# 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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## Part A.



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# 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.<br>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)<br>Acute M-factor of 1000<br>Aquatic Chronic 1 (H410)<br>Chronic M-factor of 1000 |
| **Resulting harmonised classification (future entry in Annex VI, CLP Regulation)** | Aquatic Acute 1 (H400)<br>Acute M-factor of 1000<br><br>Aquatic Chronic 1 (H410)<br>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<br><br>Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects | Acute M-factor = 1000<br>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 |

# 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

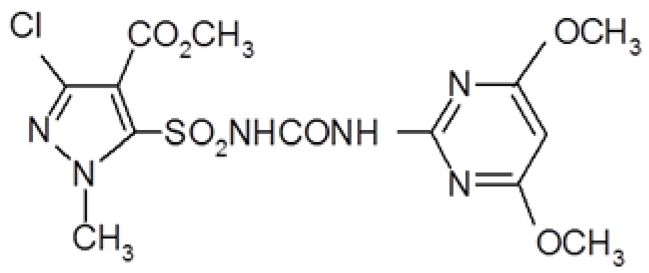
Halosulfuron-methyl (modified ISO 1750) is a sulfonylurea herbicide (IUPAC name: methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoyl-sulfamoyl)-1-methylpyrazole-4-carboxylate).

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<br>Comb, 2005 | Appearance physical state, visual observation<br>(pure: 99.9%) |
| Melting/freezing point | Mean 175.5-177.2°C (n=3) | DAR B.2.1.1<br>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<br><1.33 x 10-5 Pa at 25± 1°C | DAR B.2.1.5<br>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<br>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<br>Hirai, 1999 | EEC A6 column elution method (pure 99.9%) |
| Partition coefficient n-octanol/water | at 23 ± 2°C, measured log Kow values:<br>pH 5 1.67 (n=6)<br>pH 7 -0.0186 (n=6)<br>pH 9 -0.542 (n=6) | DAR B.2.1.19<br>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<br>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<br>Schuurman & Hooidonk, 1988 | EECA14 (technical 99.2%) |
| Self-ignition temperature | No relative self-ignition temperature. Not auto-flammable | DAR B.2.1.29<br>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<br>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<br>Pesselman, 1991i | Spectro-photometric method, (pure 99.9%) |
| Viscosity | Not applicable as substance is a solid | DAR B.2.2.11 | N/A |

# 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<br>EEC A10 method<br>GLP | Not highly flammable | Purity 99.2% | DAR B.2.1.28<br>Schuurman & Hooidonk, 1988 |
| Explosive properties<br>EEC A14 method<br>GLP | Non-explosive | Not explosive under the influence of a flame and not sensitive to shock and friction | DAR B.2.1.31<br>Schuurman & Hooidonk, 1988 |
| Self-ignition temperature<br>EEC A16 method<br>GLP | No self-ignition below 400°C | Purity 99.6% | DAR B.2.1.29<br>Comb, 2003b |
| Oxidising properties evaluation on theoretical basis<br>EEC A.17<br>GLP | No oxidizing properties | Purity 99.6% | DAR B.2.1.33<br>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 ($\alpha$) for all groups. The second phase elimination ($\beta$) 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).

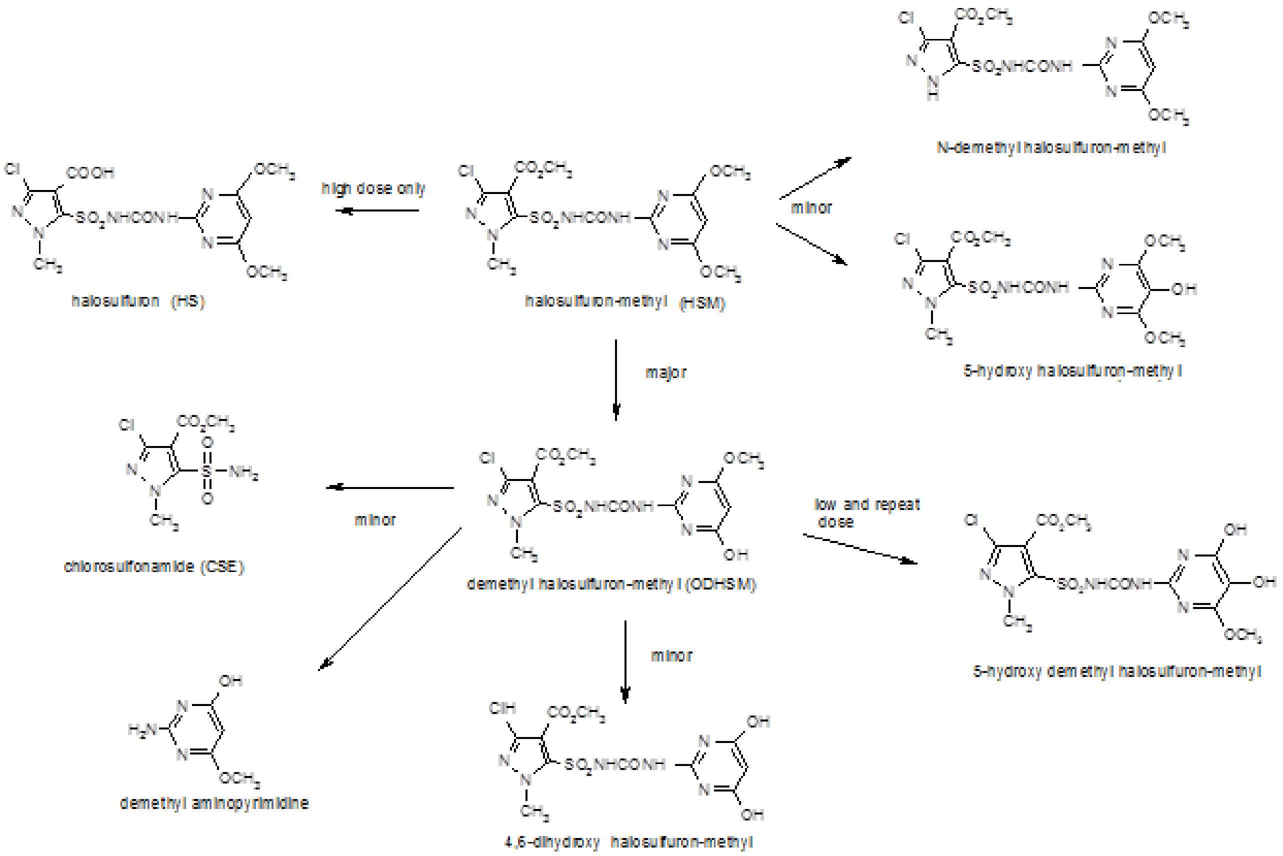
The substance is widely distributed to different organs and there is no evidence for absorption saturation.

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

## 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.

## 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<br><br>0.5g (4 hours)<br><br>OECD 404<br>GLP<br>Purity 98.5% | No signs of dermal irritation observed in any animal at any timepoint<br>Not irritant | DAR B.6.2.4<br><br>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

### 4.4.2 Eye irritation

**Table 17: Summary table of relevant eye irritation studies**

| Method | Results | Reference |
|---|---|---|
| Rabbit eye irritation<br>Rabbit, New Zealand White, 3 male and 3 female<br>0.1 ml<br>OECD 405<br>GLP<br>Purity 98.5 % | Slight to moderate conjunctival irritation resolved by 72 hours in all animals. | DAR B.6.2.5<br>Blaszcak, 1991 |

| Animal number and sex | Parameter | | Hours post instillation | Days post-instillation | | | Mean irritation score[#] |
|---|---|---|---|---|---|---|---|
| | | | 1 | 1 | 2 | 3 | |
| 0021 Male | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | + | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 2 | 1 | 0 | 0 | 0.33 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |
| 0022 Female | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | 0 | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 2 | 1 | 0 | 0 | 0.33 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |
| 0023 Male | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | + | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 2 | 1 | 1 | 0 | 0.67 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |
| 0024 Female | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | 0 | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 1 | 1 | 0 | 0 | 0.33 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |
| 0025 Male | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | 0 | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 1 | 1 | 0 | 0 | 0.33 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |
| 0026 Female | Cornea | Density | 0 | 0 | 0 | 0 | 0 |
| | | Area | 0 | 0 | 0 | 0 | |
| | Iris | | 0 | 0 | 0 | 0 | 0 |
| | Conjunctivae | Redness | 2 | 1 | 0 | 0 | 0 |
| | | Chemosis | 1 | 0 | 0 | 0 | 0.33 |
| | | Discharge | 1 | 0 | 0 | 0 | 0 |

[#] 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

#### 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.

### 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)<br>Guinea Pig, Dunkin-Hartley, 10 females/test and 10 males/test group, 20 females/ control group<br>Maximisation Study<br>OECD 406<br>GLP<br>Purity 98.5 % | No dermal reactions were seen in any of the test group or control animals | DAR 6.2.6<br>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.

### 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.

## 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)<br>Sprague Dawley rats (10/sex/group)<br>US EPA 40 CFR 158.135<br>GLP<br>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)<br>LOEL: 1000 ppm (Males 78 mg/kg/day Females: 85 mg/kg/day)<br>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<br><br>Osheroff, 1988 |
| Rat 90-day oral (dietary)<br>Sprague Dawley rats (20/sex/group)<br>US EPA FIFRA 82-1<br>GLP<br>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)<br>NOEL: 400 ppm (Males:28.8 mg/kg/day; Females:37.3 mg/kg/day)<br>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<br><br>Perry, 1990 |
| Dog 90-day oral (capsule)<br>Beagle dog (4/sex/group)<br>OECD 409<br>GLP<br>Purity 98.5 % | 0, 0.25, 1, 10, 40 mg/kg/day | NOEL: 10 mg/kg/day LOEL: 40 mg/kg/day<br>Reduced body weight gain; increased liver weight and clinical chemistry alterations | DAR B.6.3.3<br><br>Wood, 1991 |
| Dog 12-month oral (capsule)<br>Beagle dog (6/sex/group)<br>US EPA FIFRA 83-1<br>GLP<br>Purity 98.7 % | 0, 2.5, 10, 40, 160 mg/kg/day | NOAEL:10 mg/kg bw/day<br>NOEL: 1 mg/kg/day<br>LOEL: 40 mg/kg/day<br>Haematological changes | DAR B.6.3.4<br><br>Osheroff, 1991 |
| Rat 21-day dermal<br>Sprague Dawley rats (5/sex/group)<br>US EPA FIFRA 82-2<br>GLP<br>Purity 99.1 % | 0, 10, 100, 1000 mg/kg/day | NOEL: 10 mg/kg/day<br>LOEL: 100 mg/kg/day<br>Haematological changes<br>(reduced body weight gain at higher doses) | DAR B.6.3.6<br><br>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 | No treatment-related effect | | | | | 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 | | | | | | 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 | No treatment-related effect | | | | |
| Week 13 | 4.1 | 4.0 | 4.8 | 3.6 | 3.6 | | | | | |
| 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)<br>LOAEL: 1000 ppm (Males 78 mg/kg/day Females: 85 | 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 |

| | | | | |
|---|---|---|---|---|
| | | | 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)<br>NOEL: 400 ppm (Males:28.8 mg/kg/day; Females:37.3 mg/kg/day)<br>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)<br>LOEL: 2500 ppm=138.6 mg/kg/day (Females) | None at LOAEL<br>Reduced body weight gain. No histopathological changes |
| Mouse 78 week oral (dietary) | 1.7 | 17 | NOAEL: 3000 ppm=410.0 mg/kg/day (Males)<br>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<br>LOAEL: 160 mg/kg/day (f) | None at LOAEL<br>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<br>NOEL: 1 mg/kg/day<br>LOEL: 40 mg/kg/day | None at LOEL<br>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.

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

## 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<br>(dietary)<br>US EPA 83-5<br>GLP<br>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)<br>**Females**: 0, 10, 100, 1000 and 2500 ppm (0, 0.56, 5.6, 56.3, 138.6 mg/kg/day) | **Chronic toxicity:**<br>NOEL: 1000 ppm = 56.3 mg/kg/day (Females)<br>LOEL: 2500 ppm=138.6 mg/kg/day (Females) Critical effect: reduced body weight gain.<br>**Carcinogenicity:**<br>NOEL: Males: 5000 ppm Females: 2500 ppm (Males: 225.2 mg/kg/day; Females: 138.6 mg/kg/day)<br>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<br>Moore, 1992a, |
| Mouse dietary 78-week oncogenicity study<br>US EPA 83-5<br>GLP<br>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:**<br>NOAEL: 3000 ppm=410.0 mg/kg/day (Males)<br>LOAEL: 7000 ppm=971.9 mg/kg/day (Males)<br>Critical effect: Reduced male body weight gain, increased microconcretions/ mineralisation in testis and epididymal tubules.<br>**Carcinogenicity:**<br>NOEL: 7000 ppm<br>(Males: 971.9 mg/kg/day<br>Females: 1214.6 mg/kg/day)<br>LOEL>7000 ppm<br>(Males: 971.9 mg/kg/day<br>Females: 1214.6 mg/kg/day). No carcinogenic potential at any dose level | DAR B.6.5.2<br>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.

## 4.11 Toxicity for reproduction

**Table 31: Summary table of relevant reproductive toxicity studies**

| Method | Results | Dose levels | Reference |
|---|---|---|---|
| Rat two-generation (dietary)<br>US EPA FIFRA 83-4<br>GLP<br>Purity: 98.7% | NOAEL:<br>General toxicity:800 ppm (Males: 50.4 mg/kg/day: Females: 58.7 mg/kg/day)<br>Reproductive toxicity:100 ppm (Males: 6.3 mg/kg/day; Females: 7.4-11.8 mg/kg/day)<br>LOAEL:<br>General toxicity: 3600 ppm<br>Reproductive toxicity:<br>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:<br>0, 6.3, 50.4, 223.2 mg/kg/day;<br>F1 males: 0, 7.4, 61.0, 274.2 mg/kg/day;<br>F0 females:0, 7.4, 58.7, 261.4 mg/kg/day;<br>F1 females: 0, 8.9, 69.7, 319.9 mg/kg/day | DAR B.6.6.1<br>Lemen,<br>1991 |
| Rat developmental toxicity (oral gavage)<br>US EPA FIFRA 83-3<br>GLP<br>Purity: 98.5% | NOEL:<br>Maternal toxicity:250 mg/kg/day<br>Developmental toxicity: 75 mg/kg/day<br>LOEL:<br>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<br>Morseth,<br>1990a |
| Rabbit developmental toxicity (oral gavage)<br>US EPA FIFRA 83-3<br>GLP<br>Purity: 98.5% | NOEL:<br>Maternal toxicity:50 mg/kg/day<br>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.<br>LOEL:<br>15 mg/kg/day | 0, 15, 50 and 150 mg/kg/day | DAR B.6.6.3<br>Morseth,<br>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 ≤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** | 25 | 25 | 24 | 22 |
| **Total number of foetuses** | 335 | 335 | 326 | 292 |
| **Foetal weight (g)** | | | | |
| Males | 3.4 | 3.4 | 3.4 | 2.6* |
| Females | 3.2 | 3.2 | 3.2 | 2.5* |
| **External malformations:** | | | | |
| Number of foetuses affected | 0 | 0 | 0 | 4*(1.4) |
| Number of litters affected and | 0 | 0 | 0 | 3 (14) |
| **Visceral examinations:** | | | | |
| Number of foetuses examined | 165 | 165 | 163 | 146 |
| **Variations:** | | | | |
| Dilatation of lateral ventricles: | | | | |
| Number of foetuses affected | 0 | 0 | 2 (1.2) | 16*(11) |
| Number of litters affected | 0 | 0 | 2 (8.3) | 5* (23) |
| Total foetal soft tissue variations | | | | |
| Number of foetuses affected | 4 (2.4) | 8 (4.7) | 9 (5.5) | 22 *(15) |
| Number of litters affected | 3 (13) | 5 (20) | 7 (2.9) | 10 *(45) |
| **Skeletal examinations:** | | | | |
| Number of foetuses examined | 170 | 165 | 163 | 146 |
| **Malformations** | | | | |
| Vertebral anomaly with/without rib anomaly: | | | | |
| Number of foetuses affected | 0 (0) | 0 (0) | 0 (0) | 1 (0.7) |
| Number of litters affected | 0 (0) | 0 (0) | 0 (0) | 1 (4.5) |
| Filamentous tail: | | | | |
| Number of foetuses affected | 0 (0) | 0 (0) | 0 (0) | 2 (1.4) |
| Number of litters affected | 0 (0) | 0 (0) | 0 (0) | 2 (9.1) |
| Rudimentary tail: | | | | |
| Number of foetuses affected | 0 (0) | 0 (0) | 0 (0) | 1 (0.7) |
| Number of litters affected | 0 (0) | 0 (0) | 0 (0) | 1 (4.5) |
| Forked/fused ribs: | | | | |
| Number of foetuses affected | 0 (0) | 0 (0) | 0 (0) | 2 (1.4) |
| Number of litters affected | 0 (0) | 0 (0) | 0 (0) | 2 (9.1) |
| Total foetal skeletal malformations | | | | |
| 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) |

* 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) |
| 5$^{th}$ 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) |
| 6$^{th}$ 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 | | | | |
| 14$^{th}$ 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 | | | | |
| 7$^{th}$ 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 |
| <u>Overall change</u> | | | | |
| 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.

<u>Dilatation of lateral ventricles</u>

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 (%)<br>– Day 0 (%)<br>– Day 4 pre-cull<br>– Day 21 | <br>**41**<br>**42**<br>46 | <br>48<br>49<br>50 | <br>53<br>53<br>52 | <br>56<br>56<br>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 (%):<br>- males<br>- females | <br>96<br>92 | <br>95<br>95 | <br>87<br>95 | <br>95<br>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 (%)<br>– Day 0 (%)<br>– Day 4 pre-cull<br>– Day 21 | <br>47<br>46<br>48 | <br>48<br>49<br>50 | <br>44<br>45<br>45 | <br>52<br>51<br>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.

## 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<br><br>US EPA Subdivision N, 161-1 (1982)<br><br>GLP<br><br>Radiochemical purity:<br>99.1% (pyrimidine label)<br>100% (pyrazole label) | $DT_{50}$:<br>**Pyrimidine label**<br>pH 5 = 28.9 days<br>pH 7 = 13.9 days<br>pH 9 = 17.6 hours<br><br>**Pyrazole label**<br>pH 5 = 24.8 days<br>pH 7= 14.9 days<br>pH 9 = 19.5 hours<br>. | The major degradates were the rearrangement ester, the 3-chlorosulfonamide ester and the 3-chlorosulfonamide acid.<br><br>Rate of hydrolysis at pH 4 expected to be slower with a subsequent slower rate of formation of degradation | DAR B.2.1.22<br><br>Kesterton et. al., 1991 |
| Photodegradation,<br><br>US EPA Subdivision N, 161-2 (1982)GLP<br><br>Radiochemical purity:<br>98.5% (pyrimidine label)<br>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.<br><br>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<br><br>Kesterton et. al., 1993 |
| Ready biodegradability,<br>EU 92/69/EEC C.4 (1992),<br>OECD 301B (1992),<br>EPA OPPTS 835.3110 (1998)<br>GLP<br>Purity: 99.6% | 3% degradation after 29 days.<br>Not readily biodegradable | Not rapidly degradable for classification purposes. | DAR B.8.4.3.1<br><br>Barnes, 2003 |
| Water/sediment simulation study, SETAC-Europe (1995)<br><br>GLP<br><br>Radiochemical purity:<br>97.1% (pyrimidine label)<br>97.5% (pyrazole label) | $DT_{50}$ system<br>6.3 days (clay loam)<br>10.4 days (sandy loam) | Not rapidly degradable for classification purposes. | DAR B.8.4.3.2<br><br>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: $DT_{50}$ ≤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<br>GLP<br>Purity: 99.9% | LogKow:<br>pH 5: 1.67<br>pH 7: -0.0186<br>pH 9: -0.542<br>23°C | Log Kow <4 | DAR B.2.1.19<br>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<br><br>US EPA FIFRA E, 72-1<br>ASTM E 729-88<br><br>GLP | 96 hour $LC_{50}$: >131 mg/l NOEC: 131 mg/l | Purity 98.5% | DAR B.9.2.1.1<br><br>Holmes and Swigert, 1993a |
| Acute toxicity – fish *Lepomis macrochirus* Bluegill sunfish<br>US EPA FIFRA 72-1<br>ASTM E 729-88<br>GLP | 96 hour $LC_{50}$: >118 mg/l NOEC: 118 mg/l | Purity 98.5% | DAR B.9.2.1.2<br><br>Holmes and Swigert, 1993b |
| Chronic toxicity – fish early life stage *Oncorhynchus mykiss* Rainbow trout<br><br>OECD202<br>EEC C.2<br><br>GLP | LOEC: 106 mg/l NOEC: 34 mg/l | Purity 99.74% | DAR B.9.2.2.2<br><br>Graves, Mank and Swigert, 1993 |
| Acute toxicity –aquatic invertebrates *Daphnia magna*<br><br>US EPA FIFRA 72-1<br>ASTM E 729-88<br><br>GLP | 48 hour $EC_{50}$: >107 mg/l NOEC: 107 mg/l | Purity 98.5% | DAR B.9.2.4.1.1<br><br>Holmes and Swigert, 1993c |

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

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

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

| | | | |
|---|---|---|---|
| Effects on algal/plant growth<br>*Anabaena flos-aquae*<br>Blue-green alga<br>US EPA FIFRA 123-2<br>ASTM E 1218-90<br>GLP | NOEC: 0.050 mg/l | Purity 99.74%<br>$EC_{50} \leq 0.1$ mg/l | DAR B.9.2.6.2<br>Thompson and Swigert, 1994a |
| Effects on algal/plant growth<br>*Navicula pelliculosa*<br>Diatom (freshwater)<br>US EPA FIFRA 123-2<br>ASTM E 1218-90<br>GLP | 5-day $EC_{50}$: >0.350 mg/l<br>NOEC: 0.350 mg/l | Purity 99.74% | DAR B.9.2.6.3<br>Thompson and Swigert, 1994b |
| Effects on algal/plant growth<br>*Skeletonema costatum*<br>Diatom (marine)<br>US EPA FIFRA 123-2<br>GLP | 5-day $EC_{50}$: >0.400 mg/l<br>NOEC: 0.400 mg/l | Purity 99.74% | DAR B.9.2.6.4<br>Thompson and Swigert, 1994c |
| Effects on algal/plant growth<br>*Pseudokirchneriella subcapitata* Green algae | 72 hour $E_rC_{50}$: 0.0050 mg/L<br>72-hour growth rate NOEC: 0.0010 mg/L. | Purity 99.9%<br>$EC_{50} \leq 0.1$ mg/l | Additional report to DAR. Addendum, August 2012.<br>DAR B.9.2.6.9<br>Seki, 2008 |
| Effects on sediment dwellers<br>*Chironomus riparius*<br>OECD 219 (draft 2001) | 28-day $EC_{50}$: >10 mg/l<br>NOEC: 5 mg/l | Purity 99.6% | DAR B.9.2.7.2<br>Cockcroft, 2005 |
| Effects on algal/plant growth<br>*Lemna gibba* Duckweed<br>OECD 221 (draft 2002) | 7 day $E_bC_{50}$: 0.000217 mg/l<br>7 day $E_rC_{50}$: 0.000491 mg/l<br>7 day $E_{wt}C_{50}$: 0.000823 mg/l<br>NOEC: 0.00003 mg/l<br>LOEC: 0.000141 mg/l | Purity 99.6%<br>$EC_{50} \leq 0.1$ mg/l | DAR B.9.2.8.1<br>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<br><br>EU C.1<br>OECD 203<br>GLP | 96 hour $LC_{50}$: >15.3 mg/l | Purity 100% | DAR B.9.2.1.3<br><br>Jenkins, 2004a |
| Acute toxicity aquatic invertebrates *Daphnia magna*<br><br>EU C.2<br>OECD 202<br>GLP | 48 hour $EC_{50}$: >19.2 mg/l<br>NOEC: 19.2 mg/l | Purity 100% | DAR B.9.2.4.1.2<br><br>Jenkins, 2004c |
| Effects on algal/plant growth *Pseudokirchneriella subcapitata* Green alga<br><br>EU C.3<br>OECD 201<br>GLP | 72 hour $E_bC_{50}$: 17.5 mg/l<br>0-72 hour $E_rC_{50}$: >20.3 mg/l<br>NOEC: 4.9 mg/l | Purity 100% | DAR B.9.2.6.6<br><br>Jenkins, 2004d |
| **Degradation product: aminopyrimidine (AP)** | | | |
| Effects on algal/plant growth<br>*Pseudokirchneriella subcapitata*<br>EU C.3<br>OECD 201<br>GLP | 72 hour $E_bC_{50:}$ 269 mg/l<br>0-72 hour $E_rC_{50}$: 521 mg/l<br>NOEC: 62.5 mg/l | Purity 100% | DAR B.9.2.6.7<br>Flatman, 2005 |
| **Degradation product: halosulfuron (HS)** | | | |
| Effects on algal/plant growth<br>*Pseudokirchneriella subcapitata*<br>EU C.3<br>OECD 201<br>GLP | 72 hour $E_bC_{50}$: 84.7 mg/l<br>0-72 hour $E_rC_{50}$: >98 mg/l<br>NOEC: 22 mg/l | Purity 98.6% | DAR B.9.2.6.5<br>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.

<u>Findings:</u>

**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 | $R_1$<br>$R_2$<br>$R_3$ | 444<br>470<br>482 | 465 | 0.4162<br>0.4206<br>0.4219 | 0.4196 | 16.5<br>16.0<br>17.6 | 16.7 |
| 0.006$ | $R_1$<br>$R_2$<br>$R_3$ | 481<br>482<br>554 | 506<br>(0) | 0.4206<br>0.4168<br>0.4326 | 0.4234<br>(0) | 18.7<br>17.4<br>21.8 | 19.3<br>(0) |
| 0.009$ | $R_1$<br>$R_2$<br>$R_3$ | 585<br>546<br>560 | 564<br>(0) | 0.4421<br>0.4256<br>0.4372 | 0.4350<br>(0) | 24.2<br>22.3<br>22.5 | 23.0<br>(0) |
| 0.03 | $R_1$<br>$R_2$<br>$R_3$ | 477<br>583<br>505 | 521<br>(0) | 0.4162<br>0.4355<br>0.4321 | 0.4279<br>(0) | 18.7<br>25.4<br>20.2 | 21.4<br>(0) |
| 0.141 | $R_1$<br>$R_2$<br>$R_3$ | 249<br>323<br>361 | 311<br>(33) | 0.3241<br>0.3682<br>0.3861 | 0.3595<br>(14) | 13.8<br>16.5<br>17.1 | 15.8<br>(5) |
| 0.733 | $R_1$<br>$R_2$<br>$R_3$ | 63<br>83<br>69 | 72<br>(85) | 0.1261<br>0.1720<br>0.1488 | 0.1489<br>(65) | 7.4<br>7.7<br>7 | 7.4<br>(56) |
| 2.584 | $R_1$<br>$R_2$<br>$R_3$ | 21<br>40<br>46 | 36<br>(92) | 0.0411<br>0.0866<br>0.0990 | 0.0756<br>(82) | 6.7<br>5.9<br>7.7 | 6.78<br>(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

<u>*Conclusions:*</u>
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

## 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