Committee for Risk Assessment

RAC

Annex 1

Background document

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

halosulfuron-methyl (ISO); methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate

EC Number: -
CAS Number: 100784-20-1

CLH-O-0000001412-86-182/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

22 September 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Halosulfuron-methyl

EC Number: Not allocated

CAS Number: 100784-20-1

Index Number: Not available

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Version number: 2.1 **Date: 2nd June 2016**

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Halosulfuron-methyl
EC number:	Not allocated
CAS number:	100784-20-1
Annex VI Index number:	Not allocated
Degree of purity:	≥ 98.0%
Impurities:	No relevant impurities for classification. Full information is provided in the technical dossier.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not in Annex VI
Current proposal for consideration by RAC	Aquatic Acute 1 (H400) Acute M-factor of 1000 Aquatic Chronic 1 (H410) Chronic M-factor of 1000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1 (H400) Acute M-factor of 1000 Aquatic Chronic 1 (H410) Chronic M-factor of 1000

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification

Table 3: Proposed classification according to the CLP Regulation (Continued)

3.1.	Acute toxicity - dermal	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.1.	Acute toxicity - inhalation	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	None	Not included in Annex VI	Data lacking
3.4.	Skin sensitisation	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	None	Not included in Annex VI	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 - Very toxic to aquatic life Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects	Acute M-factor = 1000 Chronic M-factor =1000	Not included in Annex VI	N/A
5.1.	Hazardous to the ozone layer	Not classified	None	Not included in Annex VI	Data lacking

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

Labelling:

Signal word: Warning

Pictogram: GHS09

Hazard statements: H410; Very toxic to aquatic life with long lasting effects

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Halosulfuron-methyl is a new active substance for which in accordance with Article 6(2) of Council Directive 91/414/EEC Italy (hereinafter referred to as the RMS) received an application for approval. Complying with Article 6(3) of Directive 91/414/EEC, the completeness of the dossier was checked by the RMS. The European Commission recognised in principle the completeness of the dossier by Commission Decision 2006/586/EC.

The RMS provided its initial evaluation of the dossier on halosulfuron-methyl in the Draft Assessment Report (DAR), which was received by EFSA on 30 March 2008. The peer review was initiated on 5 October 2011 by dispatching the DAR for consultation of the Member States.

Following consideration of the comments received on the DAR, it was concluded that EFSA should conduct an expert consultation in the areas of mammalian toxicology and ecotoxicology and EFSA should adopt a conclusion on whether halosulfuron-methyl can be expected to meet the conditions provided for in Article 5 of Directive 91/414/EEC, in accordance with Article 8 of Commission Regulation (EU) No 188/2011.

Halosulfuron-methyl was discussed at the Pesticides Peer Review Meeting 95 in September 2012.

The conclusion on the peer review of the pesticide risk assessment of halosulfuron-methyl was published in the EFSA Journal (2012;10(12):2987).

2.2 Short summary of the scientific justification for the CLH proposal

Halosulfuron-methyl is a plant protection active substance which has been approved under Regulation (EC) No 1107/2009 (Commission Implementing Regulation (EU) No 356/2013 of 18 April 2013) and considered for inclusion in Annex I of Directive 91/414/EC. In 2012, EFSA published a conclusion on the peer review of the risk assessment for the active substance. This highlighted a concern for reproductive toxicity and Aquatic acute and chronic toxicity.

In the EFSA conclusion, reproductive and developmental studies showed a higher sensitivity of the offspring to halosulfuron-methyl exposure than the adult animals. The offspring‘s NOAEL in the multigeneration reproduction toxicity study was 6.3 mg/kg bw per day based on reduced pup body weight gain, while the parental NOAEL was 50.4 mg/kg bw per day regarding the same endpoint. In this study no effect on fertility or reproduction was observed up to the highest dose level of 223.2 mg/kg bw per day. In the developmental toxicity study in rabbits, the maternal and developmental NOAELs were 50 mg/kg bw per day based on early resorptions, decreased number of fetuses and reduced maternal body weight gain. In the rat, fetal toxicity was observed in the absence of maternal toxicity: the developmental NOAEL was 75 mg/kg bw per day based on a higher incidence of visceral and skeletal variations and the maternal NOAEL was 250 mg/kg bw per day due to reduced body weight, body weight gain and food consumption. These effects suggest that classification regarding developmental toxicity would be required for halosulfuron-methyl as ‘Reprotox cat. 2, H361fd, suspected of damaging the unborn child’.

However, supplementary evidence submitted to the RMS after the EU review, in the form of detailed reviews of the reproduction and developmental toxicity studies, showed that there are no substantive data to indicate higher sensitivity of offspring to halosulfuron-methyl. Developmental effects of halosulfuron-methyl administration and consistent bodyweight effects on post-natal offspring occurred only at dosages which were also maternally toxic. The classification proposed by EFSA is

therefore not included in the proposed CLP classification. The current proposal of no classification is supported by the above-mentioned review papers prepared by LSR Associates, Ltd (1: Halosulfuron-methyl_NOAEL for offspring bodyweight 20122012; 2: Halosulfuron- methyl_Tox Classification_20122012) which respectively address, offspring bodyweight data in the reproduction study and, maternal versus fetal toxicity and fetal findings in the rat fetal toxicity study, taking into account the toxicokinetics of halosulfuron-methyl, and which concludes an absence of hazard for human health assessments.

The confidential documents are provided in chapter 13 of the IUCLID 5 technical dossier.

Acute and long-term studies are available on aquatic organisms (fish, daphnia, algae and higher plants) for halosulfuron-methyl, a formulated product and the metabolite halosulfuron-methyl rearrangement (HSMR). Algae and aquatic plants were the most sensitive organisms. Regarding the degradability, halosulfuron-methyl cannot be considered rapidly degradable. The endpoint driving the environmental classification was observed in a laboratory study with halosulfuron-methyl and the higher plant *Lemna gibba* (7 day E_bC_{50} = 0.217 µg/l).

Halosulfuron-methyl is not rapidly degradable. It is proposed to classify halosulfuron-methyl as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) based on a NOEC of 0.217 µg/l in higher aquatic plants. A harmonised acute M-Factor and chronic M-factor of 1000 in accordance with the 2nd ATP criteria is proposed.

New data have been requested following the outcome of the EU review. These will not change the proposed classification and are therefore not discussed here.

2.3 Current harmonised classification and labelling

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

No harmonised classification exists for halosulfuron-methyl in Annex VI, table 3.1 of the CLP Regulation.

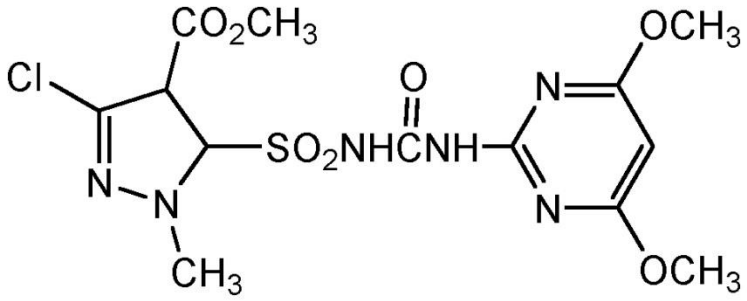
2.4 Current self-classification and labelling

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

The following entries exist on the C&L Inventory at the time of submissionClassification		Labelling		Specific Concentration limits, M-Factors	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Pictograms, Signal Word Code(s)		
Aquatic Acute 1	H400		GHS09 Wng		48
Aquatic Chronic 1	H410	H410			
Aquatic Acute 1	H400	H400	GHS09 Wng		23
Aquatic Chronic 1	H400	H410	GHS09 Wng	M=1000	1

RAC general comment

Halosulfuron-methyl is a plant protection active substance which has been approved under Regulation (EC) No 1107/2009 (Commission Implementing Regulation (EU) No 356/2013 of 18 April 2013). It is a sulfonylurea herbicide and is used against sedges and broad–leaf weeds through the inhibition of the enzyme acetolactate synthase, an essential enzyme in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine. This results in early cessation of growth followed by plant death. It has no current entry in Annex VI of the CLP Regulation and all hazard classes are open for assessment.



In 2012, the European Food Safety Authority (EFSA) published a pesticide peer review conclusion for the halosufuron-methyl (EFSA, 2012). This highlighted a concern for reproductive toxicity and aquatic acute and chronic toxicity. EFSA concluded that category 2 classification for developmental toxicity would be warranted for halosulfuron-methyl (EFSA, 2012).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Halosulfuron-methyl is an active substance in the meaning of Regulation (EC) No. 1107/2009 and according to article 36 of CLP such substances are subject to harmonised classification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

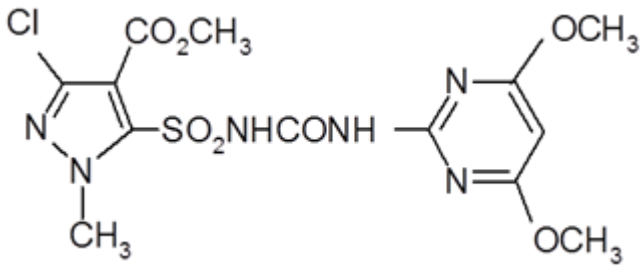
Halosulfuron-methyl (modified ISO 1750) is a sulfonylurea herbicide (IUPAC name: methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoyl-sulfamoyl)-1-methylpyrazole-4-carboxylate).

The Chemical Abstracts name is 1*H*-Pyrazole-4-carboxylic acid, 3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino]sulfonyl]-1-methyl-, methyl ester. The chemical formula is $C_{13}H_{15}ClN_6O_7S$.

Table 4: Substance identity

EC number:	None assigned
EC name:	None assigned
CAS number (EC inventory):	None assigned
CAS number:	100784-20-1
CAS name:	1*H*-Pyrazole-4-carboxylic acid, 3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino]sulfonyl]-1-methyl-, methyl ester
IUPAC name:	methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate
CLP Annex VI Index number:	None assigned
Molecular formula:	$C_{13}H_{15}ClN_6O_7S$
Molecular weight range:	434.82

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Halosulfuron-methyl	99.4% (w/w)	98-100%	The typical purity is the mean value for purity determined in the five batch analysis of halosulfuron

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity identity and levels are confidential. See confidential annex in the technical dossier.

Current Annex VI entry: Not applicable

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None	Not applicable	Not applicable	Not applicable	The substance does not contain additives

Current Annex VI entry: None

1.2.1 Composition of test material

According to the EFSA conclusion published in EFSA Journal 2012;10(12):2987 equivalence of the tested material with the technical specification was not demonstrated for the toxicity studies in the mammalian toxicology and ecotoxicology sections and was identified as requiring confirmatory information, in Commission Implementing Regulation (EU) No 356/2013. As a result, the Applicant has prepared a document that addresses this area of concern, showing that the manufacturing process used for pilot production which gave rise to the batches used for mammalian toxicological and ecotoxicological studies had not been changed for commercial production. Consequently it can be concluded that the impurity profiles would be similar to each other, i.e. equivalence of the batches used in toxicological and ecotoxicological studies and current commercial batches. To confirm the

above, 5-batch analysis data submitted and accepted by the regulatory authorities in Japan and USA for contemporary material manufactured between 1992 and 1993 are provided.

The document is provided in a confidential Annex of the technical dossier.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Pure: a fine white powder containing some crystalline particles at 20°C	DAR B.2.1.7 Comb, 2005	Appearance physical state, visual observation (pure: 99.9%)
Melting/freezing point	Mean 175.5-177.2°C (n=3)	DAR B.2.1.1 Pesselman, 1991a	OECD 102, EEC A1 capillary method, melting temperature device with metal block (technical 99.1%)
Boiling point	Value not determined.	DAR B.2.1.2	Boiling point has not been determined. Stable to 180°C. Loss of mass from 213 - 285 °C assumed to be decomposition
Relative density	$D_4^{20} = 1.57$	DAR B.2.1.4 (DAR addendum additional report B2 Volume 3 August 2012)Comb, 2008	OECD 109, EEC A3 pycnometer method (pure 99.9%)
Vapour pressure	<1 x 10-7 mmHg <1.33 x 10-5 Pa at 25± 1°C	DAR B.2.1.5 Pesselman, 1991c	EEC A4 gas saturation method (pure 99.9%).
Surface tension	70.5 mN/m (n=2) at 25ºC (90% solution). Halosulfuron-methyl is not considered surface- active.	DAR B.2.1.32 Comb 2003c	OECD 115, EEC A5 tensiometer using the OECD harmonized ring method. (technical 99.6%)
Water solubility	1.02 x 10-2 g/L at 20°C and pH 6.5 (n=3)	DAR B.2.1.14 Hirai, 1999	EEC A6 column elution method (pure 99.9%)
Partition coefficient n-octanol/water	at 23 ± 2°C, measured log Kow values: pH 5 1.67 (n=6) pH 7 -0.0186 (n=6) pH 9 -0.542 (n=6)	DAR B.2.1.19 Pesselman, 1991h	OECD 107, EEC A8 shake-flask method (pure 99.9%)
Flash point	Not required because melting point is above 40 °C	DAR B.2.1.30	N/A
Flammability	The test item in contact with the flame changed from white to brown but did not ignite. Not highly flammable	DAR B.2.1.28 Schuurman & Hooidonk, 1988	EEC A10 (technical 99.2%)
Explosive properties	Not explosive under the influence of flame and not sensitive to shock or friction	DAR B.2.1.31 Schuurman & Hooidonk, 1988	EECA14 (technical 99.2%)
Self-ignition temperature	No relative self-ignition temperature. Not auto-flammable	DAR B.2.1.29 Comb, 2003b	EEC A16 (pure 99.6%)
Oxidising properties	As neither mixture of 2:1, 1:1 or 1:2 test substance/cellulose burned to completion, halosulfuron-methyl is considered to be non-oxidising	DAR B.2.1.33 Comb, 2003d	Evaluation on theoretical basis
Granulometry	Not determined		
Stability in organic solvents and identity of relevant degradation products	Not determined		
Dissociation constant	pKa = 3.44 at 22.4ºC	DAR B.2.1.26 Pesselman, 1991i	Spectro-photometric method, (pure 99.9%)
Viscosity	Not applicable as substance is a solid	DAR B.2.2.11	N/A

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Halosulfuron-methyl is a sulfonylurea herbicide with a mean purity of 99.4% (w/w). It is stable at ambient temperature, has no explosive properties as shown in test EEC A.14 and is a solid that cannot be ignited with a flame in test EEC A.10. Halosulfuron-methyl does not self-ignite below 400°C and evaluation of its chemical structure shows that it does not possess oxidising properties (table 9, CLH report). No classification of halosulfuron-methyl for physical hazards was proposed by the DS.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

There is no data on the physical properties of halosulfuron-methyl to indicate that any classification for physical hazards is required. **RAC does not propose classification for physical hazards**.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Halosulfuron-methyl is used as a herbicide in agriculture

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of halosulfuron-methyl were assessed in the Draft Assessment Report and Proposed Decision of Italy prepared in the context of the possible inclusion of halosulfuron-methyl in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, August 2007 and subsequent addendum (2012, RMS Italy) concerning the placing of plant protection products on the market.

Halosulfuron-methyl has no explosive properties as shown in the EEC A14 test, is a solid that cannot be ignited (EEC A10) and does not possess oxidizing properties. Therefore, no classification of halosulfuron-methyl for physico-chemical properties is required.

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point		Not applicable as melting point >40°C	DAR B.2.1.30
Flammability EEC A10 method GLP	Not highly flammable	Purity 99.2%	DAR B.2.1.28 Schuurman & Hooidonk, 1988
Explosive properties EEC A14 method GLP	Non-explosive	Not explosive under the influence of a flame and not sensitive to shock and friction	DAR B.2.1.31 Schuurman & Hooidonk, 1988
Self-ignition temperature EEC A16 method GLP	No self-ignition below 400°C	Purity 99.6%	DAR B.2.1.29 Comb, 2003b
Oxidising properties evaluation on theoretical basis EEC A.17 GLP	No oxidizing properties	Purity 99.6%	DAR B.2.1.33 Comb, 2003d

3.1

3.1.1 Summary and discussion of physico-chemical hazard class

Halosulfuron-methyl is stable at ambient temperature, has no explosive properties as shown in test EEC A14 and is a solid that cannot be ignited with a flame in test EEC A10. Halosulfuron-methyl does not self-ignite below 400°C and evaluation of its chemical structure shows that it does not possess oxidising properties.

3.1.2 Comparison with criteria

There are no adverse physical properties of halosulfuron-methyl and classification for physical hazard is not required.

3.1.3 Conclusions on classification and labelling

No classification of halosulfuron-methyl for physico-chemical properties is required under CLP.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The absorption, distribution, metabolism and excretion of halosulfuron-methyl (NC-319) were studied in Sprague-Dawley rats in two separate studies (McCarthy, 1991a, DAR B.6.1.1; McCarthy 1991b, DAR B.6.1.2).

Halosulfuron-methyl labelled in the pyrimidine or pyrazole rings ([^{14}C]-Pd-halosulfuron-methyl and [^{14}C]-Pz-halosulfuron-methyl) was orally administered by gavage at single doses of 5 mg/kg body weight and 250 mg/kg body weight. A repeat oral dose of unlabelled material at 5 mg/kg body weight for 14 days followed by a single oral dose of [^{14}C]-Pd-halosulfuron-methyl or [^{14}C]-Pz-halosulfuron-methyl at 5 mg/kg body weight was also investigated. The distribution of radioactivity in tissues, biliary excretion and plasma/blood cell radioactivity kinetics were also determined in rats using a single oral dose of 5 mg/kg body weight of both radiolabelled forms of halosulfuron-methyl. Quantitative tissue distribution was also determined by autoradiography of whole body tissue sections from rats orally dosed with 5 mg/kg body weight of both radiolabelled forms of halosulfuron-methyl. In all cases halosulfuron-methyl was administered orally in a suspension of 1% Tween 80.

Absorption

Following oral administration of [^{14}C]-halosulfuron-methyl, the maximum concentrations of radioactivity in blood occurred within 0.5 hours, indicating rapid absorption. This was confirmed by autoradiography and time distribution studies that showed a much higher concentration of radioactivity throughout the body at 0.5 hours rather than at 3 hours after dosing. The pharmacokinetic parameters for whole blood after dosing rats with [^{14}C]-halosulfuron-methyl indicated that the elimination of radioactive materials from blood was biphasic with no differences observed between sexes or radiolabels for the initial phase (α) for all groups. The second phase elimination (β) was slower after dosing with [^{14}C]-Pd-halosulfuron-methyl (half lives of 53-55 hours) compared to [^{14}C]-Pz-halosulfuron-methyl (half lives of 29-38 hours). However, the areas under the concentration:time curves were similar for all groups. The slower elimination of radioactivity from the blood after dosing with [^{14}C]-Pd-halosulfuron-methyl is reflected in the apparent slower elimination from tissues containing appreciable amounts of blood (e.g. heart and spleen). There was no absorption of radiolabelled material by the red blood cells. Pre-dosing with non-radiolabelled halosulfuron-methyl for 14 days prior to dosing with [^{14}C]-halosulfuron-methyl had no effect on absorption as indicated by similar patterns of excretion.

Distribution

Following a single dose at 5.0 mg/kg of [^{14}C]-halosulfuron-methyl (report no. T42), radioactivity was widely distributed with peak concentrations of radioactivity in tissues being reached at 0.5 hours after dosing. Concentrations were highest in the plasma (8.02-14.07 μg equivalent of halosulfuron-methyl/ml) and liver (6.51-12.34 μg equivalent of halosulfuron-methyl/g).

Throughout the study the highest concentrations of radioactivity were in the liver and kidney and lowest concentrations were in the brain. By 50 hours after dosing all concentrations had decreased to <0.15 μg equivalent of halosulfuron-methyl/g. At 168 hours after administration of single or multiple oral low level doses of [^{14}C]-halosulfuron-methyl, most tissues contained radioactivity at levels comparable to background levels. For both radiolabelled forms of halosulfuron-methyl in the low dose studies, no tissue had a concentration above 0.07 μg equivalent of halosulfuron-methyl/g or ml at this time point, and there was no indication of bioaccumulation in tissues after pre-dosing with non-radiolabelled halosulfuron-methyl. At the high dose, all concentrations at 168 hours post-dosing were < 3.5μg equivalent of halosulfuron-methyl/g or ml. Less than 1% of the dose was retained in the carcass.

Autoradiography showed the uptake of radioactive material at 0.5 and 3 hours after dosing and then its rapid elimination by 96 hours. There was no uptake into foetal tissues or evidence of transplacental transfer. In pregnant rats radioactivity was observed in the kidney and intestines at 96 hours and in the intestines at 150 hours after dosing. Thus, the elimination of radioactive material from pregnant rats is slower than from males and non-pregnant females. This is not unexpected as pregnancy can affect metabolism and biliary excretion in animals.

There were no substantial sex differences in the concentrations of radioactivity after dosing with either radiolabelled forms of halosulfuron-methyl. The only difference after dosing with the two radiolabelled forms of halosulfuron-methyl was the significantly higher concentration of radioactivity in whole blood in male and female rats dosed with the high dose level of [^{14}C]-Pd-halosulfuron-methyl (2.3-3.5 μg equivalent of halosulfuron-methyl/ml) than that found after dosing with [^{14}C]-Pz-halosulfuron-methyl (0.18-0.21 μg equivalent of halosulfuron-methyl/ml).

Excretion

Following oral administration of [^{14}C]-halosulfuron-methyl, excretion of radioactive material was rapid (report no. T41). The majority of the dose was excreted within 12 hours in urine and two days in faeces. The mean total excretion over seven days was 79-102% of the dose with the mean urinary excretion accounting for 32-55% of the dose and the mean faecal excretion accounting for 35-55% of the dose. The routes and rates of excretion were independent of the dose, sex or radiolabelled form of halosulfuron-methyl. A biliary excretion study showed rapid transfer of radioactive material into the bile after an oral dose of [^{14}C]-halosulfuron-methyl (report no. T42). The total excretion in bile over eight hours was 28.8-49.7% of the dose, the mean being 35.6%.

For operator exposure risk assessment (AOEL) purposes, estimations of exposure via the oral route require the derivation of an internal systemic dose to allow for incomplete absorption from the gastrointestinal tract (Point 5.11.5; Annex III, Section 3, Point 7.3). Results of the biliary excretion study with halosulfuron-methyl (Annex II, Section 3, Point 5.1.1.2) show that absorption from the gastrointestinal tract after a single oral dose of 5 mg/kg was 32.5% and 28.8% respectively for male and female rats dosed with [^{14}C]-Pd-halosulfuron-methyl and 31.4% and 49.7% for rats given [^{14}C]-Pz-halosulfuron-methyl respectively. Therefore, the mean absorption for both radiolabels and sexes was 35.6%. However, there was no measurement of urinary excretion in this study. Consequently, absorption is underestimated. An estimate of total absorption is obtained by adding the 0-168 hour urinary excretion values from the other toxicokinetic study (Annex II, Section 3, Point 5.1.1.1) to the biliary excretion values. This is justified as the dosing regimes were comparable in both studies.

Mean cumulative radioactivity recovered in urine up to 168 hours after dosing (expressed as % of applied dose), was:

[^{14}C]-Pd-halosulfuron-methyl: 39.7% (males) and 32.6% (females)

[^{14}C]-Pz-halosulfuron-methyl: 41.5% (males) and 37.6% (females)

The mean of both radiolabels and sexes was 37.9%. Adding mean biliary excretion (35.6%) to this urinary value gives an estimated total absorption of 73.5%. As this absorbed dose was <80 % of the applied dose, a correction factor of 0.735 is needed to derive the internal (systemic) dose, $AOEL_{sys}$ (EU guidance document 7531/VI/95 rev.6 (draft 2001).

Metabolism

There was extensive metabolism with no halosulfuron-methyl quantified in urine and very little in faeces. The major metabolites identified in urine and faeces were demethyl halosulfuron-methyl (urine: 13.3-37.7%; faeces: 6.6-22.6% radioactivity) and 5-hydroxy demethyl halosulfuron-methyl (urine: not detected-39.9%; faeces: 1.6-24.5%). Minor metabolites were N-demethyl halosulfuron-methyl (3.9-7.3% radioactivity), 5-hydroxy halosulfuron-methyl (0.6-7.5% radioactivity), halosulfuron (2.5-6.1% radioactivity), chlorosulfonamide (<1% radioactivity) and 4,6-dihydroxy halosulfuron-methyl (<2% radioactivity). The metabolic profile in urine was generally similar to that in faeces. There were no differences in the metabolic profile in male and female rats and only a few differences in the profiles from the two radiolabelled forms of halosulfuron-methyl (e.g. <1% of chlorosulfonamide and one unidentified metabolite at <2% for each radiolabelled form). At the high dose there were quantitative differences in metabolites compared to the low dose which provided evidence of saturation of some metabolic pathways (e.g. the formation of 5-hydroxy demethyl halosulfuron-methyl from demethyl halosulfuron-methyl, the major metabolite in urine and faeces).

The studies conducted at the low and high dose and after repeat dosing with non-radiolabelled halosulfuron-methyl followed by halosulfuron-methyl radiolabelled in two separate positions in male and female rats gave very similar results. Hence, the absorption, distribution, metabolism and excretion of halosulfuron-methyl in the rat are largely independent of dose, sex and the position of the radiolabelling of the molecule.

4.1.2 Human information

No data are available.

4.1.3 Summary and discussion on toxicokinetics

Halosulfuron-methyl is rapidly absorbed (highest concentrations at 0.5 hour post-dosing) and has high bioavailability (> 80 % of dose, based on urinary and biliary excretion and residues in the carcass).

The substance is widely distributed to different organs and there is no evidence for absorption saturation.

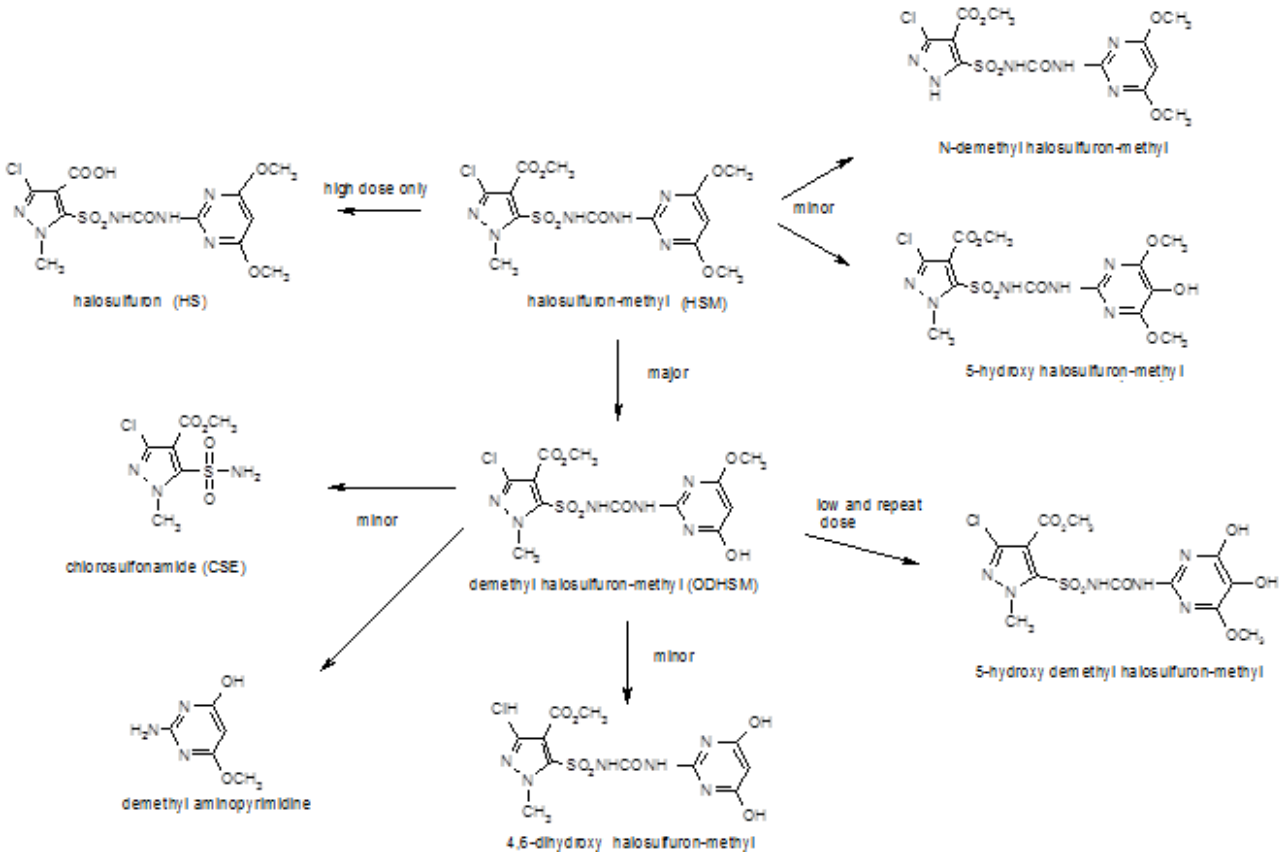
There is very low potential for accumulation (< 1% of residues 168 hours after dosing, independently on treatment regimen).

Excretion is rapid and extensive (> 70% within 12 hours in urine and within 48 hours in faeces) and between 79-102% excreted within 7 days. Urinary excretion accounts for 33-55% of the dose and 35-55% of the dose is excreted in the faeces within 7 days. 29-40% of the substance is removed by

biliary excretion within 48h.

Halosulfuron-methyl is extensively metabolised with no parent compound detected in urine and a low amount (0.6-1%) in faeces. Major pathways are demethylation and hydroxylation of the pyrimidine moiety with a minor pathway (< 3%) of cleavage between the pyrimidine and pyrazole moieties. The major metabolites are demethyl halosulfuron-methyl (urine: 13-35%; faeces: 7-8%) and 5-hydroxy demethyl halosulfuron-methyl (urine: 9-14%; faeces: 15-25%).

The proposed metabolic pathway is shown below:



4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Acute oral		
Method	**Results**	**Reference**
Rat, Sprague-Dawley, 10/sex4000, 5000, 7500 or 10,000 mg/kg. 0,5 % carboxymethyl cellulose US EPA 81-1, JMAFF 59, NohSan no. 4200 GLP Purity 98.5 %	LD50= 7758 mg/kg bw	DAR B.6.2.1.1 Osheroff, 1990a
Mice, CD-1, 10/sex 4000, 5000, 7500 or 10,000 mg/kg. 0,5 % carboxymethyl cellulose US EPA 81-1, JMAFF 59 NohSan no. 4200 GLP Purity 98.5 %	LD50= 9295 mg/kg bw	DAR B.6.2.1.2 Osheroff, 1990b
Acute dermal		
Method	**Results**	**Reference**
Rat, Sprague-Dawley, 10/sex 2000 mg/kg US EPA 81-1, JMAFF 59 NohSan no. 4200 GLP Purity 98.5 %	LD50>2000 mg/kg	DAR B.6.2.2 Osheroff, 1990c
Acute inhalation		
Method	**Results**	**Reference**
Rat, Sprague-Dawley, 5/sex 6.0 mg/L (4 h) OECD 402 GLP Purity 99.7%	LC50>6 mg/l	DAR B.6.2.3 Bechtel, 1991

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Rat

Groups of 10 male and 10 female fasted Sprague Dawley rats were given a single oral dose, by gavage, of 5000 mg/kg of halosulfuron-methyl in 0.5% carboxymethyl cellulose. Then similar sized groups were dosed with 4000, 5000, 7500 or 10,000 mg/kg.

Mortality occurred at all dose levels.

Table 11: Mortality of rats after oral administration of halosulfuron-methyl

Sex	Dose level (mg/kg)				
	5000 (initial test)	4000	5000	7500	10000
Males	1/10	0/10	2/10	2/10	5/10
Females	1/10	1/10	2/10	5/10	7/10

The principal clinical signs were slightly depressed or depressed (although not specified in the original report, depressed is taken to mean hypoactive, a common non-specific finding in toxicity studies), urine stains, hunched posture, red stains on nose and/or eyes and soft faeces.

Table 12: Group incidence of clinical signs in rats after oral administration of halosulfuron-methyl

Clinical sign[a]	Dose level (mg/kg)									
	5000 (initial test)		4000		5000		7500		10000	
	M	F	M	F	M	F	M	F	M	F
Slightly depressed[b]	8	7	3	1	4	2	4	1	6	10
Urine stains	1	1	3	8	2	5	2	1	2	2
Hunched	6	5	1	-	-	1	3	1	2	1
Salivating	2	-	-	-	-	-	1	1	-	-
Red stains on nose and/or eyes	5	4	1	-	-	-	6	1	2	2
Soft faeces	1	1	6	2	5	2	7	1	8	2
Wheezing	-	-	-	-	-	-	-	-	-	-
Thin	-	1	-	-	1	-	-	-	-	-
Compound-coloured urine	-	-	-	-	-	-	1	-	-	-
Depressed[b]	-	-	-	-	-	-	1	-	5	1
Ataxia	-	-	-	-	1	-	1	-	-	1
Tremors	-	-	-	-	-	1	-	-	-	1
Lacrimation	-	-	1	-	-	-	-	-	-	-
Alopecia	-	-	-	1	-	1	-	-	-	-

[a] highest number of rats affected in each group at each time point

[b]The meaning of depressed is not clarified in the original (1990) report. It is taken to mean hypoactive, a common non-specific finding in toxicity studies. It is unlikely to be a specific reference to depression of the CNS or narcosis.
- clinical sign not observed

Body weight gain was unaffected. All groups exhibited a variety of commonly noted necropsy findings. Observable gross pathology findings involved the lungs, liver, kidneys and spleen (disclouration) and the stomach and intestines (discolouration, abnormal content and distension).

The rat acute oral LD_{50} values were 10435 mg/kg in males and 7758 mg/kg in females

Mouse

Groups of 10 male and 10 female fasted CD-1 mice were given a single oral dose, by gavage, of 5000 mg/kg of halosulfuron-methyl in 0.5% carboxymethyl cellulose. Then similar sized groups were dosed with, 4000, 5000, 7500 or 10,000 mg/kg.

Mortality was dose-related and occurred at all dose levels.

Table 13: Mortality of mice after oral administration of halosulfuron-methyl

Sex	Dose level (mg/kg)				
	5000 (initial test)	4000	5000	7500	10000
Males	0/10	1/10	1/10	2/10	3/10
Females	1/10	0/10	1/10	4/10	5/10

The principal clinical sign at 4000 and 5000 mg/kg was slight depression (although not specified in the original report, depressed is taken to mean hypoactive, a common non-specific finding in toxicity studies) . At higher dose levels, signs also included urine stains, hunched posture, soft faeces, depression, ataxia and tremors.

Table 14: Group incidence of clinical signs in mice after oral administration of halosulfuron-methyl

Clinical sign[a]	Dose level (mg/kg)									
	5000 (initial test)		4000		5000		7500		10000	
	M	F	M	F	M	F	M	F	M	F
Slightly depressed[b]	-	1	2	-	-	3	10	10	8	5
Depressed[b]	-	-	-	-	1	-	9	4	7	4
Tremors	-	-	-	-	1	1	10	10	1	6
Hunched	-	-	-	-	-	-	7	6	-	-
Ataxia	-	-	-	-	1	1	4	4	5	6
Urine stains	-	-	-	-	-	-	6	7	1	1
Convulsions	-	-	-	-	-	-	-	-	-	1
Squinted right eye	-	1	-	-	-	-	-	-	-	1
Rough coat	-	-	-	-	1	-	3	-	-	-
Labored respiration	-	-	-	-	-	-	-	-	2	-
Soft faeces	-	-	-	-	-	-	1	-	-	-

[a] highest number of rats affected in each group at each time point

[b]The meaning of depressed is not clarified in the original (1990) report. It is taken to mean hypoactive, a common non-specific finding in toxicity studies. It is unlikely to be a specific reference to depression of the CNS or narcosis.
- clinical sign not observed

Body weight gain was unaffected. All groups exhibited a variety of commonly noted necropsy findings. Observable gross pathology findings involved the lungs, liver, kidneys and spleen

(disclouration) and the stomach and intestines (discolouration, abnormal content, distension and thin walls).

The mouse acute oral LD_{50} values were 16156 mg/kg in males and 9295 mg/kg in females.

4.2.1.2 Acute toxicity: inhalation

Groups of 5 male and 5 female Sprague Dawley CD rats were exposed in a whole body inhalation system for 4 hours to 6.0 mg/l of milled test substance and then observed for 14 days. The percentage of particles less than 1 µm was 3.6 (MMAD= 4.3 µm).

There were no mortalities. During exposure hypoactivity was noted whilst hypoactivity, laboured respiration, nasal discharge, nasal encrustation, perioral wetness and periocular encrustation were noted post exposure. All rats were normal by Day 3.

Table 15: Summary of observations during post-exposure period

Clinical observation	Dose 6.0 mg/l			
	No. of males affected	No. of females affected	First day observed	Last day observed
Hypoactive	5	2	0	0
Labored respiration	5	4	0	0
Red/pink nasal discharge	5	5	0	0
Red/brown perinasal encrustation	2	4	1	2
Perioral wetness	5	5	0	0
Periocular encrustation	0	1	0	0

All animals achieved anticipated body weight gains and no macroscopic abnormality was evident at necropsy. No histopathology was conducted as is typical for acute toxicity (LC_{50} or LD_{50}) studies.

The rat acute oral LC_{50} value of the test substance was >6.0 mg/l.

4.2.1.3 Acute toxicity: dermal

A group of 10 male and 10 female Sprague Dawley CD rats was given a single topical application of 2000 mg/kg of halosulfuron-methyl for 24 hours. They were observed for 14 days.

There was no mortality and no clinical signs (either systemic or at the application site) were seen.

The rat acute dermal LD_{50} value was >2000 mg/kg/day.

4.2.1.4 Acute toxicity: other routes

Not applicable

4.2.2 Human information

No acute toxicity data available.

4.2.3 Summary and discussion of acute toxicity

Low acute toxicity has been observed when halosulfuron-methyl was administered by oral, dermal and inhalation routes.

4.2.4 Comparison with criteria

Based on the oral LD_{50} of 10435 mg/kg in male rats and 7758 mg/kg in female rats, the oral LD_{50} of 16156 mg/kg in male mice and 9295 mg/kg in female mice, which are all greater than the range of ATE = 300 – 2000 mg/kg (Acute tox. 4), halosulfuron-methyl does not meet the criteria for classification and is therefore unclassified for acute oral toxicity. For acute dermal toxicity, a limit test in rats is available showing no signs of toxicity and mortality at 2000 mg/kg bw. therefore as the acute dermal LD_{50} for halosulfuron-methyl has exceeded the range of ATE = 1000-2000 mg/kg (Acute tox. 4), halosulfuron-methyl does not meet the criteria for classification and is therefore unclassified for acute dermal toxicity. In the acute inhalation test in rats, no mortality or treatment-related findings regarding body weight or pathology were observed at a limit test concentration of 6.0 mg/l (LC_{50} >6.0 mg/l). Note that the limit test conducted is 20% higher than the classification limit of 5.0 mg/l. Because the acute inhalation LC_{50} for halosulfuron-methyl has exceeded the range of ATE = 1.0 - 5.0 mg/L (Acute tox. 4), halosulfuron-methyl does not meet the criteria for classification and is therefore unclassified for acute inhalation toxicity.

The criteria for acute oral, dermal and inhalation toxicity classification under CLP were not met.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity through the oral, dermal and inhalatory route is warranted based on the criteria of the CLP.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity of halosulfuron-methyl

The results of two guideline (US EPA 81-1, 1984) and GLP compliant studies (Osheroff, 1990a; Osheroff, 1990b) were presented by the DS. The former was performed with rats, the latter with mice (table 10, CLH report). Oral dosing was by gavage in both studies.

The Osheroff (1990a) study was conducted with ten fasted male and female Sprague Dawley rats. The acute oral LD_{50} values for halosulfuron-methyl (purity 98.5%) were

greater than the limit dose of 2000 mg/kg bw. Mortality occurred at all dose levels. The principal clinical signs were described as common, non-specific findings and included: a vague clinical sign termed "depression", urine stains, hunched posture, red stains on nose and/or eyes and soft faeces. There was no evidence of a dose-related increase in clinical signs (table 12, CLH report). Body weight gain was unaffected, with all surviving animals gaining weight up to day of termination. All groups exhibited a variety of commonly noted necropsy findings with no evidence of a dose-related increase in any finding. The rat acute oral LD_{50} values were 10435 mg/kg bw in males and 7758 mg/kg bw in females.

The Osheroff (1990b) study was conducted with ten male and female fasted CD-1 mice. The acute oral LD_{50} values for halosulfuron-methyl (purity 98.5%) were greater than the limit dose of 2000 mg/kg bw. Mortality occurred at all dose levels. Similar clinical signs were described as for the rat. In general, increased incidences in clinical signs were confined in the two highest doses. However, there was no evidence of a dose-related increase in clinical signs (table 14, CLH report). Body weight gain was unaffected, all surviving animals gained weight up to the day of termination. All groups exhibited a variety of commonly noted necropsy findings with no evidence of a dose-related increase in any finding. The mouse acute oral LD_{50} values were 16156 mg/kg bw in males and 9295 mg/kg bw in females.

The DS did not propose classification for acute oral toxicity.

Acute inhalation toxicity of halosulfuron-methyl in rats

The results of a single GLP and guideline (OECD TG 402, 1984) compliant, acute inhalation toxicity study was presented by the DS. All exposures were for 4 hours using five Sprague Dawley CD rats/sex. The Bechtel (1991) study used a whole body inhalation system exposed to 6.0 mg/L of milled test substance. There were no deaths during the testing period. During exposure, hypoactivity was evident whilst generalised clinical signs such as hypoactivity, laboured respiration, nasal discharge, nasal encrustation, perioral wetness and periocular encrustation were noted post exposure (table 15, CLH report). All clinical signs resolved by day 3. The study did not include histopathology. There were no visible treatment-related lesions at necropsy. All rats gained weight from day 2 to termination with all animals exceeding their pre-exposure weights. The LC_{50} was > 6 mg/L/4h.

The DS did not propose classification for acute inhalation toxicity. No mortality or treatment-related findings regarding body weight or pathology were observed at the highest dose, which was higher than the classification limit of 5.0 mg/L.

Acute dermal toxicity of halosulfuron-methyl in rats

The results of a single GLP compliant, acute dermal toxicity study using 10 Sprague Dawley CD rats/sex was presented by the DS (Osheroff, 1990c). All animals were given a single topical application of 2000 mg/kg bw of halosulfuron-methyl for 24 hours. All animals survived to termination (day 14). There were no clinical signs, animals gained weight and no gross lesions were observed in any animal at necropsy. The LD_{50} was estimated to be > 2000 mg/kg bw.

The DS did not propose classification for acute dermal toxicity on the basis that no effects were seen in male and female rats in the study at the limit dose.

Comments received during public consultation

None for this section.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

The oral LD_{50} was 10435 mg/kg bw in male rats and 7758 mg/kg bw in female rats, and the oral LD_{50} was 16156 mg/kg bw in male mice and 9295 mg/kg bw in female mice. According to CLP, LD_{50} values for acute oral toxicity > 2000 mg/kg bw do not warrant classification. RAC is in agreement with the DS, that halosulfuron-methyl does not meet the criteria for classification and is therefore **not classified for acute oral toxicity**.

Acute Inhalation Toxicity

An inhalation 4 hour LC_{50} of > 6 mg/L was derived from a study conducted in rats. According to CLP, LC_{50} values for acute inhalation > 5 mg/L for dust/mist do not warrant classification. RAC is in agreement with the DS. **No classification for acute toxicity via inhalation is warranted** for halosulfuron-methyl.

Acute Dermal Toxicity

A limit test in rats showed no signs of toxicity or mortality at 2000 mg/kg bw. According to CLP, LD_{50} values for acute dermal toxicity > 2000 mg/kg bw do not warrant classification. RAC is in agreement with the DS. **No classification for acute dermal toxicity is warranted** for halosulfuron-methyl.

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

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

In the acute oral toxicity in the rat (DAR: Osheroff, 1990a), the principal clinical signs seen shortly after dosing (4000-10000 mg/kg) were non-specific and included slight depression* or depression (highest dose), urine stains, hunched posture, red stains on nose and/or eyes and soft faeces which were broadly dose-related in incidence. The majority (11 out of 20) of rats given the initial 5000 mg/kg/dose and rats given 7500 or 10,000 mg/kg were also hunched (4 out of 20 and 3 out of 20 respectively). Body weight gain was unaffected and observable gross pathology findings in males and females involved the lungs, liver kidneys and spleen (discoloration) and the stomach and intestines (discoloration, abnormal contents and distension).

In the second acute oral study, mice were administered doses of substance at 4000–10000 mg/kg mg/kg (DAR: Osheroff, 1990b). At 4000 and 5000 mg/kg, the principal clinical sign was slight depression. The principal clinical signs seen shortly after dosing at 7500 and 10,000 mg/kg included depressed or slightly depressed, urine stains and ataxia which were broadly dose-related in incidence. The majority (13 out of 20) of mice given 7500 mg/kg were hunched and all mice at this dose level had tremors. Body weight gain was unaffected and observable gross pathology findings involved the

lungs, liver and spleen (discoloration) and the stomach and intestines (discoloration, abnormal content distension and thin walls). There was no clear evidence of target organ toxicity based on clinical signs or macroscopic pathology since the findings were typical of common non-specific signs of acute oral exposure to high doses of test materials in rodents.

In the acute dermal toxicity study in rats at the dose level of 2000 mg/kg bw (DAR: Osheroff, 1990c) there was no mortality and no clinical signs, either systemic or at the application site. No evidence of target organ toxicity was found.

In the acute inhalation study with rats (DAR: Bechtel, 1991) the maximum tested nominal concentration was 6.0 mg/l air, which resulted in no mortality. Signs observed immediately after exposure were laboured respiration, hypoactivity, red/pink nasal discharge, periorbital wetness, perinasal and periorbital encrustations. The encrustations persisted up to and including Day 2 post exposure but all rats were normal by Day 3. No evidence of target organ toxicity was found. The study did not include histopathology which would have characterised the effects on the morphology of the lung. However, it is considered that the findings were typical of common non-specific signs of acute inhalation exposure to extremely high doses of test materials in rodents.

Furthermore, halosulfuron-methyl is neither classified as an eye irritant (see 4.4.2), a skin irritant (see 4.4.1) nor a skin sensitiser (see 4.6). It therefore does not have innately sensitising, irritating or corrosive properties. Given the extremely high concentrations in air, the non-specific clinical signs and rapid post exposure recovery of the animals from their clinical signs, the lack of irritancy in eye and skin irritation tests and the lack of activity in a skin sensitisation test, the weight of evidence does not support halosulfuron-methyl having respiratory irritant properties. Therefore, it is considered that halosulfuron-methyl should not be considered as a respiratory tract irritant.

* The meaning of 'depressed' is not clarified in the original (1990) report. Here it is taken to mean hypoactive, a common non-specific finding in toxicity studies. It is unlikely to be a specific reference to depression of the CNS or narcosis.

4.3.2 Comparison with criteria

Based on the clinical observations and macroscopic pathology findings from two acute oral toxicity studies, one acute dermal and one acute inhalation study, there is no evidence of target organ toxicity associated with acute exposure to halosulfuron-methyl. STOT-SE Category 1 or 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity at low or moderate doses. Using expert judgement and a weight of evidence approach there is insufficient evidence of specific target organ toxicity at low or moderate doses via oral, dermal or inhalation routes. With respect to STOT SE Category 3 (transient effects), there was insufficient evidence of respiratory tract irritation in an acute inhalation toxicity study (evidence of respiratory tract irritation is discussed in sections 4.3.1 and 4.4.3.1) and there was no evidence of narcotic effects in any acute or repeat-dose toxicity studies. Therefore effects observed in the available acute toxicity studies via the three different routes do not fulfil the classification criteria for STOT SE categories 1, 2 or 3. .

4.3.3 Conclusions on classification and labelling

No classification for STOT-SE categories 1, 2 or 3 is required with regard to acute oral, dermal or inhalation toxicity.

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

Summary of the Dossier Submitter's proposal

There were no specific clinical signs or changes in organs observed in any of the acute studies described by the DS in the CLH report that could be attributed to toxicity of halosulfuron-methyl. Clinical signs were observed at very high doses and were typically non-specific and included a presumed "hypoactivity" (not clear from the original study reports, and not considered to indicate narcosis), urine stains, hunched posture, red stains on nose and/or eyes and soft faeces. All surviving animals in all studies continued to gain weight until study termination. There were some general macroscopic pathology findings from the acute oral studies but nothing to note from the other studies. There was no evidence of target organ toxicity associated with acute exposure to halosulfuron-methyl. The DS did not propose classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Halosulfuron-methyl showed a low acute toxicity independent of the route of exposure. The clinical signs after acute oral administration of up to 5000 mg/kg bw of halosulfuron-methyl to mice and/or rats were a slight depression/'hypoactivity', urine stains and soft faeces. At higher dose levels, hunched posture, red staining on the nose and/or eyes, ataxia and tremors were also seen. Dermal administration to rats elicited no clinical signs and during inhalation exposure signs were consistent with exposure to a dusty atmosphere. Post-exposure, only transient red/brown peri-nasal encrustation was observed. Based on the clinical observations and macroscopic pathology findings from two acute oral toxicity studies, one acute dermal and one acute inhalation study, there is no evidence of target organ toxicity associated single exposure (STOT SE) to halosulfuron-methyl.

Based on observations in animal studies, STOT SE classification is assigned on the basis of findings of 'significant' and/or 'severe' toxicity at generally low doses (Cat. 1) or with significant toxicity at more moderate doses (Cat. 2). Using expert judgement and a weight of evidence approach, there is insufficient evidence of specific target organ toxicity at low or moderate doses via oral, dermal or inhalation routes. Accordingly, RAC agrees with the DS that no classification for STOT SE 1 or 2 is warranted.

With respect to STOT SE Category 3 (transient effects; narcotic effects and respiratory tract irritation), there was no specific data available and insufficient evidence of respiratory tract irritation. In general, data from single and repeated dose inhalation toxicity tests may provide useful information for this hazard category. In an acute inhalation study in rats (DAR: Bechtel, 1991) the maximum tested nominal concentration was 6.0 mg/L air, and resulted in non-specific signs observed immediately after exposure such as laboured respiration, hypoactivity, red/pink nasal discharge, periorbital wetness, perinasal and periorbital encrustations. The encrustations persisted up to and including day 2 post-

exposure but all rats were normal by day 3. At necropsy, there were no abnormalities noted. The clinical signs observed do not provide sufficient evidence for irritation of the respiratory tract. There were no short term inhalation studies performed with halosulfuron-methyl. The DS did not propose classification. There was no evidence of narcotic effects in any acute or repeat-dose toxicity studies. Accordingly, RAC agrees with the DS that **no classification for STOT SE 3 is warranted.**

4.4 Irritation

4.4.1 Skin irritation

Table 16: Summary table of relevant skin irritation studies

Method	Results	Reference
Rabbit, New Zealand White, 6 male 0.5g (4 hours) OECD 404 GLP Purity 98.5%	No signs of dermal irritation observed in any animal at any timepoint Not irritant	DAR B.6.2.4 Mercier, 1990a

4.4.1.1 Non-human information

A group of 6 New Zealand White rabbits was given a single topical application of halosulfuron-methyl for 4 hours. They were observed for dermal irritation daily for 3 days.
No dermal irritation was observed.

4.4.1.2 Human information

No human skin irritation data available.

4.4.1.3 Summary and discussion of skin irritation

No dermal irritation was observed. Therefore no CLP classification and labelling is required

4.4.1.4 Comparison with criteria

The criteria for classification under CLP were not met.

4.4.1.5 Conclusions on classification and labelling

No classification is required with regard to skin irritation under CLP

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of halosulfuron-methyl was investigated in one standard guideline (OECD TG 404, 1981) and GLP compliant study in rabbits (Mercier, 1990a, table 16, CLH report). The test substance (0.5 g) moistened with distilled water was applied for 4 hours to the intact skin of six male New Zealand White rabbits, using a patch of 2.4 x 2.4 cm, which was covered with semi occlusive dressing. No cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0 for erythema and oedema. The DS did not propose classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No oedema or erythema was observed over the time points relevant for classification (24, 48 and 72 hours); therefore, RAC agrees with the DS that **no classification for skin corrosion/irritation is warranted**.

4.4.2 Eye irritation

Table 17: Summary table of relevant eye irritation studies

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Rabbit eye irritation
Rabbit, New Zealand White, 3 male and 3 female
0.1 ml
OECD 405
GLP
Purity 98.5 %</td><td>Slight to moderate conjunctival irritation resolved by 72 hours in all animals.
<table>
<tr><th rowspan="2">Animal number and sex</th><th rowspan="2" colspan="2">Parameter</th><th>Hours post instillation</th><th colspan="3">Days post-instillation</th><th rowspan="2">Mean irritation score#</th></tr>
<tr><th>1</th><th>1</th><th>2</th><th>3</th></tr>
<tr><td rowspan="6">0021 Male</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>+</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Conjunctivae</td><td>Redness</td><td>2</td><td>1</td><td>0</td><td>0</td><td>0.33</td></tr>
<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="6">0022 Female</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Conjunctivae</td><td>Redness</td><td>2</td><td>1</td><td>0</td><td>0</td><td>0.33</td></tr>
<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="6">0023 Male</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>+</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Conjunctivae</td><td>Redness</td><td>2</td><td>1</td><td>1</td><td>0</td><td>0.67</td></tr>
<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="6">0024 Female</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Conjunctivae</td><td>Redness</td><td>1</td><td>1</td><td>0</td><td>0</td><td>0.33</td></tr>
<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="6">0025 Male</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
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<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="6">0026 Female</td><td rowspan="2">Cornea</td><td>Density</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">0</td></tr>
<tr><td>Area</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="2">Iris</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Conjunctivae</td><td>Redness</td><td>2</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Chemosis</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0.33</td></tr>
<tr><td>Discharge</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
Mean of scores on Days 1, 2 and 3 days (equivalent to 24, 48 and 72 hours) post-instillation according to EU evaluation criteria (93/21/EEC)
+ Slight deepening of rugae or slight hyperaemia of circumcorneal blood vessels</td><td>DAR B.6.2.5
Blaszcak, 1991</td></tr>
</table>

4.4.2.1 Non-human information

An acceptable acute irritation study is available.. A group of 3 male and 3 female New Zealand White rabbits was given a single ocular instillation. They were observed mild transient conjunctival irritation but all reactions had resolved by 72 hours. All rabbits exhibited slight to moderate

conjunctival irritation (redness, chemosis, discharge); two animals exhibited iridial changes at one hour only. No corneal effects were seen throughout the study. All six animals were free of all ocular irritation by 72 hours.

Halosulfuron-methyl is shown to be mildly irritating to the eyes, but not severe enough for classification.

4.4.2.2 Human information

No human eye irritation data are available.

4.4.2.3 Summary and discussion of eye irritation

In a primary eye irritation study in the rabbit mild transient conjunctival irritation was seen but all reactions had resolved by 72 hours. Halosulfuron-methyl is shown to be mildly irritating to the eyes, but not severe enough for classification.

4.4.2.4 Comparison with criteria

Since the scores were <1, and had resolved by 72 hours the criteria for classification under CLP were not met.

4.4.2.5 Conclusions on classification and labelling

No classification is required with regard to eye irritation and corrosion under CLP.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of halosulfuron-methyl was investigated in a standard guideline (OECD TG 405, 1987) and GLP compliant study (Blaszcak, 1991), using three male and three female New Zealand White rabbits. Each animal was administered 0.1 mL test substance (58.1 mg). No wash was performed after application. All rabbits exhibited slight to moderate conjunctival irritation (redness, chemosis and discharge); two animals exhibited iridial changes at 1 hour only. No corneal effects were seen throughout the study. All six animals were free of all ocular irritation by 72 hours post application. No single animal scored in excess of the CLP criteria trigger values. The DS did not propose classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No effects in the iris or cornea were noted. The effects observed in conjunctiva (redness) were reversed within 72 hours. The mean scores for each animal calculated over 24, 48

and 72 hours for erythema and oedema of the conjunctivae were less than the CLP criteria values for classification. RAC supports the DS conclusion that no classification is warranted for serious eye damage/eye irritation.

Supplemental information - In depth analyses by RAC

Table 1: Summary of rabbit ocular data

Mean values for ocular lesions 24, 48 and 72 hours after instillation

Animals	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Chemosis
1. male 0021	0	0	0.3	0
2. female 0022	0	0	0.3	0
3. male 0023	0	0	0.7	0
4. female 0024	0	0	0.3	0
5. male 0025	0	0	0.3	0
6. female 0026	0	0	0.3	0
CLP Criteria: Eye Irrit. (Cat. 2)	≥ 1	≥ 1	≥ 2	≥ 2
CLP Criteria: Eye Dam. (Cat. 1)	≥ 3	> 1.5	na	na

No single animal scored in excess of the CLP criteria trigger values.

4.4.3 Respiratory tract irritation

No specific studies to identify respiratory irritation were carried out. Guidance on the application of the CLP criteria acknowledges that there are no validated animal tests that deal specifically with respiratory tract irritation but data from single and repeated dose inhalation toxicity tests may provide useful information. An acute inhalation study in rats was carried out on halosulfuron-methyl. The evidence for respiratory tract irritation is discussed in section 4.3.1 and 4.4.3.1.

4.4.3.1 Non-human information

No specific studies to identify respiratory irritation were carried out. In an acute inhalation study in rats (DAR: Bechtel, 1991) the maximum tested nominal concentration was 6.0 mg/l air, and resulted in non-specific signs observed immediately after exposure such as laboured respiration, hypoactivity, red/pink nasal discharge, periorbital wetness, perinasal and periorbital encrustations (see table 15, section 4.2.1.2. The encrustations persisted up to and including Day 2 post exposure but all rats were normal by Day 3. At necropsy there were no abnormalities noted. The clinical signs observed do not provide sufficient evidence for irritation of the respiratory tract (see section 4.3.1). No other toxicity studies by the inhalation route were conducted.

4.4.3.2 Human information

No human respiratory irritation data are available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No specific studies to identify respiratory irritation were carried out. In an acute inhalation study there were non-specific signs of toxicity but no clear evidence of irritation of the respiratory tract (see section 4.3.1). There are no human data. Halosulfuron-methyl is not irritant or corrosive to skin or eye.

4.4.3.4 Comparison with criteria

No clear evidence of respiratory tract irritation was observed in the acute inhalation study at any dose level and therefore the classification criteria for respiratory irritation category 1, 2 or 3 were not met.

4.4.3.5 Conclusions on classification and labelling

No classification is required with regard to respiratory irritation.

4.5 Corrosivity

Table 18: Summary table of relevant corrosivity studies

Method	Results	Reference
Not relevant		

4.5.1 Non-human information

No signs of corrosivity were observed in the available irritation studies conducted with Halosulfuron-methyl (see Section 4.4).

4.5.2 Human information

No human skin irritation/corrosion data are available.

4.5.3 Summary and discussion of corrosivity

The skin irritation study shows no signs of corrosion.

4.5.4 Comparison with criteria

There was no observed dermal irritation and therefore the criteria for skin corrosion under CLP are not met.

4.5.5 Conclusions on classification and labelling

No classification is required with regard to skin corrosion.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 19: Summary table of relevant skin sensitisation studies

Method	Results	Reference
Guinea pig skin sensitisation (Magnusson and Kligman maximisation test) Guinea Pig, Dunkin-Hartley, 10 females/test and 10 males/test group, 20 females/ control group Maximisation Study OECD 406 GLP Purity 98.5 %	No dermal reactions were seen in any of the test group or control animals	DAR 6.2.6 Mercier, 1990b

4.6.1.1 Non-human information

A Magnusson and Kligman maximisation test for skin sensitisation was conducted. Based on preliminary dose range finding, groups of 10 male and 10 female Dunkin-Hartley guinea pigs were given intradermal injections of 2% w/w of halosulfuron-methyl in Freund's Complete Adjuvant and a 5% w/w suspension in water on Day 1 followed by a topical application of 70% w/w of halosulfuron-methyl in water on Day 8 (induction phase). At challenge (11 days post-induction), a single topical application of a 70% w/w of halosulfuron-methyl in water was administered on Day 22.

A positive control group given dichloronitrobenzene, DNCB (intradermal injection of 0.05% w/w and 0.1% w/w in polypropylene glycol: 0.05% w/w at challenge), was included in parallel. It induced positive reactions in 19/20 (95%) of animals.

Halosulfuron-methyl showed no potential for skin sensitisation in any animal.

4.6.1.2 Human information

No data on sensitisation in humans is available.

4.6.1.3 Summary and discussion of skin sensitisation

In a maximisation study on guinea pigs with halosulfuron-methyl, no dermal reactions were seen in any of the test group or control animals. Furthermore, no reactions of cutaneous intolerance were seen in the test group.

4.6.1.4 Comparison with criteria

A positive reaction in 30% of the test group is required in a maximisation test to indicate a sensitisation potential. There were no such positive reactions in the guinea pig study conducted with halosulfuron-methyl.

4.6.1.5 Conclusions on classification and labelling

Testing for sensitising properties by the method of Magnusson & Kligman did not show an allergenic potential. There are no human data to suggest the potential for skin sensitisation. No classification is required for skin sensitisation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of halosulfuron-methyl was investigated in a standard GLP and guideline compliant (OECD TG 406, 1981) guinea pig Maximization Test based on the method of Magnusson and Kligman (Mercier, 1990b) using 10 male and 10 female Dunkin-Hartley guinea pigs. Intradermal induction was performed at a test substance concentration of 2%, and the challenge concentration was 70%. There was a zero incidence of sensitisation. A positive control group given dichloronitrobenzene, DNCB (intradermal injection of 0.05% w/w and 0.1% w/w in polypropylene glycol: 0.05% w/w at challenge), was conducted in parallel to this study and gave a 95% positive induction incidence (19/20 animals). The DS did not propose classification.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Halosulfuron-methyl showed no potential for skin sensitisation in any animal. There were no human data to suggest the potential for skin sensitisation. RAC supports the DS's conclusion that **no classification is warranted for skin sensitisation**.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data available.
No non-human specific studies to identify respiratory sensitisation potential have been conducted. At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available.

4.6.2.2 Human information

No human information is available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data available.

4.6.2.4 Comparison with criteria

No data available..

4.6.2.5 Conclusions on classification and labelling

No classification is possible due to the absence of human or non-human respiratory hypersensitivity data.

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal No data available. **Comments received during public consultation** No comments were received during public consultation. **Assessment and comparison with the classification criteria** The assessment of respiratory sensitisation is not possible due to the absence of human and/or non-human respiratory hypersensitivity data.

4.7 Repeated dose toxicity

Table 20: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Results	Reference
Rat 28-day oral (dietary) Sprague Dawley rats (10/sex/group) US EPA 40 CFR 158.135 GLP Purity 98.5 %	0, 300, 1000, 3000, 10000 ppm (0, 23, 78, 231, 777 mg/kg bw/d in males; 0, 25, 85, 241, 888 mg/kg bw/d in females)	NOAEL: 300 ppm (Males:23 mg/kg/day; Females:25 mg/kg/day) LOEL: 1000 ppm (Males 78 mg/kg/day Females: 85 mg/kg/day) Reduced body weight gain and overall food consumption, some clinical chemistry changes. At higher doses degeneration/necrosis of pancreatic acinar cells	DAR B.6.3.1 Osheroff, 1988
Rat 90-day oral (dietary) Sprague Dawley rats (20/sex/group) US EPA FIFRA 82-1 GLP Purity 98.6 %	0, 100, 400, 1600, 6400 ppm (0, 7.4, 28.8, 116, 497 mg/kg bw/d in males; 0, 8.9, 37.3, 147, 640 mg/kg bw/d in females)	NOAEL: 1600 ppm (Males:116 mg/kg bw/day;Females:147 mg/kg bw/day) NOEL: 400 ppm (Males:28.8 mg/kg/day; Females:37.3 mg/kg/day) LOAEL: 6400 ppm (Males:497 mg/kg bw/day; Females:640 mg/kg bw/day) Reduced body weight gain, cholesterol, total bilirubin; increased (haemosiderin) pigmentation of renal tubular epithelium; mild vacuolation in the liver	DAR B.6.3.2 Perry, 1990
Dog 90-day oral (capsule) Beagle dog (4/sex/group) OECD 409 GLP Purity 98.5 %	0, 0.25, 1, 10, 40 mg/kg/day	NOEL: 10 mg/kg/day LOEL: 40 mg/kg/day Reduced body weight gain; increased liver weight and clinical chemistry alterations	DAR B.6.3.3 Wood, 1991
Dog 12-month oral (capsule) Beagle dog (6/sex/group) US EPA FIFRA 83-1 GLP Purity 98.7 %	0, 2.5, 10, 40, 160 mg/kg/day	NOAEL:10 mg/kg bw/day NOEL: 1 mg/kg/day LOEL: 40 mg/kg/day Haematological changes	DAR B.6.3.4 Osheroff, 1991
Rat 21-day dermal Sprague Dawley rats (5/sex/group) US EPA FIFRA 82-2 GLP Purity 99.1 %	0, 10, 100, 1000 mg/kg/day	NOEL: 10 mg/kg/day LOEL: 100 mg/kg/day Haematological changes (reduced body weight gain at higher doses)	DAR B.6.3.6 Osheroff, 1990d

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat oral 28 days dietary study

In the rat 28-day dietary study, animals were assigned to dose groups using random numbers and then individually identified. Five groups of rats (10/sex/group) were given dietary concentrations of 0, 300, 1000, 3000 or 10000 ppm of halosulfuron-methyl for 4 weeks. The test diets containing the appropriate level of test material were prepared freshly each week and fed *ad libitum* throughout the study except for the evening before terminal sacrifice when animals were fasted.

Animals were observed twice daily for moribundity and mortality and once daily for signs of toxicity. Detailed observations were conducted weekly. Individual body weight and food consumption were recorded weekly. Ophthalmological examinations were conducted on all animals before the start of the treatment and during Week 4. At termination of treatment, blood for haematology and chemistry determinations and urine samples were collected from all surviving animals. At termination, a full necropsy, including organ weight analysis, was performed on all animals. Preserved tissues from control and high dose groups, along with lungs, liver and kidney from all low and mid-dose animals were examined microscopically. Gross lesions from all rats were also examined.

Body weight, body weight change, total food consumption, clinical pathology parameters (except cell morphology and urinalysis) and organ weight data from treated and control groups were compared using Levene's test (homogeneity of variance), ANOVA (analysis of variance) or Dunnett's test. Data were transformed where variances were heterogeneous. A 5 % two-tailed probability level was used unless otherwise stated.

Results

Results are summarised in Table 21 and described below.

No treatment-related mortality occurred. However, one male treated with 3000 ppm and one female given 10000 ppm were found dead on the day of scheduled sacrifice. Both appeared normal and death was considered not to be related to the test material.

There were no clinical observations suggestive of a compound effect and there were no treatment-related ophthalmic lesions observed in any animal.

At 10000 ppm, mean body weights of both sexes in Weeks 1, 2 and 4 were significantly lower than mean control values. Mean body weight gains for males treated with 10000 ppm and females treated with 3000 ppm and 10000 ppm were significantly reduced from Weeks 0-4 when compared with concurrent controls. In addition body weight gain of males fed 3000 ppm was reduced for Week 0-1. A significant reduction in food consumption was noted for females receiving 3000 ppm and males receiving 10000 ppm compared to the control group. Dietary concentrations of 0, 300, 1000, 3000 and 10000 ppm corresponded to mean achieved intakes of 0, 23, 78, 231 and 777 mg/kg/day of halosulfuron-methyl in males and 0, 25, 85, 241 and 888 mg/kg/day in females, respectively.

Females treated with 300, 1000 and 10000 ppm showed a significant decrease in protein, albumin and globulin. In addition, mean glucose values were significantly reduced in both sexes at 10000 ppm. Chloride ion concentration of female groups treated with 3000 and 10000 ppm was significantly increased.

Significant increases in haemoglobin in females treated with 10000 ppm and in haematocrit in those treated with 300 and 10000 ppm compared to control values were observed. However, the differences

were very slight, they were associated to a high variability of individual values and this, together with the lack of dose–dependence, questions the toxicological significance of the observed alterations.

There were no treatment-related findings in urinalysis and no treatment-related macroscopic findings were found at necroscopy.

Female kidney/terminal body weight ratio and male liver/terminal body weight and testes-epididymides/terminal body weight ratios were higher than control values in the group receiving 10000 ppm.

Microscopic investigations showed a marked increased incidence of individual cell degeneration/necrosis of pancreatic acinar cells in animals receiving 3000 and 10000 ppm.

Table 21: Summary of rat dietary 28-day toxicity study with halosulfuron-methyl

Parameter	**Dose level (ppm)**									
	Males					**Females**				
	0	**300**	**1000**	**3000**	**10000**	**0**	**300**	**1000**	**3000**	**10000**
Achieved intake (mg/kg/day)	0	23	78	231	777	0	25	85	241	888
Body weight (g)										
Start	204.0	201.9	203.1	204.1	203.3	165.2	162.2	162.0	164.3	163.2
Week 1	265.5	261.7	262.3	255.3	241.0*	192.9	188.9	190.8	184.6	177.3*
Week 2	321.7	315.3	317.4	309.0	288.5*	213.4	209.6	214.0	203.5	194.6*
Week 3	364.4	358.8	361.9	353.8	326.0	231.2	229.7	232.1	220.7	206.8
Week 4	388.6	393.7	400.2	385.2	351.9*	250.7	246.1	245.9	234.1	218.7*
Body weight gain (g)										
Week 1	61.5	59.8	59.1	51.2*	37.7*	27.7	26.7	28.8	20.3*	14.1*
Week 2	117.7	113.4	114.3	104.9	85.2	48.2	47.4	52.0	39.2	31.4
Week 3	160.3	156.9	158.8	145.7	122.8	66.0	67.5	70.1	56.3	43.6
Week 4	184.6	191.8	197.0	181.1	148.6*	85.5	83.9	83.9	69.8*	55.5*
Food consumption (g)										
Week 1	174.1	167.0	167.6	164.2	143.1	121.8	122.7	125.2	116.1	110.7
Week 2	190.7	183.3	187.7	177.2	170.8	129.2	133.0	137.1	120.3	132.8
Week 3	182.2	181.6	184.4	177.0	171.8	128.8	131.7	133.9	118.5	125.4
Week 4	175.8	169.3	167.7	169.3	163.1	126.3	123.2	123.8	116.0	125.2
Total	722.8	701.3	716.5	687.8	648.7*	506.1	510.7	520.1	471.0*	494.1
Haematology										
HGB (g/dl)	16.7	16.9	16.4	16.7	17.2	16.4	16.9	16.7	16.6	17.2*
HCT (%)	48.1	48.5	47.2	48.2	49.1	44.8	47.8*	47.1	47.1	48.5*
Clinical chemistry										
Chloride (mmol/l)	109.2	110.6	110.0	110.7	111.1	110.2	110.9	111.7	112.3*	114.1*
Protein (g/dl)	5.9	5.9	5.8	6.0	5.7	6.9	6.2*	6.1*	6.6	6.0*
Albumin (g/dl)	3.8	3.8	3.8	3.8	3.7	4.5	4.0*	4.0*	4.4	3.9*
Globulin (g/dl)	2.1	2.1	2.1	2.2	2.0	2.4	2.2*	2.1*	2.3	2.1*
Glucose (mg/dl	120	120	114	110	100*	123	126	121	112	106*
Organ weight										
Relative weight (%)										
Kidney	0.740	0.767	0.706	0.757	0.778	0.739	0.737	0.774	0.790	0.827
Liver	2.907	2.845	2.752	2.979	3.106	3.077	2.828	2.835	3.198	3.212
Testis/epididymis	1.114	1.216	1.159	1.226	1.298	-	-	-	-	-
Histopathology										
Total incidence of pancreatic acinar cell degeneration/necrosis	0/10	1/10	1/10	8/10	10/10	1/10	2/10	1/10	8/10	10/10

* $p \leq 0.05$
HGB = Haemoglobin
HCT = Haematocrit
Total incidence = Number affected/number examined

Conclusion

In the rat 28-day dietary study, body weight gain was reduced in both sexes at 10000 ppm and in females given 3000 ppm, for which also a significant reduction in food consumption was reported. Some changes in clinical chemistry parameters (lower protein, albumin, globulin and glucose and higher chloride ion) were also recorded in females starting from 300 ppm. The major finding was an increased incidence of individual cell degeneration/necrosis of pancreatic acinar cells at 3000 ppm and above. However, this effect was not found in any other repeated oral toxicity study with the rat even at higher dose levels. The NOEL of halosulfuron-methyl was not determined, due to the slight effects in clinical chemistry observed in females at 300 ppm (corresponding to 23 mg/kg/day in males and 25 mg/kg/day in females). This dosage can be considered as the NOAEL, in view of the absence of any histopathological lesion.

Rat oral 90 days dietary study

In the rat 90-day study, animals were randomly allocated to treatment groups on the day of arrival. Groups of 20 male and 20 female rats were fed halosulfuron-methyl for 13 weeks with 0, 100, 400, 1600 or 6400 ppm.

Animals were examined twice daily for mortality and once daily for reaction to treatment. A detailed clinical examination was performed at least once every week. Individual body weight and cage group food consumption were recorded weekly commencing one week before the start of treatment until the end of dosing period. Water consumption was monitored throughout by visual inspection of the water bottles. An ophthalmoscopic examination was conducted on the control and high dose groups pre-trial and during Week 12 of treatment. Blood samples for haematology and clinical chemistry and urine samples were taken during the Week 13 from 10 males and 10 females per group. At the end of dosing, all surviving animals were necropsied, organ weights recorded and a range of tissue samples preserved. All tissues except one eye, spinal cord, rectum, rib, sternum and nasal cavity were examined histopathologically from all rats.

Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the 'F-max' test. With the exception of body weight, if the group variances were homogeneous, a parametric ANOVA was used and pair-wise comparison made via Student's t-test using Fisher's F-protected LSD. Organ weights were also analysed by analysis of covariance with body weight. Histopathology data were analysed using Fisher's Exact Probability.

Results

Results are summarised in Table 22 and described below.

No treatment-related mortality occurred. One female died during Week 10, but there was no evidence that the death was attributable to the treatment.

No clinical signs were attributed to administration of halosulfuron-methyl and there were no ophthalmic treatment-related findings.

Both sexes receiving 6400 ppm of halosulfuron-methyl showed a significant reduction in body weight compared to the controls. Females receiving 6400 ppm of halosulfuron-methyl showed a slight reduction in food consumption and food efficiency compared to the controls. There was no effect in food consumption in males, but there was an apparent reduction in food efficiency ratio over the first four weeks of the dosing period when compared with control. Dietary concentrations of 0, 100, 400, 1600 or 6400 ppm corresponded to mean achieved intakes of 0, 7.4, 28.8, 116 and 497 mg/kg/day of halosulfuron-methyl in males and 0, 8.9, 37.3, 147 and 640 mg/kg/day in females, respectively.

In males fed 6400 ppm, there were significant reductions in cholesterol (37%), total bilirubin (46%) and a significant increase in ALT (25%). In females at this dose level, significant reductions in cholesterol (29%) and total bilirubin (26%) were found.

At the same dose, other reductions in some parameters were observed (7% in total protein, 8% in albumin, and 5% in calcium). Although statistically significant due to the small magnitude of the changes they were considered of limited toxicological significance.

Haematology, urinalysis and macroscopic examination showed no treatment-related findings.

In males and females there were intergroup differences in the absolute organ weights in the high dose group, which were no more evident after adjustment for final body weight. Other spotted differences were also seen in absolute organ weights between control rats and animal treated with 100, and 400 ppm but they were considered to be due to the observed body weight differences.

Mild liver vacuolation was described in 6 out of 10 males in the high dose group. Increased pigmentation in the tubular epithelium of the kidney was present in groups receiving 1600 and 6400 ppm of halosulfuron-methyl. The effect seen at 1600 ppm although evident,did not attained statistical significance. The pigment deposits contained haemosiderin.

In the rat 90-day study report, body weight gain was reduced at 6400 ppm of halosulfuron-methyl, the highest dose level. Reductions in cholesterol (37% in males and 29% in females) and in total bilirubin (46% in males and 26% in females) as well as increased pigmentation of the renal tubular epithelium due to haemosiderin deposition and mild vacuolation in the liver were also seen at this dose level. Increased haemosiderin pigmentation of kidney tubules was observed also at 1600 ppm.

Table 22: Summary of rat dietary 90-day toxicity study with halosulfuron-methyl

Parameter	Dose level (ppm)									
	Males					Females				
	0	**100**	**400**	**1600**	**6400**	**0**	**100**	**400**	**1600**	**6400**
Achieved intake (mg/kg/day)	0	7.4	28.8	116	497	0	8.9	37.3	147	640
Body weight (g)										
Week 0	193	198	203	202	194	140	144	142	139	141
Week 4	364	371	380	367	316***	224	220	223	218	189***
Week 8	448	457	466	450	397***	266	259	265	256	224***
Week 13	511	521	532	512	458***	295	282	293	282	241***
Body weight gain (g)										
Weeks 0-13	318	323	329	310	264	155	138	150	143	100
% of control	-	102	103	97	83	-	89	97	92	65
Food consumption (g/rat/week)										
Week 1	189	201	198	193	181	139	141	140	134	151
Week 4	195	210	203	200	185	160	150	162	155	159
Week 8	190	205	199	193	180	153	153	156	151	135
Week 13	203	194	191	193	185	137	136	142	133	127
Total food consumed (Weeks 1-13)	2483	2620	2566	2509	2562	1937	1850	1980	1896	1818
% of control	-	106	103	101	103	-	96	102	98	94
Food efficiency (g/rat/week)										
Week 1	0.29	0.28	0.29	0.27	0.18	0.17	0.18	0.18	0.17	0.09
Week 4	0.17	0.15	0.18	0.18	0.15	0.11	0.11	0.10	0.11	0.07
Week 8	0.09	0.07	0.09	0.08	0.08	0.06	0.06	0.06	0.05	0.05
Week 13	0.04	0.04	0.05	0.04	0.04	-0.01	-0.04	nd	-0.02	0.01
Clinical chemistry										
ALT (IU/l)	57	56	49	52	71**	49	51	45	53	55
Cholesterol (mmol/l)	2.7	2.6	2.7	2.4	1.7***	3.1	2.8	2.7	2.7	2.2***
Total bilirubin (µmol/l)	2.8	2.6	2.7	2.5	1.5***	3.1	3.2	3.3	3.3	2.3**
Histopathology (total incidence)										
Kidneys tubular pigmentation (haemosiderin)	3/20	1/20	2/20	6/20	18/20***	8/20	6/20	10/20	12/20	17/20**

** $p \leq 0.01$; *** $p \leq 0.001$ Total incidence: Number affected / total examined

Since the increased haemosiderin pigmentation of kidney tubules was the only effect seen at 1600 ppm, it was not statistically significant and not associated with any other toxic effect, 1600 ppm (corresponding to 116 and 147 mg/kg/day of halosulfuron-methyl in males and females, respectively), can be considered as the NOAEL derived from this study. The NOEL was 400 ppm (28.8 mg/kg/day in males and 37.3 mg/kg/day in females).

For long-term toxicity assessment in rodents see section 4.10.1.1 and Tables 29 and 30.

Dog oral 90 days capsular study

In the rat 90-day study, dogs were assigned to dose groups using a randomisation procedure based on stratified body weight.

Groups of 4 male and 4 female Beagle dogs were given a daily oral dose, by capsule, of 0, 2.5, 10, 40 or 160 mg/kg/day of halosulfuron-methyl for 13 weeks.

All animals were observed daily. An ophthalmoscopic examination was conducted on all animals prior to the start of treatment and during Week 13 of treatment. Body weights were recorded weekly. Food consumption was estimated daily. Blood samples for clinical chemistry and haematology and urine samples were obtained prior to the start of treatment and in Weeks 2, 6, and 13. Clotting time was measured in Week 7. All animals were necropsied at the end of the study, a full myelogram was performed on bone marrow smears and their organs weighed. Tissues (as requested by OECD TG n° 409) were fixed and subsequently examined histopathologically. Additional section of liver, kidney and spleen were stained with Prusssian blue for the presence of iron.

For each parameter analysed statistically, analysis was performed using a 2-way analysis of variance (ANOVA) where possible. Where group differences were found at 5% significance level, each treated group was compared against the control group for the given sex using t-test. Where necessary a single sex ANOVA was performed to avoid variance heterogeneity. If ANOVA could not be performed, due to variance heterogeneity, the Kruskall-Wallis test was carried out for each sex. If there was significance at 5% level then pair-wise Wilcoxon Rank sum tests were performed to compare treated and control groups.

Results

Results are summarised in Table 23 and described below.

There were no deaths during the study.

There were no treatment-related clinical signs although one female animal in the high dose group showed signs consistent with anemia (i.e. pallor of the gums and ear, cold to touch) during weeks 6 and 7; the health conditions progressively improved without withdrawal of treatment. Other clinical observation (vomiting, loose faeces, hair loss) were spotted within the different groups (including control animal), without any dose- or time-dependence and were therefore considered not directly related to the treatment.

Ophthalmological examination showed no treatment-related findings.

Although not statistically significant, body weight gain at 160 mg/kg/day was lower than control in both sexes; in females the effect was evident also at 40 mg/kg/day.

There were no treatment-related findings for food consumption.

For all animals receiving 40 or 160 mg/kg/day, cholesterol levels were lower than those in pre-dose period and in the control group.

For females in the high dose group, total protein and albumin levels were significantly lower than the control values, starting from week 2 throughout the study. There was some evidence of the same alterations in males at the same dose at week 6, but at study termination a total recover was evident.

In addition, calcium levels for the high dose group (both genders) were statistically lower than the control ones. Some other spotted difference between control and treated animals related to different parameters were statistically significant; however, the absence of dose- and time–dependence questions their toxicological significance in relation to the treatment.

Haemoglobin levels and associated with this, erythrocyte counts and packed cell volumes, were generally lower in females receiving 160 mg/kg/day halosulfuron-methyl than in the controls, starting from Week 2. Total white blood cell counts were lower in males only during Week 13. This was generalised and not related to a specific cell type. During Week 6, a female was anaemic but recovered without withdrawal of treatment, questioning the relationship with the treatment itself. None of the other parameters were considered to have been affected by the treatment.

Myelography showed that the proportion of total erythropoietic cells, principally late erythroblasts, was significantly lower in males treated with 160 mg/kg/day. This resulted in significantly higher proportion of total granulopoietic cells and myeloid/erythroid ratios. No similar alterations were observed in females.

There were no treatment-related findings in urinalysis and faecal occult blood was not affected by treatment.

There were no treatment-related macroscopic findings.

At 160 mg/kg/day, the absolute and relative liver weights in both males and females were higher than those in the control group; the relative liver weight was higher also in the animals treated with 40 mg/kg/day.

Histopathological analysis gave similar results in control and treated animals. There was no morphological evidence of hepatotoxicity associated with the treatment-related increased in liver weight.

Table 23: Summary of capsular dog 90-day toxicity study with halosulfuron-methyl

Parameter	Dose level (mg/kg/day)									
	Males					Females				
	0	2.5	10	40	160	0	2.5	10	40	160
Body weight gain (g)										
Weeks 0-13	2.97	2.60	2.54	2.63	2.39	2.63	2.20	2.26	1.91	1.56
% of control	100	88	86	89	80	100	84	86	73	59
Clinical chemistry										
Cholesterol (mg/dl)										
Pre-dose	137	136	111	128	126	147	122	121	139	115
Week 2	133	127	102*	94**	82***	132	112	108	96*	69***
Week 6	123	120	109	83**	61***	116	111	106	91	64***
Week 13	118	121	103	87*	75**	113	110	111	99	60***
Total protein (g/dl)										
Pre-dose	4.9	5.1	5.1	5.2	4.9	5.1	5.2	4.9	5.0	4.9
Week 2	5.0	5.2	5.1	5.1	4.8	5.3	5.2	5.0	5.2	4.6***
Week 6	5.0	5.0	5.0	4.7*	4.5**	5.1	5.2	5.0	4.9	4.2***
Week 13	5.3	5.5	5.4	5.2	4.9	5.3	5.4	5.4	5.1	4.7**
Albumin (g/dl)										
Pre-dose	2.9	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	3.0
Week 2	2.9	3.1	3.0	3.1	2.7	3.2	3.1	3.0	3.1	2.7***
Week 6	3.2	3.2	3.3	3.0*	2.8***	3.4	3.4	3.2	3.2	2.7***
Week 13	3.4	3.4	3.5	3.5	3.1*	3.7	3.5	3.6	3.4*	3.1***
Haematology										
Haemoglobin (g/dl)										
Week 1	No treatment-related effect					13.7	13.9	14.2	13.8	13.5
Week 2						14.6	14.4	14.1	14.1	12.9**
Week 6						14.5	14.6	14.7	14.1	#10.8
Week 13						16.1	15.9	16.0	15.4	14.0**
WBC 1000/cmm (%)										
Week 13	14.1	10.8*	10.7*	11.3*	9.4**	No treatment-related effect				
Myelography										
LE (%) Week 14	30.5	31.0	26.7	25.3	17.4**	No treatment-related effect				
ETOT (%) Week 14	49.3	47.3	45.0	41.6	35**					
MTOT (%) Week 14	49.1	51.4	52.1	57.9	64.3**					
M/E ratio Week 14	1.0	1.1	1.2	1.5	1.8**					
Organ weights										
Liver Absolute (g)	286.8	296.4	310.8	310.4	338.9	243.8	254.4	272.6	263.0	315.7**
Relative (%)	2.98	3.14	3.19	3.39*	3.66**	2.88	3.16	3.30*	3.38*	4.06***

*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$
#: Includes value for an anaemic female

Conclusion

In the 90-day capsular study, 40 or 160 mg/kg bw/day of halosulfuron-methyl reduced body weight gain and increased liver weight. The highest dose level, 160 mg/kg bw/day, induced also a variety of haematological and clinical chemistry changes: depression in red cell parameters (erythrocyte and packed cell volume) for females, depression in total white cell counts and a shift towards myeloid cells in bone marrow of males, reduction in cholesterol levels. The NOEL was 10 mg/kg bw/day.

Dog oral 12-month capsular study

In the 12 month dog study, animals were assigned to groups randomly and then individually identified. Daily doses administered via gelatine capsules, were based on the most recently recorded bodyweight for approximately 52 weeks.

Groups of 6 male and 6 female Beagle dogs were given daily oral doses via capsule of 0.25, 1, 10 or 40 mg/kg/day of halosulfuron-methyl for 52 weeks. A similar sized control group was given an empty capsule (control group).

All dogs were observed twice daily for mortality and moribundity (approximately 1-3 hours post dose) and once daily for clinical signs. Detailed physical examinations were performed weekly throughout the study. Individual body weight and food consumption was recorded prior to treatment, weekly for Weeks 1-16 and once every 4 weeks thereafter. Ophthalmoscopic examinations were performed once during the acclimatisation period and prior to termination. Clinical pathology evaluations (clinical chemistry, haematology and urinalysis) were performed prior to initiation and during Weeks 13, 26 and 52. All surviving dogs were killed and subjected to a complete necropsy including organ weight evaluations. Histopathology was conducted on a range of tissues, as requested by the above mentioned Guidelines.

Body weight, total food consumption, clinical pathology (except cell morphology gradings and urinalysis data) and organ weight data were compared with control group of the same sex. Tests for homogeneity of variances and analysis of variance (ANOVA) were evaluated at the 5% probability level. Control means versus treated group mean comparisons were routinely evaluated at the 5% two-tailed probability level.

Results

Results are summarised in Table 24 and described below.

There was no treatment-related mortality. One male receiving 40 mg/kg/day was found dead during Week 50 of the study but there were no clinical signs, gross necropsy findings or histomorphologic lesions which could indicate that death was treatment-related.

There were no clinical observations suggestive of a treatment-related effect. Similarly, there were no treatment-related findings arising from Ophthalmological examination.

Body weight was unaffected by treatment at study termination. However, mean body weight gain of males given 10 and 40 mg/kg/day was lower (21 and 23%, respectively) than controls during the first 16 weeks of treatment, although the values did not reach the statistical significance. There were no treatment-related findings in food consumption.

For males treated with 10 or 40 mg/kg/day, a statistically significant depression in mean total cholesterol values was evident at Weeks 26 and 52. This effect was not paralleled by any hepatic

effect (either increased weight or histopathological findings). No treatment-related effects to clinical chemistry were evidenced in females.

Haematology showed that females receiving 40 mg/kg/day of halosulfuron-methyl had a significant depression in mean erythrocytes (Weeks 26 and 52), haemoglobin and haematocrit values (Week 52). Males receiving 40 mg/kg/day displayed a significant depression in the mean lymphocyte value at Week 26 and, although not statistically significant, also displayed a decreased mean lymphocyte value at Week 52.

Some other spotted difference between control and treated animals related to different parameters were statistically significant; however, the absence of dose- and time–dependence questions their toxicological significance in relation to the treatment.

There were no treatment-related findings in urinalysis and there were no gross pathology findings that could be attributed to the administration of halosulfuron-methyl.

There were no treatment-related findings to organ weights.

Pituitary Cysts were observed during microscopic examination, but this is a common findings in Beagle dogs and the very low incidence (0, 1, 0, 1, 2 in the Group 1-5 females, respectively) are within the range of historical control data at the site where the test was carried out. Therefore it can be concluded that there were no significant treatment-related findings.

Table 24: Summary of dog oral (capsular) 52-week toxicity study with halosulfuron-methyl

Parameter	Dose level (mg/kg/day)									
	Males					Females				
	0	**0.25**	**1**	**10**	**40**	**0**	**0.25**	**1**	**10**	**40**
Body weight change (kg)										
Week 0-16	4.4	4.3	4.1	3.5	3.4	3.2	3.4	3.1	3.0	2.9
Week 0-28	5.2	5.2	4.8	4.4	4.8	4.0	4.3	3.8	3.7	3.6
Week 0-52	5.8	5.4	5.3	5.0	5.4	4.6	4.8	4.2	4.4	3.9
Clinical chemistry										
Cholesterol (mg/dl)										
Week -1	161	175	153	157	164	No treatment-related effect				
Week 26	173	147	146	136*	126*					
Week 52	166	156	144	140*	121*					
Haematology										
RBC (mi/ul)										
Week -1						5.83	6.26	6.26	6.41*	6.03
Week 13						6.36	6.65	6.98*	6.71	6.30
Week 26						6.83	6.96	7.01	6.90	6.17*
Week 52						6.95	6.88	7.00	7.31	6.02*
HGB (g/dl)										
Week -1						12.8	13.8	13.5	13.4	12.9
Week 13	No treatment-related effect					14.2	15.2	15.8*	14.9	14.3
Week 26						15.8	16.2	16.1	15.8	14.5
Week 52						16.2	16.3	16.3	17.1	14.5*
HCT (%)										
Week -1						38.1	41.1	40.2	40.4	38.7
Week 13						41.5	44.1	45.7*	43.1	41.5
Week 26						45.9	46.7	46.8	45.7	42.0
Week 52						46.3	46.0	46.0	48.3	41.3*
Lymph (TH/UL)										
Week -1	4.8	4.9	6.1	4.5	4.3					
Week 13	4.1	4.0	4.8	3.6	3.6	No treatment-related effect				
Week 26	3.7	2.8	3.3	2.9	1.6*					
Week 52	2.7	2.5	2.8	1.9	1.9					

*: $p \leq 0.05$

Conclusion

The report of the 12-month dog study by capsule concluded that 40 mg/kg bw/day of halosulfuron-methyl reduced haematological parameters. Mean body weight gain of males given 10 and 40 mg/kg/day was reduced for the first 16 weeks of treatment although statistical significance was not attained and body weight was unaffected at study termination. Based on haematological changes observed in both sexes at 40 mg/kg/day, the NOAEL values is considered to be 10 mg/kg/day; the NOEL was 1 mg/kg/day.

4.7.1.2 Repeated dose toxicity: inhalation

A repeated inhalation toxicity study was not carried out, according to EU directive 94/79/EC, based on physico-chemical and toxicological properties of halosulfuron-methyl as well as its intended use.

4.7.1.3 Repeated dose toxicity: dermal

Rat dermal

In the rat 21-day dermal toxicity study, animals were assigned randomly using a computerised weight randomisation program and individually identified. Groups of 5 male and 5 female Sprague Dawley rats received a 6-hour dermal application of 0, 10, 100 or 1000 mg/kg/day of halosulfuron-methyl. It was applied as a powder to a gauze patch and then moistened with a saline solution. The gauze patch was applied to the shaved trunk of each animal and wrapped with self-adherent bandage secured with waterproof tape. The application site was roughly 10% of the body surface. At the end of the 6-hour exposure period the application site was washed with distilled water and wiped.

The animals were observed twice daily for mortality and moribundity. Cage side observations were made after each animal had been unwrapped. Clinical observations and signs of dermal irritation were recorded weekly. Individual body weights were recorded at initiation of the dosing, on Days 7 and 14 and at termination. Food consumption was recorded daily. Clinical pathology evaluations (haematology and clinical chemistry) were performed in all animals at termination. Animals found dead and those killed at termination were necropsied. Liver, kidneys and testes with epididymides were weighed and samples of treated and untreated skin, liver, kidneys and target organs were examined microscopically.

For each parameter analysed statistically, data from treated groups were compared with control values of the same sex. If the variances were heterogeneous, the data were transformed. If the variances were still not homogeneous, they were rank-transformed. Group mean comparisons were routinely evaluated at the 5% two-tailed probability level

Results

Results are summarised in Table 25 and described below.

One female treated with 100 mg/kg/day died on Day 14, but the severity of the histopathological findings in the kidney and liver did not account for its death.

There were no systemic treatment-related clinical signs neither any indication of dermal irritation at the site of application.

There was a reduction in body weight gain (19%) at the highest dose with respect to the control animal; no other effect of treatment on body weight and body weight gain was recorded. No treatment-related effects were seen on food consumption.

There were no treatment-related effects to clinical chemistry.

Mean haemoglobin values were significantly increased in males treated with 1000 mg/kg/day. Haematocrit values in males treated with 100 or 1000 mg/kg/day were statistically significantly increased compared with control, not paralleled by an increase in mean erythrocyte count.

Some gross pathology findings were recorded in the liver, kidney, urinary bladder and ureter. However, the lack of dose-response and the low incidence could not allow associating them to the treatment and were considered spurious.

Absolute and organ weights relative to body weight were unaffected by treatment.

The spotted microscopic findings were considered to be incidental and of no toxicological significance.

Table 25: Summary of rat dermal 21-day toxicity study with halosulfuron-methyl

Parameter	Dose level (mg/kg)							
	Males				Females			
	0	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Haematology								
HGB (g/dl)	16.4	17.1	17.2	17.3*	16.9	16.7	17.3	17.0
HCT (%)	48.6	50.4	51.1*	51.2*	48.3	48.5	50.8	49.7

* $p < 0.05$

Conclusion

According to the rat 21-day dermal toxicity study report, there was no evidence of irritation at the treated skin sites; at the highest dose a reduction in body weight gain was observed and a statistically significant increase in haemoglobin and haematocrit values in males treated with 100 or 1000 mg/kg/day was also reported. The NOEL was = 10 mg/kg/day.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No human information available.

4.7.1.6 Other relevant information

Two long-term oral toxicity studies are available: a 2-year combined chronic toxicity/carcinogenicity study in the rat and a 78-week carcinogenicity study in the mouse.

The 2-year dietary rat combined chronic toxicity/carcinogenicity study was conducted at dietary concentrations up to 5000 ppm (males) and 2500 ppm (females). The critical findings were reduced mean body weight throughout the study in males receiving 5000 ppm and between Weeks 13 and 52 in females fed 2500 ppm. The NOEL for chronic toxicity was therefore 1000 ppm, based on body weight reduction seen in females, corresponding to 56.3 mg/kg/day halosulfuron-methyl. Results are summarised in table 29 in section 4.10.1.1.

The 78 week study in mice was conducted at dietary concentrations up to 7000 ppm. At 7000 ppm, male body weight gain was significantly reduced over Weeks 0 to 13 whilst mean body weight was significantly reduced at Weeks 4, 13 and 24. Furthermore, at the same dose there were increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in controls; testis: 12/63 compared with 5/70 in controls). Results are summarised in table 30 in section 4.10.1.1.

On the basis of these results observed in male at the highest dose, the NOEL of halosulfuron-methyl for chronic toxicity (non-neoplastic end-points) was 3000 ppm, corresponding to mean achieved daily intakes of 410.0 mg/kg/day.

4.7.1.7 Summary and discussion of repeated dose toxicity

The most prominent effect observed upon repeated dose toxicity testing with halosulfuron-methyl upon short-term and long-term exposure was reduction of body weight gain in dogs, rats and mice. In dogs, which were the most sensitive species, changes in clinical chemistry, haematological parameters and liver weight were also observed. The relevant short-term NOAEL was 10 mg/kg bw per day from the 90-day and 1-year studies in dogs (Table 20 and the long-term NOAEL was 56.3 mg/kg bw per day from the 2-year rat study (Table 28).

The table below provides a systematic overview of the repeated dose toxicity studies relevant for the STOT-RE classification. The only study where the LOAEL fell below the cut-off values for triggering classification was a 28-day rat toxicity study (pancreatic effects). Pancreatic acinar cell degenerative changes of individual cells were noted in this study at 3000 ppm (231 and 241 mg/kg/day (M and F respectively). However, in a subsequent 90-day oral toxicity study in the rat and a 21-day dermal study there was no evidence of the pancreas being a target organ at higher doses. Aside from the findings in the rat 28 day study, there were no changes observed in any of the test species and different durations that indicated effects considered to be clear functional disturbance, serious or significant toxic changes to specific organs. The changes observed were addressed according to criteria for hazardous properties and a suitable NOAEL identified. None of the target organs were affected at sub-toxic doses and none of the effects warrants classification as STOT-RE. The weight of evidence from the studies conducted in three species shows that there is no consistent evidence of significant or severe effects at doses below the cut-off values in any species tested.

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

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

The effects observed in the battery of repeated administration tests completed for halosulfuron-methyl were limited to reduction of body weight gain, changes in clinical chemistry, haematological parameters and liver weight, and increased haemosiderin pigmentation in the renal tubular epithelium (rat). None of the observed changes were significantly or severely adverse and none triggered the STOT-RE classification.

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

Table 26 in 4.7.1.7 provides a systematic overview of the repeated dose toxicity studies relevant for the STOT-RE classification. The only study where the LOAEL fell below the cut-off values for triggering classification was a 28-day rat toxicity study (pancreatic effects). Pancreatic acinar cell degenerative changes of individual cells were noted in this study at 3000 ppm (231 and 241 mg/kg/day (M and F respectively). However, in a subsequent 90-day oral toxicity study in the rat and a 21-day dermal study there was no evidence of the pancreas being a target organ at higher doses. Aside from the findings in the rat 28 day study, there were no changes observed in any of the test species and different durations that indicated effects considered to be clear functional disturbance, serious or significant toxic changes to specific organs. The changes observed were addressed according to criteria for hazardous properties and a suitable NOAEL identified. None of the target organs were affected at sub-toxic doses and none of the effects warrants classification as STOT-RE. The weight of evidence from the studies conducted in three species from sub-acute to chronic exposure shows that there is no consistent evidence of significant or severe effects at doses below the cut-off values in any species tested.

Therefore halosulfuron-methyl does not meet the classification criteria for STOT-RE

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

Classification of halosulfuron-methyl for STOT-RE is not required.

Table 26: Summary of repeated dose toxicity studies with halosulfuron-methyl and comparison with STOT-RE criteria

	STOT RE 1 (mg/kg/day)	STOT RE 2 (mg/kg/day)	NOAEL and LOAEL	Significant/severe effects at LOAEL
Rat 21 day dermal	80	800	NOAEL >1000 mg/kg/day	None at LOEL. Increased haemoglobin and haematocrit at 100 mg/kg/day not considered to be adverse
Rat 28 day oral (dietary)	30	**300***	NOAEL: 300 ppm (Males:23 mg/kg/day; Females:25 mg/kg/day) LOAEL: 1000 ppm (Males 78 mg/kg/day Females: 85 mg/kg/day)	None at LOAEL Reduced body weight gain and overall food consumption, some clinical chemistry changes. At 3000 ppm (231 mg/kg/day M; 241 mg/kg/day F) degeneration/necrosis of pancreatic acinar cells

Rat 90 day oral (dietary)	10	100	NOAEL: 1600 ppm (Males:116 mg/kg bw/day;Females:147 mg/kg bw/day) NOEL: 400 ppm (Males:28.8 mg/kg/day; Females:37.3 mg/kg/day) LOAEL: 6400 ppm (Males:497 mg/kg bw/day; Females:640 mg/kg bw/day)	None at LOAEL Reduced body weight gain, cholesterol, total bilirubin; increased (haemosiderin) pigmentation of renal tubular epithelium; mild vacuolation in the liver>
Rat 2 year oral(dietary)	1.25	12.5	NOEL: 1000 ppm = 56.3 mg/kg/day (Females) LOEL: 2500 ppm=138.6 mg/kg/day (Females)	None at LOAEL Reduced body weight gain. No histopathological changes
Mouse 78 week oral (dietary)	1.7	17	NOAEL: 3000 ppm=410.0 mg/kg/day (Males) LOAEL: 7000 ppm=971.9 mg/kg/day (Males)	Reduced male body weight gain, increased microconcretions/ mineralisation in testis and epididymal tubules.
Dog 90 day oral	10	100	NOEL: 10 mg/kg/day LOEL: 40 mg/kg/day LOAEL: 160 mg/kg/day (f)	None at LOAEL Reduced body weight gain; increased liver weight and clinical chemistry alterations. Low haemoglobin, red blood cells and white blood cell counts. No histopathological changes.
Dog 1 year oral	2.5	25	NOAEL:10 mg/kg bw/day NOEL: 1 mg/kg/day LOEL: 40 mg/kg/day	None at LOEL Low haemoglobin, red blood cells and lymphocyte counts in males. Low cholesterol. No histopathological changes

*The figure in **bold** identifies where the study LOAEL fell below the cut-off value for STOT-RE for that study type.

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

Summary of the Dossier Submitter's proposal

Overview

The short-term and long-term repeated dose toxicity of halosulfuron-methyl was evaluated by the DS in rats, mice and dogs in studies from 21 days duration (repeated dermal toxicity study in rats) up to 1 year in the case of the dog (table 20, CLH report), along with chronic studies in both rats and mice.

No clear target organ or tissue was identified for the short-term toxicity of halosulfuron-methyl. The most prominent effect observed upon repeated dose toxicity testing with halosulfuron-methyl was a reduction of body weight gain in dogs, rats and mice. In dogs, which were the most sensitive species, changes in clinical chemistry, haematological parameters and liver weight were also observed, although none of these were of sufficient

magnitude or severity to meet the criteria for either specific target organ toxicity repeated exposure (STOT RE) category 1 or 2 (table 2, CLH report).

Rat Studies

In the rat 28-day dietary study, (Osheroff, 1988), body weight was reduced at the highest dose of halosulfuron-methyl. Overall body weight gain was reduced in both sexes at the highest dose (M/F; 777/888 mg/kg bw/day) and in females receiving 241 mg/kg bw/day, for which also a significant reduction in food consumption was reported. Some changes in clinical chemistry parameters (decreased glucose in males and lower protein, albumin, globulin and glucose; increased chloride ion in females) were also recorded in females at 25, 85 and 241 mg/kg bw/day, but these changes did not lie outside normal physiological reference values and thus do not warrant concern for organ/tissue functional disturbances. An increased incidence of individual cell degeneration/necrosis of pancreatic acinar cells at 231 mg/kg bw/day and above was described by the DS and is below the reference trigger value for STOT RE 2. The RAC Rapps note that this is not a consistent feature of halosulfuron-methyl. Furthermore, the RAC Rapps confirmed that this anomaly was not replicated in other studies or other species. Therefore, this effect is not proposed as a basis to justify classification for STOT RE 2 (see notes, supplemental information).

In the rat 90-day study report (Perry *et al.*, 1990), body weight gain was reduced at 497/640 mg/kg bw/day (M/F) halosulfuron-methyl, the highest dose level. Reductions in cholesterol and in total bilirubin, as well as increased pigmentation of the renal tubular epithelium due to haemosiderin deposition and mild vacuolation in the liver were also seen at this dose level (table 2, CLH report). Increased pigmentation was not associated with changes in clinical chemistry or functional disturbance. There were no effects on the pancreas.

A rat 90-day neurotoxicity study (Lemen, 1992) dosed males up to a maximum of 706 mg/kg bw/day and females to 316 mg/kg bw/day. There were no histopathology findings in neural tissues associated with the active substance, and no effects on the pancreas. The lowest observed adverse effect level (LOAEL) value was based on reductions in bodyweight gain.

According to the rat 21-day dermal toxicity study report (Osheroff, 1990d), there was no evidence of irritation at the treated skin sites; at the highest dose a reduction in body weight gain was observed, statistically significant increases in haemoglobin and haematocrit values in males treated with 100 or 1000 mg/kg bw/day were also reported. These effects were not paralleled by an increase in mean erythrocyte count. There were no treatment-related effects on clinical chemistry parameters. Some minor pathology findings were recorded in the liver, kidney, urinary bladder and ureter. However, the lack of dose-response, lack of consistency of the effects and their low incidence result in no support for an association with halosulfuron-methyl exposure.

In the rat 2-Generation Reproductive toxicity study (Lemen, 1991), the LOAEL was determined by generally small reductions in bodyweight indices at the top doses (223.2 to 261.4 mg/kg bw/day, F0 M:F pre-gestation body weights). Food consumption was reduced approximately by 10%. There was no substance related histopathology observed. F1 generation adults showed similar effects on bodyweight parameters.

Dog Studies

In the dog 90-day oral (capsule) study (Wood, 1991), groups of 4 male and 4 female Beagle dogs were given a daily oral dose, by capsule, of 0, 2.5, 10, 40 or 160 mg/kg bw/day of halosulfuron-methyl for 13 weeks. There were no deaths during the study and no treatment-related clinical signs. There were various changes in clinical chemistry and haematology parameters and some were statistically significant at the highest dose tested (table 23, CLH report). Generally, an absence of both dose- and time- dependence with substance exposure was observed. There were thus no clear substance specific toxicity, no treatment-related findings in urinalysis and there were no treatment-related macroscopic or histopathological findings, even though liver weights were increased. The LOAEL was considered to be 160 mg/kg bw/day based on reduced body weight gain and increased liver weight.

In the dog oral 12-month capsular study (Osheroff, 1991), groups of 6 male and 6 female Beagle dogs were given a daily oral dose, by capsule, of 0, 0.25, 1, 10 or 40 mg/kg bw/day of halosulfuron-methyl for 52 weeks. There was no treatment-related mortality or clinical observations suggestive of a treatment-related effect. Body weight was unaffected by treatment at study termination. However, mean body weight gain of females given 40 mg/kg bw/day was lower (15%, not statistically significant) than controls by the end of the study. No treatment-related findings in food consumption were observed. There was some limited clinical chemistry in males for cholesterol with no effects on clinical chemistry in females. Females showed significant but minor depressions in some haematology indices at the highest dose tested (table 24, CLH report; table 2 below). There were no treatment-related findings in urinalysis and no treatment-related macroscopic or histopathological findings. Based on haematological changes observed at 40 mg/kg bw/day, the LOAEL is set at this value.

Chronic Studies

The DS also described two long-term oral toxicity studies that were available for halosulfuron-methyl: a 2-year combined chronic toxicity/carcinogenicity study in the rat and a 78-week carcinogenicity study in the mouse.

The 2-year dietary rat combined chronic toxicity/carcinogenicity study (Moore, 1992a) was conducted at dietary concentrations up to 225.2/138.6 mg/kg bw/day for males and females, respectively. The critical findings were reduced mean body weights throughout the study in males at the highest dose and between weeks 13 and 52 in females at 138.6 mg/kg bw/day. A closer look by the RAC Rapps at the histopathology data showed no effect on the pancreas or any other organ or tissue except for seminal vesicle atrophy in males without any other pathology (no effects on prostate or testes for example). Further details by the RAC Rapps can be found in the supplemental information section later. The NOAEL for chronic toxicity was proposed to be 56.3 mg/kg bw/day, based on body weight reduction seen in females at the next higher dose.

The 78-week oral study in mice (Moore, 1992b), was conducted at dietary concentrations up to 972/1215 mg/kg bw/day for males and females, respectively. At the highest dose, male body weight parameters were significantly reduced at certain time points. At the same dose, there were increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in

controls; testis: 12/63 compared with 5/70 in controls). On the basis of these effects, the NOAEL was set at the next lower dose of 410 mg/kg bw/day in males.

Repeated Dose Toxicity Summary

The DS evaluated a variety of sub-chronic and chronic studies from rats, dogs and mice, including one short term (14 days) repeated dose dermal toxicity study in rats and presented a detailed summary of the effects in table 20 of the CLH report.

The most prominent effect observed upon repeated dose toxicity testing with halosulfuron-methyl upon short-term and long-term exposure was reduction of body weight gain in dogs, rats and mice. The only study, where effects were observed below the cut-off values for triggering classification (for STOT RE 2), was a 28-day rat oral toxicity study (pancreatic effects). Pancreatic acinar cell degenerative changes of individual cells are described in more detail later and are not considered by RAC to be indicative of halosulfuron-methyl toxicity because no consistent evidence is available from the other rat studies even when higher doses of the active substance were tested.

Overall, the DS concluded that there was no consistent evidence of significant or severe effects at doses below the cut-off values in any species tested and did not propose a classification for STOT RE.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The oral guidance cut-off values for a classification for STOT RE in category 2 under CLP are:

- ≤ 300 mg/kg bw/day from subacute studies on rat (28 days),
- ≤ 100 mg/kg bw/day from subchronic studies on rat (90 days),
- ≤ 25 mg/kg bw/day from one year studies and
- ≤ 12.5 mg/kg bw/day from 2-year studies.

In dermal studies the cut-off values are 2-fold greater than those above.

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity following repeated exposure in animals by the oral, dermal or inhalation routes **at or below** the given guidance values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

Criteria for Specific Target Organ Toxicity – Repeated Exposure

There is no human data for halosulfuron-methyl to substantiate classification in Category 1. Furthermore, animal experimental data is unconvincing with respect to significant and/or severe toxic effects at generally low exposure concentrations. What remains is consideration of category 2 or no classification.

The effects observed in a battery of repeated administration tests completed for halosulfuron-methyl in rats, mice and dogs were generally limited to reduction of body weight gain, small changes in clinical chemistry, small changes in haematological parameters and liver weight, and increased haemosiderin pigmentation in the renal tubular epithelium (rat only). None of these observed changes occurred within the guidance value range for STOT RE 2 (< 10 mg/kg bw/day concentration ≤ 100 mg/kg bw/day for a 90 day study) except some parameters in the dog oral (capsule) 90-day study. However, none of these effects were considered to be significantly or severely adverse to warrant classification.

The only study in the rat where pancreatic effects were observed within the guidance value range for STOT RE 2 was an oral 28-day rat toxicity study. Pancreatic acinar cell degenerative changes of individual cells were noted in this study at 231 and 241 mg/kg bw/day (males and females respectively). However, the histopathological data as described in the remaining studies, confirmed that the pancreatic acinar cell degeneration was an effect specific and limited only to this one study. It was not corroborated, even amongst those studies with significantly higher doses or longer duration of treatment.

Overall, RAC considers that the weight of evidence presented by the data from the studies conducted in three species from sub-acute to chronic exposure did not show consistent evidence of significant or severe effects at doses below the cut-off values relevant for classification.

RAC agrees with the DS and considers that **classification for STOT RE is not warranted**.

Supplemental information - In depth analyses by RAC

Below is a summary of all the short-term and long-term study data with respect to relevant effects. The reductions in body weight parameters as a consequence of exposure to halosulfuron-methyl determines the LOAEL in many cases in the draft assessment report (DAR). Overall, the effects observed are not considered specific to the active substance, consistent or severe enough to warrant classification with STOT RE.

Table 2: Summary of relevant NOAELs and LOAELs from repeated dose toxicity studies. In general, relevant effects occurred above the guidance value range for STOT RE 2 or the effect was not considered significant in the context of organ function or tissue damage.

Study	Guidance value (dose) for STOT RE 2	NOAEL	LOAEL	Effects at LOAEL (M/F)	Reference
		mg/kg bw/d			
Oral studies					
Rat SD 28-day	≤ 300	M: 78	**231**	↑ degeneration/necrosis of pancreatic acinar cells ([8/10]/[8/10])	Osheroff, 1988
		F: 85	**241**		
	≤ 100	M: 116	497	↓ body weight gain (17/35%)	

Rat SD 90-day		F: 147	640	↓ cholesterol (37/29%) ↓ total bilirubin (46/26%) ↑ pigmentation, kidney ([18/20]/[17/20]	Perry, 1990
Rat SD 90-day Chronic neurotox	≤ 100	M: 62.8	706	↓ body weight gain (19/11%)	Lemen, 1992
		F: 82.5	315.9		
Rat 2 year oral (dietary)	≤ 12.5	M: 108.3	225.2	↓ body weight gain (25/16%) ↓ bodyweight (18.4/--%)	Moore, 1992a
		F: 56.3	138.6		
Mouse 78 week oral (dietary)	≤ 17	M: 410	971.9	[ctrl vs top dose] ↑ microconcretions /mineralisation; epididymis ([0/40] vs [5/44] ↑ microconcretions /mineralisation; testes ([7/70] vs [12/63]	Moore, 1992b
		--	--		
Rat SD 2-Gen	≤ 300	M: 50.4_{F0}	223.2	↓ body weight (5/<10%) ↓ body weight gain (7.4/19.5%)	Lemen, 1991
		F: 58.7_{F0}	261.4		
Dog 90-day	≤ 100	M: 10	**40**	↓ body weight gain (11/27%) ↓ cholesterol (26/12%) ↓ albumin (--/8%) ↓ WBC (20/--%) ↑ liver wt. rel. (14/17%)	Wood, 1991
		F: 10	**40**		
Dog **52-week**	≤ 25	M: 10 F: 10	40 40	↓ body weight gain (--/15%) ↓ cholesterol (16/--%) ↓ RBC (--/13%) ↓ HGB (--/10.5%) ↓ HCT (--/11%)	Osheroff, 1991
Dermal studies					
Rat SD 21-day	≤ 600	100	1000	↓ body weight gain (17/--%) ↑ HGB (5/--%) ↑ HCT (5/--%)	Osheroff, 1990d
-- no effect					

Notes

1. Rat 28-day oral (dietary) study. The change in body weight parameters are either not biologically significant or are accompanied by a reduction in food consumption. Small haematological changes are seen in the high dose females only (HGB ↑ 5%; HCT ↑ 8%); clinical chemistry shows variable results with little to no dose response, glucose is significantly reduced by approximately 17% in both sexes only at the highest dose. The clinical chemistry changes, while statistically significant are within the normal physiological range at the time the study protocol was carried out (e.g. glucose, table 3 below). Therefore, these changes are not considered as toxicologically significant changes relevant for human health by affecting the function or morphology of a tissue/organ or producing serious changes to the biochemistry or haematology of the organism. Changes in female plasma proteins determined the LOAEL in the DAR but the changes were identical for both the 300 ppm and 1000 ppm dose groups. These changes were also within normal physiological reference ranges as reported by Osheroff (1988) and are not considered adverse. The 1000 ppm dietary group is thus indicated in table 2 above as a NOAEL. The most relevant adverse effect is the incidence of pancreatic acinar cell degeneration/necrosis at 3000 ppm which determines a clear LOAEL of 231 and 241 mg/kg bw/day for males and females respectively. Control and low dose incidence is typically 1-2/10 animals. There was no effect on liver weight and no effect on liver enzymes.

Table 3: Blood glucose levels in the rat 28-day dietary study following exposure to halosulfuron-methyl.

Dose M/F mg/kg	**0**	**23/25**	**78/85**	**231/241**	**777/888**
Males: normal range: 68 – 162 mg/dl					
mean	120	120	114	110	100*
sd	15.7	11.6	11.4	9.3	9.2
n	10	10	10	9	10
Females: normal range: 72 – 181 mg/dl					
mean	123	126	121	112	106*
sd	16.0	13.4	16.0	12.1	17.3
n	10	10	10	10	10

* significantly different from control at $p \leq 0.05$

Pancreatic Effects

Pancreatic acinar cell degenerative changes of individual cells were noted in the rat 28-day dietary study at 3000 ppm (231 and 241 mg/kg bw/day, M and F respectively) and at 1000 ppm (777 and 888 mg/kg bw/day, M and F, respectively). However, in a subsequent 90-day oral toxicity study in the rat and a 21-day dermal study there was no evidence of the pancreas being a target organ at higher doses. Aside from the findings in this 28-day rat oral study, there were no changes observed in any of the other rat studies or test species that indicated specific effects on the pancreas.

The compound-related increase in pancreatic lesions were observed in the 2 highest dose groups, in both males and females. They were described as an increased incidence of individual cell degeneration/necrosis of pancreatic acinar cells. A histopathological analysis of the low and mid dose groups showed one incidence of this effect per dose. There was a

progression to increased severity with increased dose at the two highest doses tested (table 4).

Table 4: Summary of pancreatic data for increased individual cell degeneration/necrosis

Dose M/F mg/kg bw/day	**0**	**23/25**	**78/85**	**231/241**	**777/888**
Males:					
no. examined	10	10	10	10	10
Finding not present:	10	9	9	2	0
- minimal:	0	1	1	7	2
- slight:	0	0	0	1	6
- moderate:	0	0	0	0	2
Females:					
no. examined	10	10	10	10	10
Finding not present:	9	8	9	2	0
- minimal:	1	2	1	7	4
- slight:	0	0	0	1	5
- moderate:	0	0	0	0	1

In comparison, the highest dose in the rat 2-year combined chronic toxicity/carcinogenicity study (Moore, 1992) corresponded with the penultimate dose in the 28-day study and did not show any substance related effect on the pancreas.

2. Rat 90-day oral (dietary) study. The high dose treatment reduced body weight gain, alterations in clinical chemistry parameters (cholesterol and total bilirubin) of both sexes and a slight reduction in food consumption by females and mild liver vacuolation in males (4/20 vs. 2/20 for controls) and increased pigmentation (all grades) in the tubular epithelium of the kidney due to haemosiderin (18/20 males vs. 3/20 in controls; 17/20 females vs. 8/20 in controls) were observed.

3. Dog 90-day oral (capsule) study. Food consumption was not affected by treatment. The majority of adverse effects were observed in the high dose group (160 mg/kg bw/day). These were reduced weight gain (both sexes, ↓ 20 – 41% males and females, respectively, not statistically significant) and also induced a variety of haematological and clinical chemistry changes: depression in red cell parameters (erythrocyte and packed cell volume, females), depression in total white cell counts (↓ 33% males) and a shift towards myeloid cells in the bone marrow of males along with a reduction in cholesterol levels (↓ 23/47%, M/F) and albumin (females ↓ 16%). There was also increased liver weight in both sexes (absolute: males ↑ 18%, females ↑ 29%; relative: males ↑ 23%, females ↑ 41%). Effects seen at 40 mg/kg bw/day are reduced weight gain (both sexes, ↓ 11–27% males and females, respectively, not statistically significant) and reduced white blood cell count (males; ↓ 20%) and slightly increased liver weight (absolute: males ↑ 8%, females ↑ 8%; relative: males ↑ 14%, females ↑ 17%). Effects seen at 10 and 2.5 mg/kg bw/day were limited to depression in total white cell counts in males (↓ 24 and 23%, respectively). There was no liver histopathology associated with treatment.

4. Dog 12-month oral (capsule) study. The statistically significant effects observed were reduction in cholesterol levels (males only, ↓ 18 and 27%, at 10 and 40 mg/kg

bw/day, respectively) and in females mean erythrocytes (↓ 12%, 40 mg/kg bw/day) and haemoglobin and haematocrit values on week 26 of treatment only (both ↓ 11% at 40 mg/kg bw/day). The experts at the European Food Safety Authority pesticides peer review (EFSA PPR) 95 (Sept 2012) agreed with the Rapporteur Member State (RMS) proposal that reduced body weight gain observed at 10 mg/kg bw/day in males (↓ 21%) was not considered to be treatment related but due to a low value outlier in the data.

5. Rat 2-year combined chronic toxicity/carcinogenicity study. In general, histopathology was unremarkable with no observed effect on the pancreas. An increased incidence of seminal vesicle atrophy was noted in males without any other accompanying pathology such as cell necrosis or inflammation being present or changes in other tissues such as the prostate and testes. For the control group through to the high dose group, the total incidence (and %) of seminal vesicle atrophy was:

- Group 1 (0 mg/kg bw/day): 11/54 (20%)
- Group 2 (0.44 mg/kg bw/day): 14/52 (27%)
- Group 3 (4.4 mg/kg bw/day): 11/50 (22%)
- Group 4 (43.8 mg/kg bw/day): 17/54 (31.5%)
- Group 5 (108.3 mg/kg bw/day): 18/52 (34.6%)
- Group 6 (225.2 mg/kg bw/day): 25/51 (49%)

These incidences do not include the 30 animals of both sexes from 3 interim sacrifices. Interim sacrifices were performed on 10 animals of each sex at 26 weeks, 52 weeks and 78 weeks. There were no incidences of seminal vesicle atrophy at 26 or 52 weeks. Minor incidences (0, 1, 1, 0, 2, 1; groups 1 to 6, respectively) were observed at 78 weeks but with no dose response. There was no prostate atrophy or testes effects noted at any dose level. There was no effect on seminal vesicle weight which is a more sensitive metric for androgen imbalance. Seminal vesicle involvement was not noted in any of the other rat studies.

6. Rat 2-Generation Reproductive toxicity study. Over 14 to 16 days in both the F0 and F1 generations there was no effect on the seminal vesicles at any dose level. There were no histopathological data reported for the pancreas. The highest dose tested was very similar to that of the rat 2-year combined chronic toxicity/carcinogenicity study. There was no substance related histopathology reported in any tissue.

7. Mouse 18-month combined chronic toxicity/carcinogenicity study. In general, histopathology was unremarkable. At the highest dose tested there were increased incidences of microconcretions/mineralisation within the lumen of both the epididymal and testis tubules (taking into account just the animals from the main study, from unscheduled sacrifice and terminal sacrifice only: epididymis: 5/52 compared with 0/50 in controls; testis: 10/52 compared with 5/50 in controls). An expanded analysis is provided below in table 5, data was taken from the original study report. Note the similar incidence at both the lowest and highest doses (> 2 orders of magnitude dosing regimen) with variable incidences at the other dose levels. A brief statistical analysis of the lesion incidence was not significant (chi-square, p = 0.53).

Table 5: Histopathological changes (male mice, CD-1) in an 18-month. Feed Study with halosulfuron-methyl. Doses in mg/kg bw/day.

Lesion / dose	0	4.0	41	410	972

Epididymis:					
- microconcretions/mineralisation	0	0	0	1	5
- animals examined	50 (0%)	50 (0%)	50 (0%)	51 (2%)	52 (10%)
Testes:					
- microconcretions/mineralisation	5	10	7	6	10
- animals examined	50 (10%)	50 (10%)	50 (14%)	51 (12%)	52 (20%)

Animals examined includes all animals on the main study from both unscheduled and scheduled terminal sacrifice and excludes those from the interim sacrifices.

The interim sacrifices showed no evidence of microconcretions/mineralisation in the epididymis.

There is no dose-response evident in the incidence of this lesion in the testes tubules at the interim sacrifices (table 6 below). The significance of this effect from a toxicological point of view is unknown though it is generally recognised as an age-related event in other tissues (e.g. kidney tubules and brain stem).

Historical control data (HCD) were only available for this lesion in epididymal tissue, and amongst 13 studies conducted from 1984 to 1988 there were incidences of 1, 1, and 2 cases from three of these studies (4 animals out of 594, i.e. 0.7%).

Table 6: Histopathological changes (male mice, CD-1) at interim sacrifice in an 18-month. Feed Study with halosulfuron-methyl. Dose mg/kg bw/day.

Lesion / dose	**0**	**4.0**	**41**	**410**	**972**
Testes: 27 weeks					
- microconcretions/mineralisation	0	0	0	1	0
- animals examined	10	10	10	10	10
Testes: 54 weeks					
- microconcretions/mineralisation	0	0	2	0	2
- animals examined	15	15	15	14	13

4.9 Germ cell mutagenicity (Mutagenicity)

Table 27: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data			
Method	**Dose levels**	**Result**	**Reference**
In vitro Bacterial reverse mutation: *Salmonella typhimurium*: TA1535, TA1537, TA1538, TA98 and TA100 *Escherichia coli:* WP2 *uvr*A$^-$ US EPA FIFRA 84-2 GLP Purity: 98,5% and 98,7%	First test: -/+ S9 mix: 1, 10, 100, 500, 1000, 2500, 5000 and 10000 µg/plate (*S. typhimurium)* 333, 667, 1000, 3330, 6670 and 10000 µg/plate (*E. coli)* Second test: -/+ S9 mix: 1, 10, 100, 500, 1000, 2500, 5000, and 9999 µg/plate (*S. typhimurium)* 333, 667, 1000, 3330, 6670 and 10000 µg/plate (*E. coli)*	Negative	DAR B.6.4.1 Jagannath and Lawlor, 1988
In vitro Chromosome aberrations (clastogenicity): Chinese hamster ovary cells US EPA FIFRA 84-2 GLP Purity: 98,5%	-S9 mix: 451, 903, 1020, 1050 and 1810 µg/ml +S9 mix: 449, 899, 1350 and 1800 µg/ml	Negative	DAR B.6.4.2 Murli, 1988
In vitro Mammalian cell gene mutation: Chinese hamster ovary cells (HGPRT assay) US EPA FIFRA 84-2 GLP Purity: 98,5%	First test: -/+ S9 mix: 100, 200, 500, 700 and 900 µg/ml Second test: -/+ S9 mix: 50, 100, 200, 500 and 700 µg/ml	Negative	DAR B.6.4.3 Stegeman, Costello and Garrett, 1993
In vitro Unscheduled DNA synthesis: Rat hepatocytes US EPA FIFRA 84-2 GLP Purity: 98,5% and 98,7%	Trial 1: 25, 50, 100, 250, 500 and 1000 µg/ml Trial 2: terminated Trial 3: 5.06, 10.1, 25.3, 50.6, 101 and 253 µg/ml	Negative	DAR B.6.4.5 Cifone, 1988
Micronucleus test, Male and Female (5sex/groups) ICR mice bone marrow erythrocytes US EPA FIFRA 84-2 GLP Purity: 98,5%	0, 500, 1667, 5000 mg/kg	Negative	DAR B.6.4.4 Ivett, 1989

4.9.1 Non-human information

4.9.1.1 In vitro data

One valid and acceptable gene mutation study with Salmonella typhimurium and Escherichia coli is available. Halosulfuron-methyl dissolved in dimethyl sulfoxide, was evaluated for mutagenic activity in the Ames Salmonella/microsome reverse mutation assay with strains TA98, TA100, TA1535,

TA1537 and TA1538 and in the Escherichia coli WP2uvrA-/mammalian-microsome reverse mutation assay in the presence and absence of exogenous metabolic activation system. In the preliminary toxicity test with TA100 and WP2uvrA, the test material did not induce changes in the appearance of the background lawn of growth at any dose, but a decrease in revertant colonies was observed at the top dose with TA100. Based on these findings, the dose ranges 1–10,000 and 333–10,000 µg/plate were selected for the main experiments in S.typhimurium and E.coli, respectively. In the main mutagenicity assays halosulfuron-methyl did not increase in number of revertant colonies/plate either of S. typhimurium strains or E. coli WP2uvrA- compared with solvent control values. The positive controls produced large increases in revertant colony numbers, which demonstrated the effectiveness of the S9-mix activation system and the ability of the test system to detect known mutagens. Halosulfuron-methyl technical, dissolved in dimethyl sulfoxide, showed no evidence of mutagenic activity in the Ames Salmonella/microsome reverse mutation assay or E. coli/mammalian microsome reverse mutation assay.

Further in vitro genotoxicity testing was investigated in a study to determine clastogenicity in mammalian cells by measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. Following a preliminary range-finding study, duplicate cultures of Chinese hamster ovary (CHO) cells were incubated with concentrations of either 451, 903, 1020, 1050 or 1810 µg/ml of halosulfuron-methyl in the absence of metabolic activation (S-9 mix) or with 449, 899, 1350 or 1800 µg/ml in its presence. Cells were incubated for 17.25 hours in the absence of activation and for 2 hours in the presence of S-9 mix. Halosulfuron-methyl did not cause any significant increases in the number of aberrant cells at any dose level either with or without metabolic activation. Halosulfuron-methyl dissolved in dimethyl sulfoxide, did not induce chromosome aberrations in cultured Chinese hamster ovary cells in either the presence or absence of metabolic activation.

The mutagenic potential of halosulfuron-methyl was assessed in cultured Chinese Hamster Ovary (CHO) cells at the HPRT gene locus. Following a range-finding experiment, an initial test was performed with concentrations of 0, 100, 200, 500, 700 and 900µg/ml (limit of solubility in culture medium) of Halosulfuron-methyl in both the presence and absence of S9 mix fortified with 1, 5 and 10% v/v S-9. This was followed by a confirmatory test with 0, 50, 100, 200, 500 and 700 µg/ml of halosulfuron-methyl without S9 or with 5% S-9 mix. Cells were treated for 3 hours both with and without S-9 mix. No significant cytotoxicity and no significant increases in mutant frequency were found either with or without S-9 mix. Therefore halosulfuron-methyl was not mutagenic in CHO cells in either the absence or presence of metabolic activation when tested to the limit of its solubility.

The ability of halosulfuron-methyl to cause unscheduled DNA synthesis (UDS) in cultured primary rat hepatocytes was evaluated in three trials. In trial 1, rat hepatocytes were exposed to concentrations between 0.025 and 1000 µg/ml of halosulfuron-methyl in the presence of tritiated thymidine (3HTdr). Treatments were moderately toxic, with >70% of survival at top dose. No induction of UDS was observed in treated cultures. As the repeat test (trial 2), higher toxicity than in trial 1 was observed, and the experiment was terminated. A third trial, with a new halosulfuron-methyl sample, was conducted using concentrations between 1.01 and 1010µg/ml of halosulfuron-methyl. Also in this trial there was no significant increase in UDS. In trials 1 and 3, there was no significant increase in UDS. Therefore, halosulfuron-methyl showed no potential to induce UDS in rat primary hepatocytes.

4.9.1.2 In vivo data

An in vivo mouse micronucleus assay was conducted to investigate the genotoxic potential of halosulfuron-methyl in somatic cells. Groups of 5 male and 5 female ICR mice were given a single oral dose by gavage of 0 (negative control), 500, 1667 and 5000 mg/kg of bodyweight of halosulfuron-methyl. A positive control group was given 80 mg/kg of cyclophosphamide. Bone

marrow samples were taken from the treated groups killed 24, 48 and 72 hours after dosing. Samples from the negative and positive control groups were taken 24 hours post-dosing. Halosulfuron-methyl did not induce a significant increase in micronuclei in mouse bone marrow polychromatic erythrocytes.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available

4.9.4 Summary and discussion of mutagenicity

The mutagenic potential of halosulfuron-methyl was evaluated in the regulatory battery of genotoxicity tests comprising in vitro tests for bacterial and mammalian cell gene mutation, chromosome aberrations and UDS and an in vivo mouse micronucleus test for chromosome damage.

All tests gave negative results, therefore halosulfuron-methyl showed no evidence of genotoxic activity either in vitro or in vivo.

4.9.5 Comparison with criteria

Based on the results of the available genotoxicity studies, classification of halosulfuron-methyl is not warranted according to CLP criteria.

4.9.6 Conclusions on classification and labelling

Halosulfuron-methyl was concluded to be non-genotoxic, and consequently no classification for mutagenic hazard is required.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Introduction

The DS reported that halosulfuron-methyl was tested in several *in vitro* studies and one *in vivo* study. In the CLH report, each specific study is summarised in table 27, section 4.9. According to the EFSA conclusion on pesticide peer review (EFSA, 2012), halosulfuron-methyl did not present a genotoxic potential either in *in vitro* or *in vivo* studies. There were no studies in germ cells. The DS agreed with the EFSA assessment and did not propose to classify halosulfuron-methyl as a germ cell mutagen.

Results* – In Vitro *Tests

The genotoxicity of halosulfuron-methyl was investigated in an Ames test (using *Salmonella typhimurium* strains: TA1535, TA1537, TA1538, TA98 and TA100 in addition to *Escherichia coli*: WP2 uvrA$^-$ in the presence and absence of exogenous metabolic activation

system), in an *in vitro* mammalian clastogenicity study using CHO cells, in an *in vitro* mammalian cell gene mutation study accessed at the HPRT gene locus using CHO cells and in an unscheduled DNA synthesis (UDS) assay in cultured primary rat hepatocytes. Positive controls were included in all assays and behaved as expected. The results from all these assays were negative (table 7, below). There was no significant cytotoxicity except for rat hepatocytes in the *in vitro* UDS assay.

Results* – In Vivo *Tests

An *in vivo* mouse micronucleus assay was conducted to investigate the genotoxic potential of halosulfuron-methyl in somatic cells. The results were negative, halosulfuron-methyl did not induce a significant increase in micronuclei in mouse bone marrow polychromatic erythrocytes. Groups of five male and five female ICR mice were given a single oral dose by gavage of 0, 500, 1667 or 5000 mg/kg bw the active substance in 0.5% carboxymethylcellulose (CMC). No deaths or cytotoxicity were observed. Exposure of the bone marrow to halosulfuron-methyl was assumed based on absorption distribution metabolism and excretion (ADME) and toxicokinetics studies with radiolabelled active substance (which indicated that halosulfuron-methyl was well absorbed orally with levels in bone similar to levels in muscle and spleen but about 25-20% that found in blood).

Negative results were obtained in all studies with halosulfuron-methyl. There is no evidence of genotoxicity for this substance.

Table 7: Summary of genotoxicity tests with halosulfuron-methyl adapted from the CLH report.

Study	Result	Test System	Reference
***In vitro* studies:**			
Bacterial mutagenicity	negative	GLP, US EPA FIFRA 84-2 (1984) *Salmonella* Strains: TA1535, TA1537, TA1538, TA98, TA100 *E. coli* WP2 uvrA$^-$	Jagannath and Lawlor, 1988
Mammalian cell mutagenicity	negative	GLP, US EPA FIFRA 84-2 (1984) CHO (HPGRT locus)	Stegeman *et al.*, 1993
Clastogenicity	negative	GLP, US EPA FIFRA 84-2 (1984) CHO cells	Murli, 1988
UDS	negative	GLP, US EPA FIFRA 84-2 (1984) Male rat (F344) hepatocytes	Cifone, 1988
***In vivo* studies:**			
Micronucleus	negative	GLP, US EPA FIFRA 84-2 (1984) Mouse (ICR) bone marrow (short term)	Ivett, 1989

Comments received during public consultation

No comments were received for this hazard endpoint.

Assessment and comparison with the classification criteria

No human data are available for halosulfuron-methyl, therefore a classification with Muta. 1A is not supported. Halosulfuron-methyl is negative in acceptable *in vitro* tests and *in vivo* somatic cell mutagenicity guideline tests in mammals. Data are not available for the induction of mutagenic effects in germ cells (a criterion for Category 1B). Overall, RAC agrees with the DS that **classification for genotoxicity is not warranted**.

4.10 Carcinogenicity

Table 28: Summary table of relevant carcinogenicity studies

Method	Dose levels (ppm)Remarks	Results	Reference
Rat 2-year combined chronic toxicity and carcinogenicity study (dietary) US EPA 83-5 GLP Purity: 98.7%	**Males**: 0, 10, 100, 1000, 2500 and 5000 ppm (0, 0.44, 4.4, 43.8, 108.3, 225.2 mg/kg/day) **Females**: 0, 10, 100, 1000 and 2500 ppm (0, 0.56, 5.6, 56.3, 138.6 mg/kg/day)	**Chronic toxicity:** NOEL: 1000 ppm = 56.3 mg/kg/day (Females) LOEL: 2500 ppm=138.6 mg/kg/day (Females) Critical effect: reduced body weight gain. **Carcinogenicity:** NOEL: Males: 5000 ppm Females: 2500 ppm (Males: 225.2 mg/kg/day; Females: 138.6 mg/kg/day) LOEL> Males 5000 ppm Females: 2500 ppm (Males: 225.2 mg/kg/day; Females: 138.6 mg/kg/day). No carcinogenic potential at any dose level	DAR B.6.5.1 Moore, 1992a,
Mouse dietary 78-week oncogenicity study US EPA 83-5 GLP Purity: 98.7%	0, 30, 300, 3000 and 7000 ppm (Males: 0, 4, 41.1, 410.0 and 971.9 mg/kg/day; Females: 0, 5.2, 51.0, 509.1 and 1214.6 mg/kg/day) (Purity: 98.7%)	**Chronic toxicity:** NOAEL: 3000 ppm=410.0 mg/kg/day (Males) LOAEL: 7000 ppm=971.9 mg/kg/day (Males) Critical effect: Reduced male body weight gain, increased microconcretions/ mineralisation in testis and epididymal tubules. **Carcinogenicity:** NOEL: 7000 ppm (Males: 971.9 mg/kg/day Females: 1214.6 mg/kg/day) LOEL>7000 ppm (Males: 971.9 mg/kg/day Females: 1214.6 mg/kg/day). No carcinogenic potential at any dose level	DAR B.6.5.2 Moore, 1992b,

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat 2 year dietary toxicity/oncogenicity study

A combined chronic toxicity and oncogenicity dietary study in rats was conducted over two years with halosulfuron-methyl.

Groups of 85 male Sprague Dawley CD rats were given dietary concentrations of 0, 10, 100, 1000, 2500 or 5000 ppm of halosulfuron-methyl whilst similar sized groups of females were fed 0, 10, 100, 1000 or 2500 ppm. Criteria evaluated for treatment-related effects included: survival, clinical signs, body weight and body weight gain, food consumption, clinical haematology and serum chemistry, urinalysis, ophthalmoscopic findings, organ weight, gross pathology and histopathological findings. After 27, 53 and 79 weeks of treatment, 10 males and 10 females from each group were killed to assess the chronic toxicity of halosulfuron-methyl. The remaining animals were killed after 105 or 106 weeks of treatment to assess carcinogenic potential. The mean bodyweight was reduced in the males given 5000 ppm (reduction range from 5.3% at Week 4 to 18.4% at Week 104).

Results

Results are summarised in Table 29 and described below.

Achieved test material intake

Overall mean achieved intakes (Weeks 1-104) in males fed 10, 100, 1000, 2500 and 5000 ppm of halosulfuron-methyl were 0.44, 4.4, 43.8, 108.3 and 225.2 mg/kg/day, respectively. Corresponding values for the females receiving 10, 100, 1000 and 2500 ppm were 0.56, 5.6, 56.3 and 138.6 mg/kg/day, respectively.

Mortality

There was no treatment-related effect on survival. Survival in males given 1000 ppm significantly lower than controls. Since survival in males and females was not dose-related , the lower survival in males given 1000 ppm was considered to be unrelated to treatment.

Clinical signs

There were no treatment-related clinical signs.

Body weight

At 5000 ppm halosulfuron-methyl, overall male group mean body weight was low throughout the study. Values were significantly lower than controls in Weeks 4, 13, 24, 52, 76 and 104. For the females given 2500 ppm, body weight was low compared to controls occasionally gaining statistical significance.

Food consumption

Intergroup statistical comparisons of food consumption data showed no consistent adverse effects at 2500 ppm or below for the males and 1000 ppm or below for the females.

For the males fed 2500 and 5000 ppm of halosulfuron-methyl, mean food consumption was significantly lower during Week 1. Additionally, significantly lower mean intakes for the males were noted during Weeks 4 and 52. Mean total food consumption was also significantly lower than the concurrent controls during Weeks 4-13 in males given 2500 and 5000 ppm and during Weeks 13-24 for males at 100 ppm.

For females, mean food consumption was low at 1000 and 2500 ppm during Week 13. At 100 and 2500 ppm it was reduced during Week 52 compared with controls. Mean total food consumption was significantly lower in the females fed 100 ppm during Weeks 4-13, 13-24. At 2500 ppm, it was low during Weeks 4-13, 24-52 and 52-76.

Ophthalmological examination

No findings were considered to be treatment-related.

Clinical chemistry

There were no clearly consistent changes related to treatment with halosulfuron-methyl.

Table 29: Summary table of 2 year rat toxicity/carcinogenicity study findings

	Males						Females				
Dietary level (ppm)	0	10	100	1000	2500	5000	0	10	100	1000	2500
Number allocated per group/sex	85	85	85	85	85	85	85	85	85	85	85
Scheduled deaths 1st kill week 27 (unscheduled deaths up to week 27)	10 (0)	10 (0)	10 (0)	10 (2)	10 (0)	10 (3)	10 (0)	10 (0)	10 (0)	10 (0)	10 (0)
Scheduled deaths 2nd kill week 53 (unscheduled deaths up to week 53)	10 (2)	10 (0)	10 (3)	10 (4)	10 (0)	10 (2)	10 (3)	10 (3)	10 (2)	10 (4)	10 (2)
Scheduled deaths 3rd kill week 79 (unscheduled deaths up to week 79)	10 (8)	10 (8)	10 (7)	10 (12)	10 (9)	10 (6)	10 (8)	10 (13)	10 (10)	10 (10)	10 (17)
Scheduled deaths final kill week 105/6 (unscheduled deaths up to week 105/6)	27 (18)	25 (22)	26 (19)	18 (19)	21 (25)	21 (23)	23 (21)	22 (17)	18 (25)	20 (21)	22 (14)
Total unscheduled deaths	28	30	29	37	34	34	32	33	37	35	33
Survival to 104 weeks (%)	49	45	47	33*	38	38	42	40	33	36	40
Incidence of clinical signs	No effects						No effects				
Mean body weight at 13 weeks (g)	582.9	580.0	582.6	578.0	584.9	549.6*	324.3	327.2	319.9	326.0	322.7
Mean body weight at 24 weeks (g)	680.9	677.5	674.3	670.0	680.7	640.5*	371.6	370.1	358.3	361.6	359.3
Mean body weight at 52 weeks (g)	826.1	826.2	816.3	827.1	826.4	768.4*	474.4	463.3	446.5	453.4	426.1*
Mean body weight at 76 weeks (g)	859.9	844.7	844.4	842.2	853.4	784.4*	529.1	521.3	505.5	516.7	470.3
Mean body weight at 104 weeks (g)	813.2	742.6	815.8	794.6	826.8	663.4*	577.4	510.4	517.6	509.6	507.4
Mean total food consumption Weeks 4-13 (g)	1895.3	1900.2	1883.1	1851.4	1823.9*	1835.8*	1434.3	1408.9	1371.1*	1391.2	1354.0
Mean total food consumption Weeks 24-52 (g)	1519.7	1514.1	1476.4	1511.3	1498.9	1467.5	1149.5	1125.8	1115.0	1139.0	1086.9*
Mean total food consumption Weeks 76-104 (g)	1483.5	1451.8	1481.1	1442.7	1442.7	1341.3	1259.3	1160.5	1219.9	1225.2	1180.8
Ophthalmology	No effects						No effects				
Haematology	No effects						No effects				
Clinical chemistry	No effects						No effects				
Urinalysis	No effects						No effects				
Absolute organ weights	No effects						No effects				
Body weight relative organ weights	No effects						No effects				
Brain weight relative organ weights	No effects						No effects				
Macroscopic findings	No effects						No effects				
Microscopic non-neoplastic findings – Seminal vesicle atrophy (unscheduled deaths)	9/27	11/28	9/24	13/36	15/32	18/30	-	-	-	-	-
Microscopic non-neoplastic findings – seminal vesicle atrophy (terminal kill)	2/27	3/24	2/26	4/18	3/20	7/21	-	-	-	-	-
Microscopic neoplastic findings	No effects						No effects				

*Significantly different from control value p≤0.05 (ANOVA); Data from treated groups were compared with data from controls of the same sex using Levene's test for homogeneity of variances. If variances of untransformed data were heterogeneous, a series of transformations was performed to achieve variance homogeneity. Analysis (ANOVA) were performed on rank-transformed data. If variances were still heterogeneous, Dunnett's test was performed for equal and unequal variances. Cumulative survival data were analysed using National Cancer Institute (USA) package.

Haematology

There were no treatment-related effects.

Urinalysis

There were no treatment-related effects.

Macroscopic findings at necropsy

Macroscopic examination revealed no treatment-related findings for decedent animals or those sacrificed at Weeks 27, 53, 79, 105 and 106.

Organ weights

Although differences in the absolute and/or body weight-relative or brain-relative weights of some organs of treated rats attained statistical significance ($p \leq 0.05$), values were incidental with no time or dose-related trends and were not associated with histopathological change.

Microscopic findings

There was no histopathological evidence of toxicity or oncogenicity.

Conclusion

Dietary administration of 5000 ppm of halosulfuron-methyl to male rats and 2500 ppm to female rats for 104 weeks reduced body weight gain and food consumption.

The critical findings in a 104-week dietary rat combined chronic toxicity and oncogenicity study were reduced mean body weight in males treated with 5000 ppm in the diet throughout the study and between Weeks 13 and 52 in females fed 2500 ppm.

The NOEL for chronic toxicity was therefore 1000 ppm, based on body weight reduction seen in females, corresponding to 56.3 mg/kg/day halosulfuron-methyl.

The NOEL for carcinogenicity was 5000 ppm (corresponding to 225.2 mg/kg/day) in males and 2500 ppm (138.6 mg/kg/day) in females.

Mouse 78 week dietary oncogenicity study

A 78-week carcinogenicity dietary study in mice was conducted to determine the oncogenic potential of halosulfuron-methyl. Groups of 75 male and 75 female CD-1 mice were given dietary concentrations 0, 30, 300, 3000 or 7000 ppm of halosulfuron-methyl for 78 weeks. During Weeks 27 and 53/54, at least 10 mice of each sex from each group were killed to assess the chronic toxicity of halosulfuron-methyl. The remaining animals were killed after 78 or 79 weeks of treatment to assess oncogenic potential.

Results

Results are summarised in Table 30 and described below:

Table 30: Summary table of 78-week mouse toxicity/carcinogenicity study findings

	Males					Females				
Dietary level (ppm)	0	30	300	3000	7000	0	30	300	3000	7000
Number allocated per group/sex	75	75	75	75	75	75	75	75	75	75
Scheduled deaths 1st kill week 27 (unscheduled deaths up to week 27)	10 (0)	10 (0)	10 (0)	10 (3)	10 (0)	10 (0)	10 (0)	10 (0)	10 (0)	10 (0)
Scheduled deaths 1st kill week 53 (unscheduled deaths up to week 53)	15 (2)	15 (2)	15 (2)	14 (3)	13 (2)	15 (0)	15 (3)	15 (3)	14 (3)	14 (3)
Scheduled deaths terminal kill week 79 (unscheduled deaths up to week 79)	40 (8)	42 (6)	41 (7)	37 (8)	45 (6)	38 (12)	35 (12)	41 (6)	45 (3)	42 (7)
Total unscheduled deaths	10	8	9	14	8	12	15	9	6	10
Survival to 78 weeks (%)	80	84	82	76	87	76	70	82	88	82
Incidence of clinical signs	No effects					No effects				
Mean body weight at 13 weeks (g)	35.7	35.6	34.6*	35.0	34.3*	27.1	28.0*	28.1*	27.6	27.5
Mean body weight at 24 weeks (g)	37.1	37.1	36.2	36.7	35.8*	29.0	29.7	29.5	29.2	29.0
Mean body weight at 78 weeks (g)	38.5	38.7	38.9	38.4	38.4	32.1	32.9	32.3	32.9	31.7
Mean total food consumption Weeks 1-4 (g)	143.2	137.4*	137.0*	137.2*	138.2*	140.3	136.9	133.7*	132.3*	132.6*
Mean total food consumption Weeks 13-24 (g)	213.7	209.7	207.8*	209.6	203.6*	220.9	214.6	208.5*	210.4*	207.9*
Mean total food consumption Weeks 52-78 (g)	266.0	262.7	260.5	267.4	269.3	277.9	264.2	269.0	262.4	268.0
Haematology	No effects					No effects				
Clinical chemistry	Not conducted					Not conducted				
Urinalysis	Not conducted					Not conducted				
Absolute organ weights	No effects					No effects				
Body weight relative organ weights	No effects					No effects				
Brain weight relative organ weights	No effects					No effects				
Macroscopic findings	No effects					No effects				
Microscopic non-neoplastic findings – Epididymis microconcretion/mineralization	0/40	0/42	0/41	1/37	5/44	-	-	-	-	-
Microscopic non-neoplastic findings – Testis microconcretion/mineralization	4/40	8/42	6/41	6/37	9/44	-	-	-	-	-
Microscopic neoplastic findings	No effects					No effects				

*Significantly different from control value p≤0.05 (ANOVA); Data from treated groups were compared with data from controls of the same sex using Levene's test for homogeneity of variances. If variances of untransformed data were heterogeneous, a series of transformations was performed to achieve variance homogeneity. Analysis (ANOVA) were performed on rank-transformed data. If variances were still heterogeneous, Dunnett's test was performed for equal and unequal variances. Cumulative survival data were analysed using National Cancer Institute (USA) package.

Achieved test material intake

Overall achieved mean intakes at 30, 300, 3000 and 7000 ppm of halosulfuron-methyl were 4, 41.1, 410.0 and 971.9 mg/kg/day respectively for males and 5.2, 51.0, 509.1 and 1214.6 mg/kg/day respectively for females.

Mortality

There were no treatment-related effects on survival. The adjusted cumulative survival rate was 70% or greater in all groups.

Clinical signs

There were no clinical signs related to treatment with halosulfuron-methyl.

Body weight

At 7000 ppm, male body weight gain was significantly reduced over Weeks 0 to 13 whilst mean body weight was significantly reduced at Weeks 4, 13 and 24.

Food consumption

Food consumption was generally unaffected by treatment.

Food consumption in Weeks 1-4 was slightly but significantly lower in both male and female groups given 300 ppm or greater. As the study progressed the statistical differences from control became fewer. By weeks 52-78 there were no differences from control in either male or female groups.

Haematology

There were no treatment-related effects. Indeed, the significantly higher mean neutrophil count and lower mean lynphocyte and monocyte counts in males of highest dose group with respect to control values only at Week 26, being not present at longer times, very low in magnitude and within the normal range for animals of that age and strain, were considered not relevant and not attributable to the treatment.

Organ weights

Organ weights were unaffected.

The few group mean organ weights of treated mice that were significantly different from control values did not show any consistent treatment-related effects.

Macroscopic findings at necropsy

There were no treatment-related findings.

Microscopic findings

Increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in controls; testis: 9/44 compared with 4/40 in controls). None of the other spotted morphological abnormalities recorded were treatment-related and there was no treatment-related increase in the incidence of neoplasia.

Conclusion

During 78 weeks of treatment with 0, 30, 300, 3000 or 7000 ppm of halosulfuron-methyl to mice, there were no treatment-related mortalities or clinical signs of toxicity. In addition, the chronic treatment revealed no evidence of oncogenic potential. At 7000 ppm, male body weight gain was significantly reduced over Weeks 0 to 13 whilst mean body weight was significantly reduced at Weeks 4, 13 and 24. Furthermore, at the same dose there were increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in controls; testis: 9/44 compared with 4/40 in controls).

On the basis of these results observed in males at the highest dose, the NOEL of halosulfuron-methyl for chronic toxicity (non-neoplastic end-points) was 3000 ppm, corresponding to mean achieved daily intakes of 410.0 mg/kg/day.

The NOEL for oncogenicity was 7000 ppm, corresponding to mean achieved daily intakes of 971.9 and 1214.6 mg/kg/day of halosulfuron-methyl in males and females respectively.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No human information is available.

4.10.3 Other relevant information

No other relevant information is available.

4.10.4 Summary and discussion of carcinogenicity

Two long-term oral toxicity studies are available: a 2-year combined chronic toxicity/carcinogenicity study in the rat and a 78-week carcinogenicity study in the mouse.

The 2-year dietary rat combined chronic toxicity/carcinogenicity study was conducted at dietary concentrations up to 5000 ppm (males) and 2500 ppm (females). The critical findings were reduced mean body weight throughout the study in males receiving 5000 ppm and between Weeks 13 and 52 in females fed 2500 ppm. The NOEL for chronic toxicity was therefore 1000 ppm, based on body weight reduction seen in females, corresponding to 56.3 mg/kg/day halosulfuron-methyl.

There was no evidence of oncogenic activity in the rat at any dose level.

The 78 week study in mice was conducted at dietary concentrations up to 7000 ppm. At 7000 ppm, male body weight gain was significantly reduced over Weeks 0 to 13 whilst mean body weight was significantly reduced at Weeks 4, 13 and 24. Furthermore, at the same dose there were increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in controls; testis: 12/63 compared with 5/70 in controls).

On the basis of these results observed in male at the highest dose, the NOAEL of halosulfuron-methyl for chronic toxicity (non-neoplastic end-points) was 3000 ppm, corresponding to mean achieved daily intakes of 410.0 mg/kg/day.

There was no evidence of oncogenic activity in the mouse at any dose level.

4.10.5 Comparison with criteria

In the absence of any oncogenic activity at any dose level in rodents and in the absence of any human information, Halosulfuron-methyl did not meet the CLP criteria for classification as a carcinogen category 1 or 2.

4.10.6 Conclusions on classification and labelling

No classification as a carcinogen is required for halosulfuron-methyl, according to the CLP Regulation.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two guideline and GLP compliant long-term oral toxicity studies were available to the DS: a 2-year combined chronic toxicity/carcinogenicity study in the rat (Moore, 1992a) and a 78-week carcinogenicity study in the mouse (Moore, 1992b). Study details were summarised in Table 28 in the CLH report. The DS concluded that there was no evidence of oncogenic potential in either study.

Rat 2-year dietary toxicity/oncogenicity study

The chronic toxicity and carcinogenicity of halosulfuron-methyl was investigated in SD rats for at least 104 weeks (Moore, 1992a). Administration of halosulfuron-methyl did not affect survival amongst males (except for a spurious finding of lowered survival in the 44 mg/kg bw/day group, 33%) or females at any dose level. Any clinical abnormalities were described as incidental with no evidence of a dose response and no consistent pattern of occurrence. There were scheduled necropsies conducted on 10 animals/sex from each dose group in 3 interim sacrifices during weeks 27, 53 and 79; and on all surviving animals at study termination.

Achieved doses (mg/kg bw/day)

Males:	0	0.44	4.4	43.8	108.3	225.2
Females:	0	0.56	5.6	56.3	138.6	--

Only male rats were tested at the highest dietary concentration of 5000 ppm (calculated mean exposure intake = 225.2 mg/kg bw/day). Females were only tested up to 2500 ppm from the initial study design.

Critical effects were observed on body weight parameters. Compared to controls, a treatment related depression of body weight was evident throughout the study in the high

dose males. Compared to controls, the male high dose group mean body weight was statistically significantly lower at weeks 4, 13, 24, 52, 76 and 104 but only biologically relevant at 104 weeks (18.4% depressed). Mean body weight change in this group was also significantly depressed during weeks 0-4 and 4-13, and mean total body weight gain over the whole length of the study was biologically significant at 25% lower compared with controls.

The only microscopic findings of note were non-neoplastic in nature, and corresponded to seminal vesicle atrophy in males with increasing dose. The DS concluded that there was no evidence of substance-related oncogenic activity in the rat at any dose level.

Mouse 18-month dietary oncogenicity study

The carcinogenicity of halosulfuron-methyl was investigated in the CD-1 mouse for at least 78 weeks (Moore, 1992b). Administration of halosulfuron-methyl did not affect survival amongst males or females at any dose level (the adjusted cumulative survival rate was 70% or greater in all groups). There were no clinical signs related to treatment with halosulfuron-methyl. There were scheduled necropsies conducted on 10 animals/sex from each dose group in 2 interim sacrifices during weeks 27, and 53/54; and on all surviving animals at study termination.

Achieved doses (mg/kg bw/day)

Males:	0	4.0	41	410	972
Females:	0	5.2	51	509	1215

Some effects were observed on body weight parameters at the initial stages of the study. A treatment-related depression of body weight gain was evident over weeks 0-13 in the high dose males whilst mean body weight was significantly reduced at weeks 4, 13 and 24 as compared to controls. Food consumption was generally unaffected by treatment. There were no treatment-related effects on haematology or organ weights. There were no treatment-related macroscopic findings at necropsy. The DS summarised the key data for the 78-week mouse toxicity/carcinogenicity study in table 30 of the CLH report.

The main microscopic findings of note were observed in males at the highest dose, and were non-neoplastic in nature; these have been discussed in the STOT RE section above (increased incidences of microconcretions/mineralisation within the lumen of both epididymal and testis tubules).

The DS concluded that there was no evidence of substance related oncogenic activity in the mouse at any dose level in any tissue.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Relevance of neoplastic changes observed in the rat study.

RAC confirmed the DS assessment of the findings by checking the original histopathological reports. There was no evidence of substance-related oncogenic activity in the rat at any dose level. There were some neoplasms of particular note but these were considered typical, incidental and/or occurring at high and variable background levels in this particular animal strain (see tables below).

Table 8: Neoplasms (male rats, SD) in a 2-year feed study of halosulfuron-methyl. Dose mg/kg bw/day.

Tumour	0	0.44	4.4	43.8	108.3	225.2
Pituitary Gland:						
- adenoma	37	41	43	33	43	32
- carcinoma	0	0	0	0	0	0
- animals examined	54	55	55	55	55	54

Animals examined includes all animals on the main study from both unscheduled and scheduled terminal sacrifice.

Table 9: Neoplasms (female rats, SD) in a 2-year feed study of halosulfuron-methyl. Dose mg/kg bw/day.

Tumour	0	0.56	5.6	56.3	138.6
Pituitary Gland:					
- adenoma	41	41	42	41	42
- carcinoma	3	1	4	3	3
- animals examined	55	54	54	55	55
Mammary Gland:					
- fibroadenoma	28	26	31	28	27
- adenoma	3	0	2	3	0
- carcinoma	6	12	14	14	9
- animals examined	54	53	53	55	53

Animals examined include all animals on the main study from both unscheduled and scheduled terminal sacrifice.

Pituitary tumours are well known in this strain of rat and were evident in both males and females at all doses. There was no dose-response, the incidences were highly variable, and there was a large background level in concurrent controls. There was no evidence for a substance-related effect.

There was a small increase in the incidence of mammary carcinoma noted at all treatments in the female rat compared with those observed in the concurrent controls. A closer examination of the data from the 3 interim sacrifices did not show any effect on tumour latency (see table 10 below). A brief statistical analysis of the carcinoma incidence was not significant (chi-square, $p = 0.25$). In addition, the lack of a dose-response relationship in tumour incidence does not support any argument for a substance-related effect.

Table 10: Mammary carcinoma (female rats, SD) incidence at each interim sacrifice. Dose mg/kg bw/day.

Period/dose	0	0.56	5.6	56.3	138.6
Interim sacrifice:					
No. 1; 27 weeks	0/10	0/10	0/10	0/10	0/10
No. 2; 53 weeks	0/10	0/10	0/10	**2/10**	0/9
No. 3; 79 weeks	0/6	0/10	**1/10**	0/10	**2/10**

In summary, RAC agrees with the DS; there is no evidence of substance-related oncogenic activity in the rat at any dose level.

Neoplastic changes in mice

RAC checked the original histopathological reports: no substance related tumours were seen in any tissue at any dose reported. There was no evidence for neoplastic potential in mice by halosulfuron-methyl.

Conclusion

In the absence of any oncogenic activity at any dose level in rodents and in the absence of any human information, RAC agrees with the DS, that **halosulfuron-methyl does not meet the CLP criteria for classification as a carcinogen in either category 1 or 2**.

4.11 Toxicity for reproduction

Table 31: Summary table of relevant reproductive toxicity studies

Method	Results	Dose levels	Reference
Rat two-generation (dietary) US EPA FIFRA 83-4 GLP Purity: 98.7%	NOAEL: General toxicity:800 ppm (Males: 50.4 mg/kg/day: Females: 58.7 mg/kg/day) Reproductive toxicity:100 ppm (Males: 6.3 mg/kg/day; Females: 7.4-11.8 mg/kg/day) LOAEL: General toxicity: 3600 ppm Reproductive toxicity: marginal LOAEL of 100 ppm (corresponding to 6.3 mg/kg/day for males and 7.4-11.8 mg/kg/day for females)	0, 100, 800and 3600 ppm F0 males: 0, 6.3, 50.4, 223.2 mg/kg/day; F1 males: 0, 7.4, 61.0, 274.2 mg/kg/day; F0 females:0, 7.4, 58.7, 261.4 mg/kg/day; F1 females: 0, 8.9, 69.7, 319.9 mg/kg/day	DAR B.6.6.1 Lemen, 1991
Rat developmental toxicity (oral gavage) US EPA FIFRA 83-3 GLP Purity: 98.5%	NOEL: Maternal toxicity:250 mg/kg/day Developmental toxicity: 75 mg/kg/day LOEL: 750 mg/kg/day. Based on clinical signs, reduced maternal and fetal body weight, slight increase in early embryonic resorptions, dilated brain ventricles and reduced ossification	0, 75, 250 and 750 mg/kg/day	DAR B.6.6.2 Morseth, 1990a
Rabbit developmental toxicity (oral gavage) US EPA FIFRA 83-3 GLP Purity: 98.5%	NOEL: Maternal toxicity:50 mg/kg/day Developmental toxicity: not defined due to the increased mean early resorptions (15.3%, 10.0%, 24.4% vs 9.7% in controls) and decreased number of fetuses (21.3%, 16.0%, 19.2% less than controls) at 15, 50 and 150 mg/kg/day. LOEL: 15 mg/kg/day	0, 15, 50 and 150 mg/kg/day	DAR B.6.6.3 Morseth, 1990b

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In a 2-generation reproductive toxicity study in the rat, Groups of 26 males and 26 female Sprague Dawley CD rats were given dietary concentrations of 0, 100, 800 or 3600 ppm of halosulfuron-methyl continuously throughout the two generations (F0 and F1). One litter was derived from the F0 generation and two litters from the F1 generation.

Results

Results are summarised in Table 32 and described below:

Table 32: Mean bodyweight (g) of offspring in the 2-generation reproduction study

Stage	Sex	Dosage ppm	Day 0	Day 14	Day 21	F1 gen Week 0	F1 gen Week 14
F0-F1	Males	0	6.57	33.35	55.04	96.0	594.3
		100	6.51	31.97	52.16	93.8	614.4
		800	6.55	29.57 *	48.29 **	88.3	595.5
		3600	6.43	28.05 **	45.69 **	82.7**	551.8**
	Females	0	6.20	32.45	52.64	85.1	328.5
		100	6.14	30.46	49.64	83.6	344.3
		800	6.28	28.92 *	46.76 *	82.1	326.7
		3600	6.05	27.03 **	43.96 **	74.5**	303.0
F1-F2a	Males	0	6.84	34.97	57.13		
		100	6.86	33.15	54.55		
		800	6.64	34.64	56.86		
		3600	6.40**	32.71	52.91		
	Females	0	6.48	33.94	55.38		
		100	6.48	33.11	51.96		
		800	6.29	33.54	54.88		
		3600	6.09*	31.18	50.55 *		
F1-F2b	Males	0	6.86	34.65	58.15		
		100	6.81	35.66	60.50		
		800	6.87	36.26	60.98		
		3600	6.41**	33.78	55.52		
	Females	0	6.50	35.36	58.80		
		100	6.44	34.12	57.22		
		800	6.56	34.25	56.66		
		3600	6.10*	33.15	54.01		

* P≤0.05 ** P≤0.01

At 3600 ppm of halosulfuron-methyl, parental and pup body weights were significantly reduced. At 800 ppm, although minimal and transient reductions in body weight were seen, the overall body weight gain was not affected in either generation. In F0 generation, an unusually low pregnancy rate (65%) and females with litters 17 out of 26 paired was noted.

The NOAEL for general toxicity was 800 ppm, corresponding to mean achieved intakes of 50.4 mg/kg/day of halosulfuron-methyl for males and 58.7 mg/kg/day for females of the F0 generation. For the F1 generation, corresponding intakes were 61.0 mg/kg/day of halosulfuron-methyl in males and 69.7 mg/kg/day in females.

The NOAEL for reproductive toxicity was not defined due to reduced pup viability indices for F0 females at 100, 800 and 3600 ppm (2.7%, 5.1% 8.1% less than controls respectively). A marginal LOAEL of 100 ppm (corresponding to 6.3 mg/kg/day for males and 7.4-11.8 mg/kg/day for females) was defined.

The NOAEL for fertility was not defined due to: reduced number of females with litters in F1 females (first littering) at 100, 800 and 3600 ppm (22.8%, 31.8%, 13.2% less than controls respectively); reduced pregnancy rate in F1 females (first littering) at 100, 800 and 3600 ppm (23.6%, 27.1%, 9.5% less than controls respectively; reduced number of females with litters in F1 females (second littering) at 100, 800 and 3600 ppm (13.7%, 18.2%, 22.8% less than controls respectively); reduced pregnancy rate in F1 females (second littering) at 100, 800 and 3600 ppm (4.4%, 8.7%, 4.4% less than controls respectively). Even if these last data did not show a clear dose-response curve they are considered of biological significance. A marginal LOAEL of 100 ppm (corresponding to 6.3 mg/kg/day for males and 7.4-11.8 mg/kg/day for females) was defined.

4.11.1.2 Human information

No human information is available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Two studies are available for developmental toxicity, one investigating effects with the rat and the other with the rabbit. Both studies were conducted using oral administration.

Rat

Groups of 25 time-mated female rats were given a daily oral dose by gavage, of 0, 75, 250 or 750 mg/kg/day halosulfuron-methyl at a dose volume of 3 ml/kg of body weight from Days 6 to 15 of gestation. The control group received the vehicle alone, 0.1% Tween® 80 and 0.5% carboxymethylcellulose in distilled water. The rats were killed on Day 20 of gestation.

Results

Results are summarised in Table 33 and described below:

Table 33: Summary of rat developmental toxicity study with halosulfuron-methyl

Parameter	Dose level (mg/kg/day)			
	0	**75**	**250**	**750**
Disposition of females:				
Number mated	25	25	25	25
Number pregnant	25	25	24	22
Number with no viable foetuses at Day 20	0	0	0	2
Clinical signs[a]:				
Alopecia	0	1	1	8
Urine stains	0	0	0	5
Body weight (g)				
Day 0	251.0	254.6	252.5	246.1
Day 6	281.2	284.6	281.6	276.9
Day 8	286.2	290.3	286.7	278.5
Day 12	305.2	308.6	307.2	289.3*
Day 16	330.5	333.8	331.0	309.8*
Day 20	383.5	385.3	383.7	357.3*
Body weight change (g)				
Day 0 to 6	30.20	30.00	29.13	30.75
Days 6 to 8	5.04	5.72	5.08	1.63
Days 8 to 12	18.96	18.28	20.54	10.75*
Days 12 to 16	25.28	25.20	23.75	20.58
Days 16 to 20	53.04	51.48	52.75	47.42
Days 6 to 16	49.28	49.20	49.38	32.96*
Days 6 to 20	102.32	100.68	102.13	80.38*
Days 0 to 20	132.52	130.68	131.25	111.13*
Food consumption (g/animal/day)				
Day 0 to 6	21.19	21.68	21.56	22.31
Days 6 to 8	21.94	22.98	21.63	19.13*
Days 8 to 12	22.49	23.54	22.22	18.26*
Days 12 to 16	23.53	24.25	23.70	22.59
Days 16 to 20	24.28	25.19	25.10	25.98
Days 6 to 16	22.80	23.71	22.68	20.42*
Days 6 to 20	23.22	24.08	23.34	22.02
Days 0 to 20	22.62	23.24	22.82	22.11
Gravid uterine weight and terminal body weight (g)				
Absolute gravid uterus weight	73.7	73.0	74.3	60.7
Maternal terminal body weight adjusted for gravid uterine weight	309.9	312.3	309.4	296.6*
Adult necropsy	No treatment-related effects			

* $p \leq 0.05$

[a] Total number affected

Table 33: Summary of rat developmental toxicity study with halosulfuron-methyl (continued)

Parameter	Dose level (mg/kg/day)			
	0	**75**	**250**	**750**
Total number of litters **Total number of foetuses**	25 335	25 335	24 326	22 292
Foetal weight (g) Males Females	 3.4 3.2	 3.4 3.2	 3.4 3.2	 2.6* 2.5*
External malformations: Number of foetuses affected Number of litters affected and	 0 0	 0 0	 0 0	 4*(1.4) 3 (14)
Visceral examinations: Number of foetuses examined **Variations:** <u>Dilatation of lateral ventricles</u>: Number of foetuses affected Number of litters affected <u>Total foetal soft tissue variations</u> Number of foetuses affected Number of litters affected	 165 0 0 4 (2.4) 3 (13)	 165 0 0 8 (4.7) 5 (20)	 163 2 (1.2) 2 (8.3) 9 (5.5) 7 (2.9)	 146 16*(11) 5* (23) 22 *(15) 10 *(45)
Skeletal examinations: Number of foetuses examined **Malformations** Vertebral anomaly with/without rib anomaly: Number of foetuses affected Number of litters affected <u>Filamentous tail:</u> Number of foetuses affected Number of litters affected <u>Rudimentary tail:</u> Number of foetuses affected Number of litters affected <u>Forked/fused ribs:</u> Number of foetuses affected Number of litters affected <u>Total foetal skeletal malformations</u> Number of foetuses affected Number of litters affected	 170 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0)	 165 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0)	 163 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0)	 146 1 (0.7) 1 (4.5) 2 (1.4) 2 (9.1) 1 (0.7) 1 (4.5) 2 (1.4) 2 (9.1) 6*(4.1) 4*(18)

* $p \leq 0.05$

Percent incidence in parenthesis

Table 33: Summary of rat developmental toxicity study with halosulfuron-methyl (continued)

Parameter	Dose level (mg/kg/day)			
	0	75	250	750
Skeletal variations				
Unossified hyoid body:				
Number of foetuses affected	22 (13)	13 (7.9)	16 (9.8)	56*(38)
Number of litters affected	11 (44)	12 (48)	8 (33)	18*(82)
Incomplete ossification of skull:				
Number of foetuses affected	4 (2.4)	7 (4.2)	8 (4.9)	83*(57)
Number of litters affected	3 (12)	6 (24)	4 (17)	21*(95)
Bipartite vertebral centrum (A):				
Number of foetuses affected	3 (1.3)	2 (1.2)	1 (0.6)	26*(18)
Number of litters affected	3 (12)	2 (8.0)	1 (4.2)	13*(59)
Unossified vertebral centrum (A):				
Number of foetuses affected	1 (0.6)	0 (0)	3 (1.8)	77*(53)
Number of litters affected	1 (4.0)	0 (0)	3 (13)	19*(86)
Hemicentrum (A):				
Number of foetuses affected	0 (0)	0 (0)	0 (0)	7*(4.8)
Number of litters affected	0 (0)	0 (0)	0 (0)	5*(23)
Incomplete ossification of vertebral arch/es:				
Number of foetuses affected				
Number of litters affected	20 (12)	12 (7.3)	31 (19)	103*(71)
Less than 4 caudal vertebrae ossified:	9 (36)	10 (40)	11 (46)	21*(95)
Number of foetuses affected				
Number of litters affected	34 (20)	35 (21)	56*(34)	109*(75)
5th sternebra unossified:	13 (52)	18 (72)	16 (67)	20*(91)
Number of foetuses affected				
Number of litters affected	51 (30)	71*(43)	59 (36)	136*(93)
6th sternebra unossified:	17 (68)	19 (76)	19 (79)	22*(100)
Number of foetuses affected				
Number of litters affected	12 (7.1)	5 (3.0)	11 (6.7)	103*(71)
Other sternebrae unossified:	9 (36)	5 (20)	7 (29)	20*(91)
Number of foetuses affected				
Number of litters affected	2 (1.2)	1 (0.6)	4 (2.5)	31*(21)
Other sternebra/e, incomplete ossification:	2 (8.0)	1 (4.0)	3 (13)	14*(64)
Number of foetuses affected				
Number of litters affected				
14th rudimentary rib/s:	1 (0.6)	3 (1.8)	2 (1.2)	26*(18)
Number of foetuses affected	1 (4.0)	3 (12)	2 (8.3)	13*(59)
Number of litters affected				
7th cervical rib/s:	1 (0.6)	3 (1.8)	1 (0.6)	23*(16)
Number of foetuses affected	1 (4.0)	2 (8.0)	1 (4.2)	8*(36)
Number of litters affected				
Incomplete ossification of rib/s:	1 (0.6)	1 (0.6)	2 (1.2)	12*(8.2)
Number of foetuses affected	1 (4.0)	1 (4.0)	2 (8.3)	7*(32)
Number of litters affected				
	0(0)	0(0)	0(0)	5*(3.4)
	0(0)	0(0)	0(0)	3 (14)

* $p < 0.05$

Percent incidence in parenthesis

Table 33: Summary of rat developmental toxicity study with halosulfuron-methyl (continued)

Parameter	Dose level (mg/kg/day)			
	0	75	250	750
Skeletal variations continued				
Less than three metacarpals ossified:				
Number of foetuses affected	0 (0)	0 (0)	0 (0)	6*(4.1)
Number of litters affected	0 (0)	0 (0)	0 (0)	4*(18)
Less than four metacarpals ossified:				
Number of foetuses affected	0 (0)	1 (0.6)	4 (2.5)	30*(21)
Number of litters affected	0 (0)	1 (4.0)	3 (13	12*(55)
Unossified pubis/es:				
Number of foetuses affected	1 (0.6)	1 (0.6)	2 (1.2)	11*(7.5)
Number of litters affected	1 (4.0)	1 (4.0)	2 (8.3)	8*(36)
Incomplete ossification of ischium (A):				
Number of foetuses affected	0 (0)	0 (0)	0 (0)	9*(6.2)
Number of litters affected	0 (0)	0 (0)	0 (0)	7*(32)
Total foetal skeletal variations				
Number of foetuses affected	105 (62)	115 (70)	114 (70)	146*(100)
Number of litters affected	23(92)	25 (100)	23 (96)	22 (100)

* $p \leq 0.05$

Percent incidence in parenthesis

At 750 mg/kg/day, maternal toxicity was characterised by alopecia, yellow stained fur, reduced body weight and bodyweight gain. Fetal toxicity was indicated by low fetal weight, dilated ventricles of the brain and reduced ossification of the bones. In addition, the overall incidence of abnormalities (3.1% of fetuses) was higher than in controls (0.6%).

The NOEL for maternal and developmental toxicity was 250 mg/kg/day.

The NOEL for developmental toxicity was 75 mg/kg/day due to increased number of fetuses and litters with soft tissue variations and less than 4 caudal vertebrae ossified at 250 and 750 mg/kg/day.

Rabbit

Groups of 17 mated New Zealand White female rabbits were given a daily oral dose, by gavage, of either 0, 15, 50 or 150 mg/kg/day of halosulfuron-methyl on gestation Days 7 to 19 at a dose volume of 3 ml/kg body weight. Controls received the vehicle alone, 0.1% Tween® 80 and 0.5%w/v carboxymethylcellulose in distilled water. They were killed on Day 29 of gestation.

Results

Results are summarised in Table 34 and described below:

Table 34: Summary of rabbit developmental toxicity study with halosulfuron-methyl

Parameter	Dose level (mg/kg/day)			
	0	**15**	**50**	**150**
Disposition:				
Females mated	17	17	17	17
Number pregnant	14	14	11	15
Number with viable foetuses at Day 29	13	10	11	13
Pregnancy rate (%)	82	82	65	88
Accidental death	1	1	0	0
Number aborted	0	2	0	2
Mean body weight	No treatment-related effects			
Mean body weight change (g):				
Day 0 to 7	166.85	144.64	242.82*	169.15
Days 7 to 9	18.77	25.73	24.82	-5.31
Days 9 to 11	40.38	40.36	42.36	7.38
Days 11 to 15	69.85	86.27	46.55	11.08
Days 15 to 20	127.77	120.91	131.45	55.85
Days 20 to 24	70.08	111.00	69.45	181.85*
Days 24 to 29	23.08	-100.39	41.18	139.15
Overall change				
Days 7 to 20 (treatment period)	256.77	273.27	245.18	69.00
Days 20 to 29 (post-treatment period)	93.15	10.61	110.64	321.00*
Food consumption (g/animal):				
Days 7 to 20	2369.92	2428.55	2455.18	1975.69
Days 0 to 29	4929.36	4717.99	5098.82	4475.55
Gravid uterine weight and terminal body weight:	No treatment-related effects			
Adult macroscopic necropsy findings:	No treatment-related effects			

*p ≤0.05

Table 34: Summary of rabbit developmental toxicity study with halosulfuron-methyl (continued)

Parameter	Dose level (mg/kg/day)			
	0	**15**	**50**	**150**
Litter data:				
Mean live foetuses per litter (%)	7.2 (88.3)	6.6 (76.4)	7.2 (89.2)	5.8 (69.4)
Mean early resorptions (%)	0.8 (9.7)	0.9 (15.3)	0.6 (10.0)	2.0 (24.4)
Mean late resorptions (%)	0.2 (2.0)	0.5 (7.0)	0.1(0.8)	0.6 (5.5)
Mean total resorption (%)	1.0 (11.7)	1.5 (22.3)	0.7 (10.8)	2.6 (29.9)
Number of litters	13	10	11	13
Number of foetuses	94	74	79	76
Number of dead foetuses	0	1	0	1
Foetal weight:	No treatment-related effects			
Foetal skeletal and visceral examinations:	No treatment-related effects			

One female at 15 mg/kg/day and two females at 150 mg/kg/day were killed on Days 23-25 due to abortion. In addition, one control and one female at 15 mg/kg/day died due to a dosing error. There were no clinical signs of toxicity during the dosing period.

At 150 mg/kg/day, maternal toxicity was indicated by reduced body weight gain and embryofetal toxicity was indicated by a higher incidence early embryonic deaths. There was no evidence of teratogenicity.

The NOEL for maternal toxicity was 50 mg/kg/day.

The NOEL for developmental toxicity was not defined due to the increased mean early resorptions (15.3%, 10.0%, 24.4% vs 9.7% in controls) and decreased number of fetuses (21.3%, 16.0%, 19.2% less than controls) at 15, 50 and 150 mg/kg/day. A marginal LOEL of 15 mg/kg/day was defined.

4.11.2.2 Human information

No data are available.

4.11.3 Other relevant information

No other relevant information is available.

4.11.4 Summary and discussion of reproductive toxicity

According to the EU peer review (EFSA Journal 2012;10(12):2987), reproductive and developmental studies showed a higher sensitivity of the offspring to halosulfuron-methyl exposure than the adult animals. The offspring‘s NOAEL in the multigeneration reproduction toxicity study was 6.3 mg/kg bw per day based on reduced pup body weight gain, while the parental NOAEL was 50.4 mg/kg bw per day regarding the same endpoint. In this study no effect on fertility or reproduction was observed up to the highest dose level of 223.2 mg/kg bw per day. In the developmental toxicity study in rabbits, the maternal and developmental NOAELs were 50 mg/kg bw per day based on early resorptions, decreased number of fetuses and reduced maternal body weight gain. In the rat, fetal toxicity was observed in the absence of maternal toxicity: the developmental NOAEL was 75 mg/kg bw per day based on a higher incidence of visceral and skeletal variations and the maternal NOAEL was 250 mg/kg bw per

day due to reduced body weight, body weight gain and food consumption. These effects suggest that classification regarding developmental toxicity would be required for halosulfuron-methyl as 'Reprotox cat. 2, H361fd, suspected of damaging the unborn child'

With regard to the conclusion that offspring are more sensitive to halosulfuron-methyl exposure than adult animals, the Applicant has provided the following responses:

Applicant comments on Reproduction study: bodyweight of offspring and NOAEL:

The Applicant considers that there is no consistent evidence in this 2-generation study overall for a higher sensitivity of the offspring to halosulfuron-methyl exposure than the adult animals.

Reproduction study: bodyweight of offspring and NOAEL

The Peer Review Report (page 373) indicates that: "The offspring NOAEL is 100 ppm (6.3 mg/kg bw per day), based on decreased pup bodyweight gain at 800 ppm in F1, F2a, F2b generation."

However, this is not supported by the study data.

As indicated in Table 32, some effect on bodyweight was seen in all 3 littering stages in the highest dosage group (3600 ppm), although the pattern differed between F1 and F2 litters. In the intermediate group (800 ppm), however, there was an apparent effect on the bodyweight gain of F1 male and female offspring only and during lactation only.

Slightly low bodyweights of the selected F1 generation at Week 0 (usually about 4 weeks of age) were not statistically significant, their bodyweight gains thereafter showed no effect of treatment and their bodyweights at Week 14, before pairing, were clearly similar to Control values.
Bodyweights and bodyweights gain during lactation in the F2a and F2b litters were similar to Control values throughout, showing no effect of treatment.

There was therefore no consistent evidence of effect on offspring bodyweight or bodyweight gain in the group receiving 800 ppm, and the effect seen in F1 litters during lactation must be considered equivocal.

Contemporary historical control data from the laboratory conducting the study show the pup body weights from halosulfuron-methyl treated animals are within the historical control range for the strain and conditions specific to the laboratory (Table 35).

In such clear absence of any confirmatory effect on bodyweight in F2a and F2b litters at 800 ppm, it is not considered necessary or appropriate to designate 800 ppm as an adverse effect level for offspring.

It is therefore considered that the NOAEL for offspring is 800 ppm, equivalent to 50.4 mg/kg bw/day on the basis of F0 parental mean intake.

Table 35: Two-generation rat study historical control data pup weights. Studies started January 1987 – December 1989 – Hazleton HWA 2096-163 (T32)

Study Ref	Mean pup weights (g)												
	Litter bred	Day 0 M	Day 0 F	Day 4 pre M	Day 4 Pre F	Day 4 post M	Day 4 post F	Day 7 M	Day 7 F	Day 14 M	Day 14 F	Day 21 M	Day 21 F
1[$]	F1	6.26	5.98	8.24	7.83	8.30	7.81	12.39	11.29	23.79	21.39	38.36	35.85
2[$]	F1	6.16	5.91	9.57	9.09	9.69	9.11	14.86	13.90	26.53	24.81	37.18	34.62
3[$$]	F1	6.53	6.45	10.31	10.04	10.53	10.15	17.46	16.25	33.52	31.64	53.35	50.41
3A[$$]	F2a	6.56	6.42	8.20	7.96	8.23	8.11	12.49	12.06	27.87	27.60	43.61	43.42
3B[$$]	F2b	6.64	6.30	8.50	7.86	8.62	8.03	12.28	11.37	27.35	25.53	45.24	43.05
4[$$]	F1	6.35	6.07	9.41	8.94	9.41	8.92	14.97	14.38	30.12	29.18	47.61	46.39
4A[$$]	F2	6.55	6.27	9.81	9.65	9.85	9.62	15.50	14.96	29.98	28.92	48.69	46.71
5[$$]	F1	6.75	6.40	9.56	9.08	9.51	9.09	15.38	14.62	31.47	30.40	51.08	49.39
5A[$$]	F2	6.47	6.06	8.73	8.21	8.78	8.21	13.61	12.78	28.63	27.18	44.83	43.25
6[$$]	F1	6.49	6.11	8.85	8.27	8.84	8.33	13.86	13.24	28.78	27.92	44.51	42.98
6A[$$]	F2	6.01	5.73	8.08	7.77	8.06	7.78	12.39	11.88	26.61	26.61	41.41	40.93
7[$]	F1	6.14	5.76	9.61	8.99	9.55	9.06	14.92	14.44	30.00	29.19	47.09	45.53
N		12	12	12	12	12	12	12	12	12	12	12	12
Mean		6.409	6.122	9.073	8.641	9.114	8.685	14.176	13.431	28.721	27.531	45.247	43.544
S.D.		0.22	0.25	0.73	0.76	0.76	0.75	1.62	1.58	2.56	2.74	4.78	4.76
Min.		6.01	5.73	8.08	7.77	8.06	7.78	12.28	11.29	23.79	21.39	37.18	34.62
Max		6.75	6.45	10.31	10.04	10.53	10.15	17.46	16.25	33.52	31.64	53.35	50.41

Study types: [$]Pilot reproduction [$$]2-generation study

End of Applicant comments on Reproduction study: bodyweight of offspring and NOAEL.

Applicant comments on Developmental toxicity:

Proposed classification

There was no effect on fertility, so H361**f**d should not be applied. As discussed below, the Applicant considers that halosulfuron-methyl should not be classified as H361d.

Toxicokinetics of halosulfuron-methyl

Halosulfuron-methyl has pKa of 3.44 and acidic characteristics. In the rat stomach it would be un-ionised (low pH medium) and readily absorbable (blood concentrations peaking by 0.5 hours; >80% of administered dose absorbed at both 5 and 250 mg/kg (Report T41). In the blood, however, halosulfuron-methyl and the principal metabolites would be highly ionised and diffusion across cell membranes would be impeded. This is in accord with the autoradiography study with pregnant rats dosed at 5 mg/kg where trans-placental transfer of radioactivity into foetal tissue was not evident (Report T42). This supports the view that the effect on fetuses at 750 mg/kg bw/day was secondary to effect on maternal physiology at a level of manifest maternal toxicity.

Maternal vs foetal toxicity at 750 mg/kg bw/day

Mean maternal bodyweight gain at 750 mg/kg bw/day), corrected by subtraction of gravid uterus weight, was reduced by 14.4% compared with Controls, and was therefore in the range of toxicologically adverse effect for animals supporting foetal development through gestation. Clinical signs in this group (alopecia and urine stains) indicate that they were stressed.

In another study, in female rats receiving 3000 or 10000 ppm in the diet in the 28-day toxicity study (mean achieved dosage 241 and 888 mg/kg bw/day respectively), bodyweight gain was reduced to 82 or 65% respectively of controls, with some necrosis of pancreatic acinar cells. This lends support to the view that 750 mg/kg bw/day by gavage in the developmental toxicity study would be in the range of adverse toxic effect, with secondary effect on fetuses.

Table 36: Analysis of foetal bodyweights

Dosage (mg/kg bw/day)	Foetal bodyweights (g)			
	Group Mean (Mean of litter means ±SD)	Range of litter means	Range of individual foetal weights	Range in which most fetuses lie
0	3.3 ±0.3	2.6 – 3.9	1.9 – 4.3	3 - 4
75	3.3 ±0.2	2.9 – 3.9	1.6 – 4.1	3 - 4
250	3.3 ±0.3	2.7 – 3.9	1.8 – 4.2	3 - 4
750	2.6 ±0.3	2.1 – 3.1	1.6 – 3.4	2 - 3

SD: standard deviation

The effect on foetal bodyweight at 750 mg/kg bw/day was notably uniform, again suggesting an effect on the dams and consequent effect on litter development, rather than direct effect on

individual fetuses. As indicated in Table 36 most fetuses in this group were in a range about 1g lighter than in other groups, with the majority of visceral and skeletal findings being associated with this relative immaturity.

Foetal findings at 250 mg/kg/day

In the intermediate dosage group (250 mg/kg bw/day), the stated increased incidence of visceral and skeletal variants was attributed to:

- A Dilatation of lateral brain ventricles in 2 fetuses
- Slightly increased incidence of renal pelvic cavitation.
- Increased incidence of fetuses with less than four caudal vertebrae ossified

These findings, which typically associate with smaller or otherwise immature fetuses, are further discussed below.

Dilatation of lateral ventricles

It is evident from Table 37 that the two fetuses with this finding in the intermediate group were much smaller than the group mean, being at the bottom end of the range of control foetal weights (see Table 36, and it is considered likely that the slight dilatation of one or both lateral brain ventricles in these fetuses was associated with their immaturity.

The percentage foetal incidence in this intermediate group (1.2%) was below the overall background incidence (2.573% in 229 studies; max 87.84%) reported for "Cerebral Ventricle, Enlargement" in this strain of rat in the1992-1994 period (MARTA and MTA 1996). It is thus evident that this is a relatively frequent control finding in this strain, and therefore should not be considered to represent a noteworthy adverse change in the intermediate group.

Table 37: Dilatation of lateral ventricles in Group 3: 250 mg/kg bw/day

Group/ Dosage (mg/kg bw/day)	Female	Fetus number	Fetus weight (g)	Finding: Dilatation of lateral ventricles	Fetal incidence %
Gp 3: 250	B81638	6	2.1	Slight, both	2/163: 1.2
	B81650	4	2.4	Slight, right	
Background control data					
Report T27: Addendum I, dated May 1991					Mean: 0.7
Data from 12 studies (see table 38)					Range: 0.0-4.7
Incidence of cerebral ventricle enlargement (MARTA and MTA, 1996)					Mean: 2.573
in this strain of rat in the period 1992-1994. Data from 229 studies					Max: 87.84

Table 38: Historical Control data – rat fetal dilated lateral ventricles of the brain (variation) from teratology studies started Jan 1986 – June 1989 Hazleton HWA 2096-150 (T27)

Dilated lateral ventricals	Coded Study No												High	Low	Mean
	1	2	3	4	5	6	7	8	9	10	11	12			
No Fetuses examined	98	144	158	171	107	130	163	174	92	172	163	173	174	92	145.4
No with observation	0	1	0	0	5	1	0	3	0	1	0	0	11	0	0.9
Percent with observation	0.0	0.7	0.0	0.0	4.7	0.8	0.0	1.7	0.0	0.6	0.0	0.0	4.7	0.0	0.7
No litters examined	23	21	24	24	22	18	23	25	30	23	21	23	30	18	23.1
No with observation	0	1	0	0	5	1	0	3	0	1	0	0	5	0	0.9
Percent with observation	0.0	4.8	0.0	0.0	23.0	5.6	0.0	12.0	0.0	4.3	0.0	0.0	23.0	0.0	4.1

Slightly increased incidence of renal pelvic cavitation.

Slightly increased incidence of renal pelvic cavitation - as indicated in Table 39, occasionally severe but usually moderate and single-sided renal pelvic cavitation was recorded in all groups, with incidence slightly higher than Control in the intermediate dosage group (250 mg/kg bw/day) and slightly higher again at 750 mg/kg/day. In the intermediate group (250 mg/kg bw/day) 6 of the 7 affected fetuses were less than 3.0 g although remaining well within the range of individual foetal weights in the Control group (see Table 32). It is therefore considered unlikely that the small increase in incidence of this finding in the group receiving 250 mg/kg bw/day indicates a noteworthy adverse response to treatment.

The percentage foetal incidence in this intermediate group (4.3%) compares with the overall background incidence and range (1.174% in 229 studies; max 19.66%) reported for "Renal Pelvis, Dilated" in this strain of rat in the1992-1994 period (MARTA and MTA 1996). It is thus evident that this is a relatively frequent control finding in this strain, and the incidence in the intermediate group should not be considered to represent a noteworthy adverse change.

Table 39: Incidence of renal pelvic cavitation

Group/ Dosage (mg/kg bw/day)	Female	Fetus number	Fetus weight (g)	Increased renal pelvic cavitation,	Fetal incidence %
Gp 1: 0	B81581	15	3.0	Moderate, both	2.4
	B81584	4	2.7	Moderate, left	
	B81587	8	3.2	Moderate, both	
		13	2.8	Severe, right; moderate, left	
Gp 2: 75	B81617	4	3.3	Severe, left	2.4
		8	3.1	Moderate, right	
	B81620	11	3.0	Moderate, right	
	B81628	15	3.0	Moderate, both	
Gp 3: 250	B81633	9	3.3	Moderate right	4.3
	B81634	1	2.9	Moderate, right	
		13	2.8	Moderate, right	
	B81635	14	2.7	Moderate, right	
	B81636	1	2.5	Moderate, right	
		3	2.9	Moderate, right	
	B81652	1	2.7	Severe, right	
Gp 4: 750	B81665	14	2.7	Moderate right	6.2
	B81666	11	2.8	Moderate, right	
	B81668	6	2.7	Moderate, right	
		10	2.6	Moderate, right	
		14	2.3	Severe, right; moderate, left	
	B81672	8	2.8	Moderate, left	
		15	2.8	Moderate, both	
	B81673	13	3.1	Moderate, right	
	B81675	4	2.5	Moderate, right	

Background control data	
Report T27: Addendum I, dated May 1991	Mean: 3.2
Data from 12 studies (see table 40)	Range: 0.0-8.2
Incidence of cerebral ventricle enlargement (MARTA and MTA, 1996)	Mean: 1.174
in this strain of rat in the period 1992-1994. Data from 229 studies	Max: 19.66

Table 40: Historical Control data – rat fetal renal pelvic cavitation (variation) from teratology studies started Jan 1986 – June 1989 Hazleton HWA 2096-150 (T27)

Renal pelvic cavitation	Coded Study No												High	Low	Mean
	1	2	3	4	5	6	7	8	9	10	11	12			
No Fetuses examined	98	144	158	171	107	130	163	174	92	172	163	173	174	92	145.4
No with observation	7	8	13	7	4	1	2	8	0	4	2	0	13	0	4.7
Percent with observation	7.1	5.6	8.2	4.1	3.7	0.8	1.2	4.6	0.0	2.3	1.2	0.0	8.2	0.0	3.2
No litters examined	23	21	24	24	22	18	23	25	30	23	21	23	30	18	23.1
No with observation	5	4	10	4	4	1	2	7	0	3	1	0	10	0	3.4
Percent with observation	22.0	19.0	41.7	16.7	18.0	5.6	8.7	28.0	0	13.0	4.8	0.0	41.7	12.23	14.8

Increased incidence of fetuses with less than four caudal vertebrae ossified

The rate of ossification shows normal variation between fetuses and between ossification centres, with some delay generally being associated with smaller weight and / or relative immaturity.

In the present study, Table 41 shows that on average fetuses showing less than four caudal vertebrae ossified were about 10% lighter than the group mean foetal weight in Groups 1, 2 and 3 (0, 75 and 250 mg/kg bw/day), although some affected fetuses in all 3 groups were at the upper end of the weight range (see Table 36 for comparison with group mean and range).

In most cases, 3 caudal vertebrae were ossified. The range of findings across all groups and weight ranges suggests normal variation within and between fetuses in the rate of ossification.
In the intermediate group in particular (250 mg/kg bw/day), affected fetuses were in a weight range very similar to that in the Control group.

It is considered therefore that the higher incidence in the group receiving 250 mg/kg bw/day does not indicate noteworthy adverse effect on the rate of ossification.

Table 41: Less than four caudal vertebrae ossified

Group/ Dosage (mg/kg bw/day)	Fetuses showing less than four caudal vertebrae ossified			
	Number of fetuses	Mean foetal bodyweight	Range of individual foetal weights	Fetal incidence %
Gp 1: 0	34	3.01	2.4-3.5	20
Gp 2: 75	35	3.09	2.6[a]-3.6	21
Gp 3: 250	56	2.98	2.2[b]-3.6	34
Gp 4: 750	109	2.45	1.6-3.2	75
Background control data				
Historical control data provided by the laboratory for the period 1994-				Mean: 35.55
1998 (earliest now available). Data from 13 studies				Range: 1.1-64

[a]: single outlier (1.6g); [b]: single outlier (1.8g)

The available toxicokinetic, toxicological and developmental information supports the view that at 750 mg/kg bw/day by gavage, halosulfuron-methyl had adverse effects on pregnant rats with consequent secondary effects on fetuses, mainly seen as small foetal weight and immaturity and associated visceral and skeletal findings. Findings suggest that this is likely to be a high-dose effect only, at a dosage (750 mg/kg bw/day) considerably higher than achieved in the 90-day, 2-year and multigeneration studies.

It is therefore considered that classification regarding developmental toxicity (as Reprotox cat. 2, H361d, suspected of damaging the unborn child') is not required.

The RMS and other experts expressed concern that increased incidences of visceral and skeletal variants occurred in the intermediate group (250 mg/kg bw/day) in the absence of maternal toxicity, and consequently considered this to be an effect level.

However, as has been detailed above, the relatively small number and slight or small increases in variants seen in this group were mostly associated with low foetal weight (below the group mean but remaining within the concurrent control range) and were generally attributable to slight immaturity. Historical control data is available for 2 of the 3 principal findings and shows that their incidence in the halosulfuron-methyl study at 250 mg/kg bw/day was well within the range of background control values. It is considered therefore that the type, range and degree of findings were not sufficient for this to be considered an adverse effect level.

It is therefore considered that 250 mg/kg bw/day is the appropriate NOAEL for foetal development as well as for maternal toxicity.

End of Applicant comments on Developmental toxicity.

4.11.5 Comparison with criteria

Reproductive function and fertility

In the rat 2-generation study there were no adverse effects on fertility, reproductive performance, pup survival or pup viability at doses up to 3600 ppm. Minor changes in reproduction and fertility parameters in treated groups were not dose-related, generally within background historical control data range for the laboratory and did not represent an adverse effect of treatment. In tables 42, 43, 44, 47 and 48, values outside the historical control range are in bold.

Table 42: Summary of pregnancy and litter data from rat 2-gen study halosulfuron-methyl – F_0 females

Parameter	Dose level (ppm)			
	0	**100**	**800**	**3600**
Number of females paired	26	26	26	26
Number of females with litters	17	21	24	24
Pregnancy rate (%)	**65**	81	92	92
Male and female fertility rates - males and females (%)	65	91	96	96
Mean duration of gestation (days)	22.0	22.0	21.8	22.2
Live birth indices (%)	99	100	98	98
Pup viability indices (%)	99	97	94	91
Weaning indices (%)	98	93	94	95
Mean number of offspring born/litter	13.59	14.05	13.67	14.33
Number of male offspring (%)				
– Day 0 (%)	54	51	52	55
– Day 4 pre-cull	55	52	50	55
– Day 21	50	53	51	53

Table 43: Summary of pregnancy and litter data from rat 2-gen study halosulfuron-methyl l– F_1 females (first littering)

Parameter	Dose level (ppm)			
	0	**100**	**800**	**3600**
Number of females paired	26	26	26	26
Number of females with litters	22	17	15	19
Pregnancy rate (%)	85	65	62	77
Male and female fertility rates - males and females (%)	85	74	80	83
Mean duration of gestation (days)	22.0	22.1	21.9	22.1
Live birth indices (%)	94	100	98	**93**
Pup viability indices (%)	90	99	95	97
Weaning indices (%)	92	93	97	98
Mean number of offspring born/litter	13.00	13.24	13.27	13.37
Number of male offspring (%)				
– Day 0 (%)	**41**	48	53	56
– Day 4 pre-cull	**42**	49	53	56
– Day 21	46	50	52	51

Table 44: Summary of pregnancy and litter data from rat 2-gen study halosulfuron-methyl F_1 females (second littering)

Parameter	Dose level (ppm)			
	0	**100**	**800**	**3600**
Number of females paired	25	26	25	25
Number of females with litters	22	19	18	17
Pregnancy rate (%)	92	88	84	88
Male and female fertility rates (%):				
- males	96	95	87	95
- females	92	95	95	91
Mean duration of gestation (days)	22.0	22.0	22.1	22.2
Live birth indices (%)	97	96	97	96
Pup viability indices (%)	93	93	91	91
Weaning indices (%)	89	90	94	97
Mean number of offspring born/litter	13.09	12.47	12.83	**11.65**
Number of male offspring (%)				
– Day 0 (%)	47	48	44	52
– Day 4 pre-cull	46	49	45	51
– Day 21	48	50	45	50

Table 45: Two-generation rat study historical control litter data. Studies started January 1987 – December 1989 – Hazleton HWA 2096-163 (T32)

Study Reference	Litter bred	Pregnant %	Day 0 livebirth index %	Day 4 Viability index %	Day 21 Weaning index %	Mean No. Live pups Day 0	% Males Day 0	% Males Day 21
1[$]	F1	100	99	99	98	14.00	50	47
2[$]	F1	93	100	98	98	13.56	51	48
3[$$]	F1	88	99	98	99	13.78	58	52
3A[$$]	F2a	86	97	93	87	13.30	43	46
3B[$$]	F2b	82	99	92	90	13.57	47	50
4[$$]	F1	100	98	98	99	15.00	46	47
4A[$$]	F2	92	99	95	97	14.29	51	50
5[$$]	F1	77	98	92	92	12.85	43	45
5A[$$]	F2	92	98	93	87	12.65	56	53
6[$$]	F1	85	96	97	93	12.25	47	49
6A[$$]	F2	85	100	91	95	13.65	53	56
7[$]	F1	80	98	99	100	11.75	53	50
N		12	12	12	12	12	12	12
Mean		88.3	98.4	95.4	94.6	13.388	49.8	49.4
S.D.		7.3	1.16	3.06	4.70	0.90	4.78	3.15
Min.		77	96	91	87	11.75	43	45
Max		100	100	99	100	15.00	58	56

Therefore it is concluded that halosulfuron-methyl does not fulfil the criteria for classification as H361f Category 2 suspected of damaging fertility

Development

In the rat 2-generation toxicity study it is considered that the NOAEL for offspring and adults is 800 ppm, equivalent to 50.4 mg/kg bw/day on the basis of F0 parental mean intake.
Some effect on bodyweight was seen in all 3 littering stages in the highest dosage group (3600 ppm), although the pattern differed between F1 and F2 litters. In the intermediate group (800 ppm), however, there was an apparent effect on the bodyweight gain of F1 male and female offspring only and during lactation only.

Table 46: Summary of data from rat 2-gen study halosulfuron-methyl – F_0 generation

Sex	Male				Female			
Dose level (ppm)	**0**	**100**	**800**	**3600**	**0**	**100**	**800**	**3600**
F_0 generation								
Mortality	0/26	0/26	1/26	0/26	0/26	0/26	0/26	0/26
Clinical signs	No treatment-related effects							
Body weight (g)								
Week 3	380.2	385.4	382.6	371.3	243.5	237.0	242.4	230.5**
Week 8	503.1	505.8	508.6	489.0	291.4	286.9	288.4	271.0**
Week 16	587.3	580.0	588.8	559.3*	335.0	353.4	354.7	322.0
Week 20	627.3	614.3	626.8	597.0*	-	-	-	-
Week 25	646.0	635.3	647.6	614.7*	-	-	-	-
Maternal body weight (g)								
Gestation								
Day 0	-	-	-	-	318.2	318.8	323.5	293.4**
Day 7	-	-	-	-	352.8	350.9	353.8	321.7**
Day 14	-	-	-	-	378.5	378.3	381.2	349.3**
Day 20	-	-	-	-	446.2	447.1	448.3	421.8*
Lactation								
Day 0	-	-	-	-	353.1	352.4	359.0	322.0**
Day 7	-	-	-	-	352.2	353.7	352.4	326.7**
Day 14	-	-	-	-	368.4	366.1	369.0	341.4**
Day 21	-	-	-	-	363.6	367.5	369.5	346.9
Rest phase								
Week 3	-	-	-	-	350.6	342.0	343.0	317.1**
Week 7	-	-	-	-	363.9	353.4	355.3	326.4**
Reproduction parameters	No treatment-related effects							
Litter size, pup live birth and viability, sex ratio	No treatment-related effects							
Pup body weight - covariate adjusted (g)								
Day 7	17.22	16.01	15.37*	14.98**	16.39	14.99	14.77	14.07**
Day 14	33.35	31.97	29.57	28.05**	32.45	30.46	28.92*	27.03**
Day 21	55.04	52.16	48.29**	45.69**	52.64	49.64	46.76*	43.96**
Adult and offspring necropsy and histopathology	No treatment-related effects							

* $p < 0.05$ ** $p < 0.01$

Slightly low bodyweights of the selected F1 generation at Week 0 (usually about 4 weeks of age) were not statistically significant, their bodyweight gains thereafter showed no effect of treatment and their bodyweights at Week 14, before pairing, were clearly similar to Control values.
Bodyweights and bodyweight gain during lactation in the F2a and F2b litters were similar to Control values throughout, showing no effect of treatment.

Table 47: Summary of data from rat 2-gen study halosulfuron-methyl – F_1 generation (first littering)

Sex	**Male**				**Female**			
Dose level (ppm)	**0**	**100**	**800**	**3600**	**0**	**100**	**800**	**3600**
F_1 generation (first littering)								
Mortality	0/26	0/26	0/25	0/26	1/26	0/26	1/26	1/26
Clinical signs	No treatment-related effects							
Body weight (g)								
Week 0	96.0	93.8	88.3	82.7**	85.1	83.6	82.1	74.5**
Week 8	491.0	491.1	477.0	442.2**	286.0	296.1	280.1	263.7**
Week 16	615.9	633.8	614.2	574.8*	337.8	356.4	335.0	313.1*
Week 25	678.0	698.1	692.8	628.6*	-	-	-	-
Maternal body weight (g)								
Gestation								
Day 0	-	-	-	-	338.8	360.8	339.9	306.5**
Day 7	-	-	-	-	372.2	385.2	368.9	332.2**
Day 14	-	-	-	-	399.8	415.6	394.7	357.9**
Day 20					470.0	484.6	466.1	429.1**
Lactation								
Day 0	-	-	-	-	383.1	400.3	385.7	338.8**
Day 7	-	-	-	-	377.9	388.5	370.1	336.1**
Day 14	-	-	-	-	387.2	395.6	376.9	352.4**
Day 21	-	-	-	-	375.5	376.6	358.2	341.6**
Reproduction parameters (first littering)	No treatment-related effects							
Litter size, pup live birth and viability, sex ratio	No treatment-related effects							
Pup body weight - covariate adjusted (g)								
Day 0	**6.84**	**6.86**	6.64	6.40**	**6.48**	**6.48**	6.29	6.09*
Day 21	**57.13**	**54.55**	56.86	52.91	**55.38**	**51.96**	54.88	50.55*
Offspring gross pathology	No treatment-related effects							

* $p < 0.05$ ** $p < 0.01$

Table 48: Summary of data from rat 2-gen study halosulfuron-methyl – F_1 generation (second littering)

Sex	**Male**				**Female**			
Dose level (ppm)	**0**	**100**	**800**	**3600**	**0**	**100**	**800**	**3600**
F_1 generation (second littering)								
Mortality	0/26	0/26	1/25	0/26	0/26	1/26	1/25	1/25
Clinical signs	No treatment-related effects							
Body weight - males (g)								
Week 26	682.5	702.8	692.3	631.8*				
Week 31	701.7	719.0	706.2	653.6*				
Week 36	743.8	750.6	738.1	684.0*				
Maternal body weight (g)								
Premating								
Week 3	-	-	-	-	360.7	380.8	355.0	327.3**
Week 5	-	-	-	-	376.5	396.2	374.4	340.4**
Week 7	-	-		-	388.5	410.5	394.9	342.7*
Week 10	-	-	-	-	485.5	463.8	406.3*	336.0**
Gestation								
Day 0	-	-	-	-	373.4	387.1	371.2	338.5*
Day 7		-	-	-	406.5	410.6	401.2	359.9**
Day 14	-	-	-	-	429.2	437.0	430.3	377.4**
Day 20	-	-	-	-	501.4	501.5	504.8	430.1**
Lactation		-	-	-				
Day 0	-	-	-	-	430.1	444.6	421.8	372.4**
Day 7	-	-	-	-	421.1	428.7	413.4	357.9**
Day 14	-	-	-	-	422.8	431.7	420.1	371.9**
Day 21	-	-		-	409.4	411.5	396.3	365.0**
Rest phase			-					
Week 3	-	-	-	-	386.2	410.8	389.6	346.6**
Week 5	-	-		-	400.4	424.1	403.5	355.7**
Reproduction parameters	No treatment-related effects							
Litter size, pup live birth and viability, sex ratio	No treatment-related effects							
Pup body weight - covariate adjusted (g)								
Day 0	**6.86**	**6.81**	**6.87**	6.41**	**6.50**	6.44	**6.56**	6.10*
Day 21	**58.15**	**60.50**	**60.98**	**55.52**	**58.80**	**57.22**	**56.66**	**54.01**
Adult and offspring necropsy and histopathology	No treatment-related effects							

* $p < 0.05$ ** $p < 0.01$

There was therefore no consistent evidence of effect on offspring bodyweight or bodyweight gain in the group receiving 800 ppm, and the effect seen in F1 litters during lactation must be considered equivocal.

Contemporary historical control absolute pup weight data from the laboratory conducting the study show the covariate adjusted pup body weights from halosulfuron-methyl treated animals are not lower than the historical control range for the strain and conditions specific to the laboratory (see table 49).

In such clear absence of any confirmatory effect on bodyweight in F2A and F2B litters at 800 ppm, it is not considered necessary or appropriate to designate 800 ppm as an adverse effect level for offspring.

It is therefore considered that the NOAEL for offspring is 800 ppm, equivalent to 50.4 mg/kg bw/day on the basis of F0 parental mean intake. No consistent evidence of effects on rat fetal bodyweights was seen in the absence of maternal toxicity in the 2-generation study.

The available toxicokinetic, toxicological and developmental information supports the view that at 750 mg/kg bw/day by gavage, halosulfuron-methyl had adverse effects on pregnant rats in the developmental study with consequent secondary effects on fetuses, mainly seen as small foetal weight and immaturity and associated visceral and skeletal findings. Findings suggest that this is likely to be a high-dose effect only, at a dosage (750 mg/kg bw/day) considerably higher than achieved in the 90-day, 2-year and multigeneration studies.

Table 49: Two-generation rat study historical control data pup weights. Studies started January 1987 – December 1989 – Hazleton HWA 2096-163 (T32)

Study Ref	Mean pup weights (g)												
	Litter bred	Day 0 M	Day 0 F	Day 4 pre M	Day 4 Pre F	Day 4 post M	Day 4 post F	Day 7 M	Day 7 F	Day 14 M	Day 14 F	Day 21 M	Day 21 F
1[$]	F1	6.26	5.98	8.24	7.83	8.30	7.81	12.39	11.29	23.79	21.39	38.36	35.85
2[$]	F1	6.16	5.91	9.57	9.09	9.69	9.11	14.86	13.90	26.53	24.81	37.18	34.62
3[$$]	F1	6.53	6.45	10.31	10.04	10.53	10.15	17.46	16.25	33.52	31.64	53.35	50.41
3A[$$]	F2A	6.56	6.42	8.20	7.96	8.23	8.11	12.49	12.06	27.87	27.60	43.61	43.42
3B[$$]	F2B	6.64	6.30	8.50	7.86	8.62	8.03	12.28	11.37	27.35	25.53	45.24	43.05
4[$$]	F1	6.35	6.07	9.41	8.94	9.41	8.92	14.97	14.38	30.12	29.18	47.61	46.39
4A[$$]	F2	6.55	6.27	9.81	9.65	9.85	9.62	15.50	14.96	29.98	28.92	48.69	46.71
5[$$]	F1	6.75	6.40	9.56	9.08	9.51	9.09	15.38	14.62	31.47	30.40	51.08	49.39
5A[$$]	F2	6.47	6.06	8.73	8.21	8.78	8.21	13.61	12.78	28.63	27.18	44.83	43.25
6[$$]	F1	6.49	6.11	8.85	8.27	8.84	8.33	13.86	13.24	28.78	27.92	44.51	42.98
6A[$$]	F2	6.01	5.73	8.08	7.77	8.06	7.78	12.39	11.88	26.61	26.61	41.41	40.93
7[$]	F1	6.14	5.76	9.61	8.99	9.55	9.06	14.92	14.44	30.00	29.19	47.09	45.53
N		12	12	12	12	12	12	12	12	12	12	12	12
Mean		6.409	6.122	9.073	8.641	9.114	8.685	14.176	13.431	28.721	27.531	45.247	43.544
S.D.		0.22	0.25	0.73	0.76	0.76	0.75	1.62	1.58	2.56	2.74	4.78	4.76
Min.		6.01	5.73	8.08	7.77	8.06	7.78	12.28	11.29	23.79	21.39	37.18	34.62
Max		6.75	6.45	10.31	10.04	10.53	10.15	17.46	16.25	33.52	31.64	53.35	50.41

Study types: [$]Pilot reproduction [$$]2-generation study

As has been detailed above, the relatively small number and slight or small increases in variants seen in this group were mostly associated with low foetal weight (below the group mean but remaining within the concurrent control range) and were generally attributable to slight immaturity. Historical control data is available for 2 of the 3 principal findings and shows that their incidence in the halosulfuron-methyl study at 250 mg/kg bw/day was well within the range of background control values. It is considered therefore that the type, range and degree of findings were not sufficient for this to be considered an adverse effect.

It is therefore considered that 250 mg/kg bw/day is the appropriate NOAEL for foetal development in rats as well as for maternal toxicity and there is no consistent evidence for developmental toxicity.

In the developmental toxicity study in rabbits, the maternal and developmental NOAELs were 50 mg/kg/day based on early resorptions, decreased number of foetuses and reduced maternal body weight gain. Fetal effects were considered to be secondary to maternal toxicity.

Therefore, it is concluded that halosulfuron-methyl does not fulfil the criteria for classification as H361d Category 2 suspected of damaging the unborn child

Lactation
There was no evidence of an effect on lactation in the 2-generation rat study. Therefore it is concluded that halosulfuron-methyl does not fulfil the criteria for classification as H362 May cause harm to breast-fed children.

Halosulfuron-methyl did not meet the CLP criteria classification for fertility toxicity, developmental toxicity or toxicity via lactation.

4.11.6 Conclusions on classification and labelling

Halosulfuron-methyl is not considered a reproduction or a developmental toxicant.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The adverse effects of halosulfuron-methyl on sexual function and fertility and on development were assessed in a rat two-generation reproduction toxicity study (Lemen, 1991) and developmental toxicity studies in the rat (Morseth, 1990a) and the rabbit (Morseth, 1990b). All studies were guideline (US EPA FIFRA 83-4 and FIFRA 83-3) and GLP compliant.

Each study is considered individually in terms of the observed effects in the section below.

Rat two-generation (dietary) study

General

In a 2-generation reproductive toxicity study in the rat (Lemen, 1991), groups of 26 male and 26 female Sprague Dawley CD rats were given dietary concentrations of 0, 100, 800 or 3600

ppm of halosulfuron-methyl continuously throughout the two generations (F0 and F1). One litter was derived from the adult F0 generation and two litters from the adult F1 generation.

Table 11: Dose levels (mg/kg bw/day)

Gen/dose (ppm)	0	100	800	3600
Males F0	0	6.3	50.4	223.2
Females F0	0	7.4	58.7	261.4
Males F1	0	7.4	61.0	274.2
Females F1	0	8.9	69.7	319.9

Maternal/Parental toxicity

Maternal toxicity was slight and mainly limited to effects on body weight parameters as seen in other rat studies with halosulfuron-methyl. The parental NOAEL is 800 ppm (50.4 mg/kg bw/day) based on decreased body weight/body weight gain at the highest dose. There were no treatment-related clinical signs prior to mating for all adults or for females during gestation and lactation. There were no treatment-related mortalities of the parental F0 and F1 generations. Adult necropsy and histopathology did not show treatment-related effects.

Adverse effects on sexual function/fertility

Presentation and discussion of effects on fertility/sexual function by the DS was minimal.

There were no adverse effects on any fertility parameters in the F0 parental animals. There were no treatment-related effects on pre-coital interval or gestation length. In the F0 generation, a low pregnancy rate (65%) was observed in the control group because only 17 out of the 26 females had litters. The pregnancy rate did not follow any dose response or treatment related effect and rose to 92% in the highest dose group.

Within the F1 adult female generation, there were variable and inconsistent effects observed for the number of females with litters at 100, 800 and 3600 ppm (first littering F2a: 22.8%, 31.8%, 13.2% and second littering F2b: 13.7%, 18.2%, 22.8% less than controls, respectively). Similarly, variable effects on pregnancy rate were also observed in F1 females (first littering; 23.6%, 27.1%, 9.5% and second littering: 4.4%, 8.7%, 4.4% less than controls, respectively). The DS did not consider the effects on pregnancy rates and numbers of dams with litters substance related. Confusingly, the DS considered these effects biologically significant and cited these effects for not determining a NOAEL for fertility.

Under the summary and discussion on reproductive toxicity, the DS concluded there was no effect on fertility up to the highest dose level of 223.2 mg/kg bw/day. Under the comparison with the criteria, the DS concluded that minor changes in fertility parameters in treated groups were not dose-related, generally within background historical control data range for the laboratory and did not represent an adverse effect of treatment.

Offspring developmental toxicity

Pup live birth indices

Pup live birth index was unaffected in both generations and all litters.

Pup viability indices

The DS defined a marginal LOAEL of 100 ppm (corresponding to 6.3 mg/kg bw/day for males and 7.4-11.8 mg/kg bw/day for females, the lowest tested dose) due to reduced pup viability indices for F1 litters from F0 females at 100, 800 and 3600 ppm (2.7%, 5.1% 8.1% less than controls, respectively). However, in the summary tables and under the comparison with the criteria the DS concluded that there were no treatment-related effects on pup viability at doses up to the highest tested dose.

Weaning indices

In the litters from F0 females, viability at weaning showed an insignificant decrease and without any clear dose-response (98%, 93%, 94%, 95% at 0, 100, 800 and 3600 ppm, respectively). These findings were not any more evident in the F2a and F2b litters where the weaning indices were similar or greater than the controls. The DS concluded that there was no substance-related effect.

Mean number of offspring born/litter

F1 litters from F0 parental animals showed no effect with respect to litter size at birth (table 42, CLH report). The mean numbers of offspring born per litter were 13.6, 14.1, 13.7 and 14.3 for the 0, 7.4, 58.7 and 261.4 mg/kg bw/day dose groups, respectively. Litter size at birth in the F2 generation, exhibited some variability in its incidence but overall the conclusion was in support of no substance-related adverse effects. In the F2a generation from the first mating, mean values do not differ from controls (13.0, 13.2, 13.3, 13.4). In the F2b generation from the second mating there was a small reduction in litter size at birth for the top dose vs. control group (11.7 vs. 13.1, respectively). The mean litter sizes at the two lower doses were not significantly different from the controls and did not follow a dose response. The HCD showed a range from 11.75 to 15.00. The DS concluded that there were no treatment-related effects.

Clinical signs

There were no treatment-related clinical signs in the pups. The sex ratio was unaffected by treatment. Necropsy and histopathology did not show treatment-related effects.

Pup weights

There was no biologically significant reduction in the body weight of new born pups (i.e. day 0) from any generation at any dose.

In the F1 male and female offspring, the body weight gain was decreased during lactation (days 7-21) from the mid dose and greater (≥ 800 ppm, 50.4 mg/kg bw/day). The effect on body weight gain during lactation was not consistent across generations. According to the data in the table 47 of the CLH report, the body weight was lower when compared to controls only for the high dose F2a females on lactation day 21. However, the DS concluded that the body weights and body weight gain in the F2a and F2b generations were similar to control values throughout lactation, showing no significant effect of treatment. HCD on absolute pup weight (1987 – 1989) from the laboratory conducting the study (table 35, CLH report), showed the covariate adjusted pup body weights from halosulfuron-methyl treated animals were not outside the HCD range for the strain and conditions specific to the testing laboratory.

The DS considered the effect seen in F1 litters during lactation to be equivocal as there was no consistent evidence of effect on offspring body weight or body weight gain in other offspring at 800 ppm (data not shown in table 47 of CLH report). Furthermore, the DS did not consider it necessary or appropriate to designate 800 ppm as an adverse effect level for offspring, but

rather that the NOAEL for offspring was 800 ppm (50.4 mg/kg bw/day), equivalent to the NOAEL set for parental toxicity due to decreases in body weight gain.

The DS concluded there were no data to indicate a higher sensitivity of offspring to halosulfuron-methyl in contrast to the conclusion stated by EFSA in its review report (EFSA, 2012). According to the EFSA peer review (2012), the critical effect was the lower pup weight at doses which were not maternally toxic, they had therefore set the offspring NOAEL at 100 ppm (6.3 mg/kg bw/day).

The DS considered that there were no dose-related or substance-related adverse effects on development.

Rat developmental (gavage) study

Groups of 25 time-mated female rats were given a daily oral dose by gavage of 0, 75, 250 or 750 mg/kg bw/day halosulfuron-methyl at a dose volume of 3 mL/kg bw from days 6 to 15 of gestation. The control group received the vehicle alone. The rats were sacrificed on day 20 of gestation. The results of the study were summarised in table 33 of the CLH report.

Gavage doses (mg/kg bw/day)

Group	Control (1)	2	3	4
Dams	0	75	250	750

Maternal toxicity

Compared to controls, uncorrected adult dam body weights were slightly reduced at all time points during gestation at the highest dose only. The reductions were no greater than 7% relative to controls (calculated from table 33 of the CLH report).

Overall body weight gain for gestation days 0–20 was similar in all groups except the high dose group. The uncorrected gestational weight gains (g) were 133, 131, 131 and 111* [117.5 g] (approximately -1.5%, -1.5% and -17% [-11%] relative to controls) for groups 1–4, respectively. Mean corrected body weight gains (g) were 59, 58, 57 and 50 [52] (approximately -1.7%, -3.4% and -15% [-12.3%] relative to controls).

Note: the values enclosed within square brackets are the adjusted means recalculated by RAC for the high dose group when data from two litters with no viable foetuses (due to complete early resorptions) at scheduled termination are excluded. See Additional Key Elements and Supplemental information - In depth analyses by RAC.

A significant reduction in uncorrected gestational bw gain was also evident over the dosing period on gestation days 6–16 for the high dose animals only (-33% relative to controls). There was no effect at the other dose levels. At 750 mg/kg bw/day, significantly lower food consumption was noted over gestation days 6 to 8 (-13%), 8 to 12 (-19%) and also over the entire treatment period (gestation days 6-16; -10.5%) compared to control values.

The only clinical signs of toxicity were confined to the high dose group of 750 mg/kg bw/day and evidenced by yellow stained fur (5/25 animals) and alopecia (8/25 animals). There was no mortality as a consequence of dosing. There were no macroscopic findings at necropsy.

At 750 mg/kg bw/day, mean gravid uterine weights (-17.6% [-10.6%]) and corrected maternal terminal body weights (-4.3% [-3.6%]) were lower than controls. At the other dose levels there was no difference relative to controls for either parameter.

A more quantitative description of maternal toxicity is provided in the *supplemental information section* of this opinion document.

The maternal NOAEL was set at 250 mg/kg bw/day based on decreased body weight/body weight gain and clinical signs (yellow stained fur and alopecia) at 750 mg/kg bw/day.

Adverse effects on sexual function and fertility

The DS did not present or discuss any data related to sexual function and fertility. Parameters related to fertility and development from the plant protection DAR were summarised by RAC and included in the *supplemental information section* of this opinion document.

Historical control data

An addendum to the original study report was included in the annexes of the original halosulfuron-methyl dossier. A summary of the laparohysterectomy data from twelve oral studies (initiation dates from 1986–1989) in the same strain of rat was reported and is the source of the HCD data presented in this rat developmental study. There was no data to indicate if the HCD was from the same contract research laboratory or a general compilation of studies for the same strain of rat as used in the present study by Morseth, 1990a.

Offspring developmental toxicity

The CLH report provides clear data summarised in table 33. There were significantly reduced foetal body weights at the highest dose which also lie outside of HCD. Both males and females were affected to similar extent; mean male body weights were reduced from 3.4 ± 0.3 g in controls to 2.6 ± 0.3 g (-24%) and mean female body weights were reduced from 3.2 ± 0.4 g to 2.5 ± 0.3 g (-22%). Lower dose groups were identical or similar to controls. The HCD for foetal weights are: males: 3.10–3.88 g, mean 3.55 g; females: 2.96–3.73 g, mean 3.37 g.

The DS did not discuss or present the data on increased post implantation loss and increased early resorptions at the highest dose.

There was an increase in external, skeletal and visceral malformations and variations at the top dose level of 750 mg/kg bw/day. Foetal growth retardation findings were strongly supported by other developmental effects; these included an increased number of foetuses and litters with soft tissue variations (e.g. dilated lateral brain ventricles and renal pelvis cavitation), and extensive and widespread skeletal variations at the highest dose. RAC noted there was evidence of foetal malformations at the highest dose with several rare visceral and external observations as confirmed by comparison to HCD.

In EFSA's peer review conclusion (EFSA, 2012), concern was expressed on the increased incidences of visceral and skeletal variants which occurred in the intermediate group (250 mg/kg bw/day). These variants included:

- dilated lateral brain ventricles: foetal/litter incidence of 2/2 vs. 0 in controls;
- renal pelvis cavitation: foetal/litter incidence of 7/5 vs. 4/3 in controls;
- less than 4 caudal vertebrae ossified: significant foetal/litter incidence of 56/16 relative to controls with an incidence of 34/13.

Consequently, 250 mg/kg bw/day was considered to be the effect level and 75 mg/kg bw/day was proposed as the developmental NOAEL (EFSA, 2012). The DS considered that the relatively small number and slight or small increase in variants seen in this group (250 mg/kg bw/day) were associated with low foetal weight (below the group mean but remaining within the concurrent control range) and could be attributable to slight immaturity. The DS reported

some statistics from a compendium of HCD compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA) and the Midwest Teratology Association (MTA) from developmental studies conducted between 1992 and 1994 in the same rat strain (1996). This was used to provide foetal incidence data for a number of findings at the intermediate dose:

1. Dilatation of lateral brain ventricles: 2/163 (1.2%)
   [MARTA and MTA 1996 = mean 2.6% in 229 studies; max 87.8%]
2. Renal pelvic cavitation: 7/163 (4.3%)
   [MARTA and MTA 1996 = mean 1.2% in 229 studies; max 19.7%]
3. Foetuses showing less than four caudal vertebrae ossified: 56/163 (34%)
   [Lab HCD 1994-1998 = mean 35.6% in 13 studies; range 1.1-64%]

The DS further considered that these effects were frequent control findings in this strain, as supported by the fact that their incidence in the halosulfuron-methyl study at 250 mg/kg bw/day were well within the range of historical control values. Thus, the incidences observed should not be considered to represent a noteworthy adverse change in the absence of maternal toxicity. The DS therefore considered that 250 mg/kg bw/day was the most appropriate NOAEL for foetal development (as well as for maternal toxicity).

In the summary and discussion of reproductive toxicity (section 4.11.4, CLH report), the DS included applicant comments in support of no classification. The DS supported no classification because effects (mainly the small foetal weight and immaturity and associated visceral and skeletal findings according to the DS) seen in the high dose group occurred together with maternal toxicity at that dose.

Rabbit developmental (oral gavage) study

Groups of 17 mated New Zealand White female rabbits were given a daily oral dose, by gavage, of either 0, 15, 50 or 150 mg/kg bw/day of halosulfuron-methyl on gestation days (GD) 7 to 19. Controls received the vehicle alone. All surviving does were sacrificed on GD 29. The DS summarised the results in table 34 of the CLH report.

Gavage doses (mg/kg bw/day)

Group	Control (1)	2	3	4
Dams	0	15	50	150

Maternal toxicity

One female at 15 mg/kg bw/day and two females at 150 mg/kg bw/day were sacrificed on GD 23-25 following observations of abortion. In addition, one control and one female at 15 mg/kg/day died due to a dosing error. At termination, one female given 15 mg/kg bw/day was considered to have aborted. There were no clinical signs of toxicity during the dosing period.

Mean terminal body weights were similar in treated and control groups with no statistical significance. At 150 mg/kg bw/day, substantially lower mean body weight changes were noted over the dosing period (GD 7 to 20) compared to the controls. These mean values were highly variable at different time points and not of statistical significance during the dosing period. Mean bodyweight gain increased substantially in the high dose group after the dosing period ended. The mean uterine weight and carcass weight values and both corrected and uncorrected body weight gains did not show a dose related response.

Table 12: Summary of rabbit body weights and body weight gain during gestation

Parameter	Dose level (mg/kg/day)			
	0	**15**	**50**	**150**
Mean terminal body weight (g)	3548	3469	3624	3570
Mean body weight change (g):				
Day 0 to 7	166.85	144.64	242.82*	169.15
Days 7 to 9	18.77	25.73	24.82	-5.31
Days 9 to 11	40.38	40.36	42.36	7.38
Days 11 to 15	69.85	86.27	46.55	11.08
Days 15 to 20	127.77	120.91	131.45	55.85
Days 20 to 24	70.08	111.00	69.45	**181.85***
Days 24 to 29	23.08	-100.39	41.18	139.15
Overall change				
Days 7 to 20 (treatment period)	256.77	273.27	245.18	69.00
Days 20 to 29 (post-treatment period)	93.15	10.61	110.64	**321.00***
Food consumption (g/animal):				
Days 26-28	201.08	146.18	237.36	**303.15***
Days 7 to 20	2369.92	2428.55	2455.18	1975.69
Days 20-29	1226.92	1094.54	1306.45	1464.69
Days 0 to 29	4929.36	4717.99	5098.82	4475.55
Gravid uterine weight (g)	470.1	423.9	456.9	406.8
corrected terminal body weight (g):	3548	3468.8	3624	3570
Adult macroscopic necropsy findings:	No treatment-related effects			

* $p \leq 0.05$

Adverse effects on sexual function and fertility

The DS did not discuss the effects related to sexual function and fertility, but according to the DAR as cited in the CLH report, and to provide a clear baseline, pregnancy rate, mean numbers of *corpora lutea* and uterine implantation sites were unaffected by treatment (see table 13, with some values taken from the DAR).

Table 13: Summary of rabbit fertility and developmental parameters

Parameter / dose (mg/kg bw/day)	HCD	0	15	50	150
Number of litters		13	10	11	13
Number of foetuses		94	74	79	76
Number of dead foetuses	0 - 2	0	1	0	1
Pregnancy rate (%)	81 - 100	82	82	**65**	88
Number of *corpora lutea*	9 – 12.3	12.5	11.2	12.6	13.2
Number of implantations	7 – 10.3	8.2	8.2	7.9	8.5
Preimplantation loss (%)	9.6 – 39.2	34.4	26.8	37.3	35.6
Number of early resorptions	0.1 – 1.0	0.8	0.9	0.6	**2.0**
Number of late resorptions	0.1 – 0.6	0.2	0.5	0.1	0.6
Number of dead foetuses per litter	0 – 0.13	0	0.1	0	0.08
Post implantation loss (%)	2.4 – 23.0	12.2	18.3	8.9	**31.5**
% live foetuses per litter	77.0 – 97.6	100	99	100	99
Sex ratio (% male)	43.5 – 55.1	48.6	43.1	45.9	37.4

Historical control data

An addendum to the original study report was included in the annexes of the original halosulfuron-methyl dossier. A summary of the laparohysterectomy data from eight of the nine oral studies (initiation dates from 1987–1989) in the same strain of rabbit was reported and is the source of the HCD presented. There was no data to indicate if the HCD was from the same contract research laboratory or if they were just a general compilation of studies for the same strain of rabbit used in different research organisations.

Offspring developmental toxicity

The DS did not discuss/present developmental toxicity findings or foetal abnormalities in any detail. The DS noted increased mean early resorptions (15.3%, 10.0%, 24.4% vs. 9.7% in controls) and decreased number of foetuses (21.3%, 16.0%, 19.2% less than controls) at 15, 50 and 150 mg/kg bw/day. The mean number of foetuses per litter was 7.2, 7.4, 7.2 and 5.8 from controls to high dose, respectively. A LOEL of 15 mg/kg bw/day was defined by the DS.

However, the DS considered the increase in early litter resorption and the reduction of live litter size at the top dose as the key effect, with concurrent maternal toxicity.

RAC notes the increased post implantation loss in the high dose group was more than double that of the concurrent controls, in addition it lies outside the HCD. This indicates a substance related effect in the high dose group and is attributable to increased numbers of early resorptions. There was no further data to explain the effect.

The DS concluded that in the rabbit developmental toxicity study, the maternal and developmental NOAELs were 50 mg/kg bw/day based on early resorptions, decreased number of foetuses and reduced maternal body weight gain. Foetal effects were considered to be secondary to maternal toxicity.

Overall DS conclusion on classification and labelling

According to the DS, halosulfuron-methyl did not meet the CLP criteria for classification for adverse effects on sexual function and fertility, on development or effects on or via lactation.

Comments received during public consultation

There were four comments received: three from MSCAs and one from the applicant.

One MSCA supported the DS, citing lack of evidence and the contribution of maternal toxicity as justification for no classification for reproductive toxicity.

One MSCA questioned the use of the autoradiography data (McCarthy, 1991), as supporting evidence for the effect on foetuses being secondary to maternal toxicity at 750 mg/kg bw/day when the autoradiography was performed using a single very low dose of halosulfuron-methyl (5 mg/kg bw). They also noted that the relevance of the Historical Control Data (HCD) should be discussed. They supported further consideration of classification with Repr. 2; H361d.

One MSCA made a point about reduced pregnancy rates and numbers of dams with litters as potentially relevant from the 2-generation rat study. Reproductive indices were confirmed to be correct. The MSCA suggested that this could be a reproductive effect worth considering for classification but did not make a proposal. The MSCA took note of the significant increase in external, skeletal and visceral malformations and variations at the top dose level of 750 mg/kg

bw/day and supported Repr. 2, H361d. The MSCA also notes a higher rate of early resorptions in the rabbit study, this too supporting classification.

The Applicant also provided comments on all three reproductive studies supporting the DS's conclusion and making some comparisons with HCD. They concluded that no classification was justified for halosulfuron-methyl. They noted in the rat developmental study that the relatively small number and slight or small increases in variations were mostly associated with low foetal weight and were generally attributable to slight immaturity. Hence, they concluded that there were no teratogenic effects observed in either the rat or rabbit developmental studies.

During the RAC opinion forming process the applicant submitted a rebuttal to the proposal to consider classification for reproductive effects and presented information for 2 female rats in the high dose group of the rat developmental study. This information had not been considered in the DAR/RAR or CLH report. In brief, 2 females did not carry live foetuses to term, 1 had 17 total early resorptions and 1 had only 2 implantations followed by 2 resorptions. Taking these 2 females into account the indices for maternal body weight and the post implantation loss are slightly altered, but the RAC proposal for classification for development is not changed (see supplemental information - In depth analyses by RAC).

Applicant rebuttal to potential classification for reproductive toxicity

The applicant provided a short position paper setting out their arguments against the potential Repr. classification in "Response to ODD Halosulfuron-methyl_2017-08-08.docx". They commented on both the rat embryo-foetal toxicity study and the rabbit developmental toxicity study.

<u>Rat embryo-foetal toxicity study with halosulfuron-methyl</u>

1. Early resorptions: In the highest dosage group (750 mg/kg bw/day), two pregnant females did not carry live foetuses to term (non-viable foetus; NVF). Omitting the data from these two animals, results in the group mean value for early resorption is 1.5, which is at the upper end of the background control data (range 0.3 to 1.5).
2. Maternal toxicity and effects in the foetuses from high dose dams: The applicant supplied a table with mean maternal body weight gains during gestation and argued that reduced body weight gain was an indicator of significant maternal toxicity, which accounted for secondary effects seen in the foetuses. They made reference to two published papers where it was evident that rat maternal physiology can compensate enough to be largely (Fleeman *et al.,* 2005) or partly (Ikemi *et al., 1993*) protective of the foetus even in the presence of marked body weight loss during the treatment period.

Table 14: Mean maternal bodyweight gain during gestation

Gestation days	**Measurement**	**Dose levels (mg/kg bw/day) (% relative to concurrent controls)**				
		0	**75**	**250**	**750**	**750 (excl. NVF)**
Number of dams		25	25	24	24	22
0 - 6	Mean bw change SD	30.20 8.85	30.00 9.30	29.13 6.62	30.75 6.92	30.77 (98%) 7.24
6 - 8	Mean bw change SD	5.04 4.47	5.72 4.86	5.08 4.42	**1.63*** 7.47	**1.73* (34%)** 7.69

6 - 12	Mean bw change SD	24.00 4.45	24.00 5.63	25.63 5.35	**12.38***** 10.68	**14.64***** **(61%)** 7.76
6 - 16	mean bw change SD	49.28 8.96	49.20 9.50	49.38 7.72	**32.96***** 17.30	**37.00***** **(75%)** 10.75
16 - 20	mean bw change SD	53.04 11.82	51.48 11.93	52.75 15.08	47.42 11.33	49.73 (94%) 8.48

NVF = Dams with non-viable foetuses

The applicant considered that in the rat embryotoxicity study the NOAEL for both dams and foetuses was 250 mg/kg bw/day and that foetal findings at 750 mg/kg bw/day could be attributed to maternal toxicity and that classification for development was not required.

RAC's response: RAC has provided a table of all the individual laparohysterectomy and foetal weight data from the high dose group of the rat teratology study (table 15, below). It is clear from these data that the total resorption of all implantations in animal B81655 represents an outlier amongst the other animals and should not be included with the other litters for assessment of the 750 mg/kg bw/day dose level. Accordingly, updated calculations have been provided for several reproductive parameters. Maternal toxicity is not considered significant to explain the spectrum of developmental effects noted in the high dose group animals and their offspring. Increased early resorptions, increased post implantation loss and decreased foetal body weight along with the catalogue of foetal aberrations in the absence of significant maternal toxicity were considered when assigning the hazard category for developmental toxicity.

Table 15: Individual laparohysterectomy and foetal weight data from the high dose group of the rat teratology study.

RAT TERATOLOGY STUDY WITH NC-319
INDIVIDUAL CESAREAN SECTION AND MEAN FETAL WEIGHT DATA
DOSE LEVEL: 750 MG/KG/DAY

FEMALE#	CORPORA LUTEA	IMPLANT SITES	RESORPTIONS EARLY	RESORPTIONS LATE	RESORPTIONS TOTAL	FETUSES LIVE	FETUSES DEAD	FETUSES TOTAL	SEX MALE	SEX FEMALE	AVERAGE FETAL BODY WEIGHT (gra MALES	FEMALES	LITTER
B81655	NVF 17	17	17	0	17	0	0	0	-	-	-	-	-
B81656	16	14	1	0	1	13	0	13	6	7	2.9	2.7	2.8
B81657	16	15	2	0	2	13	0	13	6	7	2.2	2.1	2.1
B81658	14	14	5	0	5	9	0	9	6	3	2.6	2.5	2.5
B81659	16	16	2	0	2	14	0	14	8	6	2.6	2.4	2.5
B81660	NVF 2	2	2	0	2	0	0	0	-	-	-	-	-
B81661	17	14	2	0	2	12	0	12	9	3	2.3	2.0	2.2
B81662	14	14	0	0	0	14	0	14	7	7	2.5	2.3	2.4
B81663	20	17	2	0	2	15	0	15	8	7	2.2	2.1	2.1
B81664	17	17	4	0	4	13	0	13	3	10	2.4	2.1	2.2
B81665	23	18	3	0	3	15	0	15	8	7	2.7	2.8	2.8
B81666	19	17	2	0	2	15	0	15	6	9	2.7	2.7	2.7
B81667	11	9	0	0	0	9	0	9	3	6	3.1	3.1	3.1
B81668	17	14	0	0	0	14	0	14	6	8	2.6	2.4	2.5
B81669	18	17	0	0	0	17	0	17	10	7	2.8	2.5	2.7
B81670	18	15	5	0	5	10	0	10	6	4	2.8	2.6	2.7
B81671	16	15	2	0	2	13	0	13	5	8	2.3	2.2	2.3
B81672	20	16	1	0	1	15	0	15	11	4	2.8	2.8	2.8
B81673	15	15	0	0	0	15	0	15	5	10	3.1	3.1	3.1
B81674	NP												
B81675	12	12	0	0	0	12	0	12	6	6	2.4	2.3	2.3
B81676	14	14	1	0	1	13	0	13	7	6	2.5	2.4	2.4
B81677	15	14	0	0	0	14	0	14	8	6	2.2	2.2	2.2
B81678	15	15	0	0	0	15	0	15	10	5	2.9	2.8	2.8
B81679	14	13	1	0	1	12	0	12	4	8	3.0	3.0	3.0
MEAN 1	15.7	14.3	2.2	0.0	2.2	12.2	0.0	12.2	6.7	6.5	2.6	2.5	2.6
S.D.	3.9	3.3	3.5	0.0	3.5	4.2	0.0	4.2	2.1	1.9	0.3	0.3	0.3
N	24	24	24	24	24	24	24	24	22	22	22	22	22
MEAN 2	16.2	14.8	1.5	0.0	1.5	13.3	0.0	13.3	6.7	6.5	2.6	2.5	2.6
S.D.	2.8	2.0	1.6	0.0	1.6	2.0	0.0	2.0	2.1	1.9	0.3	0.3	0.3
N	22	22	22	22	22	22	22	22	22	22	22	22	22

MEAN 1 includes data from all litters sacrificed at term.
MEAN 2 excludes data from litters with no viable fetuses at the term sacrifice.

NP=NOT PREGNANT; EXCLUDED
NVF=NO VIABLE FETUSES

Rabbit developmental toxicity study

The applicant noted considerably reduced body weight gain of does in the high dose group during the first few days of dosing, a period coinciding with the expected time of early resorptions. The adverse maternal response was therefore considered by the applicant responsible for the increased early resorptions and classification for reproductive toxicity was not supported by the applicant.

RAC's response: There was more than a doubling of the post-implantation loss and early resorptions with no adverse effect on implantation sites or numbers of *corpora lutea*. There was some evidence of maternal toxicity from uncorrected gestational body weight gain being reduced relative to controls during the dosing period only. There is also evidence for skeletal malformations in the form of forked/fused ribs. Overall, RAC concludes that the maternal body weight data is equivocal in rabbits and maternal toxicity is insufficient to explain the degree of severity of the effects at the high dose. The effects observed in rabbits support classification for reproductive (development) toxicity by RAC.

RAC assessment of skeletal variations in the rat developmental study

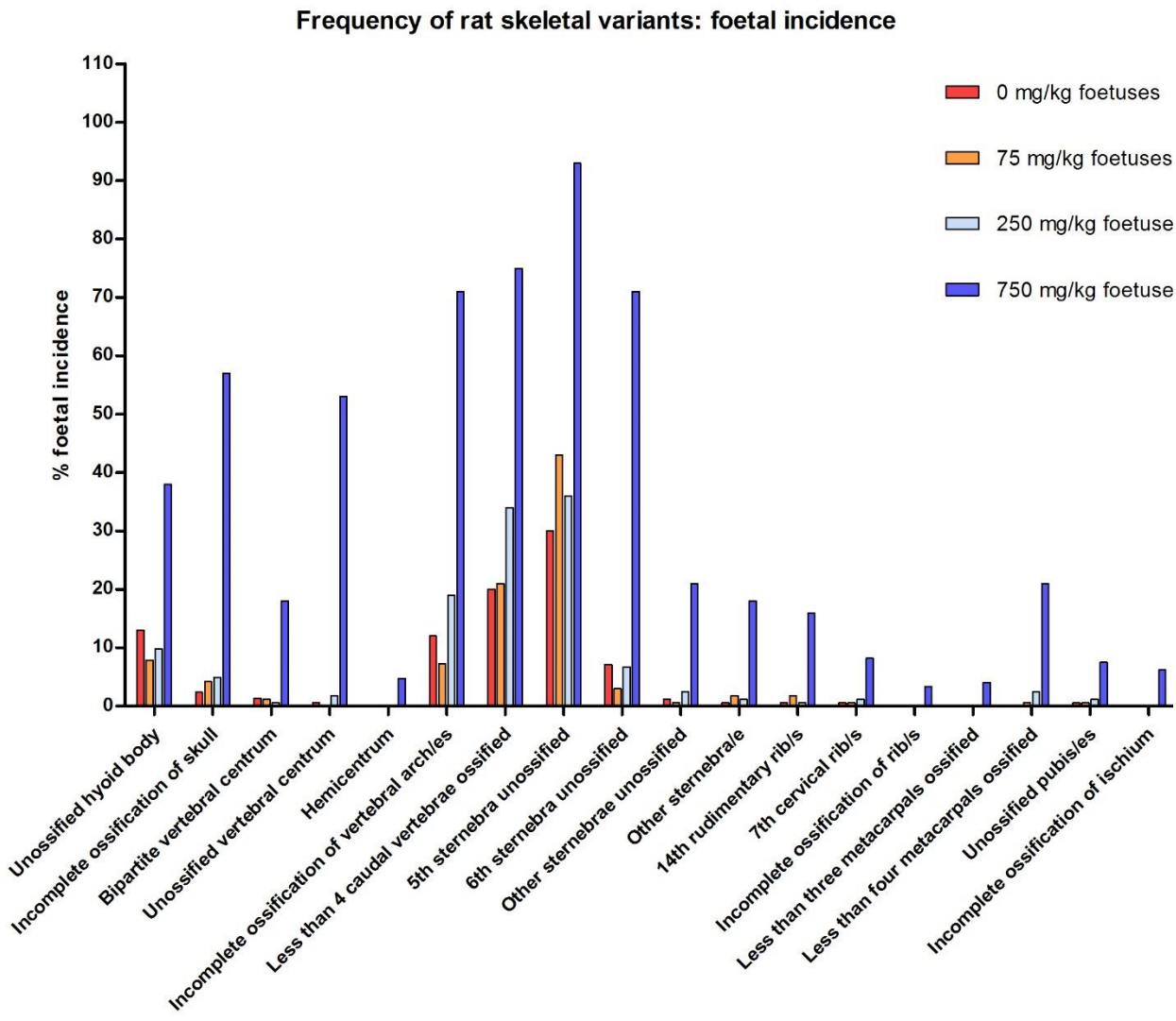
RAC noted the widespread and extensive effect on the rat skeletal system in foetuses from the high dose group as documented in table 33 of the CLH report. Reporting the overall skeletal variant incidence as [affected foetuses/litters] 105/23 – 115/25 – 114/23 – 146*/22 for controls to high dose, respectively, does not illustrate the widespread nature of the skeletal

anomalies observed at the high dose. RAC has graphed the frequency of occurrence of each anomaly with respect to dose group to illustrate more clearly the effects observed at the high dose.

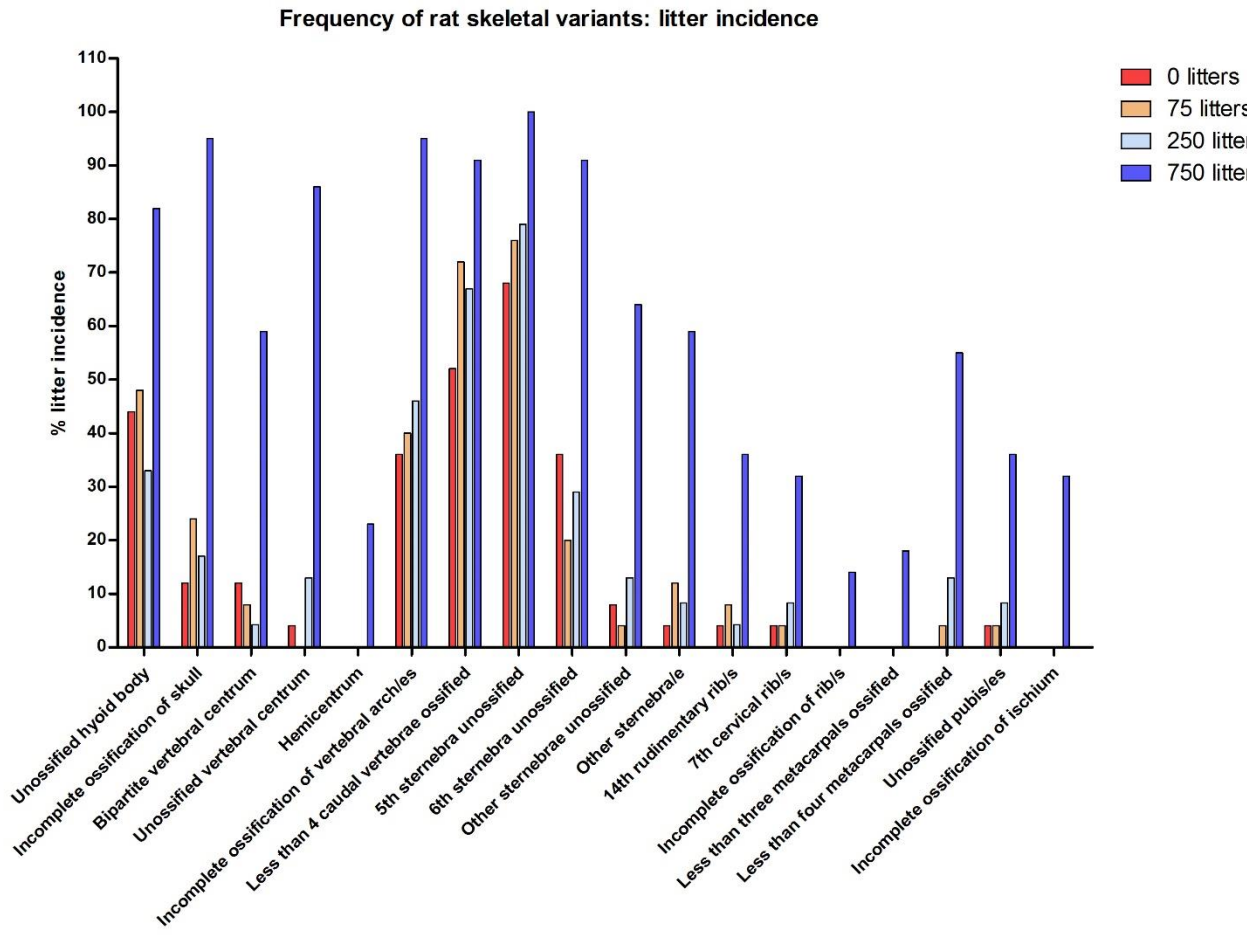
% Incidence data for skeletal variants amongst foetuses and litters:

Anomaly	**0**		**75**		**250**		**750**	
	foetuses	litters	foetuses	litters	foetuses	litters	foetuses	litters
Unossified hyoid body	13	44	7.9	48	9.8	33	38	82
Incomplete ossification of skull	2.4	12	4.2	24	4.9	17	57	95
Bipartite vertebral centrum	1.3	12	1.2	8	0.6	4.2	18	59
Unossified vertebral centrum	0.6	4	0	0	1.8	13	53	86
Hemicentrum	0	0	0	0	0	0	4.8	23
Incomplete ossification of vertebral arch/es	12	36	7.3	40	19	46	71	95
Less than 4 caudal vertebrae ossified	20	52	21	72	34	67	75	91
5th sternebra unossified	30	68	43	76	36	79	93	100
6th sternebra unossified	7.1	36	3	20	6.7	29	71	91
Other sternebrae unossified	1.2	8	0.6	4	2.5	13	21	64
Other sternebra/e, incomplete ossification	0.6	4	1.8	12	1.2	8.3	18	59
14th rudimentary rib/s	0.6	4	1.8	8	0.6	4.2	16	36
7th cervical rib/s	0.6	4	0.6	4	1.2	8.3	8.2	32
Incomplete ossification of rib/s	0	0	0	0	0	0	3.4	14
Less than three metacarpals ossified	0	0	0	0	0	0	4.1	18
Less than four metacarpals ossified	0	0	0.6	4	2.5	13	21	55
Unossified pubis/es	0.6	4	0.6	4	1.2	8.3	7.5	36
Incomplete ossification of ischium	0	0	0	0	0	0	6.2	32
Total foetal skeletal variations	62	92	70	100	70	96	100	100

Foetal incidence (% of total foetuses affected)



Litter incidence (% of total litters affected)



Assessment and comparison with the classification criteria

According to the CLP criteria, classification in Category 1A is largely based on evidence from human data, which were not present in the CLH report. Therefore, classification as Repr. 1A is not warranted.

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and shall be based on the presence of **clear** (Category **1B**) or **some** (Category **2**) evidence of an adverse effect on sexual function and fertility and/or on development. In addition, the evidence for both hazard categories shall be present in the absence of other toxic effects or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other concurrent toxic effects.

Adverse effects on development

In the rat 2-generation study there were no treatment-related adverse effects on development at doses up to 3600 ppm. The pup live birth index, litter size, pup viability (survival) and sex ratio were unaffected by treatment. There were no treatment-related clinical signs in the pups, and necropsy and histopathology did not show any treatment-related effects.

Evidence for developmental effects associated with halosulfuron-methyl were observed in both the rat (Morseth, 1990a) and rabbit (Morseth, 1990b) developmental studies:

1. Delayed development: there was a dramatic and statistically significant reduction in rat foetal body weight in both sexes:
    i. Males: 3.4 ± 0.3 vs 2.6 ± 0.3 g, controls vs. high dose (-24%)
    ii. Females: 3.2 ± 0.4 vs 2.5 ± 0.3 g, controls vs. high dose (-22%)
2. Delayed development: there was an extensive and widespread increase in rat skeletal variations:
    i. (skeletal - total variations: 105/23 – 115/25 – 114/23 – 146/22*)
3. Malformations: there was evidence for increased rat external, skeletal and visceral malformations (foetuses/litters)
    i. External – tail: 0/0 – 0/0 – 0/0 – 4/3
    ii. Skeletal – forked / fused ribs: 0/0 – 0/0 – 0/0 – 2/2
    iii. Visceral – heart / great vessel: 0/0 – 0/0 – 0/0 – 2/2
4. There was an increase in mean rat early resorptions and post-implantation loss
    i. resorptions: 1.0 vs. 1.5 (controls vs. high dose) [HCD: 0.3–1.5]
    ii. post-implantation loss: 6.9% vs. 10.1% (controls vs. high dose) [HCD: 2.9–13.6%]
5. There was a reduction in rabbit mean live litter size at the high dose:
    i. foetuses per litter: 7.2 – 7.4 – 7.2 – 5.8
6. There was a substantial increase in rabbit early resorptions and post-implantation loss:
    i. resorptions: 0.8 vs. 2.0 (controls vs. high dose) [HCD: 0.1–1.0]
    ii. post-implantation loss: 12.2% vs. 31.5% (controls vs. high dose) [HCD: 2.4–23%]
7. There was evidence of increased rabbit skeletal malformations:
    i. skeletal – forked / fused ribs: 1/1 – 0/0 – 0/0 – 4/4

Although developmental toxicity was limited in its extent in both rats and rabbits to a single (high) dose group only in each developmental study with no dose response at lower doses, RAC concludes that potency per se is not a factor that should be considered in categorisation for reproductive toxicity.

Reductions in foetal body weight were seen in only one study and species (rat), but these changes were statistically significant, outside the HCD and associated with skeletal variations. The increase in rat external, skeletal and visceral variations and a very extensive and biologically significant delayed development of the skeletal system was observed at the top dose level of 750 mg/kg bw/day and in a few cases at 250 mg/kg bw/day (in this case maturation delay without any effect from maternal toxicity or foetal body weight reductions). There was also a high incidence of lateral ventricle dilatation at the high dose.

The adverse effects on development in both rat and rabbit are not considered secondary non-specific consequences of maternal toxicity. In the rabbit developmental study, the increase in post-implantation loss at high dose was accompanied by a marked retardation of uncorrected maternal body weight gain during the dosing period, but the body weight data was highly variable and the weight change differed significantly depending on the gestational interval under the study. According to the CLP criteria, the body weight gain in rabbits may not be a useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy. In addition, there were no clinical signs of toxicity during the dosing period. Overall, RAC concludes that the maternal body weight data is equivocal in rabbits and maternal toxicity is insufficient to explain the degree of severity of the effects at the high dose. In addition, halosulfuron-methyl induced early resorptions impacting the post-implantation losses as also observed in rats in the presence of only minimal maternal toxicity. Although these effects were not statistically significant in either species, the incidences were above the concurrent control values and HCD in rabbits and above the concurrent control values in rats, these effects are considered biologically significant by RAC.

The incidences of malformations at the high dose are considered low, but the increased rat external, skeletal and visceral malformations are considered by RAC to be severe effects and toxicologically significant and relevant because the incidences were higher than in concurrent controls and above the very low HCD. The HCD show that tail malformations in rats are rare malformations with a range of 0 to 1 foetus in any single study and only 1 foetus affected out of 3787 from 12 studies, equivalent to a 0.03% foetal incidence. In the study by Morseth (1990a), 4 rat foetuses (1.4% foetal incidence) had tail malformations in the high dose group only (see tables 21 and 22 in this opinion for further detail). Also the increased rabbit skeletal malformations at the top dose level of 150 mg/kg bw/day are not common findings as HCD show forked/fused rib malformations with a range of 0 to 3 foetuses in any single study and only 8 foetuses affected out of 947 from 9 studies, equivalent to a 0.8% foetal incidence. In the study by Morseth (1990b), 4 rabbit foetuses (1.4% foetal incidence) from 4 litters had forked/fused ribs in the high dose compared to 1 rabbit foetus in the control group (see tables 25 and 26 in this opinion for further detail). These findings support similar effects in rats.

RAC also evaluated the results of a single low dose (5 mg/kg bw/day) oral gavage autoradiography study with pregnant rats, which do not provide a convincing argument against trans-placental transfer of the active substance (McCarthy, 1991b - The autoradiography, disposition in tissues and biliary excretion of NC-319 in male and female rats). Without data of concomitant plasma levels of substance in both maternal and foetal blood, it is not possible to determine the relationship of the findings manifest in both organisms. Consequently, the toxicokinetics of the substance in the foetus is unknown and the amount actually present in the foetal blood stream is also unknown, although it is assumed there would be very little restriction to the movement of the substance across the placenta for higher dosed pregnant females.

After careful consideration of all the data, RAC concludes there is sufficient evidence of a substance-mediated effect. Development of rat foetuses was impaired at high dose levels. Rat foetal body weight was dramatically reduced. There was a biologically significant increase in early resorptions which impacted on the rat post-implantation loss and this effect was also noted in the rabbit developmental study. Several widespread developmental variations were observed and there were indications of malformations in both rats and rabbits. RAC cannot exclude a direct effect on the developing foetus, the maternal toxicity is considered insufficient to explain the degree of severity of the effects observed in the foetuses from high dose dams.

Overall, RAC concludes that there is clear evidence for adverse effects on development in the absence of excessive maternal toxicity, observed in both rats and rabbits with significant severity of findings in the offspring to warrant classification for development. RAC is of the opinion that **classification with Repr. 1B – H360D** is the most appropriate classification.

Adverse effects on sexual function and fertility

In the rat 2-generation study there were no treatment-related adverse effects on fertility or reproductive performance including pre-coital interval at doses up to 3600 ppm. Gestation length was unaffected by treatment (table 16). RAC agrees with the DS that minor changes in fertility parameters in treated groups were not dose-related and do not represent an adverse effect of treatment.

RAC noted some inconsistencies associated with reduced pregnancy rates and numbers of dams with litters, but does not consider these effects as substance-related effects. Both F1 matings showed reduced pregnancy rates without a clear dose response. The pregnancy rates increased with the dose in F0 matings (65%, 81%, 92% and 92% at 0, 100, 800 and 3600 ppm), but the very low control in the F0 mating reduces confidence in this effect. All in all, the evidence on reduced pregnancy rates is not considered by RAC sufficiently robust to propose classification for fertility. In addition, there was no evidence on a reduction in the number of females pregnant or in the mean number of offspring born per litter.

Based on the available data and its interpretation, RAC agrees with the DS's assessment that **no classification for adverse effects on sexual function and fertility is warranted**.

Effects on or via lactation

In the rat 2-generation study, pup weights were not affected by treatment on day 0 of lactation following continual gestational exposure so there was no developmental delay on growth rate. However, F1 pup weights on subsequent days of lactation (days 7-21, table 17) were significantly different to controls at dam dose levels of 88.1 (800 ppm) and 429 mg/kg bw/day (3600 ppm). Effects observed in F2a and F2b pups were considered not to be consistent or biologically significant. RAC concludes, in agreement with the DS, the evidence for these effects were equivocal and classification for effects on or via lactation is not warranted.

Supplemental information - In depth analyses by RAC

Rat two-generation (dietary) study

<u>Parental toxicity</u>

General toxicity is confined to small effects on body weight parameters. The parental NOAEL is 800 ppm (50.4 mg/kg bw/day in males, 58.7 mg/kg bw/day in females) based solely on decreased body weight parameters at the highest dose. As compared to controls, the changes

in absolute weights are generally approximately 10% or less at the highest dose (table 16, below).

Table 16: Body weight effects in adults of the F0 and F1 generations.

Sex	**Females**			
Dose level (ppm)	**0**	**100**	**800**	**3600**
Mean dose level (mg/kg bw/day)	**0**	**7.4**	**58.7**	**261.4**
F0 Body weight (g)				
Week 3	243.5	237.0	242.4	230.5**
Week 8	291.4	286.9	288.4	271.0**
Week 16	335.0	353.4	354.7	322.0
F0 Gestation				
Day 0	318.2	318.8	323.5	293.4**
Day 7	352.8	350.9	353.8	321.7**
Day 14	378.5	378.3	381.2	349.3**
Day 20	446.2	447.1	448.3	421.8*
F0 Lactation				
Day 0	353.1	352.4	359.0	322.0**
Day 7	352.2	353.7	352.4	326.7**
Day 14	368.4	366.1	369.0	341.4**
Day 21	363.6	367.5	369.5	346.9
Body weight change (g)				
Week 0-14	142.5	142.5	146.1	114.8**
Day 0-20 gestation	128.06	128.35	124.83	128.46
Day 1-25 lactation	10.53	15.05	10.50	23.26
Mean Dose level (mg/kg bw/day)	**0**	**8.9**	**69.7**	**319.9**
F1 Body weight (g)				
Week 0	85.1	83.6	82.1	74.5**
Week 8	286.0	296.1	280.1	263.7**
Week 16	337.8	356.4	335.0	313.1*
F1 Gestation				
Day 0	338.8	360.8	339.9	306.5**
Day 7	372.2	385.2	368.9	332.2**
Day 14	399.8	415.6	394.7	357.9**
Day 20	470.0	484.6	466.1	429.1**
F1 Lactation				
Day 0	383.1	400.3	385.7	338.8**
Day 7	377.9	388.5	370.1	336.1**
Day 14	387.2	395.6	376.9	352.4**
Day 21	375.5	376.6	358.2	341.6**
Body weight change (g)				
Week 0-14	nc	nc	nc	nc
Day 0-20 gestation	131.14	123.81	126.27	122.63
Day 1-25 lactation	nc	nc	nc	nc

* $p < 0.05$; ** $p < 0.01$; nc = no change

Points to note: Food consumption was reduced predominantly in females at the highest dose.

- F0: Food consumption by females was statistically significantly reduced at the highest dose level at all measured time points, including gestation and lactation, this reduction was < 10% in each case.
- F0: Food consumption by males was unaffected.
- F1: Food consumption by females was statistically significantly reduced at the highest dose level at all measured time points (-7 to -15% relative to controls), except for lactation (no change).
- F1: Food consumption by males was statistically significantly reduced at the highest dose level, this reduction was < 10% in each case.

In conclusion, there was minimal parental/maternal toxicity evident in either F0 or F1 adults.

Reproductive toxicity

Several endpoints are summarised in table 17 below. The NOAEL for reproductive toxicity is the highest dose tested.

Table 17: Summary of reproductive endpoints. Values in bold are similar to the HCD or outside the HCD

Parameter	0 ppm	100 ppm	800 ppm	3600 ppm
F0 mating/litter data				
Number of females paired	26	26	26	26
Number of females pregnant	17	21	24	24
Number of females with litters	17	21	24	24
Pregnancy rate (%)	**65**	81	92	92
Mean duration of gestation (days)	22.0	22.0	21.8	22.2
Pup viability indices (%)	99	97	94	91
Weaning indices (%)	98	93	94	95
Mean number of offspring born/litter	13.6	14.1	13.7	14.3
F1 - F2A mating/litter data				
Number of females paired	26	26	26	26
Number of females pregnant	22	17	16	20
Number of females with litters	22	17	15	19
Pregnancy rate (%)	85	**65**	**62**	**77**
Mean duration of gestation (days)	22.0	22.1	21.9	22.1
Pup viability indices (%)	90	99	95	97
Weaning indices (%)	92	93	97	98
Mean number of offspring born/litter	13.0	13.2	13.3	13.4
F1 - F2B mating/litter data				
Number of females paired	25	26	25	25
Number of females pregnant	23	23	21	22
Number of females with litters	22	19	18	17
Pregnancy rate (%)	92	88	84	88

Mean duration of gestation (days)	22.0	22.0	22.1	22.2
Pup viability indices (%)	93	93	91	91
Weaning indices (%)	89	90	94	97
Mean number of offspring born/litter	13.1	12.5	12.8	11.7

Pregnancy rate = [(females pregnant / females paired) × 100]

Pregnancy rate

In the F0 dams, the pregnancy rate was very low in the control group whereas in the F1 control groups the pregnancy rate was increased (65, 85, and 92% in F0, F1A and F1B, respectively). There was an inconsistent effect on the pregnancy rate between the generations and no firm dose response. In the F0 generation these was an increase in the pregnancy rates as compared to the very low pregnancy rates in the controls. The very low control value reduces confidence in this finding for this group. In the first littering of the F1 generation, lower rates of pregnancy were observed relative to the concurrent controls but no dose response was evident. The second littering from F1 females also showed a reduction in pregnancy rate relative to controls across all doses but again no dose response was evident. Taken together only the pregnancy rates and the reductions in females with litters (see the assessment below), in the F1 generation a potential weak reproductive effect in the absence of parental toxicity was observed. However, the reduced pregnancy rates and numbers of dams with litters in the rat 2-generation study were inconsistent across the generations and there was no firm dose-response across all litters. Total HCD data (F1 and F2 litters) indicate an expected variability from 77–100%. Overall, RAC does not consider these effects as substance-related effects. Other evidence such as the reduction in the number of females pregnant or reductions in the mean number of offspring born per litter, were not observed at any dose.

Classification for fertility is not supported, the data being too variable and not sufficiently robust to justify a classification for adverse effects on sexual function and fertility.

Females with litters

An increased number of females with litters was observed in the F0 group, whereas a reduced number of females with litters were observed in the F1a and F1b groups as compared to concurrent controls. HCD were not provided. There was no convincing dose-response observed, there was a lack of a consistent response and there was a high degree of variability within controls (17-22 females with litters) and all dose groups. Thus, the data is not robust enough to conclude that there is a treatment-related response.

Adverse effects on development of the offspring

Pup live birth index was unaffected. No treatment-related clinical signs were seen in the pups. Litter size and pup viability (survival) were unaffected by treatment. The sex ratio was unaffected. Histopathology did not show treatment-related effects.

Adverse effects on or via lactation

The body weights of pups are presented in table 32 of the CLH report. Table 18 below shows the relative change in body weight with respect to the controls at actual dam exposure levels during lactation. The only effect of note was a significantly lower postnatal body weight gain in the pups during the lactation period (pup weights were not affected by treatment on day 0 of lactation following continual gestational exposure in any generation). The effect was

considered toxicologically significant and treatment-related only for the F1 pups (table 18), as the magnitude of the effect was not consistent across all litters. The **F1** pup weights during lactation were significantly different to controls at 88.1 (800 ppm) and 429 mg/kg bw/day (3600 ppm) maternal dose groups. Effects observed in the F2a and F2b second generation pups although statistically significant, were considered not toxicologically significant or treatment-related due to low magnitude of effect. Contemporary HCD from the laboratory conducting the study (1987–1989), show the absolute pup body weights from halosulfuron-methyl treated animals were within the historical control range for the strain and conditions specific to the laboratory (table 35 CLH report).

Table 18: Pup body weight change (%) relative to controls during lactation

Sex	**Males**				**Females**			
Maternal dose level (ppm)	**0**	**100**	**800**	**3600**	**0**	**100**	**800**	**3600**
Maternal dose (mg/kg)	0	11.8	88.1	429	0	11.8	88.1	429
F1 pups								
day 0	--	-1	nc	-2	--	-1	+1	-2
day 7	--	-7	**-11***	**-13****	--	-9	-10	**-14****
day 14	--	-4	-11	**-16****	--	-6	**-11***	**-17****
day 21	--	-5	**-12***	**-17****	--	-6	**-11***	**-16****
Maternal dose (mg/kg)	0	10.0	81.3	410.5	0	10.0	81.3	410.5
F2a pups								
Day 0	--	nc	-3	**-6***	--	nc	-3	**-6***
Day 14	--	-5	nc	-6	--	-2	-nc	-8
Day 21	--	-5	nc	-7	--	-6	-1	**-9***
Maternal dose (mg/kg)	0	8.5	76.5	344.5	0	8.5	76.5	344.5
F2b pups								
Day 0	--	nc	nc	**-7***	--	nc	nc	**-6***
Day 14	--	+3	+5	-3	--	-4	-3	-6
Day 21	--	+4	+5	-5	--	-3	-4	-8

Significant effects on absolute body weight: * $p < 0.05$; ** $p < 0.01$; nc = no change

Gross pathology findings for F1 pups showed that the incidence of empty stomach (pups/litters; 1/1, 0/0, 3/2 and 9/5; from controls to high dose, respectively) was greatest in the high dose group relative to all others. This effect was not observed in either of the F2 generation pups.

RAC concludes, in agreement with the DS, the evidence for lactational effects was equivocal and classification for effects on or via Lactation is not warranted.

Rat developmental (oral gavage) study

Maternal Toxicity

A quantitative description of maternal toxicity is provided below. The corrected maternal gestational body weight gains and terminal body weights for the rat at all doses are provided and a similar table for rabbits is also presented later.

Table 19: RAC Summary of Rat Maternal bodyweight indices (% change vs. control)

Parameter/dose (mg/kg bw/day)	**0**	**75**	**250**	**750**	**750†**
corrected gestational wt. gain (% relative to controls)	--	-1.7	-3.4	-14	-12
mean gravid uterine weights (% relative to controls)	--	- 1.0	+0.8	-17.6	-10.6
corrected maternal terminal body weights (% relative to controls)	--	+0.8	-0.2	-4.3	-3.6

† excludes data from 2 dams with no viable foetuses (complete early resorptions) at scheduled sacrifice.

Notes:

- no treatment related effect on corrected maternal terminal body weight (309.9, 312.3, 309.4, 296.6*g/298.7g†)
- mean maternal food consumption was not statistically significantly different over GD0 – 20 (in contrast, GD 6–8; 8–12; or 6–16 were significant at the high dose only ($p \leq 0.05$).
- mean gravid uterine weights were 73.7, 73.0, 74.3 and 60.7g/65.9g†.
- mean corrected gestational body weight gains were 59, 58, 57 and 50g/52g†.

The mean gravid uterine weights indicate an intra-uterine effect at the high dose. The effects on adjusted (corrected) maternal body weights/body weight gain are not considered to indicate significant maternal toxicity but rather a minimal maternal toxicity which is not considered sufficient to account for the developmental effects observed, such as significant reductions in foetal body weight and skeletal and visceral abnormalities and increased post implantation loss.

Adverse effects on fertility

There was no substance related effect on fertility. There was no data on sexual function. Pregnancy rate was high in all groups (100-96%), as was the number of implantations (table 20). There were a few other parameters of interest – a summary of the main fertility parameters that were not provided in the original CLH report are found below (table 20).

Table 20: RAC Summary of Rat fertility and developmental indicators

Parameter/dose (mg/kg bw/day)	**HCD**	**0**	**75**	**250**	**750[1]**	***750[2]***
Pregnancy rate (%)	80–100%	100	100	96	96	*96*
Number of *corpora lutea*	11.6–19.4	16.4	17.4	16.8	15.7	*16.2*
Number of implantations	14.0–16.5	14.4	14.2	14.5	14.3	*14.8*
Preimplantation loss (%)	7.2– 24.6	12.2	18.4	13.7	8.9	*8.6*
Number of early resorptions	0.3–1.5	1.0	0.8	0.9	**2.2**	***1.5***
Number of late resorptions	0–0.1	0	0	0	0	*0*
Post implantation loss (%)	2.9–13.6	6.9	5.6	6.2	**15.4**	***10.1***
% live foetuses per litter	86.4–97.1	91.7	94.5	94.3	82.7	*90.3*
Sex ratio (% live males)	44-51	44.4	48.5	48.0	50.5	*50.5*

[1] no litters excluded

[2] excludes data from 2 litters (dams B81655, B81660) with no viable foetuses (complete early resorptions)

Adverse effects on the development of the offspring

Early resorptions and post implantation loss were affected at the highest dose (table 20, above).

The applicant supplied a rebuttal to an initial proposal for classification for reproductive effects. In the highest dosage group (750 mg/kg bw/day), two pregnant females did not carry live foetuses to term (NVF). One of these females (B81655) lost 20 g of body weight in the day 8-12 period, suggesting excessive toxicity in this dam. In this dam, all 17 implantations were early resorptions. The second female (B81660) had only two *corpora lutea* and was therefore not a typical pregnancy. In this dam, both implantations were lost as early resorptions. RAC considered this point and recalculated several parameters based upon these two females being omitted from the high dose group. Where possible, the adjusted means excluding these two females or litters from these two dams have been included with the original values as reported by the DS in the CLH report. It is reasonable to conclude that both of the noted females represent outliers with the potential to skew the overall data for several reproductive endpoints (for example, resorption values, post-implantation loss, gravid uterine weight) and those of maternal toxicity (corrected gestational weight gain, corrected maternal terminal body weights).

The two NVF females mentioned above have hitherto been included in the analysis of body weight change during gestation and reproductive indices in the original study report and DAR. RAC notes that there was an increased post implantation loss, which is approximately 1.5 times more than that in concurrent controls and which lies just at the upper limit of the HCD. This indicates a substance-related effect in the high dose group and is only attributable to increased numbers of early resorptions. Late resorptions are not a feature when the raw data are analysed. There were no mechanistic data provided to explain the effect. Considering also the rebuttal submitted by the applicant, RAC concludes that the increased post-implantation loss and early resorptions support classification for adverse effects on development, because an increase in post implantation loss was still observed at the high dose and the observation is supported by results in the rabbit developmental study. Furthermore, these effects occured in the presence of only minimal maternal toxicity which does not indicate that the developmental effects were secondary non-specific consequences of maternal toxicity or that the maximal tolerated dose was exceeded.

There was an increase in external, skeletal and visceral malformations at the top dose level of 750 mg/kg bw/day with no dose response at lower doses. The malformations occurred at low incidences but are considered rare in the rat (table 21).

There is a very extensive and significant delayed development of the rat skeletal system at the highest tested dose of 750 mg/kg bw/day, which cannot be appreciated from just taking note of the total skeletal variations. This is a high dose effect with no apparent dose response at lower doses. Small changes in the proportions of common foetal variants observed in skeletal examinations may not necessarily warrant a classification, but this effect represents the sum of all skeletal variants that in such diversity and magnitude is considered by RAC as supporting evidence for developmental toxicity. Furthermore, in this case it is highly unlikely that maternal toxicity is sufficient to account for the degree of the effects observed in foetuses

from the high dose group. Foetal body weight was also severely adversely affected at 750 mg/kg bw/day as a consequence of intrauterine exposure. A published study by Fleeman *et al.* (2005) showed that even with drastic malnourishment of gravid females (approximately 50% diet restriction relative to controls, gives rise to a 30% reduction in gestational body weight), the foetal body weight (< 10% reduction relative to controls) was not affected to the extent that it is in this case. This suggests a direct substance-related effect during foetal development that is not considered to be a secondary non-specific consequence of maternal toxicity. The maternal toxicity as described for the rat developmental study is in fact noteworthy for its lack of severity.

The DS described the toxicokinetics of halosulfuron-methyl in the CLH report (section 4.11.4), where an argument against trans-placental transfer of the active substance is postulated based on the results of a single low dose oral gavage (5 mg/kg bw/day) autoradiography study with pregnant rats (McCarthy, 1991), and the state of ionisation of the molecule in blood. However, these arguments are not convincing considering the repeated high dose of 750 mg/kg bw/day used in the rat developmental study and the fact that the movement of active substance from the maternal to the foetal plasma compartment should not always be assumed to be an instantaneous event. In this case, a single dose study cannot predict, confirm or deny steady state concentrations of halosulfuron-methyl in both the maternal and foetal organisms.

Table 21: Summary of relevant developmental findings: (foetal/litter incidence)

Parameter/dose (mg/kg bw/day)	**0**	**75**	**250**	**750**
Number of females mated	25	25	25	25
Number of females pregnant	25	25	24	24
Number of litters[1]	25	25	24	22
Number of foetuses[2]	335	335	326	292
Mean Foetal Weight (g) corrected				
males	3.4	3.4	3.4	**2.6*** (-24%)
females	3.2	3.2	3.2	**2.5*** (-22%)
External observations:				
- Number foetuses/litters	335/25	335/25	326/24	292/22
- *Malformations:*				
- malrotated hindlimb	0	0	0	1/1
- filamentous tail	0	0	0	3/3
- rudimentary tail	0	0	0	1/1
Visceral observations:				
- Number foetuses/litters	165/24	170/25	163/24	146/22
- *Variations:*				
- lateral ventricle dilatation	0	0	2/2	16/5
- 3rd ventricle dilatation	0	0	0	1/1
- increased renal pelvic cavitation	4/3	4/3	7/5	9/6
- *Malformations:*				
- Heart/great vessel	0	0	0	2/2
- Adrenal agenesis	0	0	0	1/1
- Ectopic kidney	0	0	0	1/1
- Spinal cord agenesis	0	0	0	1/1
Skeletal observations:				
- Number foetuses/litters	170/25	165/25	163/24	146/22
- *Variations:*				

- total variations[3]	105/23	115/25	114/23	146*/22
- *Malformations:*				
- Forked/fused ribs	0	0	0	2/2
- Vertebral anomaly	0	0	0	1/1

[1] Out of 24 females pregnant at the highest dose, 2 females had no viable foetuses at term because of total early resorptions of all implantations.
[2] 2 dams had complete litter loss due to early resorptions, there was evidence of 17 implantations in one animal and only 2 implantations in the second dam.
[3] See table 33, CLH report for a more complete characterisation of skeletal variant incidence.

Foetal weights HCD: Males: 3.10–3.88 g, mean 3.55 g; Females: 2.96–3.73 g, mean 3.37 g.

Historical control data

An addendum to the original study report was included in the annexes of the original halosulfuron-methyl study report. A summary of the laparohysterectomy data from twelve oral studies (initiation dates from 1986–1989) in the same strain of rat provides further but limited data and is presented here for completeness (table 22). There was no data to indicate if the HCD was from the same contract research laboratory or a general compilation of studies for the same strain of rat that was used in the present study by Morseth, 1990. The use of HCD from unspecified laboratories is a complication, and should not always be fully relied upon. However, the data in this case identifies what may be rare malformations and other anomalies to be aware of and helps RAC to put into perspective the observed effects in the main developmental study. In this regard, the HCD is used cautiously and indicates that low incidences of rare skeletal and visceral malformations in the rat developmental study are likely due to treatment. In addition, as shown by this HCD, tail malformations are rare malformations (with a range of 0 to 1 foetuses) and in this single rat study 4 foetuses (1.4%) had tail malformations at the highest dose and are thus considered relevant and substance-related.

Table 22: Summary of rat historical control incidences of malformations & variations (12 studies, initiated between '86–'89)

Malformation/Variation	**[1]Foetuses**	**[2]Litters**	**[3]Mean L %**	**[4]F range**
External observations:				
- *Malformations:*				
- filamentous tail	1/3787	1/277	0.4	0-1
- rudimentary tail	0/3787	0/277	0	0
Visceral observations:				
- *Variations:*				
- lateral ventricle dilatation	11/1745	11/ 277	4.1	0-5
- 3rd ventricle dilatation	5/1745	5/277	1.8	0-3
- increased renal pelvic cavitation	56/1745	41/277	14.8	0-13
- *Malformations:*				
- Heart/great vessel	0/1745	0/277	0	0
- Adrenal agenesis	0/1745	0/277	0	0
- Ectopic kidney	0/1745	0/277	0	0
- Spinal cord agenesis	0/1745	0/277	0	0
Skeletal observations:				
- *Malformations:*				

- Forked/fused ribs	0/2042	0/275	0	0
- Vertebral anomaly	1/2042	1/275	0.4	0-1

[1] Foetuses = total foetal incidence observed with anomaly

[2] Litters = total litter incidence for anomaly

[3] Mean L % = mean litter incidence per study

[4] F range = number of foetuses effected per study

Conclusion

The development of rat foetuses was impaired at high dose levels. There was a significant increase in early resorptions which impacted on the rat post-implantation loss; markedly reduced foetal body weight; delayed ossification; an increase in visceral variations (dilatation of lateral ventricles); and some malformations occurring also at greater incidence than expected relative to the available HCD. RAC concludes that there is a substance-related effect on the developing foetus; the maternal toxicity is slight and RAC considers that the developmental effects are not secondary, non-specific consequences of maternal toxicity; and the maximal tolerated dose has not been exceeded.

In summary, there is clear evidence of adverse effects on development at high dose. RAC concludes that this evidence supports Repr. 1B; H360D rather than Repr. 2 H361d or no classification.

Rabbit developmental (oral gavage) study

Maternal Toxicity

A quantitative description of maternal toxicity is provided below. There were no clinical signs of toxicity during the dosing period. The corrected maternal gestational body weight gains and terminal body weights for the rabbit at all doses are provided. The picture is more complicated for the rabbit (when compared to the rat) with regards to maternal body weight, particularly during the actual dosing period. There was a marked but not statistically significant decrease in uncorrected maternal body weight gain between gestation days 7 and 20 as compared to controls (-73% compared to controls). The body weights had a high degree of variability among the individuals in the same group (the uncorrected body weight gain +68 g ± 293 g vs. 257 g ± 115 g in high dose vs. controls between GD 7-20) as is typical in rabbit studies. There was a small reduction in feed consumption during this time (not statistically significant) by about 16.6% compared to controls. Post-treatment, the mean body weight gain (uncorrected) between days 20-29 in the high dose group was significantly higher compared to the controls, illustrating a rebound effect. Hence, overall corrected maternal bw gain (GD 0-29) does not show a decrease with treatment but an increase because of the marked increase in food consumption following the cessation of substance dosing.

Table 23: RAC Summary of rabbit maternal bodyweight indices

Parameter / dose (mg/kg bw/day)	**0**	**15**	**50**	**150**
corrected gestational weight gain GD 0-29 (% relative to controls)	--	-90	+203	+226
uncorrected gestational weight gain GD7-20 (% relative to controls)	--	+6.4	-4.5	-73.1

mean gravid uterine weights (% relative to controls)	--	-9.8	-2.8	-13.5
corrected maternal terminal body weights (% relative to controls)	--	-2.5	+2.2	+0.6

Absolute values of gravid uterus, corrected terminal body weight and corrected gestational weight gain were not statistically significant relative to controls.

Notes:

- no treatment related effect on corrected maternal terminal body weight (3548, 3469, 3624, 3570 g).
- mean maternal food consumption was not statistically significantly different in any treatment group over GD 0–29; 20–29; or 7–20 (covers dosing period).
- mean gravid uterine weights were 470, 424, 457 and 407 g.
- mean corrected gestational body weight changes from GD 0-29 were 46.7, 4.6, 141.7 and 152.3 g, the rabbit data was highly variable within each dose group.

Adverse effects on sexual function and fertility

Table 24 below, summarises the data from table 34 in the CLH report and table B.6.6.3-1 from the DAR along with some data from the original study report. The pregnancy rate, mean numbers of *corpora lutea* and uterine implantation sites are presented in table 24 below.

Table 24: Summary of rabbit fertility and developmental parameters

Parameter/dose (mg/kg bw/day)	**HCD**	**0**	**15**	**50**	**150**
Number of litters		13	10	11	13
Number of foetuses		94	74	79	76
Number of dead foetuses	0-2	0	1	0	1
Pregnancy rate (%)	81-100	82	82	**65**	88
Number of *corpora lutea*	9–12.3	12.5	11.2	12.6	13.2
Number of implantations	7–10.3	8.2	8.2	7.9	8.5
Preimplantation loss (%)	9.6–39.2	34.4	26.8	37.3	35.6
Number of early resorptions	0.1–1.0	0.8	0.9	0.6	**2.0**
Number of late resorptions	0.1–0.6	0.2	0.5	0.1	0.6
Number of dead foetuses per litter	0–0.13	0	0.1	0	0.08
Post implantation loss (%)	2.4–23.0	12.2	18.3	8.9	**31.5**
% live foetuses per litter	77.0–97.6	100	99	100	99
Sex ratio (% male)	43.5–55.1	48.6	43.1	45.9	37.4

Adverse effects on the development of the offspring

There was no effect on foetal body weights.

There was a reduction in rabbit live litter size at the high dose (mean foetuses/litter: 7.2, 7.4, 7.2, 5.8).

The number of early resorptions (0.8, 0.9, 0.6 and 2.0 in controls, 15, 50 and 150 mg/kg bw/day, respectively) was increased more than 2-fold at the high dose when compared with controls and the post implantation loss was also more than 2-fold greater. Post implantation loss was judged by RAC to be a biologically significant effect in the high dose group. These

results were outside the range of HCD. Food consumption during the dosing period GD 7-20 for the high dose group was discussed earlier and found to be reduced but highly variable. There were no dose dependent incidences of abortions in pregnant females (0/13, 2/11, 0/11, 2/13 in the 0, 15, 50 and 150 mg/kg bw /day groups, respectively). RAC concludes there is some evidence of maternal toxicity but considers it insufficient to account for the large increase in early resorptions and increased post-implantation loss observed in the highest dose group. RAC finds the rabbit developmental study supportive of the rat developmental study.

Skeletal variations were well distributed across all dose groups with no observed treatment-related effect (table 25). This is in contrast to the effects seen in the rat developmental study. However, there were 4 foetal skeletal malformations (forked/fused ribs), from 4 litters in the high dose group compared with 1 foetus in 1 litter for controls. The litter incidence was 4 affected out of 13 in the high dose group. This is outside the historical control for this particular finding (> 6 fold the expected foetal incidence). Another malformation finding was described as vertebral with/without rib anomaly and discussed briefly in the original study report where this data was presented along with the forked/fused ribs. Combining the data from both anomalies, the study authors did not discuss in depth how the data could be interpreted and their final conclusion for the study as a whole was no substance induced teratogenicity. RAC notes the forked/fused ribs malformation as a distinct category of malformation with HCD provided in an appendix to the original study report. The DS did not comment in any detail with regard to rabbit foetal malformations nor were they discussed in the DAR. RAC considers this information as further support to those effects seen in the rat and sufficient for Repr. 1B – H360D.

Table 25: Summary of rabbit developmental findings: (foetal/litter incidence)

Parameter/dose (mg/kg bw/day)	**0**	**15**	**50**	**150**
Number of females mated	17	17	17	17
Number of females pregnant	14	14	11	15
Number of litters	13	10	11	13
Number of live foetuses	94	74	79	76
Mean Foetal Weight (g) corrected				
males	45.9	42.1	45.3	44.3
females	43.3	39.8	43.1	43.0
External observations:				
- Number foetuses / litters	94/13	74/10	79/11	76/13
- Variations:				
- thin skin	0	1/1	0	0
- no other observations				
- *Malformations:*				
- spina bifida	1/1	0	0	0
- cleft palate		1/1[1]	0	0
- tail reduced / rudimentary	0	1/1[1]	1/1	0
Visceral observations:				
- Number foetuses / litters	93/13	74/10	79/11	75/13
- *Variations:*				
- great blood vessel variations	4/3	10/6	4/3	3/2
- inter. lobe lung small/missing	5/4	2/2	4/3	0
- heart, atrium enlarged	0	0	0	1/1
- liver thickened	0	0	0	1/1

- *Malformations:*				
- internal hydrocephaly	0	1/1	0	0
- persistent truncus arteriosus	0	1/1	0	0
- retroesophageal subclavian artery	0	1/1	0	0
- gall bladder agenesis	0	0	2/1	0
Skeletal observations:				
- Number foetuses / litters	94/13	74/10	79/11	76/13
- *Variations:*				
- total variations	71/13	49/10	49/11	60/13
- *Malformations:*				
- forked / fused ribs	1/1	0	0	**4/4[2]**
- vertebral with/without rib anomaly	4/4	3/3	3 / 2	0
- total malformations	7/5	3/3	4 / 3	4/4

1. The same foetus. Only 1 foetus from 1 litter of rabbit E46081 from the 15 mg/kg bw/day dose group was affected and had several malformations.

2. Forked/fused ribs are the only significant malformation in the high dose group.

Table 26: Summary of rabbit historical control incidences of forked/fused ribs malformation (9 studies, initiated between '87–'89)

Malformation/Variation	[1]Foetuses	[2]Litters	[3]Mean L %	[4]F range
Skeletal observations:				
- *Malformations:*				
- Forked/fused ribs	8/947	7/124	5.6 (0–2)	0-3
- vertebral with/without rib anomaly	11/947	9/124	7.3 (0–2)	0-3

[1] Foetuses = total foetal incidence observed with anomaly
[2.] Litters = total litter incidence for anomaly
[3.] Mean L % = mean litter incidence per study/control group (range for number of litters affected)
[4.] F range = number of foetuses effected per study/control group

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

In an acute neurotoxicity study Groups of 10 male and 10 female Sprague Dawley rats were given a single oral dose, by gavage, of 0, 200, 600 or 2000 mg/kg of halosulfuron-methyl suspended in 0.1% Tween® 80 and 0.5% carboxymethylcellulose in distilled water. The dose volume was 10 ml/kg. Dose levels were based on findings from oral acute toxicity studies.

One male given 2000 mg/kg died. No treatment-related clinical signs were seen at any dose level. Transient increases in slightly to moderately uncoordinated righting reflex were seen in both sexes at 2000 mg/kg at 7 hours post-dosing. No histopathological changes were seen in the neural tissues.

There was no evidence of progressive, long-term irreversible neurotoxicity. The observed findings were indicative of systemic toxicity. The NOEL for acute neurotoxicity was 600 mg/kg of

halosulfuron-methyl, on the base of uncoordinated righting reflex seen at the higher dose (DAR B.6.7.1Wakefield, 1994).

A subchronic (90-day) neurotoxicity study was conducted with halosulfuron-methyl where groups of 10 male and 10 female young adult Sprague Dawley CD rats were treated for 13 weeks. Males were fed 0, 100, 1000 or 10,000 ppm of halosulfuron-methyl, whilst females received 0, 100, 1000 or 4000 ppm. The control animals were given untreated diet. A male mortality occurred at 100 ppm. There were no treatment-related clinical signs or evidence of any neurobehavioural changes or histopathological findings in neural tissue. At 10000 ppm, body weight gain was reduced and in males, liver weight was increased and centrilobular hepatocyte hypertrophy was found. At 4000 ppm, female body weight gain was reduced. The NOEL for neurotoxicity was 10000 ppm for males, corresponding to 706.0 mg/kg/day and 4000 ppm in females, corresponding to 315.9 mg/kg/day. The NOEL for general systemic toxicity was 1000 ppm in both sexes, corresponding to 62.8 and 82.5 mg/kg/day in males and females, respectively. (DAR B.6.7.3 Lemen, 1992)

The acute neurotoxicity study in rats did not show any progressive long term or irreversible neurotoxic changes and there was no evidence of progressive long term or irreversible neurotoxic changes in a subchronic (90-day) neurotoxicity study in rats.

No delayed neurotoxicity studies in the hen have been conducted as halosulfuron-methyl is not an organophosphorus compound.

4.12.1.2 Immunotoxicity

No data are available.

4.12.1.3 Specific investigations: other studies

Toxicological studies were provided on two metabolites. The metabolite chlorosulfonamide acid (CSA), found in plants and in groundwater, presented low acute oral toxicity (LD_{50} >5000 mg/kg DAR B6.8.1.1 McRae, 1997a); the 90-day oral NOAEL in rat was 75.8 mg/kg bw per day based on reduced body weight gain in females (DAR B.6.8.1.2 Stout and Thake, 1995). Maternal and developmental NOAELs were the limit dose of 1000 mg/kg bw per day, showing that the metabolite does not share the developmental toxicity profile of the parent, halosulfuron-methyl (DAR B.6.8.1.3 Holson, 1995). Negative results were found in an Ames test (DAR B.6.8.1.4 Stegeman, Warren and Kier, 1995) and an in vivo micronucleus test (DAR B.6.8.1.6 Stegeman, Kier, Garrett, McAdams, Warren and Schermes, 1995), however, an equivocal result in an in vitro mammalian gene mutation test (DAR B.6.8.1.5 Stegeman, Kier, McAdams and Warren, 1995) has to be clarified to conclude on the overall genotoxic potential of the metabolite.

Applicant comments on genotoxic potential of chlorosulfonamide acid:

(1) CSA gave no alerts for genotoxicity when tested by DEREK.

(2) CSA was clearly negative in the Ames test up to the maximum dose of 5 mg/plate. Therefore CSA is not mutagenic.

(3) CSA was clearly negative in the in vivo micronucleus test up to dose levels of 5000 mg/kg. There was a reduction in the PCE/NCE ratio in the female mice given the top dose indicating there had been systemic exposure including exposure to the bone marrow. Therefore CSA was clearly not clastogenic.

(4) In the XPRT assay in CHO cells, CSA was negative under all conditions except for a single dose with 5% S9. In the first test CSA was statistically positive in the presence of 5% S9 at 1400 mg/plate; however the dose higher, 1750 mg/plate was negative and at around 20% survival. Quite correctly the study was repeated and again all dose levels in the absence of S9 were negative. There was, however, a statistically significant positive result at the top dose of 1800 mg/kg, although not stated in the report, this did not constitute a true positive because according to the protocol evaluation criteria the increase must be at least 2-fold greater than the solvent control. In this case the solvent control gave a mutant frequency of 70.58 and the statistically positive response gave 104.1. Moreover, this response at 1800 mg/plate was at a relative survival of 10% which, according to current guidelines, is too high. The response at this dose level would be discounted. This leaves us with the very dubious single positive point at 1400 mg/plate; note that in the confirmatory test CSA was completely negative at this dose, amongst a wealth of negative data from both higher dose levels (1700 mg/plate in the confirmatory test) and higher and lower levels of S9.

(5) It is also interesting to note that the use of aroclor induced S9 with CHO cells can give high levels of spontaneous chromosome aberrations (Kirkland, D.J. et al, Mutat. Res. 214(1), 115 – 122, 1989) and so peculiar or spurious increases in mutation frequency are not unusual.

Given all of the above information the relevance of the single positive response in just one assay is doubtful and so CSA should be regarded as non-genotoxic.

End of Applicant comments on genotoxic potential of chlorosulfonamide acid.

Halosulfuron-methyl rearrangement (HSMR), a principal metabolite in soil and water and minor metabolite in plants, presented low acute oral toxicity (LD_{50} >5000 mg/kg; DAR B.6.8.2.1, McRae, 1997b) and negative results in an Ames test DAR B.6.8.2.2, May, 1997).

4.12.1.4 Human information

Information on humans is not available.

4.12.2 Summary and discussion

No evidence of neurotoxicity was seen in acute and short-term neurotoxicity studies, except for transient increases in uncoordinated righting reflex at 2000 mg/kg bw in the acute neurotoxicity study with chlorosulfonamide acid.

The metabolite chlorosulfonamide acid was of low oral toxicity and not mutagenic.

The metabolite halosulfuron-methyl rearrangement was of low acute oral toxicity and not mutagenic.

4.12.3 Comparison with criteria

No criteria were met as a result of the special investigations by other studies.

4.12.4 Conclusions on classification and labelling

The findings of the special investigations by ‘other’ studies did not affect the proposed classification for halosulfuron-methyl.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of halosulfuron-methyl were assessed in the Draft Assessment Report and Proposed Decision of Italy prepared in the context of the possible inclusion of halosulfuron in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, August 2007 and subsequent addenda, 2012, RMS Italy) concerning the placing of plant protection products on the market.

The summaries included in this proposal are copied primarily from the DAR (and its addenda and assessment reports when these contain relevant updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been tabulated. Detailed information is included for those studies used to derive the classification. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda.

5.1 Degradation

Table 50: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis US EPA Subdivision N, 161-1 (1982) GLP Radiochemical purity: 99.1% (pyrimidine label) 100% (pyrazole label)	DT_{50}: **Pyrimidine label** pH 5 = 28.9 days pH 7 = 13.9 days pH 9 = 17.6 hours **Pyrazole label** pH 5 = 24.8 days pH 7= 14.9 days pH 9 = 19.5 hours .	The major degradates were the rearrangement ester, the 3-chlorosulfonamide ester and the 3-chlorosulfonamide acid. Rate of hydrolysis at pH 4 expected to be slower with a subsequent slower rate of formation of degradation	DAR B.2.1.22 Kesterton et. al., 1991
Photodegradation, US EPA Subdivision N, 161-2 (1982)GLP Radiochemical purity: 98.5% (pyrimidine label) 99.0% (pyrazole label)	DT_{50}:pH 5 = 29.5 days (dark control sample) and 23.8 days for the irradiated samples, indicating minimal degradation due to the photochemical processes. pH 9 = 0.6 days for both the dark control and irradiated samples which indicated that no photodegradation occurred.	Neutral (pH 7) buffer solution was not examined since the hydrolysis products at pH 7 are a mixture of those observed under more acidic (pH 5) and more alkaline (pH 9) conditions. No unique hydrolysis products were observed at pH 7.	DAR B.2.1.23 Kesterton et. al., 1993
Ready biodegradability, EU 92/69/EEC C.4 (1992), OECD 301B (1992), EPA OPPTS 835.3110 (1998) GLP Purity: 99.6%	3% degradation after 29 days. Not readily biodegradable	Not rapidly degradable for classification purposes.	DAR B.8.4.3.1 Barnes, 2003
Water/sediment simulation study, SETAC-Europe (1995) GLP Radiochemical purity: 97.1% (pyrimidine label) 97.5% (pyrazole label)	DT_{50} system 6.3 days (clay loam) 10.4 days (sandy loam)	Not rapidly degradable for classification purposes.	DAR B.8.4.3.2 Corden, 2004

5.1.1 Stability

Hydrolysis

Halosulfuron-methyl exhibited pH sensitive hydrolytic breakdown.

At pH 5, the DT_{50} for halosulfuron-methyl was 25 to 29 days, at pH 7, the DT_{50} was 14 to 15 days, and at pH 9, the DT_{50} was 18 to 20 hours. All rates were determined at approximately 25°C. The

major (≥ 10% applied radioactivity (AR)) metabolites formed were aminopyrimidine (maximum 51.9% AR, Day 30, pH 5), chlorosulfonamide (maximum 56.9% AR, Day 30, pH 5) and halosulfuron-methyl rearrangement (maximum 79.7% AR, 46 hours, pH 9). In addition, an unknown metabolite was detected at maximum levels of 11.3% AR (14C pyrimidine label, 0 hours, pH 9) and 12.3% AR (14C pyrazole label, 0 hours, pH 9). Subsequently, these declined to 1.9% AR by 4 and 6 hours (14C-pyrimidine and 14C-pyrazole label respectively) and were not determined thereafter. Because of this extremely limited persistence, the unknown degradates were not further considered. It was not possible to calculate hydrolysis rates for aminopyrimidine, chlorosulfonamide and halosulfuron-methyl rearrangement using data from the hydrolysis study, as levels did not decline during the observation period. However, based on the results of the aqueous photolysis study (dark control, ~25°C), it is possible to propose a hydrolytic DT50 for halosulfuron-methyl rearrangement at pH 9 of 38.26 days.

Photodegradation in water

Halosulfuron-methyl was shown to be photolytically stable when exposed to natural sunlight for 30 days.

During the aqueous photolysis study, recorded degradation was attributed to hydrolysis. At pH 5, the DT_{50} for halosulfuron-methyl was 23.8 days (irradiated samples) and 29.5 days (dark control samples). At pH 9, the DT_{50} was 0.6 days in both irradiated and dark control samples. At pH 5, the major (10% AR) metabolites formed were chlorosulfonamide (23 and 22% AR, in irradiated and dark control samples respectively, Day 20) and aminopyrimidine (21 and 20% AR, in irradiated and dark control samples respectively, Day 20). At pH 9, the major metabolites formed were halosulfuron-methyl rearrangement (50 and 51% AR, in irradiated and dark control samples respectively, Day 30) and halosulfuron rearrangement (maximum 46 and 47% AR, in irradiated and dark control samples respectively, Day 30).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Biodegradation estimations are not provided since screening tests and simulation tests are available.

5.1.2.2 Screening tests

- Ready biodegradability
- Reference: DAR B.8.4.3.1 (Barnes, 2003)

The “ready” (biotic) degradability of halosulfuron-methyl was assessed using a modified Sturm test. Halosulfuron-methyl was considered not to be ready biodegradable, since 60% of the theoretical carbon dioxide production value was not achieved within 10 days of reaching 10% biodegradation.

Six 5 litre amber glass culture bottles containing 3 litres of mineral salt medium were inoculated with activated sludge (30 mg solid/litre sourced from a sewage treatment works. The mixture (10 mg Carbon[C]/l) was aerated overnight with carbon dioxide free air to remove any dissolved carbon dioxide. Halosulfuron-methyl (lot no. 011003, purity 99.6%) was added to three of the six test systems (83.7 mg/50 ml ultrapure water). The reference substance, sodium benzoate (30 ml) was added as an aqueous solution (1.72 g/litre) to one test system treated with halosulfuron-methyl and to one system containing the inoculated mineral salts medium alone. A 200 ml volume of ultrapure

water was added to the two remaining vessels that acted as controls. The vessels were continuously flushed with air (from which the carbon dioxide was removed) for 29 days and incubated at ca 20ºC.

The outlet air was trapped in three consecutive volatile traps containing 0.025N nominal barium hydroxide. Determination of CO2 in the barium hydroxide traps was measured on Days 2, 3, 4, 6, 8, 10, 14, 21, 28 and 29 by titration.

Sodium benzoate had been biodegraded by 62% after 6 days and 82% after 29 days in the absence of halosulfuron-methyl, and by 63% after 6 days in its presence, which confirmed that halosulfuron-methyl was not inhibitory to the activity of the microbial inoculum. Cumulative levels of CO2 production in the controls after 29 days (71.5 and 69.3 mg CO2) were within acceptable range for this assay system (recommended maximum = 120 mg CO2 for a three litre culture). These results confirm that the inoculum was viable and that the test was valid.

The mean cumulative CO2 production by halosulfuron-methyl at 10 mg C/l was equivalent, at most, to 3% theoretical CO2 production by the end of the test on Day 29.

Mean cumulative CO2 production by mixtures containing halosulfuron-methyl was equivalent, at most, to 3% of the theoretical value (TCO2, 110.1 mg CO2) by the end of the test on Day 29. Substances are considered to be readily biodegradable in this test if CO2 production is equal to or greater than 60% of the theoretical value within ten days of the level reaching 10%. Halosulfuron-methyl cannot, therefore, be considered to be readily biodegradable.

5.1.2.3 Simulation tests

Water/sediment

The fate of halosulfuron-methyl in water/sediment was assessed using sediment and overlaying water from two UK sites: Bury Pond, a static pond with a clay loam sediment, a pH of 8.1 and 3.6% organic carbon content and Chatsworth, a large perennial lake site with sandy loam sediment, a pH of 6.7 and 5.4% organic matter content. Sediment/water systems were treated with [^{14}C-pyrazole] or [^{14}C-pyrimidine] halosulfuron-methyl at a nominal concentration of 17 µg/l ^{14}C-halosulfuron-methyl (pyrazole or pyrimidine labelled), equivalent to an agricultural use rate of 50 g a.s./ha. The systems were incubated at approximately 20 ± 2°C under aerobic conditions in the dark for up to 100 days.

The proportion of halosulfuron-methyl in the total Bury Pond water-sediment system decreased from a mean of 93.8% AR at time zero to 39.0% AR after 7 days (29.5% AR water phase and 9.5% AR sediment phase) then to 0.2% AR after 100 days. The most significant degradation product was halosulfuron-methyl rearrangement, which accounted for a mean of 2.4% AR after 1 day, increasing to 38.4% AR after 14 days (21.0% AR water phase, 17.4% AR sediment) before declining to 4.1% AR after 100 days (1.1% AR water phase, 3.0% AR sediment). The occurrence and distribution of other degradation products (≥ 5% AR) between the water and sediment are summarised in the table 51 below A number of other minor components including halosulfuron rearrangement and O-demethyl halosulfuron-methyl were observed, each accounting for < 5% AR.

Table 51: Distribution of degradation products (≥ 5% AR) in Bury Pond system

Component	Maximum incidence (%)	Occurrence (days)	Distribution in water phase (%)	Distribution in sediment (%)
Halosulfuron-methyl rearrangement*	38.4	14	21.0	17.4
Halosulfuron*	13.6	14	8.3	5.4
Chlorosulfonamide (pyrazole label)	6.4	14	4.1	2.3
Chlorosulfonamide acid (pyrazole label)	10.3	100	5.4	4.9
Aminopyrimidine (pyrimidine label)	7.7	3	6.3	1.4

In the Chatsworth system the proportion of halosulfuron-methyl in the total water/sediment system decreased from a mean 94.7% AR at time zero to 34.6% AR after 14 days (24.6% AR water phase, 10.0% AR sediment) then to 3.2% AR after 100 days. The most significant degradation product was halosulfuron-methyl rearrangement, which accounted for a mean of 6.9% AR after 3 days, increasing to 40.8% AR after 14 days (29.2% AR water phase, 11.6% AR sediment) before declining to 7.3% AR after 100 days. The occurrence distribution of other degradation products (≥ 5% AR) between the water and sediment are summarised in the table 52 below. A number of other minor components including O-demethyl halosulfuron-methyl were also observed, each accounting for < 6% AR.

Table 52: Distribution of halosulfuron-methyl in Chatsworth system

Component	Day	Level detected (%)	Distribution in water phase (%)	Distribution in sediment (%)
Halosulfuron-methyl	0	94.70	94.70	ns
	1	92.65	86.40	6.25
	3	77.75	69.50	8.25
	7	67.35	57.60	9.75
	14	34.55	24.55	10.00
	30	9.65	6.75	2.90
	59	7.10	4.15	2.95
	100	3.20	2.05	1.15

Throughout the study bound residues increased reaching 50.8-59.6% AR in clay loam and 18.8-23.3% AR in sandy loam soil. Volatile radioactivity represented up to 5.7% AR and was identified as carbon dioxide.

The data for halosulfuron-methyl and its degradates were fitted to first order kinetics to determine the degradation rates the kinetic parameters including the DT_{50} and DT_{90}. Following application to Bury Pond and Chatsworth water/sediment systems halosulfuron-methyl was degraded rapidly in both systems. The half-lives were 6.3 days in the clay loam system and 10.4 days in the sandy loam

system. Halosulfuron-methyl disappeared rapidly from water with DT_{50} of 4.6 and 8.1 days in the pond and lake systems. It also disappeared rapidly from sediment with DT_{50} values of 1.2 and 1.6 days in the pond and lake systems. The DT_{50} and DT_{90} for halosulfuron-methyl rearrangement were slightly longer in both systems. The half-lives were 22.0 days in the clay loam system and 25.4 days in the sandy loam system. Halosulfuron-methyl rearrangement disappeared from water with DT_{50} values of 13.6 and 20.0 days in the pond and lake systems.

The degradation was due to pH dependent hydrolysis and microbial action (in the halosulfuron-methyl hydrolysis study, Point B.2.1.22), degradation was mainly to halosulfuron-methyl rearrangement, aminopyrimidine and chlorosulfonamide with lower levels of chlorosulfonamide acid). This indicates that the production of these degradates results from chemical hydrolysis rather than microbial degradation. In contrast the production of halosulfuron, halosulfuron rearrangement and O-demethyl halosulfuron-methyl is likely to be due to microbial action.

Table 53: The DT_{50} and DT_{90} values for halosulfuron-methyl

	Water phase		**Sediment phase**		**Total system**	
	DT_{50} (days)	**DT_{90} (days)**	**DT_{50} (days)**	**DT_{90} (days)**	**DT_{50} (days)**	**DT_{90} (days)**
Clay loam (Bury Pond)	4.6	15.3	1.2	4.0	6.3	20.9
Sandy loam (Chatsworth)	8.1	26.8	1.6	5.1	10.4	34.4

Calculated using mean values

Other degradation studies:

- Aerobic degradation in soil at 20°C: DT_{50} 17 days (clay loam); 33 days |(loamy sand)

Reference: DAR B.8.1.1.1 (Knight, 2004)

- Anaerobic degradation in soil at 20°C: DT_{50} 37 days (total system)

Reference: DAR B.8.1.1.2.1 (Haynes, 2004)

- Photolysis in soil at 23°C: no DT_{50} could be measured (soil photolysis did not occur)

Reference: DAR B.8.1.1.2.1 (Kesterson, 1992b)

- Soil dissipation: $DT_{50} \leq 1$ day

Reference: DAR B.1.2.2.1 (Wilson, 2004)

5.1.3 Summary and discussion of degradation

Halosulfuron-methyl exhibited pH sensitive hydrolytic breakdown with base-catalysed degradation occurring faster than acid catalysed decomposition. Three major metabolites were identified, aminopyrimidine, chlorosulfonamide and halosulfuron-methyl rearrangement, none of which underwent further degradation. Based on the results of the aqueous photolysis study (dark control,

~25°C), it is possible to estimate a hydrolytic DT_{50} for halosulfuron-methyl rearrangement at pH 9 of 38.26 days.

Halosulfuron-methyl was shown to be photolytically stable when exposed to natural sunlight for 30 days.

Halosulfuron-methyl is not readily biodegradable

In laboratory incubations in dark aerobic natural sediment water systems, halosulfuron-methyl exhibited low persistence, forming the major metabolites halosulfuron-methyl rearrangement‘ (HSMR, max 42% AR in both water and sediment, exhibiting moderate persistence) and halosulfuron rearrangement (HSR, 40% AR in water with the concentration still increasing at study end, 100 days). The unextractable sediment fraction (not extracted by acetonitrile followed by acidified acetonitrile) was a major sink for the pyrimidine and pyrazole ring 14C radiolabel, accounting for 19–60% AR at study end (100 days). Mineralisation of these radiolabels accounted for only 0.2–5.7% AR at the end of the study.

Halosulfuron-methyl degrades in water, to produce stable hydrolysis products. Halosulfuron-methyl is photolytically stable when exposed to natural sunlight for 30 days is not readily biodegradable in a biodegradation screening test. In a water/sediment degradation simulation test, the average DT_{50} of the whole system is 6.3-10.4 days. Mineralisation accounted for only 0.2–5.7% AR after 120 days.

Halosulfuron-methyl degrades in soil in aerobic and anaerobic conditions in the laboratory to produce a number of metabolites, halosulfuron-methyl re-arrangement, halosulfuron re-arrangement, chlorosulfonamide acid, chlorosulfonamide and aminopyrimidine. Although photolysis in soil was shown not to occur in the laboratory, a field dissipation study confirmed that halosulfuron-methyl is rapidly degraded in soil by microbial processes. In these studies levels of soil metabolites were shown to quickly decline in soil but this occurred because of dissipation rather than further degradation.

Based on these findings halosulfuron-methyl is considered to be not rapidly degradable (CLP) in the aquatic environment.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Two studies are available to determine the adsorption and desorption behaviour of halosulfuron-methyl and its metabolites in soil.

In the first study the adsorption and desorption of halosulfuron-methyl and three aerobic soil metabolites, chlorosulfonamide acid, chlorosulfonamide and aminopyrimidine was investigated on four different soils, silt loam, sandy loam, loamy sand and silty clay loam (DAR 8.2.1.1, Nadeau et al., 1991).

Freundlich adsorption constants for halosulfuron-methyl were in the range 31 to 199 indicating that it may be classed as having a medium to high potential for mobility in the four soils tested. Similarly, chlorosulfonamide acid was classified as having a very high potential for mobility based on Koc values of –4.92 to 9.95 for the four soils. Chlorosulfonamide has a medium potential for mobility based on Koc values of 65 to 342 and aminopyrimidine a slight to low potential for mobility based on Koc values of 259 to 8279. The soil dependent order of adsorption (highest to lowest) was generally consistent with the organic content of the soils.

Freundlich first desorption constants (Kd1) for halosulfuron-methyl were in the range 1.16 to 8.05. They were higher than the corresponding Freundlich adsorption constants (0.36 to 3.98), indicating that halosulfuron-methyl was easily desorbed and had a low affinity for each soil type. For chlorosulfonamide acid, the Freundlich first desorption constants were in the range -0.14 to 1.04. Since they were higher than the corresponding Freundlich adsorption constants (-0.03 to 0.16) in three of the four soils, this indicates that chlorosulfonamide acid was easily desorbed and had a low affinity for the soils. The Freundlich first desorption constants for chlorosulfonamide and aminopyrimidine were in the range 1.27 to 8.57 and 4.88 to 194 respectively. Again these were higher than the corresponding Freundlich adsorption constants suggesting that chlorosulfonamide and aminopyrimidine were also easily desorbed and had a low affinity for each soil type.

In a second study, the soil leaching potential of the fourth aerobic soil metabolite, halosulfuron-methyl rearrangement, was studied in soils from Spain, Italy and Japan for three soil types clay loam, loamy sand and sandy loam, respectively. Freundlich adsorption constants for halosulfuron-methyl rearrangement were in the range 0.98-2.40 indicating that it was slightly absorbed to all test soils. Adsorption constants for all three soil types appeared to correlate well with soil organic carbon content. Freundlich desorption constants were slightly higher than the corresponding value for the absorption phase indicating that halosulfuron-methyl rearrangement had a low affinity for soil and was easily removed during the desorption phase. Halosulfuron-methyl rearrangement has a medium to high potential for mobility in the three soils tested.

5.2.2 Volatilisation

Halosulfuron-methyl has a vapour pressure of <1.33 x 10-5 Pa at 25° and a Henry's law constant of 3.5 x 10-6 Pa m3 mol-1. Based on this information it is considered that halosulfuron-methyl has low potential for volatilisation from either soil, plant or water surfaces. Consequently, this is not a likely route of environmental contamination.

5.2.3 Distribution modelling

Not relevant to this dossier.

5.3 Aquatic Bioaccumulation

Table 54: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 107, EEC A8 shake-flask method GLP Purity: 99.9%	LogKow: pH 5: 1.67 pH 7: -0.0186 pH 9: -0.542 23°C	Log Kow <4	DAR B.2.1.19 Pesselman,1991h

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The estimation of bioaccumulation potential in fish is based on the partition coefficient n octanol/water (log P_{ow}) of the active substance. In the section on physico-chemical properties different values for the log P_{ow} depending on the pH were measured (see table 8)

The log P_{ow} values are then compared with the threshold values for bioaccumulation, threshold CLP ≥ 4. Since the log P_{ow} of halosulfuron-methyl is lower than both threshold values, the potential risk for bioaccumulation in tissues of aquatic organisms is low.

5.3.1.2 Measured bioaccumulation data

No data available and not required (see 5.3.1.1).

5.3.2 Summary and discussion of aquatic bioaccumulation

The measured log P_{ow} values for halosulfuron-methyl were all below the threshold value for bioaccumulation, i.e. threshold CLP ≥ 4. Therefore, no experimental bioaccumulation data are required. The potential risk for bioaccumulation of halosulfuron-methyl in tissues of aquatic organisms is considered low.

5.4 Aquatic toxicity

A brief summary of the aquatic toxicity studies listed in the DAR for the three trophic levels fish, aquatic invertebrates and algae/aquatic plants are reported below. Only reliable and acceptable ecotoxicity tests from the DAR were used.

Table 55: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Active substance: halosulfuron-methyl (HSM)			
Acute toxicity – fish *Oncorhynchus mykiss* Rainbow trout US EPA FIFRA E, 72-1 ASTM E 729-88 GLP	96 hour LC_{50}: >131 mg/l NOEC: 131 mg/l	Purity 98.5%	DAR B.9.2.1.1 Holmes and Swigert, 1993a
Acute toxicity – fish *Lepomis macrochirus* Bluegill sunfish US EPA FIFRA 72-1 ASTM E 729-88 GLP	96 hour LC_{50}: >118 mg/l NOEC: 118 mg/l	Purity 98.5%	DAR B.9.2.1.2 Holmes and Swigert, 1993b
Chronic toxicity – fish early life stage *Oncorhynchus mykiss* Rainbow trout OECD202 EEC C.2 GLP	LOEC: 106 mg/l NOEC: 34 mg/l	Purity 99.74%	DAR B.9.2.2.2 Graves, Mank and Swigert, 1993
Acute toxicity –aquatic invertebrates *Daphnia magna* US EPA FIFRA 72-1 ASTM E 729-88 GLP	48 hour EC_{50}: >107 mg/l NOEC: 107 mg/l	Purity 98.5%	DAR B.9.2.4.1.1 Holmes and Swigert, 1993c

Table 55: Summary of relevant information on aquatic toxicity (Continued)

Acute toxicity –aquatic invertebrates *Mysidopsis bahi* Mysid shrimp US EPA FIFRA 72-3 ASTM E 729-88 GLP	96 hour LC_{50}: 109 mg/l NOEC: 72 mg/l	Purity 98.5%	DAR B.9.2.4.3 Swigert and Smith, 1993
Acute toxicity –aquatic invertebrates *Lymnaea peregra* Gastropod mollusc No method available. Study based on OECD principles for aquatic toxicity tests ans on the available literature on snails GLP	96 hour LC_{50}: >89.9 mg/l NOEC: 89.9 mg/l	Purity 99.6%	DAR B.9.2.4.4 Jenkins, 2004b
Chronic toxicity –aquatic invertebrates *Daphnia magna* US EPA FIFRA 72-4 ASTM E 1193-87 GLP	Adult survival NOEC: 5.7 mg/l Reproduction LOEC: 6.9 mg/l Growth LOEC: 6.9 mg/l Overall LOEC: 6.9 mg/l	Purity 98.5%	DAR B.9.2.5.1.1 Zelinka, Martin and Swigert, 1993a
Chronic toxicity –aquatic invertebrates *Daphnia magna* US EPA FIFRA 72-4 ASTM E 1193-87 GLP	Adult survival NOEC: 7.2 mg/l Reproduction NOEC: 7.2 mg/l LOEC: >7.2 mg/l Growth NOEC: 7.2 mg/l Overall NOEC: 7.2 mg/l LOEC: >7.2 µg/l	Purity 98.3%	DAR B.9.2.5.1.2 Zelinka, Martin and Swigert, 1993b
Effects on algal/plant growth *Pseudokirchneriella subcapitata* Green alga US EPA FIFRA 123-2 US 40 CFR 797.1075 ASTM E1218-90 GLP	5-day E_yC_{50}: 0.00194 mg/l 5-day ErC_{50}: 0.00507 mg/l; 5-day EbC_{50}: 0.00203 mg/l NOEC: 0.00063 mg/l	Purity 98.5% $EC_{50} \leq 0.1$ mg/l	DAR B.9.2.6.1 Thompson and Swigert, 1993

Table 55: Summary of relevant information on aquatic toxicity (Continued)

Effects on algal/plant growth *Anabaena flos-aquae* Blue-green alga US EPA FIFRA 123-2 ASTM E 1218-90 GLP	NOEC: 0.050 mg/l	Purity 99.74% $EC_{50} \leq 0.1$ mg/l	DAR B.9.2.6.2 Thompson and Swigert, 1994a
Effects on algal/plant growth *Navicula pelliculosa* Diatom (freshwater) US EPA FIFRA 123-2 ASTM E 1218-90 GLP	5-day EC_{50}: >0.350 mg/l NOEC: 0.350 mg/l	Purity 99.74%	DAR B.9.2.6.3 Thompson and Swigert, 1994b
Effects on algal/plant growth *Skeletonema costatum* Diatom (marine) US EPA FIFRA 123-2 GLP	5-day EC_{50}: >0.400 mg/l NOEC: 0.400 mg/l	Purity 99.74%	DAR B.9.2.6.4 Thompson and Swigert, 1994c
Effects on algal/plant growth *Pseudokirchneriella subcapitata* Green algae	72 hour E_rC_{50}: 0.0050 mg/L 72-hour growth rate NOEC: 0.0010 mg/L.	Purity 99.9% $EC_{50} \leq 0.1$ mg/l	Additional report to DAR. Addendum, August 2012. DAR B.9.2.6.9 Seki, 2008
Effects on sediment dwellers *Chironomus riparius* OECD 219 (draft 2001)	28-day EC_{50}: >10 mg/l NOEC: 5 mg/l	Purity 99.6%	DAR B.9.2.7.2 Cockcroft, 2005
Effects on algal/plant growth *Lemna gibba* Duckweed OECD 221 (draft 2002)	7 day E_bC_{50}: 0.000217 mg/l 7 day E_rC_{50}: 0.000491 mg/l 7 day $E_{wt}C_{50}$: 0.000823 mg/l NOEC: 0.00003 mg/l LOEC: 0.000141 mg/l	Purity 99.6% $EC_{50} \leq 0.1$ mg/l	DAR B.9.2.8.1 Jenkins, 2005a

Table 55: Summary of relevant information on aquatic toxicity (Continued)

Degradation product: halosulfuron-methyl rearrangement (HSMR)			
Acute toxicity fish *Oncorhynchus mykiss* Rainbow trout EU C.1 OECD 203 GLP	96 hour LC_{50}: >15.3 mg/l	Purity 100%	DAR B.9.2.1.3 Jenkins, 2004a
Acute toxicity aquatic invertebrates *Daphnia magna* EU C.2 OECD 202 GLP	48 hour EC_{50}: >19.2 mg/l NOEC: 19.2 mg/l	Purity 100%	DAR B.9.2.4.1.2 Jenkins, 2004c
Effects on algal/plant growth *Pseudokirchneriella subcapitata* Green alga EU C.3 OECD 201 GLP	72 hour E_bC_{50}: 17.5 mg/l 0-72 hour E_rC_{50}: >20.3 mg/l NOEC: 4.9 mg/l	Purity 100%	DAR B.9.2.6.6 Jenkins, 2004d
Degradation product: aminopyrimidine (AP)			
Effects on algal/plant growth *Pseudokirchneriella subcapitata* EU C.3 OECD 201 GLP	72 hour E_bC_{50}: 269 mg/l 0-72 hour E_rC_{50}: 521 mg/l NOEC: 62.5 mg/l	Purity 100%	DAR B.9.2.6.7 Flatman, 2005
Degradation product: halosulfuron (HS)			
Effects on algal/plant growth *Pseudokirchneriella subcapitata* EU C.3 OECD 201 GLP	72 hour E_bC_{50}: 84.7 mg/l 0-72 hour E_rC_{50}: >98 mg/l NOEC: 22 mg/l	Purity 98.6%	DAR B.9.2.6.5 Cockcroft, 2004

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Three groups of at least 10 Rainbow trout (*Oncorhynchus mykiss*), were exposed to halosulfuron-methyl at the nominal test concentration of 120 mg/l over a 96-hour period under flow-through conditions. Fish were acclimatised for approximately 50 hours; the mean wet-weight and fork length of ten controls at dosing was 3.59 g (range = 2.75-4.28 g) and 56 mm (range = 50-61 mm) respectively. A negative control (well water) and solvent control (dimethylformamide, DMF at 1.2 ml/l) group each containing two groups of at least 10 rainbow trout were also maintained. Test media were prepared by injection of a stock solution of halosulfuron-methyl in DMF (0.1 g/ml) into the mixing chamber of the dosing apparatus where it was mixed with well water to achieve the nominal dosing concentration 120 mg/l. The mean measured concentration of halosulfuron-methyl was 131 mg/l. Mean measured levels were 110%, 109% and 108% of the nominal value at 0, 48 and 96 hours,

respectively, equivalent to a mean value of 109% of the nominal. A white precipitate was observed in the mixing chambers but not in the test chambers.

The 96-hour LC_{50} value of halosulfuron-methyl to Rainbow trout was >131 mg/l. The 96-hour NOEC of halosulfuron-methyl was 131 mg/l.

In a second study three groups of at least 10 Bluegill sunfish (Lepomis macrochirus) were exposed to halosulfuron-methyl at the nominal test concentration of 120 mg/l over a 96-hour period under flow-through conditions. Fish were acclimatised for approximately 26 hours; the mean wet-weight and fork length of ten controls at dosing was 0.61 g (range = 0.29-1.07 g) and 31 mm (range = 27-34 mm) respectively. A negative control (well water) and solvent control (DMF at 1.2 ml/l) group each containing two groups of at least 10 bluegills were also maintained. Test media were prepared by injection of a stock solution of halosulfuron-methyl in DMF (0.1 g/ml) into the mixing chamber of the dosing apparatus where it was mixed with well water to achieve the nominal dosing concentration 120 mg/l. The corrected mean measured concentration of halosulfuron-methyl over the test period was 118 mg/l. Mean measured levels were 98%, 97% and 100% of the nominal value at 0, 48 and 96 hours respectively, equivalent to a mean value of 98% of nominal. A white precipitate was observed in the mixing chambers but not in the test chambers. There were no mortalities in the negative control or solvent control groups. No mortalities occurred in the 118 mg/l treatment group. All fish (treated and control) appeared healthy and normal throughout the test.

The 96-hour LC_{50} value of halosulfuron-methyl to Bluegill sunfish was >118 mg/l. The 96-hour NOEC of halosulfuron-methyl was 118 mg/l.

Metabolite study:

The acute toxicity of halosulfuron-methyl rearrangement was investigated with the more sensitive of the fish species used to test the acute toxicity of the active substance - Rainbow trout (*Oncorhynchus mykiss*)

Measured concentrations of halosulfuron-methyl rearrangement ranged from 13.35 to 17.36 mg/l in samples of freshly prepared and expired (24 hour old) media with an overall mean measured concentration of 15.3 mg/l, which was lower than the stated limit of aqueous solubility of halosulfuron-methyl rearrangement (24 mg/l).

No treatment-related mortality was observed in the test. Based on this data the 96-hour LC_{50} was >15.3 mg/l.

Sublethal effects were observed within 2 hours of exposure and during the 96-hour exposure period effects included darkened pigmentation, hyperventilation, trailing faeces and mucus and loss of coordination. Two fish showed signs of recovery at the end of the 96-hour period.

Based on this data the 96-hour LC_{50} was >15.3 mg/l.

Comment:

The 96-hour NOEC of halosulfuron-methyl rearrangement, stated in the study report was 3.61 mg/l, based on results from the range finding test.

The RMS does not consider appropriate the use of the range finding test for the NOEC evaluation, due to the fact that only three fishes per treatment were tested. Therefore a conservative NOEC of < 15.3 mg/l halosulfuron-methyl rearrangement is proposed.

5.4.1.2 Long-term toxicity to fish

Four replicate groups of up to 30 newly fertilised rainbow trout (*Oncorhynchus mykiss*) embryos were exposed to halosulfuron-methyl under flow-through conditions at nominal test concentrations of 1.2, 3.6, 11, 33 and 100 mg/l in an 87-day fish early life stage toxicity test. A negative control group (diluent medium) and a solvent control group (DMF at 1.2 ml/l) were also maintained. The mean measured concentrations were 1.2, 3.8, 12, 34 and 106 mg/l.

There were no apparent treatment-related effects upon survival of Rainbow trout exposed to any of the test concentrations of halosulfuron-methyl during the 60-day post hatch early life stage toxicity test.

Growth was the most sensitive biological parameter in the test. Body lengths at 28 and 60 days, post hatch, and body weights at the end of the test were reduced at 106 mg/l compared to the control group.

The 87-day LOEC of halosulfuron-methyl to Rainbow trout was 106 mg/l. The 87-day NOEC of halosulfuron-methyl was 34 mg/l.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Daphnia magna (3 replicates of 10 neonates; <24 hours old) were exposed under flow-through conditions to a nominal test concentration of 120 mg/l of halosulfuron-methyl for 48 hours. A negative control (well water) and a solvent control (dimethylformamide (DMF) at 1.2 ml/l) group were also maintained. A stock solution was prepared by direct addition of the test substance to DMF solvent. An aliquot of the stock solution was then injected into the mixing chamber where it was mixed with well water to achieve the nominal dosing concentration 120 mg/l. For each test chamber approximately 13.8 volume replacements were achieved every 24 hours. Immobilisation, clinical signs and behaviour were observed at 6.5, 24 and 48 hours. Dissolved oxygen, conductivity, temperature and pH were monitored. Test aquaria were aerated. The corrected mean measured concentration of halosulfuron-methyl was 107 mg/l. Mean measured levels were 90%, 89% and 89% of the nominal value at 0, 24 and 48 hours respectively (averaged 89% of nominal). A white precipitate was observed in the mixing chambers but not in the test chambers throughout the study.

The 48-hour EC_{50} value of halosulfuron-methyl to *Daphnia magna* was greater than 107 mg/l. The 48-hour NOEC for halosulfuron-methyl was 107 mg/l.

In a second study to investigate the short-term toxicity to aquatic invertebrates mysid shrimps (≤24 hours old) were exposed to five nominal concentrations of halosulfuron-methyl at 15.6, 25.9, 43.2, 72.0 and 120 mg/l over a 96 hour period under flow-through conditions. A negative control group (seawater) and a solvent control group (DMF at 1.2 ml/l) were also maintained. The four lowest test groups, the negative control and the solvent control groups comprised two replicate 500 ml glass test vessels each containing 10 mysids and the highest test group had two replicates of 15 mysids. The test was conducted at 25 ± 1ºC with a photoperiod of 16 hours light: 8 hours dark (intensity 861 Lux) with 30 min low light at the beginning and end of each light phase.

A primary stock solution was prepared by direct addition of the test substance to DMF at a concentration of 0.1 g/l. Aliquots of the stock solution were then diluted with DMF to prepare four additional stock solutions at concentrations 0.060, 0.036, 0.022 and 0.013 g/ml. An aliquot of each stock solution was injected into the mixing chamber where it was mixed with saltwater to achieve the nominal dosing concentrations 15.6, 25.9, 43.2, 72.0 and 120 mg/l. The corrected mean measured

concentrations for 0, 48 and 96 hours were 16, 25, 43, 72 and 127 mg/l, equivalent to 97-106% of nominal values. All calculated values are based upon mean measured concentrations. There was a white precipitate in the mixing chambers of 43, 72 and 127 mg/l concentrations throughout the test. No precipitate was found in the test chambers.

The LC_{50} and NOEC values of halosulfuron-methyl to *Mysidopsis bahia* were:

72h LC_{50} = >127 mg/l

96h LC_{50} = 109 mg/l

96h NOEC = 72 mg/l

A study is available for the acute toxicity (96-hour) in gastropod molluscs. One group of twenty juvenile *Lymnaea peregra* (4 replicates of 5; shell length at dosing 4-5 mm) were exposed under semi-static conditions for 96 hours to halosulfuron-methyl at a nominal concentration of 100 mg/l, selected following a range-finding study conducted at nominal test concentrations of 0.1, 1, 10 and 100 mg/l. The test and control media were renewed after 48 hours. The test substance was added directly to dilution medium and treated with ultrasound for 20 minutes, stirred for approximately four hours and adjusted to the final volume with dilution medium.

Measured concentrations of halosulfuron-methyl in fresh and expired media ranged from 86-96% of nominal values with an overall mean measured concentration of 89.9 mg/l.

There were no mortalities of snails in the test groups. There were two deaths in the control group, one of which was attributed to accidental damage of the shell. No treatment-related effects were observed.

The 96-hour LC_{50} value of halosulfuron-methyl to *Lymnaea peregra* was >89.9 mg/l. The 96-hour NOEC of halosulfuron-methyl was ≥89.9 mg/l.

Metabolite study:

The acute toxicity to *Daphnia magna* of the degradation product, halosulfuron-methyl rearrangement, was determined. No immobilisation or adverse effects with treatment with halosulfuron-methyl rearrangement was noted. Environmental parameters remained within the OECD 202 limits during the study.

Measured concentrations of halosulfuron-methyl rearrangement ranged between 17.56 and 20.89 mg/l with an overall mean measured concentration of 19.2 mg/l. Although the mean measured concentration is less than the stated limit of aqueous solubility of halosulfuron-methyl rearrangement, a condition of maximum attainable exposure was thought to have been achieved.

The 48-hour EC_{50} value of halosulfuron-methyl rearrangement to *Daphnia magna* was greater than 19.2 mg/l. The 48-hour NOEC for halosulfuron-methyl rearrangement was 19.2 mg/l.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Daphnia magna (<24 hours old) were exposed under dynamic flow-through conditions for 21 days to halosulfuron-methyl at test concentrations of 7.5, 15, 30, 60 and 120 mg/l (based on the results of an acute toxicity study) dissolved in DMF at 1.2 ml/l. A negative control group (well water) and solvent control group (DMF at 1.2 ml/l) were also maintained. Overall mean measured concentrations were 6, 9, 14, 28, 57 and 114 mg/l. Ten replicate test compartments (300 ml glass beakers) were maintained in each treatment and control group chamber, seven containing one daphnid and three

containing five daphnids. The experimental design differs from that recommended in OECD 211 which recommends 40 animals divided into four groups of 10 animals at each test concentration or a minimum of 20 animals per concentration divided into two or more replicates with an equal number of animals.

The parental lengths for halosulfuron-methyl treatment groups were statistically significantly reduced compared to the negative control and solvent control. Also, dry weights for the treatment groups were statistically significantly reduced. There were no apparent treatment-related effects upon survival of *Daphnia magna* exposed to halosulfuron-methyl at 6.9, 14, 28 or 57 mg/l. Survival was significantly reduced at 114 mg/l.

The LOEC for neonate production was 6.9 mg/l, the lowest test concentration tested.

In a second 21-day chronic study *Daphnia* (<24 hours old) were exposed under dynamic flow-through conditions for 21 days to halosulfuron-methyl at nominal test concentrations of 0.47, 0.94, 1.9, 3.8 and 7.5 mg/l dissolved in DMF at 0.2 ml/l. Exposure concentrations were based on results of a previous 21-day chronic study. A negative control group (well water) and solvent control group (DMF at 0.2 ml/l) were also maintained. Ten 300 ml glass beakers were established per test and control group; seven containing one daphnid and three containing five daphnids. A primary stock solution was prepared by direct addition of the test substance to DMF at a concentration of 37.5 mg/ml. Aliquots of the stock solution were diluted with DMF to prepare four additional stocks at concentrations of 18.8, 9.38, 4.69 and 2.34 mg/ml. An aliquot of each stock solution was injected into the mixing chamber where it was mixed with well water to achieve the nominal dosing concentrations 0.47, 0.94, 1.9, 3.8 and 7.5 mg/l.

Overall mean measured concentrations of halosulfuron-methyl were 0.45, 0.98, 1.8, 4.0 and 7.2 mg/l for nominal test concentrations of 0.47, 0.94, 1.9, 3.8 and 7.5 mg/l, respectively, equivalent to 96, 104, 95, 105 and 96 % of nominal values. Samples taken in response to diluter malfunction indicated that test concentrations were not altered substantially. This incidence did not appear to adversely affect the results of the study.

There were no apparent treatment-related effects upon survival, growth, or reproduction of *Daphnia magna* exposed to halosulfuron-methyl at 0.45, 0.98, 1.8, 4.0 and 7.2 mg/l. The LOEC of halosulfuron-methyl to Daphnia magna was >7.2 mg/l, the highest treatment level tested, and the NOEC was 7.2 mg/l.

5.4.3 Algae and aquatic plants

Six studies are available to determine effects on algal/plant growth and growth rate.

- Green alga

Reference: DAR B.9.2.6.1 (Thompson and Swigert, 1993)

Cultures of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*; initial cell concentration approximately 3 x 10^3 cells/ml) were exposed to halosulfuron-methyl for 5 days at nominal test concentrations of 0.00063, 0.0013, 0.0025, 0.0050 and 0.010 mg/l, selected following a range finding study. A negative control group (culture medium) and a solvent control group (DMF at 0.1 ml/l) were also maintained. The test was conducted at 24 ± 2ºC under static conditions with continuous illumination (4310 lux).

At the start of the study, the mean measured levels of halosulfuron-methyl in samples of the stock solutions ranged between 112 and 135% of their nominal values. The mean measured concentration

for the 10 mg/l stability preparation on Day 0 and 5 was 10.7 and 8.13 mg/l, respectively, representing 107 and 81.3% of the nominal value. The EC_{50} value was calculated using nominal test concentrations.

The 5-day E_yC_{50}, based on nominal test concentrations was 0.0019 mg/l, the E_rC_{50} was 0.00507 µg/l and the E_bC_{50} was 0.00203 µg/l. The 5-day NOEC, based on nominal test concentrations was 0.00063 mg/l. Environmental parameters remained within acceptable limits throughout the study.

- Blue-green alga

Reference: DAR B.9.2.6.2 (Thompson and Swigert, 1994a)

Cultures of *Anabaena flos-aquae*; initial cell concentration approximately 3 x 10^3 cells/ml) were exposed to halosulfuron-methyl for 5 days at nominal test concentrations of 25, 50, 100, 200 and 400 µg a.s./l, selected following a range finding study. A negative control group (culture medium) and a solvent control group (dimethylformamide (DMF) at 20.0µl/l) were also maintained. The test was conducted at 24 ± 2ºC under static with continuous illumination (2150 lux).

At the start of the study, the mean measured levels of halosulfuron-methyl in samples of the stock solutions were between 80-91% of the nominal values. The mean measured concentration for 10 mg a.s./l on Day 0 and 5 was 10.2 and 7.89 mg a.s./l, respectively, representing 102 and 79% of the nominal value. The EC_{50} value was calculated using nominal test concentrations.

Algal growth inhibition at 25, 50 and 100 µg/l was 35, 49 and -2.8 %. According to the original study report, although algal growth was reduced at 25 and 50 µg/l, indicated by lower mean cell densities, there was no dose response and values were not statistically different from the pooled control. Algal growth at 200 and 400 µg/l was significantly reduced by 86 and 98% respectively compared to the pooled control. The study report concluded that EC_{50}, based on nominal test concentrations was 158 µg a.s./l and the 5-day NOEC, based on nominal test concentrations was 100 µg a.s./l.

Comment:

The RMS deems that dose-response is not monotonic due to anomalous results occurred in the 100 µg/l test. Raw data in fact indicate that from day 1 to day 2, Replicate A collapsed from 10,000 to 7,000 cells/ml, Replicate B showed a growth pattern similar to controls and Replicate C collapsed from 18,000 to 6,000 cells/ml. Afterwards, the growth of replicates A and C re-started exponentially until the end of test (day 5). This growth pattern is not observed in any other treatment.

Consequently, the RMS considers the EC_{50} of 158 µg a.s./l not reliable.

A conservative $NOEC_{biomass}$ can be set at nominal 50 µg a.s./l (i.e. the highest concentration without statistically significant inhibition of cell density).

- Diatom (freshwater)

Reference: DAR B.9.2.6.3 (Thompson and Swigert, 1994b)

Based on a range finding study and three times the maximum field application rate (0.157 lb/acre), five replicate 250-ml Erlenmeyer flasks containing Navicula pelliculosa cultures in sterilised, freshwater algal medium with vitamins were exposed for 5 days under static conditions to halosulfuron-methyl at the nominal test concentration of 350 µg a.s./l. A negative control group (culture medium) and a solvent control group (DMF at 35.0 µl/l) were also maintained. The initial cell concentration was approximately 3x103 cells/ml. The test was conducted at 24 ± 2ºC under continuous illumination (intensity 4310 lux).

At the start of the study, the mean measured levels of halosulfuron-methyl in samples of the stock solutions were 8924 and 88528 mg a.s./l, both equivalent to 89% of their nominal values. The mean measured concentration for 10 mg a.s./l on Day 0 and 5 was 9.7 and 7.7 mg a.s./l, respectively, representing 97 and 77% of the nominal value. The EC_{50} value was calculated using nominal test concentrations.

There were no treatment-related significant ($p<0.05$) differences between the mean cell density in the pooled control replicates and the 350 µg a.s./l treatment group. The 5-day EC_{50}, based on nominal test concentrations was >350 µg a.s./l. The 5-day NOEC, based on nominal test concentrations was 350 µg a.s./l. Environmental parameters remained within acceptable limits throughout the study.

Diatom (marine)

Reference: DAR B.9.2.6.4 (Thompson and Swigert, 1994c)

Based on a range finding study and the maximum field application rate, three replicate 250-ml Erlenmeyer flasks containing *Skeletonema costatum* cultures in sterilised, saltwater algal medium were exposed for 5 days under static conditions to halosulfuron-methyl at nominal test concentrations of 25, 50, 100, 200 and 400 µg a.s./l. A negative control group (culture medium) and a solvent control group (DMF at 20.0 µl/l) were also maintained. The initial cell concentration was approximately 10 x 10^3 cells/ml. The test was conducted at 20 ± 2ºC under continuous illumination (intensity 4310 lux).

At the start of the study, the mean measured levels of halosulfuron-methyl in samples of the stock solutions were 544, 1107, 2350, 4624, 9129 and 91831 mg a.s./l and ranged between 89-94% of the nominal values. The mean measured concentration for 10 mg a.s./l on Day 0 and 5 was 9.4 and 5.4 mg a.s./l, respectively, representing 94 and 54% of the nominal value. The EC_{50} value was calculated using nominal test concentrations.

There were no treatment-related significant differences between the mean cell density in the pooled control replicates and the 25, 50, 100, 200, and 400 µg a.s./l treatment groups. The 5-day EC_{50}, based on nominal test concentrations was >400 µg a.s./l. The 5-day NOEC, based on nominal test concentrations was 400 µg a.s./l. Environmental parameters remained within acceptable limits throughout the study.

- Green algae

Reference: Additional report to DAR. Addendum, August 2012, (Seki 2008).

Cultures of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*; initial cell concentration approximately 104 cells/mL) were exposed to halosulfuron-methyl for 3 days at nominal test concentrations of 0.032, 0.010, 0.0032, 0.0010 and 0.00032 mg/L, selected following preliminary studies. A negative control group (culture medium) and a solvent control group (DMF at 0.1 mL/L) were also maintained. Three replicates were established for each exposure concentration and six for both control groups. The test was conducted at 21-24 ± 2ºC with continuous shaking and illumination (60-120 µE/m^2/s ± 15%).

The measured concentration of halosulfuron-methyl in the exposures solutions were 86-91% of the nominal concentration at the start of exposure and 86-92% of the nominal concentration at the end of exposure period. The measured concentrations were within ± 20% of the nominal concentration during the exposure period.

At halosulfuron-methyl levels of 0.032 and 0.010 mg/l algal growth was inhibited. At 0.0032 mg/l the algal growth was logarithmic, although inhibition was observed. Algal growth at halosulfuron-methyl levels of 0.0010 and 0.00032 mg/l were similar to the control and vehicle control.

Observation of distended cells (0.032 and 0.010 mg/l) and partially aggregated cells (0.032 - 0.0032 mg/l) compared to the controls were determined. The conditions of cells in the other exposure levels were similar to the controls. In the controls, the condition of cells was normal.

The 3-day E_rC_{50}, based on nominal test concentrations was 0.0050 mg/L. The 3-day growth rate NOEC, based on nominal test concentrations was 0.0010 mg/L. Environmental parameters remained within acceptable limits throughout the study.

- Duckweed

Reference: DAR B.9.2.8.1 (Jenkins, 2005)- key study

Four replicate flasks of *Lemna gibba*, strain G3 (containing 12 fronds per replicate) were exposed for 7 days under semi-static conditions (renewal of flasks on Days 3 and 5) to halosulfuron-methyl at nominal test concentrations of 0.032, 0.1, 0.32, 1, 3.2 and 10 µg/l. Test levels were based on the results of a range-finding test conducted using concentrations 0.01, 0.1, 1 and 10 µg/l. A formulation trial was also conducted using dimethylformamide and ultra- high purity water, along with two main tests, which were invalidated due to formulation problems. Four replicate flasks of a negative control group (20X AAP culture medium) were also maintained. The test was conducted at 23.2 25.7ºC with continuous illumination (light intensity 7588-7926 lux).

The test substance was added directly to dilution medium to give a nominal stock concentration of 10 mg/l. To aid dissolution, ultrasound treatment (15 minutes) and vigorously shaking (5 minutes) were employed. An aliquot of the stock solution was then diluted in dilution medium to prepare an intermediary stock solution of 100 µg/l, which was then serially diluted to give the required nominal test concentrations.

Findings:

Analysis of test concentrations in media:

Measured levels of halosulfuron-methyl in samples of freshly prepared media ranged from 76-122% of their nominal values. Measured levels in samples of expired media ranged from 1-16% of their nominal value at 10 µg/l, from below the limit of quantification (0.03 µg a.s./l) to 13% at 1 and 3.2 µg/l, and could not be quantified at 0.032 to 0.32 µg/l.

The overall mean measured levels of halosulfuron-methyl were 0.006, 0.009 (estimated values), 0.03, 0.141, 0.733 and 2.584 µg/l.

Measured levels of halosulfuron-methyl in samples of expired media from flasks at nominal concentrations of 0.1, 0.32 and 1 µg/l, incubated without plants ranged from 31-71%. Comparison with flasks containing plants suggested that the presence of Lemna had an effect on the stability of the halosulfuron-methyl under the conditions of the test.

Plant growth:

Based on the area under the growth curve and biomass, growth appears to have been stimulated at 0.006, 0.009 and 0.03 µg/l. In the phytostatic/phytotoxic extension test, subcultures established from test cultures at 0.733 and 2.584 µg/l had re-established growth after seven days of incubation, indicating that at these levels halosulfuron-methyl was phytostatic.

Observations:

Gibbosity (humped appearance), chlorosis and reductions in frond size were observed in cultures at 0.733 and 2.584 µg/l. In cultures at 0.141 µg/l, chlorosis and reductions in root length were observed in a maximum of approximately 20% of the plants.

Environmental parameters:

Environmental parameters in control and test vessels remained within acceptable limits throughout the study but with the following exceptions:

on days 3 and 5, the difference in the light intensity over the test area (+17% and +16%, respectively) exceeded the recommended range (± 15%) on two occasions. Since the test flasks were allocated to new positions after each renewal of the test media, this variation is not thought to have affected the reliability of the results;

the pH of control and test cultures increased by more than 1.5 pH units between renewals during the test. Such deviation however does not affect the reliability of the results since the validity criteria are met.

Table 56: Inhibition of growth of *Lemna gibba* following exposure to halosulfuron-methyl for 7 days

Mean measured concentrations (µg/l)	Replicate number	Area under curve at 7 days	Mean (% I)	Growth rate (0-7 days)	Mean (% I)	Final biomass at 7 days (mg)*	Mean (% I)
Control	R_1 R_2 R_3	444 470 482	465	0.4162 0.4206 0.4219	0.4196	16.5 16.0 17.6	16.7
0.006$	R_1 R_2 R_3	481 482 554	506 (0)	0.4206 0.4168 0.4326	0.4234 (0)	18.7 17.4 21.8	19.3 (0)
0.009$	R_1 R_2 R_3	585 546 560	564 (0)	0.4421 0.4256 0.4372	0.4350 (0)	24.2 22.3 22.5	23.0 (0)
0.03	R_1 R_2 R_3	477 583 505	521 (0)	0.4162 0.4355 0.4321	0.4279 (0)	18.7 25.4 20.2	21.4 (0)
0.141	R_1 R_2 R_3	249 323 361	311 (33)	0.3241 0.3682 0.3861	0.3595 (14)	13.8 16.5 17.1	15.8 (5)
0.733	R_1 R_2 R_3	63 83 69	72 (85)	0.1261 0.1720 0.1488	0.1489 (65)	7.4 7.7 7	7.4 (56)
2.584	R_1 R_2 R_3	21 40 46	36 (92)	0.0411 0.0866 0.0990	0.0756 (82)	6.7 5.9 7.7	6.78 (59)

R_1-R_3: Replicates 1 - 3.
*: Final biomass based on dry weight of fronds.
% I: Percentage inhibition compared to the control cultures.
$: Estimated value.

Table 57: Summary of endpoints of *Lemna gibba* following exposure to halosulfuron-methyl for 7 days

	Mean measured concentration of halosulfuron-methyl (µg/l)
Area under the growth curve	
E_bC_5 (Day 7)	0.0628 (0.0401 & 0.0917)

E_bC_{50} (Day 7)	0.217 (0.169 & 0.282)
E_bC_{90} (Day 7)	1.17 (0.644 & 2.23)
Average specific growth rate	
E_rC_5 (Day 7)	0.0411 (0.0224 & 0.0705)
E_rC_{50} (Day 7)	0.491 (0.403 & 0.596)
E_rC_{90} (Day 7)	3.13 (2.16 & 4.74)
Dry weight/biomass	
$E_{wt}C_5$ (Day 7)	0.140 (0.0573 & 0.264)
$E_{wt}C_{50}$ (Day 7)	0.823 (0.455 & 1.74)
$E_{wt}C_{90}$ (Day 7)	>2.584
LOEC	0.141
NOEC	0.03

( ): 95% confidence limits

Conclusions:
After 7 days exposure of *Lemna gibba* to halosulfuron-methyl, the E_bC_{50}, E_rC_{50} and $E_{wt}C_{50}$ values for inhibition of growth were 0.217, 0.491 and 0.823 µg/l, respectively.

The NOEC of halosulfuron-methyl for area under the growth curve and growth rate was 0.03 µg/l; based on the final biomass (dry weight) the LOEC was 0.141 µg/l.

At 0.733 and 2.584 µg/l, halosulfuron-methyl was phytostatic as potential for recovery was demonstrated.

Metabolite studies:

- Green algae

Reference: DAR B.9.2.6.6 (Jenkins, 2004d).

The effect of the degradation product, halosulfuron-methyl rearrangement, on *Selenastrum capricornutum* was investigated.

At the start of the test, the measured levels of halosulfuron-methyl rearrangement were 1.29, 2.68, 5.40, 11.21 and 21.08 mg/l (corresponding to nominal values of 1.5, 3, 6, 12 and 24 mg/l, respectively) and ranged between 86-93% of nominal values. After 72 hours, the measured levels were 1.37, 2.24, 4.40, 9.29 and 19.43 mg/l and ranged between 73-91% of nominal values (81 and 106% of 0-hour measured values). The overall mean measured concentrations of halosulfuron-methyl rearrangement were 1.33, 2.46, 4.90, 10.3 and 20.3 mg/l.

The measured levels at 72 hours in flasks containing no algae were 1.31 and 22.64 mg/l (corresponding to nominal values of 1.5 and 24 mg/l, respectively), 87 and 94% of nominal values. Comparison with flasks containing algae indicated that the stability of the test substance was not affected by the presence of algal cells.

Halosulfuron-methyl rearrangement significantly inhibited algal growth at 10.3 and 20.3 mg/l.

Microscopic examination of test cultures after 48 hours exposure revealed some algal cells at 10.3 and 20.3 mg/l to be swollen compared to the control group. After 72 hours swollen cells were only noted at 20.3 mg/l.

The 72-hour EbC_{50} value (based on area under the growth curve) was 17.5 mg/l (95% confidence limits 10.3-20.3 mg/l).

The 72-hour ErC_{50} value based on average specific growth rate was >20.3 mg/l (13% inhibition).

The 72-hour NOEC was 4.90 mg/l.

The pH of control and test cultures (except highest test concentration) increased by >1.5 units. This was associated with high cell growth. All other measurements of water quality (temperature and pH) in control and test vessels remained within the OECD 201 limits throughout the study.

- Green algae

Reference: DAR B.9.2.6.7 (Flatman, 2005).

The effect of the degradation product, aminopyrimidine, on *Selenastrum capricornutum* was investigated.

At the start of the test, the measured levels of aminopyrimidine were 62.0, 123, 246, 488 and 977 mg/l (corresponding to nominal values of 62.5, 125, 250, 500 and 1000 mg/l, respectively) and ranged between 97.7-99.2% of nominal values. After 72 hours, the measured levels were 63.4, 126, 250, 503 and 1011 mg/l and ranged between 100-101% of nominal values. Endpoints have been determined using nominal values.

The measured level at 72 hours in flasks containing no algae was 257 mg/l (corresponding to a nominal value of 250 mg/l), 103% of the nominal value. Comparison with flasks containing algae indicated that the stability of the exposure solutions was not affected by the presence of algal cells.

Aminopyrimidine inhibited algal growth at concentrations tested in excess of 62.5 mg/l. Microscopic inspection of test and control cultures after 72 hours revealed no abnormalities.

The 72-hour EbC_{50} value based on biomass was 269 mg/l (95% confidence limits 255 284 mg/l). The 0-72-hour ErC_{50} value based on growth rate was 521 mg/l (95% confidence limits 510 532 mg/l). The 72-hour NOEC was 62.5 mg/l.

- Green algae

Reference: DAR B.9.2.6.5 (Cockcroft, 2004).

The effect of the degradation product, halosulfuron, on *Selenastrum capricornutum* was investigated.

At the start of the study, the measured levels of halosulfuron were 4.48, 9.68, 22.0, 45.9 and 96.9 mg/l (corresponding to nominal values of 4.6, 10, 22, 46 and 100 mg/l, respectively) and ranged between 96.8-99.9% of nominal values. After 72 hours, the measured levels were 4.38, 10.1, 22.5, 45.1 and 99.9 mg/l and ranged between 95.2-102% of nominal values. The overall mean measured concentrations of halosulfuron were 4.4, 9.9, 22, 46 and 98 mg/l, equivalent to between 96-100% of the nominal values.

The measured level at 72 hours in flasks containing no-algae was 23.0 mg/l (corresponding to a nominal value of 22 mg/l), 104% of the nominal value. Comparison with flasks containing algae indicated that the stability of the test substance was not affected by the presence of algal cells. Environmental parameters remained within acceptable limits throughout the study. pH of control cultures remained within 1 unit over the 72 hour test period.

Halosulfuron significantly inhibited algal growth at 46 and 98 mg/l. Microscopic examination of control and test cultures revealed no abnormalities or contamination The test was considered valid as cell concentrations in control cultures increased by a factor of >16 within 72 hours.

The 72-hour EbC_{50} value based on biomass (72 hours) was 84.7 mg/l (95% confidence limits 67.6 >98 mg/l).

The 72-hour ErC_{50} value based on specific growth rate (72 hours) was >98 mg/l (95% confidence limits, not calculated).

The 72-hour NOEC was 22 mg/l.

5.4.4 Other aquatic organisms (including sediment)

Chronic toxicity testing for a representative species of aquatic insects, the midge (*Chironomus riparius*), has been undertaken. Four replicates of 20 *Chironomus riparius* larvae (first instar stage), were exposed under static conditions for 28 days to ^{14}C-halosulfuron-methyl at nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg/l. Exposure concentrations were selected following a range finding study in which 10 newly hatched larvae in each of two replicate vessels were exposed for 48 hours at nominal exposure concentrations of 0.1, 1.0, 10 and 100 mg/l. In the final test, ^{14}C-halosulfuron-methyl was dispersed in Elendt M4 medium (8 cm depth) overlaying a 2 cm layer of sediment using dimethylformamide (DMF) as a vehicle. In addition four control, eight solvent control replicates (100 µl/l DMF) and four vessels (two at 10 mg/l and two 0.63 mg/l) for destructive analytical sampling were prepared. For exposure concentration, aliquots of non-radiolabelled halosulfuron-methyl were weighed into vials and dissolved in small volumes of acetone. At the highest test concentration ethyl acetate was added to aid dissolution. Aliquots of a stock solution of radiolabelled 14C-halosulfuron-methyl (0.30 or 0.25 ml) were added and the solutions mixed by gentle shaking. Following evaporation, the dried samples were stored at < -15 °C. On the day of dosing, to prepare the dosing solutions, each sample was dissolved in dimethylformamide (DMF) by sonication. Aliquots of each dosing solution were radioassayed by liquid scintillation counting (LSC). The required volume of each dose solution was dispensed into the overlying water in each test vessel.

The concentrations of ^{14}C-halosulfuron-methyl in the overlying water ranged between 70-106% of applied radioactivity (AR) during the study. At 0.63 and 10 mg/l (destructive vessels) the majority of the radioactivity (84.4–105.7% AR) was found in the overlying water phase with minimal amount (0.1–0.6% AR) in pore water. The concentration of ^{14}C halosulfuron-methyl in the sediment phase increased from 0.9-2.2% AR at Day 0 to 12.6-16% AR at Day 7. The total amount of test material accounted for in all fractions on Day 28 ranged from 105.3-112.4% AR, with 70-77.7% AR in the overlying water.

Mean emergence from control and treated groups were in the range of 66.6-87.2% and 70.3-84.1% respectively. Emergence in the untreated control group was slightly lower than the guideline-specified limit at 66.6%. However, as the results are based on comparisons with the solvent control, this is not considered to affect the validity of the study. There were no significant differences ($p>0.05$) in mean emergence and in the sex ratio of midges emerging between the solvent control and treated groups.

The mean development rate was reduced by the 5.0% at 10 mg/l, compared to the solvent control. This difference was statistically significant at $p>0.05$. In this respect, the study report suggests this

not to be of ecological importance due to the fact that the emergence of chironomids at 10 mg/l was unaffected.

The EC_{50} for emergence and development was considered to be >10 mg/l.

The NOEC for emergence and development was 10 mg/l the highest rate tested.

Comment: In contrast to the proposal made on the original study report (NOEC for development, 10 mg/l), the RMS deems to set a conservative NOEC for development at 5 mg/l, i.e. the highest rate tested without statistically significant inhibition.

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

Degradation

Halosulfuron-methyl degrades in water, to produce stable hydrolysis products. Halosulfuron-methyl is photolytically stable when exposed to natural sunlight for 30 days is not readily biodegradable in a biodegradation screening test. In a water/sediment degradation simulation test, the average DT_{50} of the whole system is 6.3-10.4 days but mineralisation accounted for only 0.2–5.7 % AR after 120 days. Based on these findings halosulfuron-methyl is considered to be not rapidly degradable (CLP) in the aquatic environment.

Aquatic bioaccumulation

The measured log P_{ow} values for halosulfuron-methyl (-0.542 to 1.67) were all below the threshold value for bioaccumulation, i.e. threshold CLP ≥4. The potential risk for bioaccumulation of halosulfuron-methyl in tissues of aquatic organisms is considered low.

Aquatic toxicity

Both acute and chronic toxicity studies were conducted for the three trophic levels.

The 96 hour acute LC_{50} for fish is higher than 118000 µg/l and the 87 day chronic NOEC is 34000 µg/l.

The 48 hour EC_{50} for aquatic invertebrates is higher than 89900 µg/l and the 21 day chronic NOEC is 7200 µg/l.

The 72 h ErC_{50} for algae is 5.0 µg/l and the 72 h NOEC is 1.0 µg/l.

The most sensitive species is aquatic plants with a 7 day EbC_{50}[1] of 0.217 µg/l and a 7 day NOEC of 0.03 µg/l.

[1] According to EFSA Peer review of the pesticide risk assessment of the active substance halosulfuron-methyl (EFSA Journal 2012;10(12):2987) the biomass endpoint is to be used instead of the growth rate endpoint.

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

In aquatic toxicity studies aquatic plants (*Lemna gibba*) were identified as the most sensitive species with E_rC_{50} of 0.217 µg/l and a NOEC of 0.03 µg/l. Halosulfuron-methyl is not rapidly degradable and the potential for aquatic bioaccumulation is low.

Proposal for classification and labelling of halosulfuron-methyl according to CLP and 2nd ATP:

Classification:

Aquatic Acute category 1 (based on ErC_{50} algae and aquatic plants ≤ 1 mg/L)
H400
M-factor = 1000 (based on 0.0001 mg/l < $L(E)C_{50}$ ≤ 0.001 mg/l) *Lemna gibba* EC_{50} 0.000217 mg/l mg/l

Aquatic Chronic category 1 (based on NOEC algae and aquatic plants ≤ 0.1 mg/L)
H410
M-factor = 1000 (based on NRD and 0.00001 mg/l < NOEC ≤ 0.0001 mg/l) *Lemna gibba* NOEC 0.00003 mg/l

Labelling:
GHS pictogram: yes
Signal word: warning
Hazard assessment: H410 Very toxic to aquatic life with long lasting effects
Precautionary statements: Prevention – P273 Avoid release to the environment
Response – P391 Collect spillage
Disposal – P501 Dispose of contents / container to … in accordance with local regulations

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Halosulfuron-methyl is an active substance in herbicides and is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008. The DS proposed to classify the substance as Aquatic Acute 1; H400 (M=1000) based on a 7d E_bC_{50} of 0.000217 mg/L for the macrophyte *Lemna gibba*, and Aquatic Chronic 1; H410 (M=1000) based on lack of rapid degradation and a 7d NOE_rC of 0.00003 mg/L for *L. gibba*.

Degradation

Halosulfuron-methyl hydrolyses with a half-life of 25 – 29 days at pH 5, 14 – 15 days at pH 7 and 18 – 20 hours at pH 9 (at around 25 °C). It is photolytically stable when exposed to natural sunlight for 30 days; degradation observed in an aqueous photolysis study was attributed to hydrolysis (based on dark control results). The major abiotic transformation

products were the rearrangement ester, the 3-chlorosulfonamide ester and the 3-chlorosulfonamide acid (depending on pH).

A modified Sturm test (OECD TG 301B) indicated 3 % degradation (at most) over 29 days. Toxicity controls demonstrated that the substance was not inhibitory to the microbial inoculum, so it is not readily biodegradable.

Two aerobic water/sediment simulation tests at approximately 20°C indicated rapid primary degradation but a low level of mineralisation over 100 days. Total system half-lives were 6.3 days in the clay loam system (pH 8.1) and 10.4 days in the sandy loam system (pH 6.7). This was attributed to both hydrolysis and microbial degradation. Mineralisation of the radiolabels accounted for only 0.2 – 5.7% of applied radioactivity (AR) at the end of the study. Several transformation products were identified, the major ones being halosulfuron-methyl rearrangement (with a half-life of 22 –25.4 days) and halosulfuron rearrangement (half-life not stated). Bound residues increased throughout the study, reaching 19–60% AR by the end.

The CLH report includes data for soil degradation but as these are not directly relevant they are not summarised here.

The DS concluded that halosulfuron-methyl is not rapidly degradable based on this information.

Bioaccumulation

The measured octanol-water partition coefficient (log K_{OW}) is in the range -0.02 to 1.67 at 23 °C, depending on pH (the pK_a is 3.44 at 22.4 °C). The DS concluded that halosulfuron-methyl is not bioaccumulative as the log K_{OW} is < 4.

Aquatic toxicity

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). All studies were performed under flow-through conditions with results expressed in terms of mean measured concentrations, unless stated otherwise.

Table 21: Summary of relevant information on aquatic toxicity

Method	**Test organism**	**Endpoint**	**Toxicity values in mg/L**	**Reference**
Short-term toxicity to fish				
US EPA FIFRA 72-1, ASTM E 729-88	*Oncorhynchus mykiss* (Rainbow Trout)	96h EC_{50}	> 131	Holmes and Swigert, 1993a
OECD TG 202, EEC C.2	*Lepomis macrochirus* (Bluegill Sunfish)	96h LC_{50}	> 118	Holmes and Swigert, 1993b
Long-term toxicity to fish				
US EPA FIFRA 72-4, ASTM E 1241-88[a]	*Oncorhynchus mykiss* (Rainbow Trout)	87d NOEC	34	Graves *et al.*, 1993
Short-term toxicity to aquatic invertebrates				

US EPA FIFRA 72-1, ASTM E 729-88	*Daphnia magna*	48h EC_{50}	> 107	Holmes and Swigert, 1993c
US EPA FIFRA 72-3, ASTM E 729-88	*Mysidopsis [Americamysis] bahia* (mysid shrimp)	96h LC_{50}	109	Swigert and Smith, 1993
N.A. ("based on OECD principles", semi-static)	*Lymnaea peregra* (gastropod mollusc)	96h LC_{50}	> 89.9	Jenkins, 2004b
Long-term toxicity to aquatic invertebrates				
US EPA FIFRA 72-4, ASTM E 1193-87	*Daphnia magna*	21d NOEC (reproduction)	< 6.9	Zelinka *et al*., 1993a
US EPA FIFRA 72-4, ASTM E 1193-87	*Daphnia magna*	21d NOEC	7.2	Zelinka *et al*., 1993b
Toxicity to algae and aquatic macrophytes				
US EPA FIFRA 123-2, US 40 CFR 797.1075, ASTM E1218-90 (static)	*Pseudokirchneriella subcapitata*	120h E_rC_{50} 120h NOE_rC	0.00507 0.00063 (nominal)	Thompson and Swigert, 1993
OECD TG 201[b] (static)	*Pseudokirchneriella subcapitata*	72h E_rC_{50} 72h NOE_rC	0.005 0.001 (nominal)	Seki, 2008
US EPA FIFRA 123-2, ASTM E 1218-90 (static)	*Anabaena flos-aquae* (blue-green alga)	120h NOE_bC	0.05 (nominal)	Thompson and Swigert, 1994a
US EPA FIFRA 123-2, ASTM E 1218-90 (static)	*Navicula pelliculosa* (diatom)	120h EC_{50} 120h NOEC (based on cell density)	> 0.35 0.35 (nominal)	Thompson and Swigert, 1994b
US EPA FIFRA 123-2 (static)	*Skeletonema costatum* (diatom)	120h EC_{50} 120h NOEC (based on cell density)	> 0.4 0.4 (nominal)	Thompson and Swigert, 1994c
OECD TG 221 (draft 2002) (semi-static)	*Lemna gibba* (duckweed)	7d E_rC_{50} 7d NOE_rC	0.000491 0.00003	Jenkins, 2005a

N.A. – data not available

Note: a – The CLH dossier cites an incorrect test method (OECD TG 202). This was an early life stage test, and the actual method is taken from the DAR.

b – The CLH dossier does not cite the test method. The actual method is taken from the DAR.

The Zelinka *et al*. (1993a) chronic toxicity study used fewer daphnids than recommended by the OECD TG. The DS did not provide a reliability rating. Although effects were observed

at the lowest concentration, the DS did not discuss this further. RAC notes that this does not influence the classification.

Concentrations were maintained close to nominal in the static algal/diatom tests, so results were reported based on nominal concentrations. Four of the tests were conducted over 5 days (120h), but 72h results are not provided. The DS did not provide any information about whether the cells were in an exponential growth phase throughout.

The key study is for *Lemna gibba*. The results were based on mean measured concentrations since measured levels in samples of expired media ranged from 1 – 16% of their nominal value at the highest dose, from below the limit of quantification (0.03 µg/L) to 13% at the next two doses and could not be quantified at the three lowest doses. The overall mean measured levels of halosulfuron-methyl were 0.006, 0.009 (estimated values), 0.03, 0.141, 0.733 and 2.584 µg/L. Additional measurements in expired media from flasks kept without plants showed concentrations in the range 31 – 71 of nominals, suggesting that the presence of *Lemna* affected the stability of halosulfuron-methyl under the conditions of the test.

A 28d NOEC of 5 mg/L is also reported for the insect *Chironomus riparius* from a static water-sediment test. The total amount of test material accounted for in all fractions at the end of the test ranged from 105.3-112.4% AR, with 70-77.7% AR in the overlying water.

Ecotoxicity data are presented for the main transformation products in several species, and none appear to be more toxic than the parent substance.

Comments received during public consultation

Four MSCAs supported the proposed environmental classification. Two MSCAs asked why the biomass end point was used for the acute classification (rather than growth rate, noting that this does not affect the proposal); the DS said that it was based on the outcome of an EFSA expert consultation without any further explanation. One MSCA asked for some minor clarifications, but these do not affect the proposal.

Assessment and comparison with the classification criteria

Degradation

The substance undergoes primary degradation via both abiotic and biotic processes with a half-life of less than 16 days at a pH of 6 and above. However, it is not readily biodegradable, and the abiotic half-life at pH 5 is above 16 days. Limited mineralisation was also observed in two water/sediment simulation tests. Therefore, RAC agrees with the DS that halosulfuron-methyl is not rapidly degradable.

Bioaccumulation

RAC agrees with the DS that halosulfuron-methyl has a low potential to bioaccumulate based on a log K_{OW} value below the CLP Regulation threshold of 4.

Aquatic toxicity

Short-term aquatic toxicity data are available for three trophic levels (10 species). The EC_{50}s for algae and aquatic macrophytes are below 1 mg/L, with the duckweed *Lemna gibba* the most sensitive species. The DS proposal is based on a 7d E_bC_{50} of 0.000217 mg/L, but the DS did not adequately explained why this is preferred to the more usual growth

rate end point (7d E_rC_{50} of 0.000491 mg/L) recommended in the Guidance on the Application of the CLP Criteria Version 4.1, June 2015 (Section 4.1.3.3.1, p. 505). RAC prefers using the 7d E_rC_{50} of 0.000491 mg/L. Nevertheless, the choice does not affect the actual classification, which is **Aquatic Acute 1 (H400)**. As $0.0001 < EC_{50} \leq 0.001$ mg/L, the acute **M-factor is 1000**.

Long-term aquatic toxicity data are available for three trophic levels (8 species). Algae and aquatic macrophytes are the most sensitive group, and the lowest result is a 7d NOE_rC of 0.00003 mg/L for the duckweed *Lemna gibba*. As this concentration is below the threshold value of 0.1 mg/L for non-rapidly degradable substances, RAC concludes that a classification as **Aquatic Chronic 1 (H410)** is justified. As $0.00001 < NOEC \leq 0.0001$ mg/L, the chronic **M-factor is 1000**. RAC notes that there is some uncertainty in the M-factor as test concentration maintenance was poor (the equivalent nominal concentration is 0.00032 mg/L, which would justify an M-factor of 100; the same M-factor would be derived for the next most sensitive species (*Pseudokirchneriella subcapitata*) with a 72h NOE_rC of 0.001 mg/L).

Overall, **RAC agrees with the DS to classify halosulfuron-methy as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 both with an M-factor of 1000**.

6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of halosulfuron-methyl according to Regulation (EC) No 1107/2009. The summaries included in this proposal are partly copied from the DAR volume 3, annex B. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR Volume 3 and its addendum.

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8 ANNEXES

None