CENTRE FOR CHEMICAL REGULATION
AND FOOD SAFETY (EUROPE)
The Lenz, Hornbeam Business Park,
Harrogate. HG2 8RE UK
T (+44) 1423 853200 F (+44) 1423 810431
info@exponent.com

TOXICOLOGY ASSESSMENT OF HALOSULFURON-METHYL AS AN ACTIVE SUBSTANCE IN AGRICULTURAL REMEDIES

Prepared by:

Dr. Anna Rowbotham and Dr. Lata Koshy

Exponent International Limited

The Lenz, Hornbeam Business Park

Harrogate, HG2 8RE, UK

Prepared for the Derogation Group consisting of:

Farm-Ag International (Pty) Ltd, ICA International Chemicals (Pty)
UPL South Africa (Pty) Ltd, Sharda International Africa (Pty) Ltd
Villa Crop Protection (Pty) Ltd, Rainbow Agrosciences (Pty) Ltd
Green Island Investments Pty Ltd

Project No.: 2403474.UK0

Exponent Report No.: 2403474.UK0 - 6756

Date: 3rd December 2024

Table of Contents

1. Executive Summary

Halosulfuron-methyl is a sulfonylurea post-emergence herbicide used to control broad-leaved weeds and sedges in a range of crops (maize, sorghum, wheat, sugarcane etc…). It is systemic and selective, and acts as an inhibitor of acetohydroxyacid synthase restricting the biosynthesis of the essential amino acids, valine and isoleucine, thus restricting plant growth.

In the Republic of South Africa regulatory jurisdiction, according to Regulation 8(1)(d) and 10(3)(e) respectively, the Registrar (Act 36 of 1947) may not grant or renew a registration after 1 June 2024 if a plant protection product contains substances of concern. In the European Union, halosulfuron-methyl has been classified with respect to reproductive toxicity as Repr.1B H360D (May damage the unborn child) in accordance with Regulation (EC) No. 1272/2008 (also known as the CLP Regulation for Classification and Labelling), and as such would be considered a substance of concern. In exceptional circumstances, the Registrar may grant a registration for a product (i.e.: an agricultural remedy) containing a substance of concern, based on a risk assessment demonstrating the safe use of the product.

Halosulfuron-methyl is registered in South Africa and in order to maintain the registration of their product containing halosulfuron-methyl, the halosulfuron-methyl Derogation Group is submitting a derogation for their water dispersible granule (WG/WDG) formulation products containing 750 g/kg halosulfuron-methyl. This derogation includes dietary and non-dietary human health risk assessments to demonstrate the safe use of these products. To support the derogation application and inform the human health risk assessments, a summary review of the toxicological profile of halosulfuron-methyl has been carried out, considering recent and relevant authoritative regulatory evaluations and the derivation of health-based reference values. Toxicological information has been sourced from evaluations conducted primarily by the European Union (EU) European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA).

The toxicology profile of halosulfuron-methyl has been comprehensively reviewed as part of authoritative regulatory evaluations undertaken in the EU by EFSA and ECHA. Assessments conducted by EFSA have incorporated hazard identification and characterisation to inform human health risk assessments, whereas ECHA and its Committee for Risk Assessment (RAC) exclusively identified human health hazards for risk management and communication purposes in the EU.

Halosulfuron-methyl has low acute toxicity via the oral, dermal and inhalation routes, is not irritating or corrosive to the skin or the eyes and is not sensitising to the skin. Halosulfuron-methyl is not genotoxic based on the findings of a standard battery of *in vitro* and *in vivo* studies, is not neurotoxic and is not considered as having endocrine disruption potential in any regulatory jurisdiction.

The most prominent effect observed upon repeated dose 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/day from the 90-day and 1-year studies in dogs and the long-term NOAEL was 43.8 mg/kg/day from the 2-year rat study.

In the respective 2-year dietary studies in male and female rats and in a 78-week study in male and female mice, no oncogenic effects were observed indicating that halosulfuron-methyl is not carcinogenic.

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/day based on reduced pup body weight gain, while the parental NOAEL was 50.4 mg/kg bw/day. In this study, no effect on fertility or reproduction was observed up to the highest dose level of 223.2 mg/kg bw/day. In the developmental toxicity study in rabbits, the maternal and developmental NOAELs were 50 mg/kg bw/day based on early resorptions, decreased number of foetuses and reduced maternal body weight gain. In the rat, foetal toxicity was observed in the absence of maternal toxicity: the developmental NOAEL was 75 mg/kg bw/day based on a higher incidence of visceral and skeletal variations and the maternal NOAEL was 250 mg/kg bw/day due to reduced body weight, body weight gain and food consumption.

In the EU, halosulfuron-methyl has been classified for reproduction toxicity in Category 1B, (Repr. 1B; H360D "May damage the unborn child") in accordance with the CLP Regulation – a hazard identification process intended for the communication of risk management measures throughout the chemical supply chain. Risk assessments conducted as part of the evaluation performed within the EU regulatory jurisdiction have included the relevant developmental hazard as part of the hazard characterisation.

Based on the review of the toxicological profile of halosulfuron-methyl, the critical human health effects have been adequately identified and characterised. The following health-based reference values are considered to be relevant to inform the dietary and non-dietary risk assessments for the water dispersible granule (WG) formulation products containing 750 g/kg halosulfuron-methyl and are sufficiently conservatively protective in respect of human health:

Reference endpoint	**Derived value**	**Source**	**Based on endpoint:**
ADI	**0.063 mg/kg bw/day**	EFSA (2012)	Based on a NOAEL of 6.3 mg/kg bw/day from a rat reproductive toxicity study (offspring toxicity), UF* = 100
ARfD	**0.5 mg/kg bw/day**	EFSA (2012)	Based on the NOAEL of 50 mg/kg bw/day from a rabbit developmental toxicity study (maternal toxicity), UF = 100
AOEL	**0.063 mg/kg bw/day**	EFSA (2012)	Based on a NOAEL of 6.3 mg/kg bw/day from a rat reproductive toxicity study (offspring toxicity), UF = 100 No correction for oral absorption required
AAOEL	Not required	-	-

*UF uncertainty factor – 100 = 10 (interspecies) x 10 (intraspecies)

2. Introduction

Halosulfuron-methyl (CAS No. 100784-20-1, EC No. 600-130-3) is a sulfonylurea post-emergence herbicide used to control broad-leaved weeds and sedges in a range of crops (maize, sorghum, wheat, sugarcane etc.). It is systemic and selective, and acts as an inhibitor of acetohydroxyacid synthase restricting the biosynthesis of the essential amino acids, valine and isoleucine, thus restricting plant growth.

In the Republic of South Africa regulatory jurisdiction, according to Regulation 8(1)(d) and 10(3)(e) respectively, the Registrar (Act 36 of 1947) may not grant or renew a registration after 1 June 2024 if a plant protection product contains substances of concern. In the European Union (EU), halosulfuron-methyl has been classified with respect to reproductive toxicity as Repr.1B (H360D "May damage the unborn child") in accordance with Regulation (EC) No. 1272/2008, and as such would be considered a substance of concern. However, in exceptional circumstances, the Registrar may grant a registration for a product (i.e.: an agricultural remedy) containing a substance of concern and the Applicant can submit a derogation to achieve this. According to Section 2.1 of the *"Guideline for the Application for a Derogation for an Agricultural Remedy Identified as a Substance of Concern"* issued by the Registrar (DALLRD, 2024),

"Before commencing an application for derogation of an agricultural remedy, the applicant must conduct a risk assessment to evaluate the risks associated with the use of the remedy according to the proposed uses for which a derogation is sought and determine whether the associated risks can be sufficiently mitigated."

The Halosulfuron-methyl Derogation Group comprising of: Farm-Ag International (Pty) Ltd, ICA International Chemicals (Pty) Ltd, UPL South Africa (Pty) Ltd, Sharda International Africa (Pty) Ltd, Villa Crop Protection (Pty) Ltd, Rainbow Agrosciences (Pty) Ltd and Green Island Investments Pty Ltd, is submitting a derogation for their water dispersible granule formulations (WG/WDG) containing 750 g/kg halosulfuron-methyl.

As part of the derogation, dietary and non-dietary human health risk assessments have been carried out to demonstrate the safe use of the products containing the active substance, halosulfuron-methyl. To support the derogation application and inform the human health risk assessment, this report provides a summary review of the toxicological profile of halosulfuron-methyl, considering recent and relevant authoritative regulatory evaluations, and the derivation of health-based reference values.

3. Regulatory evaluations of halosulfuron-methyl in Europe

Sections 4 and 5 of this report provide a summary of the toxicological profile of halosulfuron-methyl and the rationale for the derived health-based reference values, respectively, sourced from recent and relevant authoritative regulatory evaluations of the toxicological data for the substance (i.e.: for its approval as a plant protection product active substance and the consideration of associated risks to human health).

Halosulfuron-methyl has not been evaluated by the Joint Meeting on Pesticide Residues (JMPR), the expert *ad hoc* body administered jointly by the United Nations (UN) Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Toxicological information has therefore been sourced primarily from evaluations conducted by the European Union (EU) European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA).

These evaluations, and the regulatory context are summarised in the sections below.

3.1. European Union (EU) – EFSA evaluation of halosulfuron-methyl as a pesticide active substance

In the EU, halosulfuron-methyl was evaluated as a new pesticide active substance in the framework of Council Directive 91/414/EEC with Italy being the designated Rapporteur Member State (RMS). The RMS provided its initial evaluation of the dossier on halosulfuron-methyl in the Draft Assessment Report (DAR), which was received by the EFSA on 30 March 2008. (DAR, 2011: Public version).

EFSA published its conclusion on the peer review of the risk assessment of halosulfuron-methyl in 2012 (EFSA, 2012) and it was approved in the EU as a new plant protection active substance in September 2013. It is noted that the 2012 EFSA conclusion indicated that halosulfuron-methyl required classification for reproduction toxicity in Category 2 (Repr. 2 H361fd, "Suspected of damaging the unborn child.")

Subsequently, in 2017, RAC issued their opinion that halosulfuron-methyl should be classified as toxic for reproduction in the more severe hazard category: Repro. 1B (H360D "May damage the unborn child"), in accordance with Regulation (EC) No 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures (referred to as the "CLP Regulation"). This conclusion was based on the same data set initially submitted for the 2013 approval and only indicates a difference of interpretation from EFSA 2012.

An application for the renewal of halosulfuron-methyl as an active substance was submitted under Regulation (EC) No 1107/2009 in 2020 and is currently under evaluation by the RMS Italy.

In summary, the current regulatory status of halosulfuron-methyl in the EU is as follows: the substance is approved as an active substance and is currently under evaluation for renewal of approval. A Draft Renewal Assessment Report was prepared by the RMS, Italy in January 2023 and an overall decision on the approval of the renewal of halosulfuron-methyl is pending. The currently approved health-based reference values in the EU are therefore those indicated in the EFSA Conclusion 2012.

3.2. European Union (EU) – ECHA hazard identification and harmonised classification

Within the EU legislative framework, Regulation (EC) No. 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures serves as a hazard identification process, with direct risk management consequences, to ensure that the hazards presented by chemical substances are clearly communicated to workers and consumers in the European Union, across the supply chain. As such, the CLP Regulation does not facilitate the assessment of exposures to the chemical substances, the characterisation of the hazards (i.e.: via health-based reference values) or the assessment of health risks.

With respect to human health hazards, at the time of the classification review, there were no existing harmonised classifications for halosulfuron-methyl. Following the public consultation and the assessment of the available evidence against the classification criteria, RAC included a new classification for reproductive toxicity: Repro. 1B, with the hazard statement H360D: *"May damage the unborn child"* in their Opinion, that was adopted in September 2017. This conclusion was based on the same data set initially submitted for the 2013 approval and only indicates a difference of interpretation between EFSA 2012 and ECHA 2017.

4. Halosulfuron-methyl: Summary of mammalian toxicity data

This section presents a summary of the mammalian toxicological profile of halosulfuron-methyl based on the conclusions of authoritative regulatory evaluations conducted by EFSA and ECHA respectively, as part of evaluations in the EU.

As the renewal of the approval of halosulfuron-methyl is still on-going in the EU, there is currently no EFSA conclusion pertaining to this evaluation. The Applicants dossier (redacted version 1, December 2020: available from OpenEFSA (https://open-efsa.europa.eu) indicates that the toxicological data package submitted for the renewal is in the main part comparable to that submitted during the first active substance approval. While some additional studies were submitted in line with the changes in the data requirements since the new active substance approval in Europe, the findings from these studies do not impact overall on the toxicological profile of the substance.

It is noted that during the renewal evaluation, the RMS has made some changes in the interpretation in the findings of some studies in the dataset (i.e.: different No-Observed-Adverse-Effect-Levels, NOAEL have been derived in some cases) and while these changes do not impact overall on the critical effects identified as the points of departure for setting reference values, these interpretations are currently tentative subject to agreement at the level. Hence, in the sections below, relevant toxicological data has been sourced primarily from the RMS Assessment Report (DAR) prepared during the new active substance evaluation (EU, 2011: Public version DAR) as the conclusions drawn from interpretation of this dataset have informed the currently agreed EU health-based reference values.

4.1. Absorption, distribution, metabolism and excretion.

EU – EFSA evaluation and conclusions, 2012.

EFSA concluded that based on toxicokinetic studies, halosulfuron-methyl is rapidly absorbed (with the highest concentrations reached 0.5 hours post-dosing) and has high bioavailability (>80% of dose, based on urinary and biliary excretion and residues in the carcass). There was no evidence for absorption saturation.

In toxicokinetic studies, halosulfuron-methyl was widely distributed to different organs and had a very low potential for accumulation (<1% of residues were detected 168 hours after dosing, independently of the treatment regime).

Halosulfuron-methyl was found to be rapidly and extensively excreted with >70% excreted via the urine within 12 hours or via the faeces within 48 hours. Overall, between 79-102% of the administered dose was found to be excreted within 7 days. Between 32-55% of the administered dose was excreted via the urine within 7 days and 29-40% of the administered dose was excreted via the bile within 38 hours.

Halosulfuron-methyl is extensively metabolised in animals: no parent compound was detected in urine and low amounts (0.6-7%) only were detected in the faeces. The major metabolic pathway involved the demethylation and hydroxylation of the pyrimidine moiety with a minor pathway (<3%) involving the cleavage between the pyrimidine and pyrazole moieties. The major metabolites were determined to be: demethyl halosulfuron-methyl (urine: 13.3-37.7%; faeces: 6.6-22.6%) and 5-hydroxy demethyl halosulfuron-methyl (urine: not detected-39.9%; faeces: 1.6-24.5%).

EFSA concluded that the toxicologically relevant compound in animals, plants and the environment was determined to be halosulfuron-methyl.

4.2. Acute toxicity

EU – EFSA evaluation and conclusions, 2012

The acute toxicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: respective acute oral toxicity studies in rats and in mice, an acute dermal toxicity study in rats, an acute, whole body inhalation exposure study, respective skin and eye irritation and corrosion studies in rabbits and a skin sensitisation study conducted using the guinea pig maximisation test. These studies are detailed in the 2011 DAR (EC, 2011) and are summarised in the table below.

Based on the evaluation of the acute toxicity dataset, EFSA concluded that halosulfuron-methyl has low acute toxicity when administered via the oral, dermal or inhalation routes. Halosulfuron-methyl was not irritating to the skin or the eyes and did not have potential for skin sensitisation based on a Magnusson and Klingman test. The critical endpoints for the acute toxicity of halosulfuron-methyl included in Appendix A of the 2012 EFSA conclusion are indicated in bold in the table below (EFSA, 2012).

At renewal, an additional phototoxicity study was submitted indicating that halosulfuron-methyl does not have any phototoxic potential (EC, 2022).

Table 4.1: Summary of acute toxicity studies using halosulfuron-methyl

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50}	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
Acute oral US EPA 81-1 (1984); JMAFF 59 NohSan no. 4200 (1985), GLP	Rat (Sprague Dawley; 10/sex/group) Dose levels: 4000, 5000, 7500,10000 mg/kg bw 14-day observation period	LD_{50} = 10,435 mg/kg bw (males); 7758 mg/kg bw (females) **Overall LD_{50} = 7758 mg/kg bw**	Low toxicity	EC, 2011, Anon. 1990a (IIA, 5.2.1.1) **EFSA Conclusion, 2012 (LoE)**
Acute oral US EPA 81-1 (1984); JMAFF 59 NohSan no. 4200 (1985), GLP	Mouse (CD-1; 10/sex/group) Dose levels: 4000, 5000, 7500,10000 mg/kg bw 14-day observation period	LD_{50} = 16, 156 mg/kg bw (males); 9295 mg/kg bw (females) **Overall LD_{50} = 9295 mg/kg bw**	Low toxicity	EC, 2011, Anon. 1990b (IIA, 5.2.1.2) **EFSA Conclusion, 2012 (LoE)**
Acute dermal US EPA 81-1 (1984); JMAFF 59 NohSan no. 4200 (1985), GLP	Rat (Sprague-Dawley; 10/sex/group) Dose: 2000 mg/kg bw (Limit dose) 14-day observation period	**LD_{50} = > 2000 mg/kg bw**	Low toxicity	EC 2011, Anon. 1990c (IIA, 5.2.2) **EFSA Conclusion, 2012 (LoE)**
Acute inhalation US EPA 81-3 (1984);	Rat (Sprague-Dawley; 5/sex/group)	**LC_{50} > 6.0 mg/L air/4 h (whole body)**	Low toxicity	EC, 2011, Anon. 1991 (IIA, 5.2.3)

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50}	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
JMAFF 59 NohSan no. 4200 (1985); EU 92/69/EEC, B.2 (1992); (OECD 403 (1981); GLP	4h whole body exposure to 6 mg/L MMAD approx.4.3 µm 14-day observation period			**EFSA Conclusion, 2012 (LoE)**
Skin irritation OECD 404 (1981), GLP	Rabbit (NZW, 6 males) 0.5g for 4 hours (semi-occlusive)	-	**Non-irritant**	EC 2011, Anon. 1990a (IIA, 5.2.4) **EFSA Conclusion, 2012 (LoE)**
Eye irritation OECD 405 (1987) GLP	Rabbit (NZW, 3 males) 0.1 mL	-	Mild, transient ocular irritation **Non-irritant**	EC 2011, Anon. 1991 (IIA, 5.2.5) **EFSA Conclusion, 2012 (LoE)**
Skin sensitisation (Magnusson and Klingman maximisation Test) OECD 406 (1981)	Guinea pigs (Dunkin-Harley, 10/sex/group)	-	**Non-sensitiser (Magnusson and Klingman test)**	EC, 2011, Anon 1990b (IIA, 5.2.6) **EFSA Conclusion, 2012 (LoE)**

Key: LoE – List of Endpoints, NZW – New Zealand White

4.3. Short-term toxicity

EU – EFSA evaluation and conclusions, 2012

The short-term oral toxicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: 28-day and 90-day repeated dose dietary toxicity studies in rats, a 90-day repeated dose and a 12-month capsule study in dogs and a 21-day repeated dermal study in rats. These studies are detailed in the 2011 DAR (EC, 2011) and are summarised in the table below.

Based on the evaluation of the available short-term, repeated dose toxicity studies conducted using halosulfuron-methyl, EFSA concluded that the prominent effect observed was the reduction of body weight gain in dogs, rats and mice. In dogs, considered to be the most sensitive species, changes in clinical chemistry, haematological parameters and liver weight were also observed. The relevant short-term NOAEL was determined to be 10 mg/kg bw/day from the 90-day and 1-year studies in dogs (EFSA, 2012).

Summary of studies from the 2011 DAR:

In a 28-day repeated dose, dietary study, Sprague-Dawley (10/sex/group) were administered halosulfuron-methyl at 0, 300, 1000, 3000 or 10000 ppm (corresponding to 0, 23, 78, 231 and 777 mg/kg bw/day in males and 0, 25, 85, 241 and 888 mg/kg bw/day in females).

In the study, body weight gain was reduced in both sexes treated at 10000 ppm and in females treated at 3000 ppm, with a corresponding significant reduction in food consumption. Some changes in clinical chemistry parameters (lower protein, albumin, globulin and glucose and higher chloride ion) were also recorded in females treated at and above 300 ppm. The main finding observed in the study 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 rat studies even at higher dose levels. A NOEL was not determined in the study as slight effects in clinical chemistry parameters were observed in females treated at the lowest dose of 300 ppm. The NOAEL for the study was determined to be 300 ppm (corresponding to 23 mg/kg bw/day in males and 25 mg/kg bw/day in females), based on the absence of any histopathological lesions at this dose level.

In a 90-day repeated dose, dietary study, Sprague-Dawley (20/sex/group) were administered halosulfuron-methyl at 0, 100, 400, 1600, or 6400 ppm (corresponding to 0, 7.4, 28.8, 116 and 497 mg/kg bw/day in males and 0, 8.9, 37.3, 147and 640 mg/kg bw/day in females). In the study, 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 the kidney tubules was observed also at 1600 ppm. Since the increased haemosiderin pigmentation of kidney tubules was the only effect seen at 1600 ppm, was not statistically significant and not associated with any other toxic effect, the NOAEL for the study was determined to be 1600 ppm (corresponding to 116 and 147 mg/kg bw/day of halosulfuron-methyl in males and females, respectively). The NOEL was determined to be 400 ppm (28.8 mg/kg bw/day in males and 37.3 mg/kg bw/day in females).

In a 90-day capsular study, Beagle dogs (4/sex/group) were administered halosulfuron-methyl at 0, 2.5, 10, 40 or 160 mg/kg bw/day. In the study, halosulfuron-methyl administered at 40 or 160 mg/kg bw/day reduced body weight gain and increased liver weight. The highest dose level, 160 mg/kg bw/day, induced a variety of haematological and clinical chemistry changes including: a decrease in red cell parameters (erythrocyte and packed cell volume) for females, a decrease in total white cell counts and a shift towards myeloid cells in the bone marrow of males, and a reduction in cholesterol levels. The NOAEL for the study was determined to be 10 mg/kg bw/day.

In a 12-month capsule study, Beagle dogs (4/sex/group) were administered halosulfuron-methyl at 0, 0.25, 1, 10 or 40 mg/kg/day. In the study, doses at and above 40 mg/kg bw/day reduced haematological parameters. The 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 for the study was determined to be 10 mg/kg/day; the NOEL was 1 mg/kg bw/day.

In a 21-day dermal study, Sprague-Dawley (5/sex/group) were treated with halosulfuron-methyl at 0, 10, 100 or 1000 mg/kg bw/day. In the study, 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 bw/day was observed. The NOEL for the study was determined to be 10 mg/kg bw/day.

EFSA Conclusion, 2012: Appendix 2, List of Endpoints

The critical effects associated with the short-term toxicity of halosulfuron-methyl were considered to be: reduced body weight gain, liver effects and haematological changes in the dog and in the rat and increased haemosiderin pigmentation in the renal tubular epithelium in the rat. The following NOAELs were agreed for the short-term toxicity of halosulfuron-methyl:

Relevant oral NOAEL (Short-term toxicity): 90-day and 1-year, dog: 10 mg/kg bw/day; 90-day, rat: 116 mg/kg bw/day.

Relevant dermal NOAEL (Short-term toxicity): 21-day, rat: 100 mg/kg bw/day.

Relevant inhalation NOAEL (Short-term toxicity): Not required.

Table 4.2: Summary of short-term toxicity studies using halosulfuron-methyl

STUDY	SPECIES/STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
Rat 28-day oral (dietary) US EPA 40 CFR 158.135; JMAFF 59 NohSan no. 4200 (1985), GLP	Sprague-Dawley (10/sex/group) 0, 300, 1000, 3000, 10000 ppm (Males: 0, 23, 78, 231 and 777 mg/kg/day; Females: 0, 25, 85, 241 and 888 mg/kg/day) (purity: 98.5%)	NOAEL: 300 ppm (Males: 23 mg/kg/day Females: 25 mg/kg/day)	LOEL: 1000 ppm (Males 78 mg/kg/day Females: 85 mg/kg/day) Reduced body weight gain and overall food consumption, some clinical chemistry changes. At higher doses degeneration/necrosis of pancreatic acinar cells	EC 2011, Anon. 1988 (IIA, 5.3.1)
Rat 90-day oral (dietary) US EPA FIFRA 82-1 (1984); JMAFF 59 NohSan no. 4200 (1985). GLP	Sprague-Dawley (20/sex/group) 0, 100, 400, 1600, 6400 ppm (Males: 0, 7.4, 28.8, 116 and 497 mg/kg/day; Females: 0, 8.9, 37.3, 147 and 640 mg/kg/day) (purity: 98.6%)	NOAEL: 1600 ppm (Males: 116 mg/kg bw/day; Females: 147 mg/kg bw/day) NOEL: 400 ppm (Males: 28.8 mg/kg/day; Females: 37.3 mg/kg bw/day	LOEL: 6400 ppm Males: 497 mg/kg bw/day Females: 640 mg/kg bw/day) Reductions in: body weight gain, cholesterol, total bilirubin; increased (haemosiderin) pigmentation of renal tubular epithelium; mild vacuolation in the liver	EC 2011, Anon. 1990 (IIA, 5.3.2)
Dog 90-day oral (capsule) Similar to OECD 409, GLP	Beagle (4/sex/group) 0, 2.5, 10, 40 and 160 mg/kg/day (purity: 98.5%)	NOEL: 10 mg/kg/day	LOEL: 40 mg/kg/day Reduced body weight gain; increased liver weight and clinical chemistry alterations	EC 2011, Anon. 1991 (IIA, 5.3.3)
Dog 12-month oral (capsule) US EPA FIFRA 83-1 (1984); JMAFF	Beagle (6/sex/group) 0, 0.25, 1, 10 and 40 mg/kg/day (purity: 98.7%)	NOAEL: 10 mg/kg bw/day NOEL: 1 mg/kg/day	LOEL: 40 mg/kg/day Haematological changes (increased mean haemoglobin and haematocrit)	EC 2011, Anon 1991 (IIA, 5.3.4)

STUDY	SPECIES/STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
NohSan no. 4200 (1985). GLP				
Rat 21-day Dermal US EPA FIFRA 82-2 (1984). GLP	Sprague-Dawley (5/sex/group) 0, 10, 100, 1000 mg/kg/day (purity: 99.1%)	NOEL: 10 mg/kg/day	LOEL: 100 mg/kg/day Haematological changes (reduced body weight gain at higher doses)	EC 2011, Anon. 1991 (IIA, 5.3.7)

4.4. Genotoxicity

EU – EFSA evaluation and conclusions, 2012

As part of the EU toxicological assessment, the mutagenic potential of halosulfuron-methyl was evaluated in a regulatory battery of genotoxicity tests comprising: *in vitro* tests for bacterial and mammalian cell gene mutation, and chromosome aberrations and for unscheduled DNA, and an *in vivo* mouse micronucleus test for chromosome damage. These studies are detailed in the 2011 DAR (EC, 2011) and are summarised in the table below.

All the available *in vitro* and *in vivo* genotoxicity studies gave negative results, where applicable, in the presence and in the absence of a metabolic activation system, therefore, during the EU evaluation, EFSA concluded that halosulfuron-methyl did not have any genotoxic potential.

Table 4.3: Summary of genotoxicity studies using halosulfuron-methyl

STUDY	SPECIES/STRAIN AND DOSES	RESULTS	REFERENCE/STUDY NUMBER
***In vitro* studies**			
Bacterial reverse mutation US EPA FIFRA 84-2 (1984), GLP	*Salmonella typhimurium:* (TA1535, TA1537, TA1538, TA98 and TA100) *Escherichia coli:* WP2 uvrA First test: -/+ S9 mix: 1, 10, 100, 500, 100, 2500, 5001 and 10002 µg/plate (S. typhimurium) 333, 667, 1000, 3330, 6670 and 10000 µg/plate (E. coli) Second test: -/+ S9 mix: 1, 10, 100, 500, 1000, 2500, 5000, and 9999 µg/plate (S. typhimurium) 333, 667, 1000, 3330, 6670 and 10000 µg/plate (E. coli)	Negative +/- S9 mix	EC 2011, Jagannath and Lawlor, 1988 (IIA, 5.4.1)
Chromosome aberrations (clastogenicity) US EPA FIFRA 84-2 (1984), GLP	Chinese hamster ovary cells -S9 mix: 451, 903, 1020, 1050 and 1810 µg/ml +S9 mix: 449, 899, 1350 and 1800 µg/ml	Negative +/- S9 mix	EC 2011, Muri, 1988 (IIA, 5.4.2)
Mammalian cell gene mutation	Chinese hamster ovary cells (HGPRT assay)	Negative +/- S9 mix	EC 2011, Stegeman *et al.*, 1993 (IIA, 5.4.3)

STUDY	SPECIES/STRAIN AND DOSES	RESULTS	REFERENCE/STUDY NUMBER
***In vitro* studies**			
US EPA FIFRA 84-2 (1984), GLP	First test: -/+ S9 mix: 100, 200, 500, 700 and 900 µg/ml *Second test:* -/+ S9 mix: 50, 100, 200, 500 and 700 µg/ml		
Unscheduled DNA Synthesis US EPA FIFRA 84-2 (1984), GLP	Rat primary hepatocytes Trial 1: 25, 50, 100, 250, 500 and 1000 µg/ml Trial 3: 5.06, 10.1, 25.3, 50.6, 101 and 253 µg/ml	Negative	EC, 2011, Cifone, 1988 (IIA, 5.4.7)
***In vivo* studies**			
Micronucleus test US EPA FIFRA 84-2 (1984), GLP	Male and female ICR mice (5/sex/group) Bone marrow erythrocytes Doses: 0, 500, 1667 and 5000 mg/kg	Negative (no clastogenic or aneugenic potential	EC, 2011, Anon. 1989 (IIA, 5.4.4)

4.5. Chronic toxicity and oncogenicity

EU – EFSA and conclusions, 2012

The long-term toxicity and carcinogenicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: a 2-year combined chronic toxicity and carcinogenicity dietary feeding study in male and female Sprague-Dawley rats and a 78-week dietary feeding oncogenicity study in male and female CD-1 mice. These studies are detailed in the 2011 DAR (EC, 2011) and are summarised in the table below.

Based on the evaluation of long-term, repeated dose toxicity studies conducted using halosulfuron-methyl, EFSA concluded that the prominent effect observed was the reduction of body weight gain in rats and mice. The relevant long-term NOAEL was determined to be 43.8 mg/kg bw/day from the 2-year rat study.

No carcinogenic potential was observed in either rats or mice.

Summary of studies from the 2011 DAR

In a 104-week dietary combined chronic toxicity and oncogenicity study, Sprague-Dawley rats (85/sex/group) were administered halosulfuron-methyl at 0, 10, 100, 1000, 2500 or 5000 ppm in the male groups (corresponding to 0, 0.44, 4.4, 43.8, 108.3 or 225.2 mg/kg bw/day) and 0, 10, 100, 1000 or 2500 ppm in the female groups (corresponding to 0, 0.56, 5.6, 56.3 or 138.6 mg/kg bw/day). The critical effects in the study were reduced mean body weights observed throughout the study in males treated at 5000 ppm and between weeks 13 and 52 in females treated at 2500 ppm. The NOAEL for chronic toxicity (i.e.: non neoplastic end-points) was determined to be 1000 ppm, based on body weight reduction seen in females, corresponding to 56.3 mg/kg bw/day halosulfuron-methyl.

There was no evidence of oncogenic activity at any dose level. The respective NOAELs for oncogenicity was therefore determined to be: 5000 ppm in males and 2500 ppm in females, corresponding to 225.2 and 138.6 mg/kg bw/day of halosulfuron-methyl respectively.

In a 78-week dietary feeding oncogenicity study, CD-1 mice (75/sex/group) were administered halosulfuron-methyl at 0, 30, 300, 3000 or 7000 ppm (corresponding to 0, 4, 41.1, 410.0 or 971.9 mg/kg bw/day in males and 0, 5.2, 51.0, 509.1 or 1214.6 mg/kg bw/day in females).

In male mice treated at 7000 ppm, 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, there were increased incidences of microconcentrations/mineralisation 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 males treated at the highest dose, the NOAEL for the chronic toxicity (i.e.: non neoplastic end-points) of halosulfuron-methyl was determined to be 3000 ppm, corresponding to a mean achieved daily intake of 410.0 mg/kg bw/day.

No carcinogenic effects were observed in the study. The NOEL for oncogenicity was therefore determined to be 7000 ppm, corresponding to mean achieved daily intakes of 971.9 and 1214.6 mg/kg bw/day of halosulfuron-methyl in males and females, respectively i.e.: the highest dose tested.

EFSA Conclusion, 2012: Appendix A, List of Endpoints

The critical effects associated with the long-term toxicity of halosulfuron-methyl were considered to be: reduced body weight gain in rats and in mice, and increased microconcretions/mineralisation in the testis and epididymal tubules in mice. The following NOAELs were concluded for the long-term toxicity of halosulfuron-methyl:

Relevant NOAEL (Long-term toxicity): 43.8 mg/kg bw/day; 2-year, rat; 410 mg/kg bw/day; 18-month, mouse.

It was concluded that halosulfuron-methyl did not have carcinogenic potential.

Table 4.4: Summary of chronic toxicity and oncogenicity studies using halosulfuron-methyl

STUDY	SPECIES/ STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
Rat 2-year combined chronic toxicity and carcinogenicity study (dietary) US EPA 83-5 (1984); JMAFF 59 NohSan No. 4200 (1985), GLP	Sprague-Dawley (85/sex/group) **Males**: 0, 10, 100, 1000, 2500 and 5000 ppm (0, 0.44, 4.4, 43.8, 108.3, 225.2 mg/kg/day) **Females**: 0, 10, 100, 1000 and 2500 ppm (0, 0.56, 5.6, 56.3, 138.6 mg/kg/day) (Purity: 98.7%)	**Chronic toxicity:** NOEL: 1000 ppm = 56.3 mg/kg/day (Females:) **Carcinogenicity:** NOEL: Males: 5000 ppm Females: 2500 ppm (Males: 225.2 mg/kg/day Females: 138.6 mg/kg/day)	**Chronic toxicity:** LOEL: 2500 ppm=138.6 mg/kg/day (Females) Critical effect: reduced body weight gain **Carcinogenicity:** LOEL: Males: 5000 ppm Females: 2500 ppm (Males: 225.2 mg/kg/day Females: 138.6 mg/kg/day	EC 2011, Anon. 1992a (IIA, 5.5.1-5.5.2)

STUDY	SPECIES/ STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
			No carcinogenic potential at any dose level	
Mouse dietary 78-week oncogenicity study US EPA 83-5 (1984); JMAFF 59 NohSan No. 4200 (1985), GLP	CD-1 (75/sex/group) 0, 30, 300, 3000 and 7000 ppm (Males: 0, 4, 41.1, 410.0 and 971.9 mg/kg/day; Females: 0, 5.2, 51.0, 509.1 and 1214.6 mg/kg/day) (Purity: 98.7%)	**Chronic toxicity** NOAEL: 3000 ppm=410.0 mg/kg/day (Males) **Carcinogenicity:** NOEL: 7000 ppm (Males: 971.9 mg/kg/day Females: 1214.6 mg/kg/day)	**Chronic toxicity** LOAEL: 7000 ppm=971.9 mg/kg/day (Males) Critical effect: Reduced male body weight gain, increased microconcretions/ mineralisation in testis and epididymal tubules. **Carcinogenicity:** LOEL: 7000 ppm (Males: 971.9 mg/kg/day Females: 1214.6 mg/kg/day) No carcinogenic potential at any dose level	EC 2011, Anon. 1992b (IIA, 5.5.3)

4.6. Reproduction toxicity – Effects on fertility and sexual function

EU – EFSA and conclusions, 2012

The reproduction toxicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: a dietary two-generation reproduction toxicity study conducted in rats (with one litter in the first generation and two litters in the second generation. This study is detailed in the 2011 DAR (EC, 2011) and is summarised in the table below.

Summary of studies from the DAR, 2011

In a two-generation reproduction toxicity study, Sprague-Dawley rats (26/sex/group) were administered halosulfuron-methyl via the diet at 0, 100, 800 or 3600 ppm in two successive generations (corresponding to: 0, 6.3, 50.4, 223.2 mg/kg bw/day in F0 males and 0, 7.4, 61.0 or 274.2 mg/kg bw/day in F1 males; 0, 7.4, 58.7 or 261.4 mg/kg bw/day in F0 females and 0, 8.9, 69.7 or 319.9 mg/kg bw/day in F1 females.

In the study, reduced parental and pup body weights and/or body weight gains and parental food consumption were observed in both generations treated with halosulfuron-methyl at 3600 ppm. Overall, body weight gain was generally unaffected. There were no effects on fertility, reproductive performance or pup survival at any dose level. The NOAEL for general toxicity was determined to be 800 ppm, corresponding to mean achieved intakes of 50.4 mg/kg bw/day in males and 58.7 mg/kg bw/day in females. The NOAEL for effects on reproduction and fertility was 223.3 mg/kg bw/day i.e. the highest dose tested. The NOAEL for developmental

effects was determined to be 6.3 mg/kg bw/day based on decreased pup bodyweight gain at 800 ppm.

EFSA Conclusion, 2012: Appendix A, List of Endpoints

The critical effects for the reproduction toxicity of halosulfuron-methyl were concluded to be: reduced parental body weight and body weight gain and reduced pup body weight gain in the F1, F2a and F2b generation offspring. No adverse effects on reproduction or on fertility were observed. The following NOAELs were concluded for the reproductive and the developmental toxicity of halosulfuron-methyl:

Relevant parental NOAEL: 50.4 mg/kg bw/day

Relevant reproduction NOAEL: 223.2 mg/kg bw/day (the highest dose tested)

Relevant offspring NOAEL: 6.3 mg/kg bw/day

RAC Evaluation of reproductive toxicity – Effects on fertility and sexual function, 2017

The findings from the two-generation reproductive toxicity study were additionally evaluated in the consideration of the harmonised classification and labelling for halosulfuron-methyl in accordance with the CLP Regulation. On the basis that there were no treatment-related adverse effects on fertility or on reproductive performance in the study, including pre-coital interval at doses up to 3600 ppm in the study, the Dossier Submitter (DS), Italy concluded that no classification for adverse effects on sexual function and fertility was warranted.

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

Based on the available data and its interpretation, the RAC agreed with the DS's assessment that no classification for adverse effects on sexual function and fertility was warranted (ECHA, 2017).

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

Table 4.5: Summary of reproduction toxicity studies (effects on fertility and sexual function) using halosulfuron-methyl

STUDY	SPECIES/ STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
Two-generation (dietary) US EPA Guideline 83-4 (1984) JMAFF 59 NohSan No. 3850 (1984)	Rat (Sprague-Dawley Crl:CD BR; 26/sex/group) 0, 100, 800 and 3600 ppm (F0 males: 0, 6.3, 50.4, 223.2 mg/kg/day; F1 males: 0, 7.4, 61.0, 274.2 mg/kg/day; F0 females: 0, 7.4, 58.7, 261.4 mg/kg/day; F1 females: 0, 8.9, 69.7, 319.9 mg/kg/day)	NOAEL: General toxicity: 800 ppm (Males: 50.4 mg/kg/day Females: 58.7 mg/kg/day) Reproductive toxicity: 100 ppm (Males: 6.3 mg/kg/day; Females: 7.4-11.8 mg/kg/day) **EFSA Conclusion, 2012: NOAEL: Offspring: 100 ppm (males 6.3 mg/kg bw/day)**	LOAEL: General toxicity: 3600 ppm Reproductive toxicity: marginal LOAEL of 100 ppm (corresponding to 6.3 mg/kg/day for males and 7.4-11.8 mg/kg/day for females)	EC 2011, Anon. 1991 (IIA, 5.6.1)

4.7. Reproduction toxicity - Developmental effects

EU – EFSA evaluation and conclusions (2012)

The developmental toxicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: respective pre-natal, embryofoetal toxicity studies conducted in rats and in rabbits. These studies are detailed in the 2011 DAR (EC, 2011) and are summarised in the table below.

Summary of studies from the 2011 DAR

In a pre-natal developmental toxicity study, CD Crl:CD BR rats (25 females/group) were administered halosulfuron-methyl via oral gavage at 0, 75, 250 or 750 mg/kg bw/day from gestation day (GD) 6 to 15. In the study, the administration of halosulfuron-methyl at 750 mg/kg bw/day was maternally toxic causing clinical signs (alopecia and stained fur), reduced body weight and body weight gain. Developmental toxicity at this dose level was evidenced as a slight increase in early embryonic resorptions, reduced foetal weight, dilatation of the brain ventricles and reduced ossification. There was no indication of teratogenicity. The NOEL for maternal effects was determined to be 250 mg/kg bw/day. The NOEL for developmental toxicity was determined to be 75 mg/kg bw/day based on an increased number of foetuses and litters with soft tissue variations and less than 4 caudal vertebrae ossified observed in rats treated at 250 and at 750 mg/kg bw/day.

In the corresponding rabbit pre-natal developmental toxicity study, New Zealand White rabbits (17 females/group) were administered halosulfuron-methyl via oral gavage at 0, 15, 50 or 150 mg/kg bw/day from GD 7 to 19. In the study, maternal body weight was reduced at the highest dose level, 150 mg/kg bw/day. Embryofoetal toxicity was evidenced as an increased incidence of early resorptions. No indication of teratogenicity was found.

The NOEL for maternal toxicity was determined to be 50 mg/kg bw/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 foetuses (21.3%, 16.0%, 19.2% less than controls) observed at the 15, 50 and 150 mg/kg/day dose levels respectively. A marginal developmental LOEL of 15 mg/kg bw/day was defined.

EFSA Conclusion, 2012: Appendix A, List of Endpoints.

The critical effects for the developmental toxicity of halosulfuron methyl in rats were concluded to be: decreased maternal body weight/body weight gain and reduced food consumption and an increased number of foetuses and litters with visceral and skeletal variations. The following NOAELs were agreed during the EU evaluation:

Relevant maternal NOAEL (rat): 250 mg/kg bw/day

Relevant developmental NOAEL (rat): 75 mg/kg bw/day

The critical effects for the developmental toxicity of halosulfuron methyl in rabbits were concluded to be decreased maternal body weight/body weight gain and the increased in mean early resorptions and decreased number of foetuses. The following NOAELs were agreed during the EU evaluation:

Relevant maternal NOAEL (rabbit): 50 mg/kg bw/day

Relevant developmental NOAEL (rabbit): 50 mg/kg bw/day

Based on the findings of the developmental toxicity studies in rats and in rabbits, EFSA concluded that halosulfuron methyl meets the criteria for classification as reproduction toxicity in Category 2 (Repro. 2 H361fd). It was considered that the findings warranted consideration in respect of hazard classification for reproductive toxicity: the harmonised classification of halosulfuron-methyl was subsequently discussed by the ECHA RAC in the context of the human health hazard criteria indicated in the CLP Regulation (as discussed below).

EU - RAC Evaluation of developmental toxicity, 2017

The RAC evaluated the findings of the 2-generation reproduction study and the respective rat and rabbit developmental toxicity studies against the criteria indicated in the CLP Regulation:

"Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and shall be based on the presence of clear (Category 1B) or some (Category 2) evidence of an adverse effect on sexual function and fertility and/or on development. In addition, the evidence for both hazard categories shall be present in the absence of other toxic effects or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other concurrent toxic effects." (ECHA, 2017).

In their consideration of the findings in the rat 2-generation study (EC 2011, Anon. 1991 (IIA, 5.6.1), the RAC concluded there were no treatment-related adverse effects on development at doses up to 3600 ppm: the pup live birth index, litter size, pup viability (survival) and sex ratio were unaffected by treatment. There were no treatment-related clinical signs in the pups, and necropsy and histopathology did not show any treatment-related effects.

The RAC considered the following evidence for developmental effects associated with halosulfuron-methyl observed in the respective rat (Morseth, 1990a; EC 2011, Anon. 1990a (IIA, 5.6.10)) and rabbit (Morseth, 1990b; EC 2007, Anon. 1990b (IIA, 5.6.11)) developmental studies (ECHA, 2017):

1. Delayed development: there was a dramatic and statistically significant reduction in rat foetal body weight in both sexes:

i. Males: 3.4 ± 0.3 vs 2.6 ± 0.3 g, controls vs. high dose (-24%)

ii. Females: 3.2 ± 0.4 vs 2.5 ± 0.3 g, controls vs. high dose (-22%)

2. Delayed development: there was an extensive and widespread increase in rat skeletal variations:

i. (skeletal - total variations: 105/23 – 115/25 – 114/23 – 146/22)

3. Malformations: there was evidence for increased rat external, skeletal and visceral malformations (foetuses/litters)

i. External – tail: 0/0 – 0/0 – 0/0 – 4/3

ii. Skeletal – forked / fused ribs: 0/0 – 0/0 – 0/0 – 2/2

iii. Visceral – heart / great vessel: 0/0 – 0/0 – 0/0 – 2/2

4. There was an increase in mean rat early resorptions and post-implantation loss

i. resorptions: 1.0 vs. 1.5 (controls vs. high dose) [HCD: 0.3–1.5]

ii. post-implantation loss: 6.9% vs. 10.1% (controls vs. high dose) [HCD: 2.9–13.6%]

5. There was a reduction in rabbit mean live litter size at the high dose:

i. foetuses per litter: 7.2 – 7.4 – 7.2 – 5.8

6. There was a substantial increase in rabbit early resorptions and post-implantation loss:

i. resorptions: 0.8 vs. 2.0 (controls vs. high dose) [HCD: 0.1–1.0]

ii. post-implantation loss: 12.2% vs. 31.5% (controls vs. high dose) [HCD: 2.4–23%]

7. There was evidence of increased rabbit skeletal malformations:

i. skeletal – forked / fused ribs: 1/1 – 0/0 – 0/0 – 4/4

While the developmental toxicity was limited in both rats and rabbits to a single (high) dose group only in each study with no dose response observed at lower doses, the RAC concluded that potency, considered in isolation, was not a factor that should be considered in categorisation for reproductive toxicity. Although the reductions in foetal body weight were seen in only one study and species (i.e.: the rat), the RAC noted that the changes were statistically significant, outside the historical control data (HCD) range and associated with skeletal variations. The increase in rat external, skeletal and visceral variations and a very extensive and biologically significant delayed development of the skeletal system was observed at the top dose level of 750 mg/kg bw/day and in a few cases at 250 mg/kg bw/day (in this case maturation delay without any effect from maternal toxicity or foetal body weight reductions). There was also a high incidence of lateral ventricle dilatation at the high dose.

The RAC did not consider the adverse effects on development observed in the respective rat and rabbit studies to be secondary non-specific consequences of maternal toxicity. In the rabbit developmental study, the increase in post-implantation loss at high dose was accompanied by a marked retardation of uncorrected maternal body weight gain during the dosing period, but the body weight data was highly variable and the weight change differed significantly depending on the gestational interval under the study. According to the CLP criteria, the body weight gain in rabbits was not considered be a useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy. In addition, there were no clinical signs

of toxicity during the dosing period. Overall, the RAC concluded that the maternal body weight data was equivocal in rabbits and there was insufficient maternal toxicity to explain the degree of severity of the effects at the high dose. In addition, halosulfuron-methyl induced early resorptions impacting the post-implantation losses were also observed in rats in the presence of only minimal maternal toxicity. Although these effects were not statistically significant in either species, the incidences were above the concurrent control values and HCD in rabbits and above the concurrent control values in rats. Based on these considerations, the effects were considered biologically significant by the RAC.

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

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

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

Overall, the RAC concluded that there was clear evidence for adverse effects on development in the absence of excessive maternal toxicity, observed in both rats and rabbits with significant severity of findings in the offspring to warrant classification for development. The RAC adopted the opinion that classification with Repr. 1B – H360D “May damage the unborn child” was the most appropriate classification.

Table 4.6: Summary of reproduction toxicity studies (effects on development) using halosulfuron-methyl

STUDY	SPECIES/ STRAIN AND DOSES	NOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS	REFERENCE/ STUDY NUMBER
Developmental toxicity (oral gavage) US EPA FIFRA 83-3 (1984), GLP	Rat (CD Crl:CD BR; 25 females/group 0, 75, 250 and 750 mg/kg/day (GD 6 to 15)	NOEL: Maternal toxicity: 250 mg/kg/day Developmental toxicity: 75 mg/kg/day	LOEL: Maternal: 750 mg/kg/day Based on clinical signs, reduced maternal and foetal body weight, slight increase in early embryonic resorptions, dilated brain ventricles and reduced ossification Developmental: Increased number of foetuses and litters with soft tissue variations and less than 4 caudal vertebrae ossified at 250 and 750 mg/kg	EC 2011, Anon. 1990a (IIA, 5.6.10)
Developmental toxicity (oral gavage) US EPA 83-3 (1984), GLP	Rabbit (NZW, 17 females/group) 0, 15, 50 and 150 mg/kg/day (GD 7 to 19)	NOEL: Maternal toxicity: 50 mg/kg/day Developmental toxicity: not defined due to the increased mean early resorptions (15.3%, 10.0%, 24.4% vs 9.7% in controls) and decreased number of foetuses (21.3%, 16.0%, 19.2% less than controls) at 15, 50 and 150 mg/kg/day.	LOEL: Maternal: 150 mg/kg/day Based on reduced maternal body weight gain and increased early embryonic deaths	EC 2007, Anon. 1990b (IIA, 5.6.11)

4.8. Neurotoxicity

EU – EFSA evaluation and conclusions (2012)

The neurotoxicity studies conducted using halosulfuron-methyl evaluated as part of the EU toxicological assessment included: an acute oral neurotoxicity study and a 13-week subchronic dietary neurotoxicity study conducted in rats.

Summary of studies from the 2011 DAR (EC, 2011)

In an acute neurotoxicity study, rats received a single oral dose of halosulfuron-methyl at 0, 200, 600 or 2000 mg/kg bw/day. In the study, the highest dose level, 2000 mg/kg bw of halosulfuron-methyl, caused treatment-related transient increases in uncoordinated righting

reflex in both sexes at seven hours post-dosing. In addition, reduced body weight gain was seen in males during the first week post dosing. There were no microscopic neuropathological lesions. The NOAEL for acute neurotoxicity was 600 mg/kg bw/day.

In a subchronic dietary neurotoxicity study, male rats were dosed with halosulfuron-methyl at 0, 100, 1000 and 10000 ppm (0, 6.3, 62.8 and 706.0 mg/kg bw/day) whereas females were dosed at 0, 100, 1000 and 4000 ppm (0, 8.1, 82.5, 315.9 mg/kg bw/day) for 90 days. In the study, subchronic dietary treatment with up to 10000 ppm of halosulfuron-methyl in males and up to 4000 ppm in females did not induce any evidence of neurotoxicity, including any microscopic lesions of central and peripheral nervous systems. Overall body weight gain was reduced in males at 10000 ppm and a non-significant reduction was seen in females at 4000 ppm. Centrilobular hepatocyte hypertrophy in high dose males was associated with increased body weight and relative liver weight. The NOELs for neurotoxicity were 10000 ppm and 4000 ppm corresponding to 706.0 and 315.9 mg/kg bw/day of halosulfuron-methyl in males and females, respectively. The NOEL for general systemic toxicity was 1000 ppm in both sexes, corresponding to 62.8 and 82.5 mg/kg bw/day of halosulfuron-methyl in males and females, respectively.

Taking into account that the neurotoxicity study investigated a limited number of parameters compared with the repeated oral 90-day toxicity study in rats, there were no discrepancies in the data from the two respective studies.

Table 4.7: Summary of neurotoxicity studies using halosufuron-methyl

STUDY	SPECIES/ STRAIN AND DOSES	NOAEL	TARGET ORGAN/ SIGNIFICANT EFFECTS/ COMMENTS	REFERENCE/ STUDY NUMBER
Acute neurotoxicity (OECD 424, GLP)	Sprague-Dawley Single oral dose 0, 200, 600 and 2000 mg/kg	Neurotoxicity: 600 mg/kg	Decreased body weight gain and transient uncoordinated aerial righting reflexes in both sexes 7 hours post dosing, evidence of systemic toxicity. No progressive long term or irreversible neurotoxic changes were associated with treatment	Anon, 1994
90-Day neurotoxicity (OECD 424, GLP)	Sprague-Dawley Dietary Dose levels: Males 0, 100, 1000 and 10,000 ppm (0, 6.3, 62.8 and 706.0 mg/kg bw/day) Females 0, 100, 1000 and 4000 ppm (0, 8.1, 82.5, 315.9 mg/kg bw/day)	Neurotoxicity: Males 10,000 ppm, (706.0 mg/kg/day) Females 4000 ppm, (315.9 mg/kg/day) General systemic toxicity: 1000 ppm for both males and females, (62.8 and 82.5 mg/kg/day, respectively).	No evidence of neurotoxicity. Systemic toxicity: Body weight gain was reduced and, in males, centrilobular hepatocyte hypertrophy was increased at 10,000 ppm	Anon, 1992

4.9. Endocrine disrupting properties

At the current time, halosulfuron-methyl is not considered as having any endocrine disruption potential in any regulatory jurisdiction.

4.10. Summary of the toxicology profile of halosulfuron-methyl

The toxicology profile of halosulfuron-methyl has been comprehensively reviewed as part of authoritative regulatory evaluations undertaken in the EU by EFSA and ECHA. Assessments conducted by EFSA have incorporated hazard identification and characterisation to inform human health risk assessments, whereas ECHA and the RAC exclusively identified human health hazards for risk management and communication purposes in the EU.

Halosulfuron-methyl has low acute toxicity via the oral, dermal and inhalation routes, is not irritating or corrosive to the skin or the eyes and is not sensitising to the skin. Halosulfuron-methyl is not genotoxic based on the findings of a standard battery of *in vitro* and *in vivo* studies, is not neurotoxic and is not considered as having endocrine disruption potential in any regulatory jurisdiction.

The most prominent effect observed upon repeated dose 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/day from the 90-day and 1-year studies in dogs and the long-term NOAEL was 43.8 mg/kg bw/day from the 2-year rat study.

In the respective 2-year dietary studies in male and female rats and in a 78-week study in male and female mice, no oncogenic effects were observed indicating that halosulfuron-methyl is not carcinogenic.

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/day based on reduced pup body weight gain, while the parental NOAEL was 50.4 mg/kg bw/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/day. In the developmental toxicity study in rabbits, the maternal and developmental NOAELs were 50 mg/kg bw/day based on early resorptions, decreased number of foetuses and reduced maternal body weight gain. In the rat, foetal toxicity was observed in the absence of maternal toxicity: the developmental NOAEL was 75 mg/kg bw/day based on a higher incidence of visceral and skeletal variations and the maternal NOAEL was 250 mg/kg bw/day due to reduced body weight, body weight gain and food consumption.

In the EU, halosulfuron-methyl has been classified for reproduction toxicity in Category 1B, (Repr. 1B; H360D *"May damage the unborn child")* in accordance with the CLP Regulation – a hazard identification process intended for the communication of risk management measures throughout the chemical supply chain. Risk assessments conducted as part of the evaluation performed within the EU regulatory jurisdiction have included the relevant developmental hazard as part of the hazard characterisation, as indicated in the derivation of human health reference values discussed in Section 5.

5. Derivation of human health reference values

Following the evaluation of the mammalian toxicology and hazard profile of halosulfuron-methyl, the currently agreed health-based reference values adopted in the EU for use in regulatory risk assessments are summarised in Table 5.1 and discussed further in the sections below. The EU agreed reference values have been established based on the robust and critical evaluation of a comprehensive toxicological dataset for halosulfuron-methyl where the points of departure have taken into account the relevant critical effects and are considered to be adequately protective in the context of a health-based risk assessment. These reference values are therefore appropriate for informing the human health risk assessments submitted as part of the derogation application to support the safe use of the 750 WG products:

Table 5.1: Summary of health-based reference values derived for human health risk assessment (Source: EFSA Conclusion, 2012)

Reference endpoint	Derived value	Source	Based on endpoint:
ADI	**0.063 mg/kg bw/day**	EFSA (2012)	Based on a NOAEL of 6.3 mg/kg bw/day from a rat reproductive toxicity study (offspring toxicity), UF = 100
ARfD	**0.5 mg/kg bw/day**	EFSA (2012)	Based on the NOAEL of 50 mg/kg bw/day from a rabbit developmental toxicity study (maternal toxicity), UF = 100
AOEL	**0.063 mg/kg bw/day**	EFSA (2012)	Based on a NOAEL of 6.3 mg/kg bw/day from a rat reproductive toxicity study (offspring toxicity), UF = 100 No correction for oral absorption required
AAOEL	Not required	-	-

5.1. Reference values for dietary risk assessments

Derivation of the ADI

The potential health risk to consumers is considered to mainly result from the long-term exposure to residues of halosulfuron-methyl in food. In accordance with internationally accepted procedures, during the EU evaluation of halosulfuron-methyl as a pesticide active substance, the Acceptable Daily Intake (ADI) was derived, taking into account the critical effects and most relevant effects observed in the toxicological database, the NOAEL determined for the most sensitive species and an appropriate safety factor.

Following the peer review of the pesticide risk assessment of the active substance halosulfuron-methyl and expert consultation, the critical effect for the derivation of the ADI was determined to be offspring effects: reduced pup body weight gain in the F1, F2a and F2b generations observed in the two-generation reproduction toxicity study conducted in rats (EFSA, 2012). Based on these findings, the lowest NOAEL was determined to be 6.3 mg/kg bw/day. Applying a standard safety factor of 100 (i.e.: 10 for interspecies variability and 10 for intraspecies variability) to the NOAEL of 6.3 mg/kg bw/day, **the EU agreed ADI was determined to be 0.063 mg/kg bw/day.**

Derivation of an ARfD

The Acute Reference Dose (ARfD) is defined as an estimate of a substance in food or drinking water, that can be ingested over a short period, usually one day, without appreciable health risks to the consumer.

Following the peer review of the pesticide risk assessment of the active substance halosulfuron-methyl and expert consultation, the critical effect for the derivation of the ARfD was determined to be maternal toxicity observed in the rabbit pre-natal developmental toxicity study (EFSA, 2012). Based on these findings, the lowest NOAEL was determined to be 50 mg/kg bw/day. Applying a standard factor of 100 to the NOAEL of 50 mg/kg bw/day, **the EU agreed ARfD was determined to be 0.5 mg/kg bw/day.**

It is noted that as part of the on-going EU renewal evaluation of halosulfuron-methyl, the RMS has proposed the same reference values for the dietary risk assessment as are currently agreed at the EU level.

5.2. Reference values for non-dietary risk assessments

Derivation of the AOEL

The Acceptable Operator Exposure Level (AOEL) is the maximum amount of active substance to which an operator may be exposed without any adverse health effects.

Following the peer review of the pesticide risk assessment of the active substance halosulfuron-methyl and expert consultation, the critical effect for the derivation of the AOEL was determined to be offspring effects: reduced pup body weight gain in the F1, F2a and F2b generations observed in the two-generation reproduction toxicity study conducted in rats (EFSA, 2012). Based on these findings, the lowest NOAEL was determined to be 6.3 mg/kg bw/day. No correction to account for oral absorption was required. Applying a standard safety factor of 100 to the NOAEL of 6.3 mg/kg bw/day, **the EU agreed AOEL was determined to be 0.063 mg/kg bw/day.**

It is noted that as part of the on-going EU renewal evaluation of halosulfuron-methyl, the RMS has proposed the same AOEL value as currently agreed at the EU level.

During the peer review evaluation for the active substance approval in 2012, EFSA concluded that a reference value for acute operator exposures (i.e.: an Acute Acceptable Operator Exposure Level, AAOEL value) was not required. While the RMS has proposed an AAOEL value of 0.5 mg/kg bw/day (based on a comparable derivation to the ARfD) in the context of the EU renewal, this reference value is currently tentative only and subject to agreement at the EU level and has not therefore been considered in the non-dietary risk assessment submitted to support the derogation.

6. References

DALLRD (Department: Agriculture, Land Reform and Rural Development), 2024. Guideline for the Application for the Derogation for an Agricultural Remedy Identified as a Substance of Concern. Issued by the Registrar: Act No 36 or 1947, Private Bag X343, Pretoria, 0001, Republic of South Africa, April 2024.

EC (European Commission), 2011. DAR (Draft Assessment Report – Public version). Initial risk assessment by the rapporteur Member State Italy for the new active substance: Halosulfuron-methyl of the review programme referred to in Article 11(1) of Commission Regulation (EC) No. 1107/2009. Volume 3, B6 October 2011.

EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance halosulfuron (evaluated variant halosulfuron-methyl) EFSA Journal 2012;10(12):2987 and its Appendix A

EC (European Commission), 2022. Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008. Halosulfuron-methyl. Volume 3 – B.6 (AS). January 2022. Rapporteur Member State: Italy Directorate General for Hygiene, Food Safety and Nutrition Via Ribotta, 5 I – 00144 ROMA ITALY Co-Rapporteur Member State: Poland.

EC (European Commission), 2023. Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008. Halosulfuron-methyl. List of End Points. January 2023. Rapporteur Member State: Italy Directorate General for Hygiene, Food Safety and Nutrition Via Ribotta, 5 I – 00144 ROMA ITALY Co-Rapporteur Member State: Poland.

ECHA (European Chemicals Agency), 2017. Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at EU level of halosulfuron-methyl (ISO); methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate. CAS Number: 100784-20-1. CLH-O-0000001412-86-182/F Adopted 22 September 2017.

ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No. 528/2012 and (EC) No. 1107/2009. EFSA Journal 2018; 16(6): 5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311. ECHA-18-G-01-EN.

7. Supported products

Company	Product	Registration number
Farm-Ag International (Pty) Ltd	Brigadier 750 WG	L9218
ICA International Chemicals (Pty) Ltd	WeedO 750 WG	L11149
UPL South Africa (Pty) Ltd	Cyprex WG	L7665
Sharda International Africa (Pty) Ltd	Halosulfuron 750 WDG	L10855
Villa Crop Protection (Pty) Ltd	Halo 750 WDG	L8283
Rainbow Agrosciences (Pty) Ltd	Flagship 750 WDG	L10539
Green Island Investments Pty Ltd	Halo-Fron WG	L10152