	1000.5
w-7	7.5	80.4	804.0	7.8	79.5	812.1
w 1-7	8.0	81.4	839.1	8.7	85.7	885.5
Females						
Premating period litter a						
w-1	14.7	148.5	1483.4	30.4	291.4	2983.8
w-10	11.7	120.6	1277.0	12.8	126.0	1293.1
w 1-10	13.5	132.6	1387.6	17.5	172.0	1753.4
Pre-mating litter b						
w-1	12.0	121.8	1277.2	14.7	136.7	1334.5
w-2	11.2	115.0	1159.4			
Pregnancy litter a						
w-1	14.2	140.6	1417.5	13.9	138.2	1432.4
w-2	12.8	127.2	1305.3	12.8	138.3	1388.0
w-3	11.4	114.7	1160.4	12.1	120.7	1317.8
Pregnancy litter b						
w-1	13.4	135.3	1335.8			
w-2	12.7	130.2	1319.3			
w-3	11.2	116.0	1159.6	15.0☆	141.9☆	1428.1☆
Lactation litter a						
d- 0-4	18.4	158.5	1582.6	16.5	171.8	1588.8
d- 4-7	23.1	225.1	2129.4	23.1	222.8	2100.6
w-2	27.7	272.0	2682.0	29.2	294.3	2974.1
w-3	36.5	364.4	3670.7	36.0	356.0	3506.0
Lactation litter b						
d- 0-4	15.8	152.8	1470.3	17.9	172.8	1833.7
d- 4-7	19.8	207.0	1993.5	21.8	209.8	1152.4
w-2	25.4	259.0	2436.7	24.4	243.5	2725.8
w-3	32.1	319.4	3073.3	29.2	282.3	3173.1
☆ d18-21						

Mean weekly body weight gain for both sexes of both parental generations were comparable to those of controls during most of the phases of the study (see table 4.11.1.1-2).

Table 4.11.1.1.-2: Bw and bw gain during the various phase of the study

Dose levels	0 ppm		160 ppm		1600 ppm		16000 ppm	
	P	F1	P	F1	P	F1	P	F1
Males								
Premating bw (g)								
d-0	238.8	44.9	239.4	45.3	241.1	46.9	245.0	46.3
d-70	407.3	392.5	417.3	395.6	411.4	402.2	407.0	388.3
Females								
Premating bw (g)								
d-0	185.2	42.8	183.8	42.6	181.8	45.0	182.9	54.0
d-70	242.0	241.4	243.8	234.2	242.4	232.5	242.9	237.8
Gestation bw changes (g)								
Litter a								
d- 1-7	22.7	24.5	24.8	24.5	24.0	26.9	25.4	26.2
d-7-14	23.9	26.1	23.5	24.6	24.9	26.6	23.0	28.2
d-14-21	82.6	86.6	78.9	79.3	85.2	82.3	77.9	82.8
Litter b								
d- 1-7	22.8	14.4	24.4	13.4	24.1	13.3	23.9	15.2
d-7-14	20.7	24.1	22.8	20.6	21.0	22.6	21.7	23.5
d-14-21	76.5	97.0	87.6	87.7	86.6	81.1	77.0	96.5

There was no test substance related effect on sperm motility in any of the treated groups of P and F1 parental males (see table 4.11.1.1-3). There was a suspicion of test substance related effect on reproductive performance in P and F1 generations, due to lower than expected initial pregnancy rates, but following a second cohabitation using the same parental pairs, pregnancy rates were comparable with controls in all dose groups. Therefore, fertility concerns were disregarded. All pups selected to become the F1 parental generation exhibited a normal development of genitalia and there was no statistically significant difference in the mean-time interval for preputial separation or vaginal opening. Mating indices for both males and females, pregnancy rates, male fertility indices, gestation indices and parturition indices were unaffected by treatment (see table 4.11.1.1-4). For both generations, pregnancies and deliveries were not impaired by test substance; the mean gestation length for the treated and control groups were comparable. There were no treatment related gross macroscopic organ changes nor histological alterations of the reproductive organs in either the P and F1 generation parental rats.

Table 4.11.1.1-3: results of sperm analysis in P and F1 parental males

Dose group	0 ppm		160 ppm		1600 ppm		16000 ppm	
Generation	**P**	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
Progressively moving forward spermatozoa (%)	29.7	17.2	29.9	21.3	28.5	22.1	30.2	19.2
Locally motile sperm (%)	58.9	57.3	61.0	64.2	62.6	59.7	58.5	63.7
Sperm count								
Total testicular spermatids ($x10^8$)	2519	2667					2447	2608
Spermatids ($x10^8$)/g fresh tissue	1333	1403					1319	1380
Total spermatozoa in cauda epididymides ($x10^8$)	1446	1389					1357	1418

Spermatozoa ($x10^8$) / g fresh tissue	5335	5625					5369	5717

Table 4.11.1.1-4: Mating and reproductive performance

P generation	Litter F1a				Litter F1b			
	Control	160 ppm	1 600 ppm	16 000 ppm	Control	160 ppm	1 600 ppm	16 000 ppm
Females	n = 24	n = 25	n = 25	n = 25	n = 23	n = 24	n = 25	n = 25
Females inseminated 1st oestrus	13	20	14	20[1]	18	18	20[2]	18[1]
2nd oestrus	10	5	10	5	5	4	5	7
3rd oestrus	1	0	0	0	0	1	0	0
4th oestrus	0	0	1	0	0	0	0	0
5th oestrus					0	1	0	0
Female mating index (%)	100	100	100	100	100	100	100	100
Females impregnated	21	18	21	22	20	20	20	23
Pregnancy index (%)	88	72	84	88	87	83	80	92
Females littering	20	17	21	22	20	20	20	22
Mean gestation length (d)	23.2	23.3	23.0	23.2	23.3	23.3	23.1	23.2
Females rearing live pups until d- 21 of lactation	20	16	20	21	19	19	20	20
Males	n = 25[3]	n = 25	n = 25	n = 25	n = 25[3]	n = 25[3]	n = 25	n = 25
Males inseminating	25	25	25	25	25	25	25	25
Male mating index (%)	100	100	100	100	100	100	100	100
Males impregnating	22	18	21	22	22	21	20	23
Male fertility index (%)	88	72	84	88	88	84	80	92

[1] females inseminated without spermatozoa being detected
[2] 2 of these females were inseminated without spermatozoa being detected.
[3] Due to death of 1female, 1male paired with an untreated female and mated

F1 generation	Litter F2a				Litter F2b			
	Control	160 ppm	1 600 ppm	16 000 ppm	Control	160 ppm	1 600 ppm	16 000 ppm
Females	n = 25	n = 25	n = 25	n = 25	n = 25	n = 25	n = 25	n = 25
Females inseminated 1st oestrus	14	11	19	20	14	18	15[3]	11
2nd oestrus	3	9	3	3	9[3]	6[b]	5	12[b]
3rd oestrus	5	3	2	1	2[a]	1	2	0
4th oestrus	1	2	0	0	0	0	0	0
5th oestrus					0	0	0	1
Female mating index (%)	92	100	96	96	100	100	88	96
Females impregnated	22	24[1]	17	13	24	24	22	24
Pregnancy index (%)	96	96	71	54	96	96	100	100
Mean gestation length (d)	23.1	23.4	23.1	22.8	22.9	23.1	23.1	23.1
Females littering	22	23	17	13	24	24	22	24
Females rearing live pups until d- 21 of lactation	22	21	17	13	23	22	19	21
Males	n = 25	n = 25[2]	n = 25[2]	n = 25[2]	n = 24 +1[c]	n = 25[2]	n = 25[2]	n = 25[2]
Males inseminating	23	25	24	24	24	25	22	24
Male mating index (%)	92	100	96	96	100	100	88	96
Males impregnating	22	24*	18	13	23	24	22	24
Male fertility index (%)	96	96	75	54	96	96	100	100

[1] In 1 female, bw gains during mating period and decline of bw from d-21 (344g) to d-28 (304g) following insemination suggested undetected delivery. One of the 24* males was the allocated one (this pair was excluded from summary and statistics).
[2] In order to avoid sibling mating, one of these males was paired with an untreated female.
[3] One of these was inseminated without spermatozoa being detected

a The allocated male of 1 of these dams died during pairing and the dam was inseminated by a replacement male from the same group.
b One of these dams was probably impregnated without spermatozoa being detected in 1st oestrus.

c Died during pairing without impregnating the allocated female.

No treatment-related effects were detected in the F1a, F1b, F2a, and F2b offspring (see table 4.11.1.1-5). The mean number of live and dead pups at birth were comparable in all groups from all litters. At birth, the F1a, F1b, F2a and F2b pups (live and stillborn) from all treated groups were normally developed and had normal body weight; no test substance related effect was seen on sex ratio and on external abnormalities in any of the litters. During lactation, mortality was comparable in all groups including controls.

Table 4.11.1.1-5: Litter data (mean)

Dose levels	**0 ppm**				**160 ppm**				**1600 ppm**				**16000 ppm**			
Litters	**F1a**	**F1b**	**F2a**	**F2b**	**F1a**	**F1b**	**F2a**	**F2b**	**F1a**	**F1b**	**F2a**	**F2b**	**F1a**	**F1b**	**F2a**	**F2b**
Mean n° of pups at birth																
Live	12.3	10.9	13.0	13.0	11.9	11.9	12.5	12.2	12.0	11.3	12.0	11.2	11.9	10.5	11.8	12.8
dead	1.25	0.84	1.45	1.33	1.81	1.40	0.33	0.54	1.76	1.20	0.76	0.86	0.95	0.80	1.00	1.29
Dead/live (%)	10.2	11.6	11.2	10.2	16.3	11.8	2.7	4.5	14.7	10.6	6.4	7.7	8.4	13.2	8.4	10.1
Mortality during lactation																
d-1	4	1	11	5	3	10	5	3	7	2	0	1	7	7	2	16
d-2	3	1	1	1	1	3	3	1	1	1	1	0	2	1	0	3
d-3	0	0	3	0	0	1	1	2	1	0	0	0	2	1	0	3
d-4	2	0	1	1	0	0	2	10	2	0	2	3	0	1	0	1
Total d-1-4	9	2	16	7	4	14	11	16	11	3	3	4	11	10	2	23
Dead/live pups at birth (%)	3.7	1.0	5.6	2.2	2.2	5.9	4.2	5.5	4.4	1.3	1.5	1.6	4.4	4.5	1.3	7.5
Total deaths d5-21	2	6	0	55	2	9	2	52	3	3	3	48	3	11	0	44
Dead/live after culling d-4 (%)	1.3	4.1	0.0	29.3	1.6	6.0	1.3	28.7	1.8	2.0	2.3	31.2	1.9	7.3	0.0	23.9
Pup weights (g)																
d-0	6.3	6.6	6.3	6.4	6.2	6.5	6.3	6.5	6.3	6.3	6.4	6.3	6.3	6.4	6.4	6.5
d-7	15.4	15.1	16.0	15.3	15.2	16.8	15.6	15.5	14.5	12.4	15.3	12.6	15.3	13.3	14.6	13.3
d-21	42.8	44.5	44.5	45.2	43.7	47.0	44.1	46.1	45.3	42.6	47.7	44.9	47.6	46.5	45.9	45.9

No treatment related macroscopic findings were observed in the F1a, F1b, F2a and F2b pups sacrificed on day 4 (culling) or on postnatal day 21. No test substance related microscopic changes were seen in the F2b pups. All the litters examined on post-natal d-21 exhibited an age-dependent state of development of their genital organs. Alterations seen in the other tissue and organ systems of the parental animals and pups were also confirmed to be strain-dependent or spontaneous in origin.

No adverse effects were indicated from the evaluation of parental or neonatal parameters and no treatment related effects on reproductive performance were noted at dietary levels up to and including 16000 ppm. The NOAEL for reproductive toxicity (maternal and development) was 16000 ppm i.e. approximately 1175 and 1388 mg/kg bw/d in males and females, respectively.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

Table 4.11.2-1: Summary table of relevant development toxicity studies

Method	Results	Remarks	Reference
Rat oral developmental toxicity (teratogenicity) study Groups of 23 presumed pregnant Sprague-Dawley females rats (Hsd:SD; 8-10 w old at start of study) Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in an aqueous suspension of 1% w/v methyl-cellulose Dose levels: 0; 100; 315 or 1000 mg/kg bw/d by oral gavage once daily, on d-7 through d-16 of presumed gestation. OECD 414; 1981	NOAEL for maternal and developmental toxicity: 1000 mg/kg bw/d	Oral administration Mesosulfuron-methyl up to 1000 mg/kg bw during pregnancy did not induce maternal or developmental toxicity in the rat.	M-187036-01-1; 1999 (Hofmann Th., 1999b)
Rabbit oral developmental toxicity (teratogenicity) study Groups of 15 mated female Himalayan rabbits (5-10 months of age at start of the study) Mesosulfuron-methyl (AE F130060) technical (purity: 94.6%) in an aqueous suspension of 1% w/v methyl-cellulose. Dose levels: 0; 100; 315 or 1000 mg/kg bw/d by oral gavage once daily, on d-6 through d-18 of gestation. OECD 414; 1981	NOAEL for maternal and developmental toxicity: 1000 mg/kg bw/d	Oral administration Mesosulfuron-methyl up to 1000 mg/kg bw/d during pregnancy did not induce maternal or embryo/foetal toxicity nor teratogenicity in the rabbit.	M-181336-02-1; 1998 (Hofmann Th., 1998b)

4.11.2.1 Non-human information

In a developmental study in rats, conducted to OECD 414 guidelines, groups of 23 presumed pregnant Sprague-Dawley females rats were administered 0; 100; 315 or 1000 mg/kg bw/day of AE F130060 technical (94.6 % purity) in an aqueous suspension of 1% w/v methyl-cellulose, by oral gavage once daily, on day 7 through to day 16 of presumed gestation (M-187036-01-1; 1999).

Rats were observed pre-test and twice daily throughout the dosing period for mortality, behaviour and general health condition; body weights were determined on days 1, 4, 7, 10, 14, 17, 19 and 21 of pregnancy; food consumption was recorded between days1-4, 4-7, 7-10, 10-14, 14-17, 17-19 and 19-21 of pregnancy. All rats were sacrificed on day 21 of presumed gestation and foetuses were removed by Caesarean section; macroscopic examination was performed with emphasis on the uteri; gravid uterus weight was recorded; corpora lutea were counted; live and dead foetuses, conceptuses undergoing resorption, placenta were weighed and examined macroscopically; uterine implantations sites were counted; crown-rump lengths of foetuses were recorded. Half of the foetuses of each litter were necropsied, sexed and checked for stage of development and skeletal abnormalities; remaining foetuses were examined for internal organs changes.

There were no deaths, no clinical signs of toxicity, no impairment of body weight gain or food consumption and no gross lesions were observed in treated dams at necropsy compared to controls (see table 4.11.2.1-1).

All females became pregnant except 1 in the control group, 3 in the low dose group and 1 in the high dose group. The uterus weight, the number of early and late conceptuses undergoing resorption, the litter size, the number of live foetuses, the foetal and placental weights, the sex ratio and the crown-rump lengths did not differ among treated and control groups. No dead foetuses were found.

One low dose foetus exhibited multiple major defects which were considered to be an isolated event and not test substance-related. There were no statistically significant treatment-related increases in the incidences of skeletal defects, of minor defects, of variations and retardations which occurred in all groups including controls. There were no statistically significant increased incidences of visceral findings.

Oral administration of daily dose up to 1000 mg/kg in pregnant rats did not induce maternal or developmental toxicity and this dose should be considered as the NOAEL.

Table 4.11.2.1-1: Summary findings

Dose group (mg/kg bw/d)	0	100	315	1000
Dams				
N°	23	23	23	23
Killed at term	22	20	23	22
Killed non pregnant	1	3	-	1
With live foetuses at delivery	22	20	23	22
Corpora lutea	349	302	372	351
implantations	308	258	320	299
Bw gain (g) during pregnancy				
d-1-4	15.3	12.6	13.5	15.4
d-10-14	17.8	15.8	18.3	18.5
d-19-21	32.2	26.6	30.3	28.6
Uterus weight (g)	72.6	65	73.2	70.6
Preimplantation loss (%)	12.7	14.3	14.3	14.7
Post implantation loss (%)	6.7	10.0	3.6	6.3
Early resorptions	19	22	12	19
% of implantations (mean)	6.7	10.0	3.6	6.3
Late resorptions	-	-	-	-
% of implantations (mean)	-	-	-	-
Litters				
Live foetuses	289	236	308	280
Mean	13.1	11.8	13.4	12.7
Males (%)	53.3	49.2	50.6	52.5
Bw (g)	3.7	3.7	3.7	3.7
Crow-rump length (mm)	37.3	37.5	37.3	37.9
N° fœtus examined	148	121	159	145
N° litters examined	22	20	23	22
Minor defects				
Splitting of parietal bone	0	1	0	0
Fragmented thoracic vertebral centres	1	1	1	1
Longitudinally displaced sternebrae	0	1	0	0
Aplastic and fused ribs	0	1	0	0
Retardations				
Non ossified metacarpale 5 of the forepaw	4	3	8	12
Slight ossification of skull bones	2	1	0	0
Non ossified vertebral centres	1	0	1	1
Non ossified sacral vertebrae	1	0	0	0
Ossification <2 caudal vertebral centres	28	13	19	19
Non or weekly ossified sternebrae	24	24	36	34
Non ossified metatarsale 5 of the hindpaw	1	0	1	0
Visceral findings				
Distended ureter	0	1	6	2
Distended kidney pelvis	0	0	1	0

In a rabbit developmental study, conducted to OECD 414 guidelines, groups of 15 mated female Himalayan rabbits (5-10 months of age at start of the study) were administered orally (by gavage) once daily, 0 (vehicle); 100; 315 or 1000 kg/bw/d of AE F130060 technical (94.6% purity) in an aqueous suspension of 1% w/v methylcellulose on day 6 through to day 18 of gestation (M-181336-02-1; 1998).

Dose levels were determined in a range finding study in which no deaths or signs of toxicity were observed in animals (dams and pups) at up to 1000 kg/bw/d.

All rabbits were examined pre-test and twice daily during the dosing and post-dosing periods for behaviour and general health condition. Body weight and food consumption were recorded at least twice weekly from day 0 of gestation. All surviving animals were sacrificed on day 29 presumed gestation and a gross necropsy was performed on thoracic and abdominal contents with emphasis on the uterus; gravid uterus weight was determined; number of corpora lutea were counted; live and dead foetuses, conceptuses undergoing resorption, placentae were recorded, weighed and examined macroscopically; all foetuses were necropsied and checked for external and internal abnormalities (eye, brain, heart kidney and skeleton); their crown-rump lengths were recorded.

There were no treatment-related deaths, no clinical signs of toxicity and no effects on body weight development reported. A slight, but statistically significant reduction of food consumption was seen between day 8 and day 10 in the top dose group and between day 13 and day 16 in all treated groups, but this finding was not considered as treatment related because the food consumption was comparable in all treated group (no dose-dependency) and was well within the historical range (see table 4.11.2.1-3); furthermore, the difference to control values were very close and deviations in similar degree but inverse trend were seen in other study phases as well (e. g. day 3 to 5).

Table 4.11.2.1-2: Group mean bw changes (g/animal) and food consumption (g/100 g bw) during pregnancy

Dose	0 mg/kg	100 mg/kg	315 mg/kg	1000 mg/kg
Mean body weight (g)				
d-0	2746	2757	2786	2722
d-8	2850	2844	2851	2818
d-16	2958	2893	2942	2922
d-23	3001	2918	2966	2961
d-29	3118	3034	3114	3080
Food consumption (g/100 g bw)				
d-0-3	2.9	2.5	2.9	3.8
d-3-6	3.2	3.0	3.1	3.5
d-6-8	2.9	2.8	2.7	2.8
d-8-10	2.7	2.4	2.4	2.3*
d-10-13	2.7	2.5	1.6	2.4
d-13-16	2.7	2.1*	2.2*	2.2*
d-16-19	2.8	2.3	2.5	2.7
d-19-23	3.1	2.6	2.8	3.0
d-23-26	3.0	2.7	3.1	3.2
d-26-29	3.1	2.9	3.2	3.4

*: Significantly different from control

Except 1 female in each of the low and high dose group, all females became pregnant. No compound related effects were seen during necropsy except one animal at 1000 mg/kg bw which had no junction between uterus and vagina. The uterus was filled with clear liquid. Gravid uterus weight was comparable in all groups.

Mean foetal body weight, crown rump lengths, litter sizes, number of live foetuses, sex ratio and placental weights were not affected by the substance administration. There was no statistically increased incidence of dead foetuses (4 in the high dose group and 2 in the low dose group) which was within the historical range.

One foetus in the high dose group had thoracoschisis, gastroschisis with protrusion of organs, dysplasia and aplasia of numerous bones. These isolated findings were explained by strangulation of the developing thorax by the umbilical cord and thus not considered treatment-related. There were no increased incidences for skeletal and organ findings when compared to the control group, all findings being within historical ranges and not dose dependent (see table 4.11.2.1-2).

Table 4.11.2.1-3. Summary findings

Dose group (mg/kg bw/d)	**0**	**100**	**315**	**1000**
Dams				
Pregnancies	15	14	15	14
Intercurrent deaths	-	-	-	-
Females with abortion	-	-	-	-
With live foetuses at delivery	15	14	15	14
Corpora lutea	121	113	129	120
implantations	108	95	112	106
Uterus weight (g)	382.3	375.1	415.9	392.1
Preimplantation loss (%)	9.8	15.1	12.8	11.9
Post implantation loss (%)	6.2	5.2	1.8	8.2
Early resorptions	8	3	2	5
% of implantations (mean)	6.23	3.3	1.8	5.3
Late resorptions	-	2	-	4
% of implantations (mean**)**	-	1.9	-	2.9
Litters				
Live foetuses	100	90	110	97
Males (%)	50.0	47.8	43.6	50.9
Bw (g)	41.2	41.7	40.7	40.8
Crow-rump length (mm)	98.3	98.1	96.4	97.1
N° foetuses examined	100	90	110	97
N° litters examined	15	14	15	14
Minor defects				
Splitting of parietal bone	2	2	4	2
Fissure in parietal bone	1	-	2	1
Perforation in parietal bone	2	-	2	2
Epactal bone between nasal/frontal bone	4	3	2	7
Displaced, dysplasia, fused sternebrae	2	2	4	0
Fused or dislocated caudal vertebral centres	0	1	0	1
Retardations				
Non ossified /weakly ossified sternebrae	32	35	35	33
Ossification < 13 caudal vertebral centres	19	13	26	12
Weakly ossified phalanx	2	-	-	-
Non ossified phalanx II	-	-	1	-

Oral administration of AE F130060 up to 1000 mg/kg bw during pregnancy of rabbit did not induce maternal or embryo/foetal toxicity nor teratogenicity. The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw.

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

No information available.

4.11.4 Summary and discussion of reproductive toxicity

In the **multigeneration study** administration of AE F130060 at dietary concentrations of up to and including 16 000 ppm (equivalent to approximately 800 mg/kg bw/day up to 3000 mg/kg bw/day, depending on the different phases of the study) did not cause any substance related adverse effects on reproduction, fertility, mating behaviour or malformations in the offspring in a multigeneration study in rats. This lack of substance related findings was in line with the results of the **developmental toxicity studies** in rats and rabbits, performed at the same laboratory, which clearly showed no substance-related adverse findings up to and including the limit dose level of 1000 mg/kg bw/d.

It is concluded that Mesosulfuron-methyl (AE F130060) is not a developmental toxicant and is devoid of any teratogenic potential. There is no indication of reproductive toxicity even if taken into account the extremely high test substance intake, which during certain phases of dietary treatment by far exceeded the internationally accepted limit dose of 1 000 mg/kg bw/d.

4.11.5 Comparison with criteria

There is no evidence that Mesosulfuron-methyl produces reproduction or developmental toxicity and, therefore, no classification is warranted.

4.11.6 Conclusions on classification and labelling

No classification is proposed for this endpoint.

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal ***Effects on fertility***

Reproductive toxicity was tested in a two-generation dietary study in rats, which was conducted according to OECD TG 416.

There were no treatment-related effects on mortality of the parental P and F1 generations. Also no clinical findings could be attributed to treatment in any of the P1 and F1 parental animals. No effects on food consumption (except slightly higher food consumption in high dose P males) and body weight gain were observed.

No test substance related effect on sperm motility in any of the treated groups of P and F1 parental males were recorded. However, there was some indication of test substance related effects on reproductive performance in the P and F1 generations, due to lower initial pregnancy rates, but following a second cohabitation using the same parental pairs, pregnancy rates were comparable with controls in all dose groups.

All pups selected to become the F1 parental generation exhibited a normal development of genitalia and there was no statistically significant difference in the meantime interval for preputial separation or vaginal opening.

Mating indices for both males and females, pregnancy rates, male fertility indices, gestation indices and parturition indices were unaffected by treatment (see table 4.11.1.1-4 in the background document). For both generations, pregnancies and deliveries were not impaired by the test substance; the mean gestation duration for the treated and control groups were comparable. There were no treatment related gross macroscopic organ changes nor histological alterations of the reproductive organs in either the P or F1 generation parental rats.

No treatment related effects were detected in the F1a, F1b, F2a and F2b offspring. The mean number of live and dead pups at birth were comparable in all groups from all litters and they were all normally developed and had normal body weight. No test substance related effects was seen on sex ratio and on external abnormalities in any of the litters. During lactation, mortality was comparable in all groups, including controls.

No treatment related macroscopic findings were observed in F1a, F1b, F2a and F2b pups sacrificed on day 4 (culling) and on postnatal day 21. No test substance related microscopic findings were seen in F2b pups.

Overall, it can be concluded that no adverse effects were indicated from the evaluation of parental or neonatal parameters and no treatment related effects on reproductive performance were noted at dietary levels up to and including 16,000 ppm (1,175 and 1,288 mg/kg bw/d in males and females, respectively).

Developmental toxicity

Developmental toxicity was tested in single rat and rabbit gavage developmental toxicity studies conducted according to OECD TG 414.

Oral administration of daily doses up to 1,000 mg/kg bw/d in pregnant rats did not induce maternal or developmental toxicity. All females became pregnant except 1 in the control group, 3 in the low dose group and 1 in the high dose group. One low dose foetus exhibited multiple major defects which were considered to be an isolated event and not substance related.

For the rabbit study a preliminary range finding study was performed, in which no deaths or signs of toxicity were observed in dams and pups at doses up to 1,000 mg/kg bw/d.

In the main study there were no treatment related deaths, no clinical signs of toxicity and no effects on body weight gain reported. A slight but statistically significant reduction in food consumption was seen between day 8 and day 10 in the high dose group and between day 13 and day 26 in all treated groups. There was no dose-response relationship for this effect (equal among the different treated groups) and it was stated to be within the historical control range.

Apart from one female in each of the low and high dose groups of the main study, all females became pregnant. One foetus in the high dose group displayed effects which were reported to be related to strangulation with the umbilical cord and thus not considered treatment related. There were no increased incidences for skeletal and organ findings when compared to the control group, all findings being within historical control ranges and not dose dependent.

Oral administration of mesosulfuron-methyl up to a dose of 1,000 mg/kg bw/d did not induce maternal or embryo/foetal toxicity nor teratogenicity.

Summary

In the two-generation study in rats, administration of mesosulfuron-methyl at dietary concentrations of up to and including 16,000 ppm (equivalent to approximately 800 mg/kg bw/d up to 3,000 mg/kg bw/d) did not cause any substance related adverse effects on reproduction, fertility, mating behaviour or malformations in the offspring in a multigeneration study in rats. This lack of substance related findings was in line with the results of the developmental toxicity studies in rats and rabbits, performed at the same laboratory, which clearly showed no substance related adverse findings up to and including the limit dose level of 1,000 mg/kg bw/d.

It is concluded that mesosulfuron-methyl is not a developmental toxicant and is devoid of any teratogenic potential. There is no indication of reproductive toxicity even at extremely high test substance doses, which during certain phases of dietary treatment by far exceeded the internationally accepted limit dose of 1,000 mg/kg bw/d.

Comments received during public consultation

One Member State supported the proposal for no classification. No further comments for this endpoint were received during public consultation.

Assessment and comparison with the classification criteria

No findings indicating effects on fertility were seen in a 2-generation TG compliant study in rats even at high doses. In TG compliant studies in rats and rabbits, no evidence of developmental toxicity was observed.

Based on the available data, RAC agrees with the DS that **no classification for reproductive toxicity (effects on fertility or developmental toxicity)** is warranted.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Mesosulfuron-methyl is a sulfonylurea herbicide. This well-known class of compounds is devoid of any neurotoxic effects. Furthermore, the chemical structure of Mesosulfuron-methyl has no structural relationships with organophosphates or any other known neurotoxicants.

No evidence of clinical signs indicative of delayed neurotoxicity or other neurotoxic effects were seen in the acute, subacute, subchronic (90-day) or long-term toxicity studies, even at international regulatory limit dose levels. Furthermore, there were no neuropathological changes. Similarly, in the two generation reproduction toxicity study, no clinical signs were seen in either the F1 or F2 offspring or their parents.

For these reasons, acute, subchronic or developmental neurotoxicity studies were not triggered. Therefore none have been conducted.

4.12.1.2 Immunotoxicity

No information available. From the toxicity studies no evidence of any immunotoxic effects was seen.

4.12.1.3 Specific investigations: other studies

The toxicological profile had been consistently characterised in the tested species and threshold levels as well as NOAELs could be clearly defined in subchronic and chronic testing. There were no mutagenic, carcinogenic or teratogenic properties and no specific toxic effects to reproduction. Accordingly, no further testing was deemed necessary.

A comparative in-vitro metabolism study was performed in order to determine the relevance of the toxicological animal data. The results of the tests with ^{14}C-mesosulfuron-methyl demonstrated that the in vitro metabolism was only very slightly different between rats and humans. While no metabolism was found in rat liver microsomes, an unresolved peak region was detected in the 1 h HPLC-chromatogram of the human liver microsomes incubation that accounted for 8.1% of the total relative percentage. This region was not a clearly recognizable peak, but a non-dissolved region which appeared directly after elution of the active substance from the column, and which was < 10% of the TRR (which was a trigger set for this study type). The results suggest that phase I metabolism is not significantly involved in the biotransformation of mesosulfuron-methyl in rat and human liver microsomes. It is noted that only one dose (15µM) was tested in this study with incubations times 0 and 1 hour. Additionally liver microsomes from rat species were used, whereas the toxicological reference values were determined on studies conducted with dog and mouse. At the moment, no OECD Guideline is available for this study type. Thus this study is considered acceptable but only as supplementary information. Nevertheless, clarification of the results of this study would have been helpful.

The phototoxic potential of Mesosulfuron-methyl (AE F130060) technical was tested in a phototoxicity assay using BALB/c 3T3 cells. The experiment was performed twice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME). Cytotoxic effects did

not occur after exposure of the test item to the cells, neither in the presence nor in the absence of irradiation with artificial sunlight in the RFE as well as in the ME. Therefore, ED50-values or a PIF could not be calculated. The resulting MPE value was 0.048 and 0.000, respectively. Under the condition of the *in vitro* 3T3 NRU- phototoxicity test, mesosulfuron-methyl did not have any phototoxic effects on BALB/c 3T3 cells. Therefore, mesosulfuron-methyl is not considered phototoxic

A package of a standard battery of genotoxicity and mutagenicity tests *in vitro* (Ames tests, chromosomal aberrations assays in Chinese Hamster V79 cells and gene mutation assays (HPRT) in Chinese Hamster V79 cells), with the groundwater metabolites AE F147447, AE F160460 and BCS-CV14885 has been performed. No indication of genotoxicity was observed in these assays.

4.12.1.4 Human information

No reports of adverse effects on human health caused by Mesosulfuron-methyl in the work-place has been reported.

No substance-related disturbances to human health have been found in the Medical surveillance of manufacturing plant personnel (annual medical examinations of the workers and specific examinations in accordance with the requirements of the trade association of the German chemical industry (Berufsgenossenschaft Chemie). This involved physical examinations, blood chemistry, haematology and urinalysis.

No dermal allergic reactions have been detected during the handling of Mesosulfuron-methyl to date.

There are no known adverse effects resulting from production, formulation or agricultural use of Mesosufuron-methyl. One suicide attempt has been reported by the applicant, however no document was provided to support this observation. After drinking an unknown amount of a formulation containing mesosulfuron-methyl, nausea, vomiting and stomach upset were seen. No sequelae were observed in the patient.

4.12.2 Summary and discussion

Mesosulfuron-methyl a sulfonylurea herbicide, has no structural relationship to neurotoxic substances. Moreover, its very low and non-specific toxicological profile shows no evidence of neurotoxic potential. Consequently no special studies have been conducted for this.

Genotoxicity and mutagenicity tests *in vitro*, with the groundwater metabolites AE F147447, AE F160460 and BCS-CV14885 have been performed.The results of all three *in vitro* studies were negative with and without metabolic activation. Therefore, no indications for genotoxicity were observed for the three metabolites.

4.12.3 Comparison with criteria

There is no indication that Mesosulfuron-methyl is neurotoxic or immunotoxic.

4.12.4 Conclusions on classification and labelling

No classification is proposed for human health hazards.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of Mesosulfuron-methyl were assessed in the Draft Assessment Report and the Addendum to the Draft Assessment Report prepared in the context of the inclusion of Mesosulfuron in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, December 2001 and subsequent addenda, RMS France) concerning the placing of plant protection products on the market.

The results included in this proposal are taken from the DAR (and its addenda and assessment reports when these contain updated information), updated with the new data submitted in the context of the Approval Renewal and reported in the Draft Renewal Assessment Report (dRAR), when relevant. For more details on the test system and the tables of individual results, the reader is referred to the DAR, its addenda, and to the dRAR.

For several studies, the synonym AE F130060 is used for Mesosulfuron-methyl.

5.1 Degradation

Table 5.1-1: Summary of relevant information on degradation

Method	Results	Remarks	GLP study	Reference
OECD guideline 111 (1981) Directive 92/69/EEC part C.7. (1992) Under consideration of : EPA OPPTS 835.2110 and OPPTS 835.2130	Hydrolysis: pH 4 : DT_{50} 3.5 d (25° C), 7.2 d (20° C) pH 7 : DT_{50} 253 d (25° C), 23.1 d (40° C) pH 9 : DT_{50} 318 d (25° C), 27.2 d (40° C)		Yes	M-197200-01-1, 2000
SETAC Europe (1995); EPA Guideline N 162-3 (1982)	Soil photolysis: not significant		Yes	M-194849-01-1, 2000
OECD guideline (1997), EPA guideline N 161-2 (1982)	Photodegradation in water: pH 7, 25° C, DT_{50} 45.9 d (continuous artificial light), x 7.5 under real conditions (June, 50° N)	no relevant photodegradation in water	Yes	M-197103-01-1, 2000
OECD Test Guideline 101, 1981 OECD Test Guideline 316, 2008	Quantum yield in pure water: Φ = 0.024748	no relevant photodegradation in water	Yes	M-473896-01-1, 2013
OECD Test guideline 309 DRAFT SANCO 11802/2010/rev 1 in accordance with Regulation (EC) No 1107/2009	Aerobic mineralization in surface water: DT_{50} 672 d	2 concentrations studied; 62 days experiment; 20.4 °C	Yes	M-486556-01-1, 2014

Table 5.1-1: Summary of relevant information on degradation continued

BBA IV, 4-1 (1986); SETAC Europe (1995); EPA Guideline N 162-1 (1982)	Aerobic soil degradation, laboratory: geomean DT_{50} at 20°C, FC = 42.4 days	pH 5.2-7.5, values for 9 soils	Yes	M-511078-01-1, 2015
BBA part IV, 4-1, 1986; SETAC Europe, 1995	Aerobic soil degradation, field: geomean DT50 at 20°C, FC = values not normalised according to the current FOCUS guidance document	Autumn (4 sites: FR, GR, UK) Spring (6 sites: I, SP, FR, GR, UK)	Yes	M-198875-01-1, 2000 M-359355-01-1, 2009
SETAC Europe (1995); EPA Guideline N 162-2 (1982)	Anaerobic soil degradation: geomean DT50 at 20°C,= 30.3 days	Mean of 2 labels, pH 5.4	Yes	M-199429-01-1, 2000 M-198307-01-1, 2000
SETAC guideline	Water/sediment degradation: Water phase: geomean DT50 at 20°C = 33.9 days Sediment phase: geomean DT50 at 20°C = 55.2 days Total system: geomean DT50 at 20°C = 45.7 days	2 water systems	Yes	M-198526-01-1, 2000 M-511142-01-1, 2015

5.1.1 Stability

The results of a hydrolysis study (M-197200-01-1, 2000) following the OECD guideline 111 (1981) showed that Mesosulfuron-methyl is rapidly hydrolysed in buffer at pH 4 (DT_{50} 3.5 days at 25° C). Hydrolysis is much slower at pH 7 (DT_{50} 253 days at 25° C) and at pH 9 (DT_{50} 318 days at 25° C).

A photodegradation in soil study (M-194849-01-1, 2000) showed that Mesosulfuron-methyl is photolytically stable since no degradation was observed under both dark and light conditions.

The aqueous photolysis of Mesosulfuron-methyl in water was considered not significant since estimated half-lives are above 30 days (continuous artificial light) at 25° C (M-197103-01-1, 2000). A mean quantum yield of Φ = 0.024748 was determined for the direct phototransformation of Mesosulfuron-methyl in pure water using polychromatic light according to the ECETOC method. Environmental half-lives of sunlight exposed top surface water layers were estimated to above 1000 days for a direct transformation of Mesosulfuron-methyl, confirming that direct phototransformation will not contribute significantly to the dissipation of Mesosulfuron-methyl from the aquatic environment.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available.

5.1.2.2 Screening tests

No ready biodegradability test is available.

5.1.2.3 Simulation tests

Aerobic soil metabolism:

The route of degradation of Mesosulfuron-methyl in soil under aerobic conditions in the dark was investigated in a comprehensive set of laboratory studies, using soils of varying texture, physico-chemical properties and different regional provenance. Two different radiolabel positions, [phenyl-UL-^{14}C] and [pyrimidyl-2-^{14}C], were employed; the treatments were based on an application rate equivalent to 15 g/ha:

- eight soils under standard aerobic conditions at 20 °C and 50 % maximum water holding capacity (MWHC), pyrimidyl-2-^{14}C label;
- one soil under standard aerobic conditions at 20 °C and 50% MWHC, phenyl-UL-^{14}C label;
- one soil under cold aerobic conditions at 10 °C and 50% MWHC, phenyl-UL-^{14}C and pyrimidyl-2-^{14}C label

In laboratory studies using ^{14}C-radiolabels positioned in the pyrimidyl and phenyl moieties, two initial degradation routes were observed for Mesosulfuron-methyl in aerobic soil: Cleavage of the methyl ester at the phenyl ring to result in AE F154851, and ether demethylation at the pyrimidine ring to yield AE F160459. As common successor product of both intermediates, AE F160460 may be formed via metabolic loss of the respective second methyl group. Moreover, breakdown of the molecule backbone occurs via cleavage of the sulfonylurea bridge, leading to the fragments AE F099095 and AE F092944 derived from the pyrimidine moiety, and AE F140584 and its cyclisation product AE F147447 derived from the phenyl moiety.

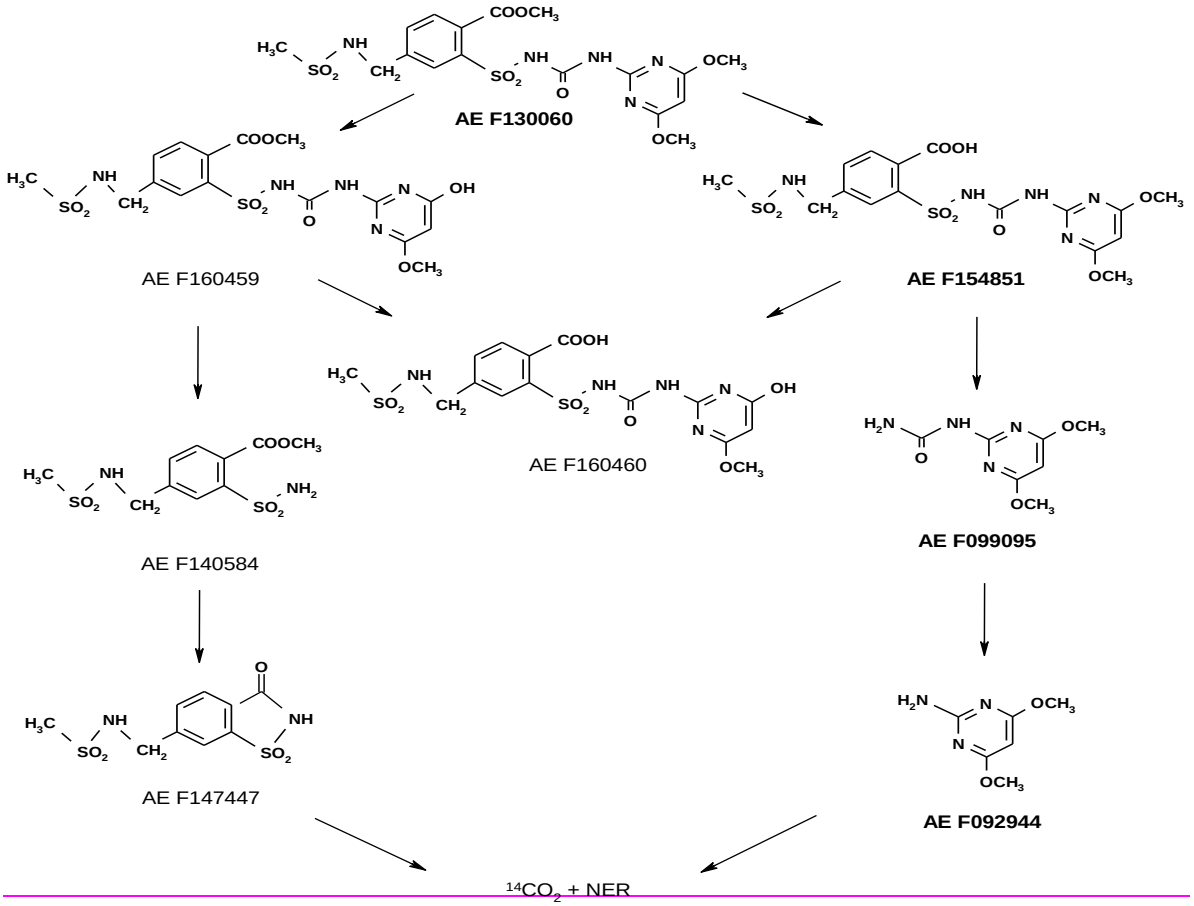
To define the maximum abundance values from the regulatory relevant data set, the following assumptions were considered by the RMS. The following endpoints were selected by the RMS related to study period and test series considered as relevant. Only the sampling data obtained for the regular time of 120 days were considered relevant by the RMS. The data determined for extension period were excluded. In addition, only the data determined in the study performed at 20°C were used.

The absolute abundance of the individual metabolites showed significant soil-to-soil variation, predominant products reaching major levels were AE F154851 (up to 16.2% observed at day 44), AE F160459 (up to 8.9% observed at day 62), AE F099095 (up to 29.2% observed at day 15), AE F092944 (up to 10.1% observed at day 62), AE F160460 (up to 8.6% observed at day 62), AE F140584 (up to 5.1% observed at day 28), and AE F147447 (up to 5.8% observed at day 120). All degradates are transient intermediates, which are either transformed to their respective metabolic downstream products, mineralized to carbon dioxide (up to 32.1% observed at day 120), or integrated into the soil matrix as non-extractable residues (up to 59.2% observed at day 120). The degradation of Mesosulfuron-methyl in soil was shown to be a microbially mediated process.

The studies were kinetically evaluated according FOCUS (2011), resulting in a geomean DT50 of 42.4 days for degradation of the parent substance Mesosulfuron-methyl.

The proposed biotransformation pathway for Mesosulfuron-methyl in aerobic soil is shown in Figure 5.1.2.3-1 below.

Figure 5.1.2.3-1: Proposed aerobic degradation pathway for Mesosulfuron-methyl in soil



Anaerobic soil degradation:

Mesosulfuron-methyl was found degradable in flooded anaerobic soil, with a half-life comparable to that observed under aerobic conditions (geometric mean DT_{50} = 30.3 days at 20 °C). The biotransformation led to the same components as observed in aerobic soils, except for the only negligible formation of CO_2 (≤ 1% AR), inherent to anaerobic condition. The proposed metabolic route therefore is equivalent to that proposed for aerobic conditions.

Photolysis on soil:

Soil photolysis of [^{14}C]mesosulfuron-methyl (mixture of pyrimidyl-2- and phenyl-UL-label) applied to the surface of thin-layers of soil using a xenon arc light source with a 12 hours light/dark cycle was investigated for 30 days (M-194849-01-1; 2000). This irradiation period corresponded to 41.4 days

natural Florida sun days. Mesosulfuron-methyl was not degraded by photolysis during 30 days of illumination. No metabolites were detected by HPLC. Volatile radioactivity (CO_2) was measured in an amount of 2.4%. Assuming a first order decay, these results would implicate a photolytic half-life of [^{14}C] mesosulfuron-methyl of > 300 days corresponding to > 414 Florida summer sunlight days.

Mesosulfuron-methyl is not photodegraded to significant extent at wavelengths >290 nm on soil surfaces. Soil photolysis will therefore not contribute notably to elimination from the terrestrial environment, and will not lead to the generation of relevant degradates.

Aerobic mineralisation in surface water

The mineralisation of [pyrimidyl-2-^{14}C] mesosulfuron-methyl in filtered surface water (no sediment) was studied under aerobic conditions in the dark in the laboratory for 62 days at 20 °C (M-486556-01-1, 2014). The test was performed in static systems consisting of Erlenmeyer flasks with baffles each containing 100 mL surface water and equipped with traps for the collection of carbon dioxide and volatile organic compounds. The test included two test item concentration levels, 11.0 µg/L (low dose), and 109.3 µg/L (high dose), a positive control for confirmation of microbial viability (benzoic acid), and sterilised water negative control.

Mesosulfuron-methyl was found not mineralized to relevant extent in surface water under aerobic conditions in the dark in the laboratory, the formation of carbon dioxide was insignificant throughout the study (< 0.1% AR at study end). AE F092944, a known hydrolysis degradate of mesosulfuron-methyl, was identified with maximum amounts of 6.5% and 6.3% AR at DAT-62 in samples of the low and high concentration, respectively.

Water/sediment degradation:

Mesosulfuron-methyl was found microbially degraded in two tested aerobic sediment/water systems. The proposed route of degradation is consistent with the route of degradation in aerobic soil. The products of predominant abundance were AE F160459, AE F147447, and AE F160460, which reached maximum abundances of 21.6% AR , 10.9% AR, and 8.4 % AR in the total systems, respectively. Terminal bioconversion led into the formation of non-extractable residues, and ^{14}C-carbon dioxide. The study was kinetically evaluated according FOCUS (2006), resulting for the parent substance Mesosulfuron-methyl in a geomean DT50 of 33.9 days for the water phase, and of 45.7 days for the total system.

5.1.3 Summary and discussion of degradation

Please refer to summaries above.

Soil

Regarding the P criteria, Mesosulfuron-methyl does not fulfil the P criteria since its half-life is lower than 120 days.

Water

Regarding the P criteria, Mesosulfuron-methyl does not fulfil the P criteria since its half-life in the water phase is lower than 40 days in the water-sediment study.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The mobility in soil of Mesosulfuron-methyl was studied by batch equilibrium tests (OECD guideline number 106) on 9 different soils (M-186653-01; 2000). An overview of the data is presented in the table. These data did not indicate a correlation of soil adsorption with soil pH.

Table 5.2.1-1: Soil adsorption data of Mesosulfuron-methyl

Component / Soil	Kf [mL/g]	Koc [mL/g]	Kom [mL/g]	Freundlich exponent 1/n
Mesosulfuron-methyl				
Hamlet	1.69	345	200.1	0.85
Horace	3.71	137	79.5	0.93
Münster	0.41	37	21.5	0.93
Speyer	0.71	31	18.0	0.91
Millington	2.28	86	49.9	0.90
Schwanheim	0.24	26	15.1	0.92
Loivre	0.60	36	20.9	0.93
Fairfield	1.22	85	49.3	0.90
Hattersheim	0.56	48	27.8	0.93
Arithmetic mean		**92.3**	**53.6**	**0.91**
Geometric mean		**63.9**	**37.1**	**0.91**

The fate and mobility of [2-^{14}C-pyrimidyl]-labelled Mesosulfuron-methyl was investigated outdoors under actual use conditions in two lysimeter studies with undisturbed monoliths of sandy soils (depth 1.0 m, surface area 0.5 m^2). Applications were made to winter wheat at a rate of 15 g/ha, twice in consecutive years. The treatment was timed either in spring (first study) or in autumn (second study). The annual average active substance concentration in the leachate did not reach or exceed the trigger of 0.1 µg/L.

5.2.2 Volatilisation

Mesosulfuron-methyl has a low vapour pressure (3.5×10^{-12} Pa at 20° C) and Henry's law constant is low (2.434×10^{-10} Pa m^3 $mole^{-1}$ at pH 5 (lower solubility of 7.24 mg/L). In addition it is likely to be rapidly degraded by oxidative photodegradation in air (DT_{50} 0.05 days; M-192037-01-1, 1999). Accordingly negligible concentrations are expected in air.

5.2.3 Distribution modelling

Not relevant for this type of dossier.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log P_{ow} of Mesosulfuron is estimated to be -0.48 (pH7). Substances with a log Pow < 4 are considered to have low potential to bioaccumulate according to Regulation EC 1272/2008). Regarding the PBT criteria, Mesosulfuron-methyl is therefore not considered as B since the log Kow is lower than 4.

5.3.1.2 Summary and discussion of aquatic bioaccumulation

No study has been conducted to determine the bioconcentration of the active substance as it was not considered necessary on the basis that the active substance has a low potential to accumulate. (See 5.3.1.1)

5.4 Aquatic toxicity

The available aquatic toxicity studies are detailed in tables 5.4-1: acute fish studies, 5.4-2: long-term fish toxicity studies, 5.4-3: acute toxicity to aquatic invertebrates, 5.4-4: long-term toxicity to aquatic invertebrates, 5.4-5 toxicity to algae and 5.4-6 toxicity to aquatic plants, respectively.

Table 5.4-1: Summary of short term fish toxicity studies

Method	Results	Remarks	Reference
Short term toxicity to fish			
The acute toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity 94.6% w/w) was assessed. Juvenile rainbow trout (*O. mykiss*) exposed for 96 hours under static conditions. Nominal concentrations of 10, 18, 32, 56 and 100 mg/L were tested plus a control group. Analytical sampling conducted at 0 and 96 hours. OECD 203, US-EPA E§72-1, EU C.1	No mortality or sub-lethal effects observed. Measured concentrations of test solutions were within 80 – 120% of nominal. Results based on nominal concentrations **96 hour LC_{50} >100 mg/L**	Conducted to GLP	M-186666-01-1, 1999
The acute toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity 94.6% w/w) was assessed. Bluegill sunfish (*L. macrochirus*) exposed for 96 hours under static conditions. A nominal concentration of 100 mg/L was tested plus a control group. Analytical sampling conducted at 0 and 96 hours. OECD 203, US-EPA E§72-1, EU C.1	No mortality or sub-lethal effects observed. Measured concentrations of test solution was within 80 – 120% of nominal. Results based on nominal concentrations. **96 hour LC_{50} >100 mg/L**	Conducted to GLP	M-186597-01-1, 1999
The acute toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity 95.7% w/w) was assessed. Sheepshead minnow (*C. variegatus*) were exposed for 96 hours under static conditions. A nominal concentration of 100 mg/L was tested plus a control group. Analytical sampling conducted at 0 and 96 hours. OECD 203, US-EPA E§72-3	No mortality or sub-lethal effects observed. Measured concentrations of test solution were within 80 – 120% of nominal. Results based on nominal concentrations. **96 hour LC_{50} >100 mg/L**	Conducted to GLP	M-238810-01-1; 2001

Table 5.4-2: Summary of long-term fish toxicity studies

Long-term toxicity to fish			
The effects of Mesosulfuron-methyl (technical substance AE F130060, purity 94.6% w/w) on the growth of fish was assessed. Juvenile rainbow trout (*O. mykiss*) exposed in a static renewal system over a 28-day exposure period. Nominal concentrations of 0.32, 1.0, 1.32, 10 and 32 mg/L were tested plus a control group. Mortality and intoxication symptoms were recorded daily throughout the study and fish growth rates were calculated at the end of the test Analytical sampling conducted at regular intervals during the test. OECD 204, ISO 10229	No mortality or intoxication symptoms were observed. The growth rate of the test fish was not statistically different in Mesosulfuron-methyl exposed fish from the growth rate in the control. Measured concentrations of test solutions were within 80 – 120% of nominal. Results based on nominal concentrations. **NOEC 28 d = 32 mg a.s./L**	Conducted to GLP	M-187567-01-1, 2000
The effects of Mesosulfuron-methyl (technical substance AE F130060, purity 96.7% w/w) was tested in a fish early life stage test using fathead minnow (*P. promelas*). Fathead minnow embryos (less than 24 hours old) were tested for a 32 day period under continuous flow through exposure conditions at nominal test concentrations of 6.3, 13, 25, 50 and 100 mg a.s./L. A control group was also tested. Hatching success, post hatch survival rates and the effects on growth (length and weight) were determined. Analytical sampling conducted at regular intervals during the test. OPPTS 850.1400, EPA OPP 72-4	Based on the results of this study, there were no adverse effects on fathead minnow from Mesosulfuron. Geometric mean measured concentrations were determined to be: 6.6, 10, 25, 46 and 95 mg a.s./L. **NOEC 32 d = 95 mg a.s./L**	Conducted to GLP	M-241475-01-1; 2003

Table 5.4-3: Summary of short term toxicity to aquatic invertebrates

Short term toxicity to aquatic invertebrates			
The acute toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity 94.6% w/w) was assessed. *Daphnia magna* were exposed for 48 hours under static conditions. A nominal concentration of 100 mg/L was tested plus a control group. Analytical sampling conducted at 0 and 48 hours. OECD 202, US-EPA E§72-2, EU C.2	No effects on mobility were recorded. Measured concentrations of test solution was within 80 – 120% of nominal. Results based on nominal concentrations. **48 hour EC_{50} >100 mg a.s./L**	Conducted to GLP	M-186707-01-1, 1999
The acute toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity 95.7% w/w) was assessed. Mysid shrimp *(Mysidopsis bahia)* were exposed for 96 hours under static conditions. A nominal concentration of 100 mg/L was tested plus a control group. Analytical sampling conducted at start and end of the study. US-EPA OPP 72-3	One mysid in the control was not found during observations at 96 hours and was thought to have been a victim of cannibalism. No additional mortality or sublethal effects were observed in the control or 100 mg/L treatments during the study. Measured concentrations of test solution were within 80 – 120% of nominal. Results based on nominal concentrations. **96 hour LC_{50} >100 mg a.s./L**	Conducted to GLP	M-238811-01-1 ; 2001

Table 5.4-4: Summary of long-term toxicity to aquatic invertebrates

Long-term toxicity to aquatic invertebrates			
The effects of Mesosulfuron-methyl (technical substance AE F130060, purity 94.6% w/w) on the reproduction of *Daphnia magna* was determined. *Daphnia* were exposed for 21 days under semi-static conditions (renewal 3 times per week). Nominal concentrations of 1.0, 1.8, 3.2, 5.6, 10 and 18 mg/L were tested plus a control group. The mortality of adults and the number of juveniles produced were recorded 3 times per week. Analytical sampling conducted at regular intervals during the test. OECD 202, US-EPA E§72-4, EU C.2	No immobilisation was observed. The number of living juvenile at each day of assessment and their cumulative number after 21 days did not differ from the control at any treatment level. Measured concentrations of test solutions were within 80 – 120% of nominal. Results based on nominal concentrations. **NOEC 21 d = 1.8 mg a.s./L for effects on size (weight and length) of females** NOEC 21 d =32 mg a.s./L for reproduction	Conducted to GLP	M-197785-02-2, 2000

Table 5.4-5: Summary of toxicity to algae

Toxicity to algae			
The toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity = 94.6%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions over an exposure period of 96 hours. Nominal concentrations of 0.032, 0.056, 0.1, 0.18 and 0.32 mg/L were tested plus a control group. Cell density was determined every 24 hours during the test. Analytical sampling conducted at 0 and 96 hours. OECD 201, US-EPA J§123-2, EU C.3	The mean measured concentrations ranged from 54.8 to 91.6% and were: 0.018, 0.041, 0.084, 0.165, and 0.292 mg/L. Results based on mean measured concentrations. **E_rC_{50} 72/96 h > 0.29 mg a.s./L** **NOErC = 0.018 mg a.s./L**	Conducted to GLP	M-143500-01-1, 1998
The toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity = 97.4%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions over an exposure period of 96 hours. Nominal concentrations of 0.143, 0.458, 1.46, 4.69 and 15 mg/L were tested plus a control group. Cell density was determined every 24 hours during the test. Analytical sampling conducted at 0 and 96 hours. OECD: 201**;** OCSPP Guideline 850.4500: Algal Toxicity (January 2012)	The mean measured concentrations ranged from 96.7 to 108% and were: 0.154, 0.492, 1.56, 4.87, and 14.5 mg/L. Results based nominal concentrations. **E_rC_{50} 72 h = 3.99 mg a.s./L** **NOErC = 0.143 mg a.s./L**	Conducted to GLP	M-516540-01, 2015

Table 5.4-5: Summary of toxicity to algae continued

Toxicity to algae continued			
The toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity = 94.6%) to the diatom species *Navicula pelliculosa* was determined under static conditions over an exposure period of 96 hours. Nominal concentrations of 10, 18, 32, 56 and 100 mg/L were tested plus a control group. Cell density was determined every 24 hours during the test. Analytical sampling conducted at 0 and 96 hours. OECD 201, US-EPA J§123-2, EU C.3	Measured concentrations of test solutions were not within 80 – 120% of nominal. Mean measured concentrations were determined to be 7.6, 14.1, 25.4, 47.4 and 74.9 mg/L. Based on mean measured: **E_rC_{50} 96 h > 74.9 mg/L** **NOErC = 74.9 mg/L**	Conducted to GLP	M-187975-01-1, 2000
The toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity = 95.7%) to the blue-green algal species *Anabaena flos-aquae* was determined under static conditions over an exposure period of 96 hours. Nominal concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L were tested plus a control group. Cell density was determined every 24 hours during the test. Analytical sampling conducted at 0 and 96 hours. OECD 201, US-EPA J§123-2	Mean measured concentrations were 105 – 112% of nominal therefore results based on nominal concentrations. **96 h E_rC_{50} = 4.1 mg a.s./L** **NOErC = 1 mg a.s./L**	Conducted to GLP	M-238869-01-1 ; 2001
The toxicity of Mesosulfuron-methyl (technical substance AE F130060, purity = 95.7%) to the marine diatom *Skeletonema costatum* was determined under static conditions over an exposure period of 96 hours. Nominal concentrations of 13, 22, 36, 60 and 100 mg/L were tested plus a control group. Cell density was determined every 24 hours during the test. Analytical sampling conducted at 0 and 96 hours. OECD 201, US-EPA J§123-2	Mean measured concentrations were 94 – 106% of nominal therefore results based on nominal concentrations. **72 h E_rC_{50} > 100 mg a.s./L** **NOErC = 60 mg a.s./L**	Conducted to GLP	M-238809-01-1; 2001

Table 5.4-6: Summary of toxicity to aquatic plants

Toxicity to aquatic plants			
The effects of Mesosulfuron-methyl (technical substance AE F130060, purity = 95.3%) on the growth of the duckweed *Lemna gibba* G3 was determined under renewal conditions over a 7 day period. Nominal test concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 µg/L were tested plus a control group. Test media was renewed on days 3 and 5 of the test. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded. Analytical sampling conducted at each media renewal during the test. Draft OECD guideline, US-EPA J§123-2, ASTM 1415-91 (1991)	Mesosulfuron-methyl concentrations in fresh test media could not be measured in the control (spiked) and 0.32 µg/L samples at day 5, because of strong interference of the media on the analysis that could not be explained. Therefore, mean recovery in control (recovery of spiked samples) and 0.32 µg/L was re-calculated from the mean of days 0 and 3 recovery. Results based on nominal concentrations. **E_rC_{50} 7 days > 1.0 µg/L** **NOE_rC 7 days = 0.18 µg/L**	Conducted to GLP	M-195390-01-1; 2000
The effects of Mesosulfuron-methyl (technical substance AE F130060, purity = 98.1%) on the growth of the duckweed *Lemna gibba* G3 was determined under renewal conditions over a 7 day period. Nominal test concentrations of 1.0, 1.8, 3.2, 5.6 and 10 µg/L were tested plus a control group. Test media was renewed on days 3 and 5 of the test. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded. Analytical sampling conducted at each media renewal during the test. Draft OECD guideline, US-EPA J§123-2, ASTM 1415-91 (1991)	Time-weighted average concentrations for 1.0, 1.8, 3.2, 5.6 and 10 µg/L were 77.36%, 78.42%, 95.71%, 90.36% and 94.06% of nominal, respectively. Since time-weighted average concentrations were below 80% of nominal at the two lowest treatment levels the biological results were based on time-weighted average concentrations. **E_rC_{50} (frond number) 7 days = 1.717 µg/L** **NOE_rC 7 days < 0.77 µg/L**	Conducted to GLP	M-206814-01; 2002

Table 5.4-6: Summary of toxicity to aquatic plants continued

Toxicity to aquatic plants continued			
The new *Lemna* study (Bruns 2013; M-445139-01-1) was performed according to the currently valid guideline OECD 221 (2006) measuring two endpoints, frond number and frond area. This study can be considered as a fully valid study without restrictions. This 8-week study was designed to mimic the exposure of an outdoor-pond study and to obtain 8-week effect data for *Lemna* – a species that could not be kept in outdoor ponds. Beside the 8-week endpoints, effect data were calculated on a weekly basis. The endpoints obtained from the first 7-day period can be used for tier-1 risk assessments. The effects of Mesosulfuron-methyl (technical substance AE F130060, purity = 97.4%) on the growth of the duckweed *Lemna gibba* G3 was determined under renewal conditions over an 8 week period. Nominal initial test concentrations were 0.194, 0.388, 0.755, 1.55 and 3.10 µg/L. Test media was renewed at the start of each 7 day period of the test. Frond number and frond area were assessed twice during each 7 day test period. Analytical sampling conducted at each media renewal during the test. OECD 221	Results are based on nominal initial concentrations. Results below represent the results following the first 7 day exposure period of the study only: **$E_rC_{50\ (frond\ area)}$ 7 days = 1.29 µg/L** $E_rC_{50\ (frond\ number)}$ 7 days = 1.61 µg/L **NOE_rC 7 days = 0.388 µg/L**	Conducted to GLP	M-445139-01-1

5.4.1 Fish

5.4.1.1 Short term toxicity to fish

Three acute toxicity tests with fish are available, one with rainbow trout, the second with bluegill sunfish, and a third one with sheepshead minnow. In all studies the LC_{50} was >100 mg a.s./L, the maximum concentration tested.

5.4.1.2 Long-term toxicity to fish

A study to assess the effects of Mesosulfuron-methyl on the growth of juvenile rainbow trout over a 28 day period is available. There were no effects of the test substance on the growth of the fish up to the maximum concentration tested. The 28 day NOEC was therefore determined to be 32 mg a.s./L.

One chronic study on early life stage exposure with fathead minnow was performed. The maximum tested mean measured concentration was 95 mg a.s./L. No relevant treatment related effects were observed at this maximum dose level, resulting in a NOEC of 95 mg a.s./L.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

An acute toxicity to *Daphnia magna* study is available. No test substance related effects on mobility were recorded in the test. The EC_{50} was determined to be >100 mg a.s./L, the maximum concentration tested.

One acute study on *Mysidopsis bahia* was performed. No mortality or sublethal effects were observed at the concentration of 100 mg a.s./L, resulting in a LC50 >100 mg a.s./L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A 21-day *Daphnia magna* reproduction study is available. No test substance related effects on reproduction were recorded but significant effects on the size of the adults at the end of the test were determined. The lowest NOEC value from the study was 1.8 mg a.s./L.

5.4.3 Algae and aquatic plants

Five studies are available to assess the toxicity of Mesosulfuron-methyl to algae, using *Pseudokirchneriella subcapitata, Navicula pelliculosa, Anabaena flos-aqua* and *Skeletonema costatum*. In the algae study M-143500-01-1 (1998), only 'greater than' information for ErC50 could be obtained. A definite information on ErC50 for green algae *Pseudokirchneriella subcapitata* could however be derived from a repeated test M-516540-01-1 (2015) using a higher dosing regime. Endpoints for green algae *Pseudokirchneriella subcapitata* from this latest study are more relevant. The lowest EC50 and the lowest NOEC values were determined in the study with *P. subcapitata* (72-hr ErC50 = 3.99 mg/L; 72-hr NOErC = 0.143 mg/L).

The inhibition on 7-day growth rate-figures has been studied within three Lemna-tests with mesosulfuron-methyl. The lowest 7-day ErC50 definitive value of the studies available, was 1.29 µg

a.i./L based on frond area (data of study KCA 8.2.7/09), thereby representing the most critical toxicity value out of all of the studies with aquatic organisms. This study is summarised below.

This study is considered to be relevant and reliable and is carried forward for classification purposes with a 7-day ErC50 of 1.29 a.i./L and 7-day NOErC of 0.388 µg a.i./L. It can be noted that a lower NOErC of 0.18 µg a.i./L was available from another Lemna test (M-195390-01-1; 2000). This NOErC is in the same range for classification purpose and did not change classification or M-factors. NOEC by definition depends on dose spacing in the test. It was considered more robust to take into account the NOErC from the same study from which the lowest reliable ErC50 was issued (M-445139-01-1)Therefore, a more detailed summary for this study is reported below:

Effect of Mesosulfuron-methyl on *Lemna gibba*. Bruns E (2013)

A study to assess the growth inhibition of the duckweed *Lemna gibba* was performed with Mesosulfuron-methyl (AE F130060; substance, technical, 97.4%), under semi-static conditions according to the OECD 221 guideline under GLP.

This 8-week study was designed to mimic the exposure of an outdoor-pond study and to obtain 8-week effect data for *Lemna* – a species that could not be kept in outdoor ponds. Beside the 8-week endpoints, effect data were calculated on a weekly basis. The endpoints obtained from the first 7-day period have therefore been used for tier-1 risk assessment.

Triplicate *Lemna* cultures with an initial frond number of 12 fronds per replicate were exposed to the test substance in 20X-AAP medium at five initial nominal treatment levels of 0.194, 0.388, 0.775, 1.55 and 3.10 µg/L for an initial period of 7 days. A control group was also included. The test media was not renewed during this 7 day exposure period. Frond number and frond area was assessed on days 3, 5 and 7 of the test period. The test was conducted in glass dishes (diameter of 10 cm and a height of 6 cm) with 200 mL of test or control medium. The test was maintained at a temperature of 24 ± 2 °C and under constant illumination of 6500 - 10000 lux.

Chemical analyses of the test media were conducted at the start and the end of the 7 day exposure period. Analyses of freshly prepared test media for AE F130060 resulted in test substance concentrations ranging from 68 - 95% of the nominal concentrations. Analyses of aged test media for AE F130060 after the initial 7-day exposure resulted in test substance concentrations ranging from 79 - 116% of the nominal concentrations. The results have been based on initial nominal concentrations.

The level of 50% growth inhibition for frond number (E_rC_{50}) after 7 days was calculated to be 1.61 µg/L (95% confidence limits 1.06 – 2.91 µg/L). The level of 50% growth inhibition for total frond area (E_rC_{50}) after 7 days was determined to be **1.29 µg/L** (95% confidence limits 0.866 – 1.98 µg/L). A significant inhibition of growth in terms of frond number and frond area was observed at initial nominal concentrations of 0.775 µg/L and above. The no observed effect concentration (NOErC), defined as no significant growth inhibition and no changes in plant appearance and development, was considered to be **0.388 µg/L**.

5.4.4 Other aquatic organisms (including sediment)

No information available.

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

Summary of the relevant toxicity and fate data for Mesosulfuron-methyl

Short term toxicity		
Fish	*Oncorhynchus mykiss*	96 h LC_{50} >100 mg a.s./L
	Lepomis macrochirus	96 h LC_{50} >100 mg a.s./L
	Cyprinodon variegatus	96 h EC_{50} >100 mg a.s./L
Invertebrates	*Daphnia magna*	48 h EC_{50} >100 mg a.s./L
	Mysidopsis bahia	96 h EC_{50} >100 mg a.s./L
Algae	*Pseudokirchneriella subcapitata*	72 h E_rC_{50} = 3.99 mg a.s./L
Aquatic plants	***Lemna gibba***	**7 d E_rC_{50} = 0.00129 mg a.s./L**
Long term toxicity		
Fish	*Oncorhynchus mykiss*	28 d NOEC = 32 mg a.s./L
Invertebrates	*Daphnia magna*	21 d NOEC = 1.8 mg a.s./L
Algae	*Pseudokirchneriella subcapitata*	72 h NOEC = 0.143 mg a.s./L
Aquatic plants	***Lemna gibba***	**7 d NOEC = 0.000388 mg a.s./L**

Degradation/ Persistence

For the purposes of chronic aquatic hazard classification, Mesosulfuron-methyl must be assessed against the criteria for “rapid biodegradability”. The assessment criteria are discussed below in Table 5.5-1.

Table 5.5-1 Assessment of Rapid Degradability

Rapid degradation criteria	Data	Substance meets criteria
The substance is demonstrated to be readily biodegradable	No data available	No
The substance is ultimately degraded in a surface water simulation test with a DT_{50} <16 days (corresponding to 70% degradation after 28 days)	Mesosulfuron-methyl degrades in water/sediment systems with a DT50 > 16 days	No
The substance is primarily degraded, biotically or abiotically, in the aquatic environment with a half-life <16 days (corresponding to 70% degradation after 28 days), and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.	Mesosulfuron-methyl significantly degrades if degradation is microbially driven. However, half-lives are above 16 days.	No

In conclusion, Mesosulfuron-methyl does not meet any of the assessment criteria and is therefore **not rapidly degradable** in the aquatic environment.

PBT/vPvB Assessment

Under the CLP regulation, an assessment of persistence must also be made against the PBT and vPvB criteria.

Persistence

Soil

Mesosulfuron-methyl does not fulfil the P or vP criteria since its half-life is lower than 120 days.

Water

Mesosulfuron-methyl does not fulfil the P or vP criteria since its half-life in the water phase is lower than 40 days in the water-sediment study.

Bioaccumulation

According to the guidance on the application of the CLP criteria, substances with a log $K_{ow} \leq 4$ are considered to have low potential to bioaccumulate. Therefore, Mesosulfuron-methyl does not fulfil this criterion since the log K_{ow} is lower than 4. **Mesosulfuron-methyl does not have the potential to bioaccumulate.**

CLP Acute aquatic hazard

L(E)C_{50} values are available for fish, aquatic invertebrates, algae and plants. The lowest EC_{50} value obtained for Mesosulfuron-methyl is 0.00129 mg a.s./L for aquatic plants. Mesosulfuron-methyl therefore fulfils the criteria for classification as Aquatic Acute Cat. 1 (L(E)C_{50} ≤ 1 mg/L).

CLP Chronic aquatic hazard

NOEC values are available for fish, aquatic invertebrates, algae and plants. The lowest NOEC value obtained for Mesosulfuron-methyl is 0.000388 mg a.s./L for aquatic plants. Mesosulfuron-methyl therefore fulfils the criteria for classification as Aquatic Chronic Cat. 1 (chronic NOEC ≤ 0.1 mg/L).

M-factor

Acute M-factor: A comparison of the L(E)C_{50} values obtained from short term aquatic toxicity tests indicates that the lowest value falls within the >0.001 to ≤0.01 mg/L band. Based on this information the acute M-factor is 100.

Chronic M-factor: The substance is not rapidly degradable. A comparison of the NOEC values obtained from long term aquatic toxicity tests indicates that the lowest value falls within the >0.0001 to ≤0.001 mg/L band. Based on this information the chronic M-factor is 100.

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

Table 5.6-1 Conclusion on environmental classification

Hazard Class and Category Code(s)	Hazard statement Code(s)
Aquatic Acute 1, H400	Very toxic to aquatic life
Aquatic Chronic 1, H410	Very toxic to aquatic life with long lasting effects

Labelling:

Pictogram		Hazardous to the environment
Signal word	Warning	
Hazard statements	H410	Very toxic to aquatic life with long lasting effects
Precautionary statements	P273	Avoid release to the environment

P391	Collect spillage
P501	Dispose of contents/container in accordance with local/regional/national/international regulations

Acute M-factor: 100

Chronic M-factor: 100

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Mesosulfuron-methyl is an herbicide that has been reviewed as a new active substance. All data presented are derived from the methyl ester mesosulfuron-methyl and the proposed classification is for mesosulfuron-methyl.

The dossier submitter (DS) indicated aquatic plants to be the most sensitive trophic level. Based on the available data, the DS proposed environmental hazard classification as Aquatic Acute 1 (H400) with an M-factor of 100 based on acute aquatic toxicity for *Lemna gibba* (7d E_rC_{50} = 0.00129 mg/L), and Aquatic Chronic 1 (H410) with an M-factor of 100, based on chronic aquatic toxicity for *Lemna gibba* (7d NOE_rC = 0.00038 mg/L) and being not rapidly degradable.

Degradation

There are no available data on biodegradation as well as no available ready biodegradability screening tests. The results of a hydrolysis study (OECD TG 111) showed that mesosulfuron-methyl is rapidly hydrolysed at pH 4 (DT_{50} 3.5 days) but was not rapidly hydrolysed at pH 7 and pH 9 (respectively DT_{50} 253 and 318 days). Based on the two water/sediment degradation studies (SETAC, GLP) mesosulfuron-methyl was microbially degraded in the tested aerobic sediment/water systems. The water phase geomean DT_{50} at 20°C was 33.9 days and sediment phase geomean DT_{50} at 20°C was 55.2 days. The total system geomean DT_{50} at 20°C was 45.7 days. Based on the aerobic mineralisation in a surface water study (OECD TG 309, GLP), mesosulfuron-methyl did not mineralise to any relevant extent in dark laboratory conditions. The formation of carbon dioxide was insignificant throughout the study (< 0.1% AR at study end). Therefore, the DS concluded that mesosulfuron-methyl does not meet the assessment criteria for ready biodegradability and should be considered as not rapidly degradable in the aquatic environment.

Bioaccumulation

The estimated log P_{ow} for mesosulfuron–methyl was -0.48 at pH 7. This value is below the CLP trigger value of 4 intended to identify substances with a potential to bioaccumulate.

As the log P_{ow} value indicates a low potential to bioaccumulate, no study has been conducted to determine the BCF of mesosulfuron-methyl. Therefore, the DS proposed considering the substance as having a low potential for bioaccumulation.

Aquatic Toxicity

The ecotoxicological tests results from available acute and chronic studies for all trophic levels of mesosulfuron–methyl are summarised in the following table and sections.

Test organism / guideline, test method	**Short-term result (endpoint)**	**Long-term result (endpoint)**	**Reference**
Toxicity to fish			
Rainbow trout (*Oncorhynchus mykiss*) / OECD TG 203, GLP	96h LC_{50} > 100 mg/L	-	M-186666-01-1, 1999
Bluegill sunfish (*L. macrochirus*) / OECD TG 203, GLP	96h LC_{50} > 100 mg/L	-	M-186597-01-1, 1999
Sheepshead minnow (*C. variegatus*)	96h LC_{50} > 100 mg/L	-	M-238810-01-1; 2001
Rainbow trout (*Oncorhynchus mykiss*) / OECD TG 204, GLP	-	28d NOEC = 32 mg/L	M-187567-01-1, 2000
Fathead minnow (*P. promelas*) / OPPTS 850.1400, EPA OPP 72-4, GLP	-	32d NOEC = 95 mg/L	M-241475-01-1; 2003
Toxicity to aquatic invertebrates			
Daphnia magna / OECD TG 202, GLP	48h EC_{50} > 100 mg/L	-	M-186707-01-1, 1999
Mysid shrimp (*Mysidopsis bahia*) / US-EPA OPP 72-3, GLP	96h LC_{50} > 100 mg/L	-	M-238811-01-1 ; 2001
Daphnia magna / OECD TG 202, GLP	-	21d NOEC = 1.8 mg/L	M-197785-02-2, 2000
Toxicity to algae			
Selenastrum capricornutum (*Pseudokirchneriella subcapitata*) / OECD TG 201, GLP	72/96h E_rC_{50} > 0.29 mg/L	NOE_rC = 0.018 mg/L	M-143500-01-1, 1998

Selenastrum capricornutum (*Pseudokirchneriella subcapitata*) / OECD TG 201, GLP	72h E_rC_{50} = 3.99 mg/L	NOE_rC = 0.143 mg/L	M-516540-01, 2015
Navicula pelliculosa / OECD TG 201, GLP	96h E_rC_{50} > 74.9 mg/L	NOE_rC = 74.9 mg/L	M-187975-01-1, 2000
Anabaena flos-aquae / OECD TG 201, GLP	96h E_rC_{50} = 4.1 mg/L	NOE_rC = 1 mg/L	M-238869-01-1 ; 2001
Skeletonema costatum / OECD TG 201, GLP	72h E_rC_{50} > 100 mg/L	NOE_rC = 60 mg/L	M-238809-01-1; 2001
Toxicity to aquatic plants			
Lemna gibba / Draft OECD guideline, US-EPA J§123-2, ASTM 1415-91, GLP	7d E_rC_{50} > 0.001 mg/L	7d NOE_rC = 0.00018 mg/L	M-195390-01-1; 2000
Lemna gibba / Draft OECD guideline, US-EPA J§123-2, ASTM 1415-91, GLP	7d E_rC_{50} = 0.001717 mg/L	7d NOE_rC < 0.00077 mg/L	M-206814-01; 2002
Lemna gibba / OECD TG 221, GLP	**7d E_rC_{50} = 0.00129 mg/L** 7d E_rC_{50} = 0.00161 mg/L	**7d NOE_rC = 0.000388 mg/L**	M-445139-01-1; 2013

The DS confirmed that all the provided studies are reliable and proposed classification of mesosulfuron-methyl based on aquatic toxicity studies using *Lemna gibba* (2013). The study of *Lemna gibba* (2013) was considered as fully valid without any restrictions.

Acute toxicity

The DS proposed classification of mesosulfuron-methyl as Aquatic Acute 1 (H400), M-factor = 100, based on acute toxicity in aquatic plants (*Lemna gibba*): 7d E_rC_{50} = 0.00129 mg/L (growth inhibition for total frond area).

Chronic toxicity

The DS proposed classification of mesosulfuron-methyl as Aquatic Chronic 1 (H410), M-factor = 100, based on chronic toxicity in aquatic plants (*Lemna gibba*): 7d NOE_rC = 0.000388 mg/L (growth inhibition and no changes in plant appearance and development).

Comments received during public consultation

Three Member State Competent Authorities (MSCA) submitted comments on the environmental part of the DS's proposal. All of them support the proposed classification as Aquatic Acute 1 and Aquatic Chronic 1, as well as the corresponding M-factors of 100.

However, one MSCA asked for clarification on the substance identity as three substances identities are given in CLH report (mesosulfuron-methyl, mesosulfuron and mesosulfuron-methyl-sodium). It not was clear why the other two substances were mentioned in this dossier as the IUCLID Dossier and the cover page of the CLH report indicate that the substance is known only as mesosulfuron-methyl. In answer, the DS clarified that Mesosulfuron is the ISO common name. The active substance manufactured is the variant mesosulfuron-methyl. All data are related to this variant. The DS agreed that for better clarity, only mesosulfuron-methyl shall be considered in the CLH report proposal.

One MSCA pointed out that it would be helpful if, in the assessment, key and supportive studies were identified, and also if the reliability of the studies were indicated. Another MSCA noted that with the exception of the more detailed description for the study on *Lemna gibba*, little information is given for the other reported studies on aquatic toxicity. This makes it difficult to make an objective evaluation of the environmental hazards and to decide which studies should be regarded as key studies. Nevertheless, they agree that aquatic plants (*Lemna gibba*) are the most sensitive trophic level. In answer, the DS confirmed that all studies reported in the CLH report are reliable. The key study was described as follows: "This study is considered to be relevant and reliable and is carried forward for classification purposes with a 7 day E_rC_{50} of 1.29 µg/L and 7 day NOE_rC of 0.388 µg/L" with a detailed summary of the study included in the CLH report. A detailed description of the rest of the study was reported in the RAR volume CA-B9.

Assessment and comparison with the classification criteria

Degradation

RAC notes that there are no available data on biodegradation estimation as well as no available ready biodegradability screening tests and therefore the substance must be considered to be not readily biodegradable. RAC agrees that mesosulfuron-methyl does not fulfil the criteria necessary to be considered ultimately degraded in a surface water simulation test (DT_{50} < 16 days, corresponding to 70% degradation after 28 days). RAC agrees that mesosulfuron-methyl does not fulfil the criteria for primarily degradation, biotically or abiotically, in the aquatic environment (DT_{50} < 16 days corresponding to 70% degradation after 28 days), when it can be demonstrated that the primary degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. RAC notes that mesosulfuron-methyl degraded in water/sediment systems, however with a DT_{50} > 16 days. In conclusion, RAC agrees that mesosulfuron-methyl does not meet any of the assessment criteria and should be considered as not rapidly degradable in the aquatic environment.

Bioaccumulation

RAC agrees that the estimated log P_{ow} for mesosulfuron–methyl is -0.48 (at pH 7) which is below the CLP log P_{ow} trigger value of 4. As this value shows a low potential to bioaccumulate no study to determine the BCF of the mesosulfuron-methyl was required.

Aquatic toxicity

RAC agrees that there are adequate acute and chronic aquatic toxicity data for all trophic levels (fish, invertebrates, algae/aquatic plants). RAC agrees that aquatic plants (*Lemna gibba*) are the most sensitive trophic level for the purpose of aquatic acute and chronic classification.

Acute toxicity

RAC agrees that the aquatic plant (*Lemna gibba*) 7 day E_rC_{50} = 0.00129 mg/L (based on growth inhibition for total frond area) is the lowest reliable acute/short-term endpoint for aquatic acute classification purposes.

Chronic toxicity

RAC agrees that the aquatic plant (*Lemna gibba*) 7 day NOE_rC = 0.000388 mg/L (based on growth inhibition no changes in plant appearance and development) is the lowest reliable long-term endpoint for aquatic chronic classification purposes.

Conclusion on classification

Mesosulfuron-methyl is considered as not rapidly degradable and does not fulfil the criteria for having a high potential for bioaccumulation. Based on the available and most reliable information, RAC is of the opinion that mesosulfuron-methyl should be classified as:

Aquatic Acute 1 based on E_rC_{50} = 0.00129 mg/L for *Lemna gibba*. As this acute toxicity value falls within the 0.001 < $L(E)C_{50}$ ≤ 0.01 mg/L range, the **acute M-factor is 100**.

Aquatic Chronic 1 based on NOE_rC = 0.000388 mg/L for *Lemna gibba*. As this chronic toxicity value falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the **chronic M-factor is 100**.

6 OTHER INFORMATION

No other relevant information

7 REFERENCES

European Commission. Draft Assessment Report Mesosulfuron-Methyl, prepared by France in December 2001, with updated addendum of April and May 2003.

European Commission. Draft Renewal Assessment Report Mesosulfuron-Methyl, prepared by France in July 2015

8 ANNEXES

IUCLID dataset for Mesosulfuron.