**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

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

CLH-O-0000001412-86-182/F

**Adopted**

**22 September 2017**

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CLH-O-0000001412-86-182/F

# OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

**EC Number:** **Not allocated**

**CAS Number:** **100784-20-1**

The proposal was submitted by **Italy** and received by RAC on **11 July 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

# PROCESS FOR ADOPTION OF THE OPINION

**Italy** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 August 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 September 2016**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan MURRAY**

Co-Rapporteur, appointed by RAC: **Steve DUNGEY**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **22 September 2017** by **consensus**.

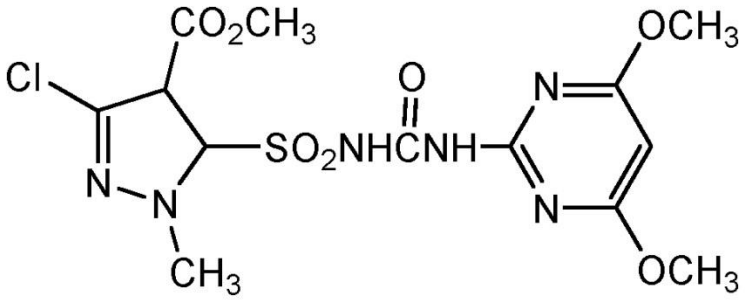
**Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)**

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard state-ment Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | 613-RST-VW-Y | halosulfuron-methyl (ISO); methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate | - | 100784-20-1 | Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | GHS09<br>Wng | H410 | | M=1000<br>M=1000 | |
| RAC opinion | 613-RST-VW-Y | halosulfuron-methyl (ISO); methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate | - | 100784-20-1 | Repr. 1B<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H360D<br>H400<br>H410 | GHS08<br>GHS09<br>Dgr | H360D<br>H410 | | M=1000<br>M=1000 | |
| Resulting Annex VI entry if agreed by COM | 613-RST-VW-Y | halosulfuron-methyl (ISO); methyl 3-chloro-5-{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-1-methyl-1H-pyrazole-4-carboxylate | - | 100784-20-1 | Repr. 1B<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H360D<br>H400<br>H410 | GHS08<br>GHS09<br>Dgr | H360D<br>H410 | | M=1000<br>M=1000 | |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC general comment

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




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

## RAC evaluation of physical hazards

### Summary of the Dossier Submitter's proposal

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

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

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

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

#### *Acute oral toxicity of halosulfuron-methyl*

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

The Osheroff (1990a) study was conducted with ten fasted male and female Sprague Dawley rats. The acute oral $LD_{50}$ values for halosulfuron-methyl (purity 98.5%) were greater than the limit dose of 2000 mg/kg bw. Mortality occurred at all dose levels. The principal clinical signs were described as common, non-specific findings and included: a vague clinical sign termed "depression", urine stains, hunched posture, red stains on nose and/or eyes and soft faeces. There was no evidence of a dose-related increase in clinical signs (table 12, CLH report). Body weight gain was unaffected, with all surviving animals gaining weight up to day of termination. All groups exhibited a variety of commonly noted necropsy findings with no evidence of a dose-related increase in any finding. The rat acute oral $LD_{50}$ values were 10435 mg/kg bw in males and 7758 mg/kg bw in females.

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

The DS did not propose classification for acute oral toxicity.

#### *Acute inhalation toxicity of halosulfuron-methyl in rats*

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

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

***Acute dermal toxicity of halosulfuron-methyl in rats***

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

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

## Comments received during public consultation

None for this section.

## Assessment and comparison with the classification criteria

***Acute Oral Toxicity***

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

***Acute Inhalation Toxicity***

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

***Acute Dermal Toxicity***

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

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

## Summary of the Dossier Submitter's proposal

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

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

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

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

With respect to STOT SE Category 3 (transient effects; narcotic effects and respiratory tract irritation), there was no specific data available and insufficient evidence of respiratory tract irritation. In general, data from single and repeated dose inhalation toxicity tests may provide useful information for this hazard category. In an acute inhalation study in rats (DAR: Bechtel, 1991) the maximum tested nominal concentration was 6.0 mg/L air, and resulted in non-specific signs observed immediately after exposure such as laboured respiration, hypoactivity, red/pink nasal discharge, periorbital wetness, perinasal and periorbital encrustations. The encrustations persisted up to and including day 2 post-exposure but all rats were normal by day 3. At necropsy, there were no abnormalities noted. The clinical signs observed do not provide sufficient evidence for irritation of the respiratory tract. There were no short term inhalation studies performed with halosulfuron-methyl. The DS did not propose classification. There was no evidence of narcotic effects in any acute or repeat-dose toxicity studies. Accordingly, RAC agrees with the DS that **no classification for STOT SE 3 is warranted.**

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier Submitter's proposal

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

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

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

## RAC evaluation of serious eye damage/irritation

### Summary of the Dossier Submitter's proposal

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

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

No effects in the iris or cornea were noted. The effects observed in conjunctiva (redness) were reversed within 72 hours. The mean scores for each animal calculated over 24, 48 and 72 hours for erythema and oedema of the conjunctivae were less than the CLP criteria values for classification. RAC supports the DS conclusion that no classification is warranted for serious eye damage/eye irritation.

## RAC evaluation of respiratory sensitisation

### Summary of the Dossier Submitter's proposal

No data available.

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

The assessment of respiratory sensitisation is not possible due to the absence of human and/or non-human respiratory hypersensitivity data.

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

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

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

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

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

### Summary of the Dossier Submitter's proposal

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

No clear target organ or tissue was identified for the short-term toxicity of halosulfuron-methyl. The most prominent effect observed upon repeated dose toxicity testing with halosulfuron-methyl was a reduction of body weight gain in dogs, rats and mice. In dogs, which were the most sensitive species, changes in clinical chemistry, haematological parameters and liver weight were also observed, although none of these were of sufficient magnitude or severity to meet the criteria for either specific target organ toxicity repeated exposure (STOT RE) category 1 or 2 (table 2, CLH report).

Rat Studies

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

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

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

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

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

Dog Studies

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

urinalysis and there were no treatment-related macroscopic or histopathological findings, even though liver weights were increased. The LOAEL was considered to be 160 mg/kg bw/day based on reduced body weight gain and increased liver weight.

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

Chronic Studies

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

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

The 78-week oral study in mice (Moore, 1992b), was conducted at dietary concentrations up to 972/1215 mg/kg bw/day for males and females, respectively. At the highest dose, male body weight parameters were significantly reduced at certain time points. At the same dose, there were increased incidences of microconcretions/mineralisation both within the lumen of both the epididymal and testis tubules (epididymis: 5/44 compared with 0/40 in controls; testis: 12/63 compared with 5/70 in controls). On the basis of these effects, the NOAEL was set at the next lower dose of 410 mg/kg bw/day in males.

***Repeated Dose Toxicity Summary***

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

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

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

## Comments received during public consultation

No comments were received during public consultation.

## Assessment and comparison with the classification criteria

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

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

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

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

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

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

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

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

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

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

#### *Introduction*

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

#### In Vitro *Tests*

The genotoxicity of halosulfuron-methyl was investigated in an Ames test (using *Salmonella typhimurium* strains: TA1535, TA1537, TA1538, TA98 and TA100 in addition to *Escherichia coli*: WP2 uvrA$^-$ in the presence and absence of exogenous metabolic activation system), in an *in vitro* mammalian clastogenicity study using CHO cells, in an *in vitro* mammalian cell gene mutation study accessed at the HPRT gene locus using CHO cells and in an unscheduled DNA synthesis (UDS) assay in cultured primary rat hepatocytes. Positive controls were included in all assays and behaved as expected. The results from all these assays were negative (table 7, below). There was no significant cytotoxicity except for rat hepatocytes in the *in vitro* UDS assay.

#### In Vivo *Tests*

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

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

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

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

### Comments received during public consultation

No comments were received for this hazard endpoint.

### Assessment and comparison with the classification criteria

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

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

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

#### *Rat 2-year dietary toxicity/oncogenicity study*

The chronic toxicity and carcinogenicity of halosulfuron-methyl was investigated in SD rats for at least 104 weeks (Moore, 1992a). Administration of halosulfuron-methyl did not affect survival amongst males (except for a spurious finding of lowered survival in the 44 mg/kg bw/day group, 33%) or females at any dose level. Any clinical abnormalities were described as incidental with

no evidence of a dose response and no consistent pattern of occurrence. There were scheduled necropsies conducted on 10 animals/sex from each dose group in 3 interim sacrifices during weeks 27, 53 and 79; and on all surviving animals at study termination.

Achieved doses (mg/kg bw/day)

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

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

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

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

***Mouse 18-month dietary oncogenicity study***

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

Achieved doses (mg/kg bw/day)

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

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

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

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

## Comments received during public consultation

No comments were received during public consultation.

## Assessment and comparison with the classification criteria

***Relevance of neoplastic changes observed in the rat study.***

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

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

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

#### *Rat two-generation (dietary) study*

General

In a 2-generation reproductive toxicity study in the rat (Lemen, 1991), groups of 26 male and 26 female Sprague Dawley CD rats were given dietary concentrations of 0, 100, 800 or 3600 ppm of halosulfuron-methyl continuously throughout the two generations (F0 and F1). One litter was derived from the adult F0 generation and two litters from the adult F1 generation.

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

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

Maternal/Parental toxicity

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

Adverse effects on sexual function/fertility

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

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

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

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

Offspring developmental toxicity

*Pup live birth indices*

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

*Pup viability indices*

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

*Weaning indices*

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

*Mean number of offspring born/litter*

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

*Clinical signs*

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

*Pup weights*

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

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

The DS considered the effect seen in F1 litters during lactation to be equivocal as there was no consistent evidence of effect on offspring body weight or body weight gain in other offspring at 800 ppm (data not shown in table 47 of CLH report). Furthermore, the DS did not consider it necessary or appropriate to designate 800 ppm as an adverse effect level for offspring, but rather that the NOAEL for offspring was 800 ppm (50.4 mg/kg bw/day), equivalent to the NOAEL set for parental toxicity due to decreases in body weight gain.

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

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

***Rat developmental (gavage) study***

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

Gavage doses (mg/kg bw/day)

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

Maternal toxicity

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

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

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

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

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

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

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

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

Adverse effects on sexual function and fertility

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

*Historical control data*

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

Offspring developmental toxicity

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

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

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

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

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

Consequently, 250 mg/kg bw/day was considered to be the effect level and 75 mg/kg bw/day was proposed as the developmental NOAEL (EFSA, 2012). The DS considered that the relatively small number and slight or small increase in variants seen in this group (250 mg/kg bw/day) were associated with low foetal weight (below the group mean but remaining within the concurrent control range) and could be attributable to slight immaturity. The DS reported some statistics from a compendium of HCD compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA) and the Midwest Teratology Association (MTA) from developmental studies conducted between 1992 and 1994 in the same rat strain (1996). This was used to provide foetal incidence data for a number of findings at the intermediate dose:

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

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

In the summary and discussion of reproductive toxicity (section 4.11.4, CLH report), the DS included applicant comments in support of no classification. The DS supported no classification because effects (mainly the small foetal weight and immaturity and associated visceral and

skeletal findings according to the DS) seen in the high dose group occurred together with maternal toxicity at that dose.

***Rabbit developmental (oral gavage) study***

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

Gavage doses (mg/kg bw/day)

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

Maternal toxicity

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

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

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

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

* $p \leqslant 0.05$

Adverse effects on sexual function and fertility

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

*Table 13: Summary of rabbit fertility and developmental parameters*

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

*Historical control data*

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

Offspring developmental toxicity

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

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

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

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

***Overall DS conclusion on classification and labelling***

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

## Comments received during public consultation

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

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

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

One MSCA made a point about reduced pregnancy rates and numbers of dams with litters as potentially relevant from the 2-generation rat study. Reproductive indices were confirmed to be correct. The MSCA suggested that this could be a reproductive effect worth considering for classification but did not make a proposal. The MSCA took note of the significant increase in external, skeletal and visceral malformations and variations at the top dose level of 750 mg/kg bw/day and supported Repr. 2, H361d. The MSCA also notes a higher rate of early resorptions in the rabbit study, this too supporting classification.

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

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

***Applicant rebuttal to potential classification for reproductive toxicity***

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

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

1. Early resorptions: In the highest dosage group (750 mg/kg bw/day), two pregnant females did not carry live foetuses to term (non-viable foetus; NVF). Omitting the data from these two animals, results in the group mean value for early resorption is 1.5, which is at the upper end of the background control data (range 0.3 to 1.5).

2. Maternal toxicity and effects in the foetuses from high dose dams: The applicant supplied a table with mean maternal body weight gains during gestation and argued that reduced body weight gain was an indicator of significant maternal toxicity, which accounted for secondary effects seen in the foetuses. They made reference to two published papers where it was evident that rat maternal physiology can compensate enough to be largely (Fleeman *et al.,* 2005) or partly (Ikemi *et al., 1993*) protective of the foetus even in the presence of marked body weight loss during the treatment period.

*Table 14: Mean maternal bodyweight gain during gestation*

| **Gestation days** | **Measurement** | **Dose levels (mg/kg bw/day) (% relative to concurrent controls)** | | | | |
|---|---|---|---|---|---|---|
| | | **0** | **75** | **250** | **750** | **750 (excl. NVF)** |
| Number of dams | | 25 | 25 | 24 | 24 | 22 |
| 0 - 6 | Mean bw change | 30.20 | 30.00 | 29.13 | 30.75 | 30.77 (98%) |
| | SD | 8.85 | 9.30 | 6.62 | 6.92 | 7.24 |
| 6 - 8 | Mean bw change | 5.04 | 5.72 | 5.08 | **1.63*** | **1.73* (34%)** |
| | SD | 4.47 | 4.86 | 4.42 | 7.47 | 7.69 |
| 6 - 12 | Mean bw change | 24.00 | 24.00 | 25.63 | **12.38***** | **14.64*** (61%)** |
| | SD | 4.45 | 5.63 | 5.35 | 10.68 | 7.76 |
| 6 - 16 | mean bw change | 49.28 | 49.20 | 49.38 | **32.96***** | **37.00*** (75%)** |
| | SD | 8.96 | 9.50 | 7.72 | 17.30 | 10.75 |
| 16 - 20 | mean bw change | 53.04 | 51.48 | 52.75 | 47.42 | 49.73 (94%) |
| | SD | 11.82 | 11.93 | 15.08 | 11.33 | 8.48 |

NVF = Dams with non-viable foetuses

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

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

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

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

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

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

NP=NOT PREGNANT; EXCLUDED
NVF=NO VIABLE FETUSES

Rabbit developmental toxicity study

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

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

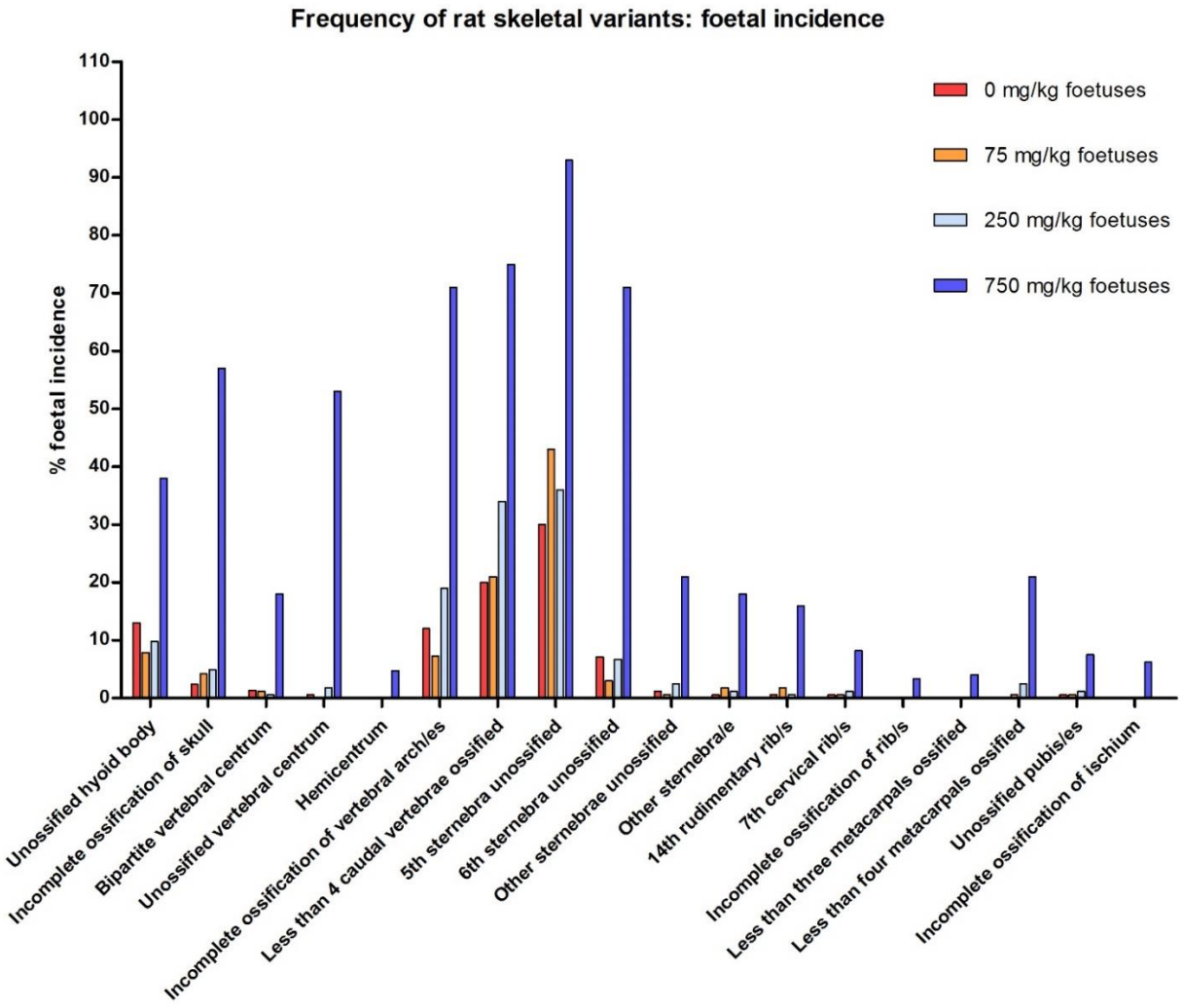
***RAC assessment of skeletal variations in the rat developmental study***

RAC noted the widespread and extensive effect on the rat skeletal system in foetuses from the high dose group as documented in table 33 of the CLH report. Reporting the overall skeletal variant incidence as [affected foetuses/litters] 105/23 – 115/25 – 114/23 – 146*/22 for controls to high dose, respectively, does not illustrate the widespread nature of the skeletal anomalies observed at the high dose. RAC has graphed the frequency of occurrence of each anomaly with respect to dose group to illustrate more clearly the effects observed at the high dose.

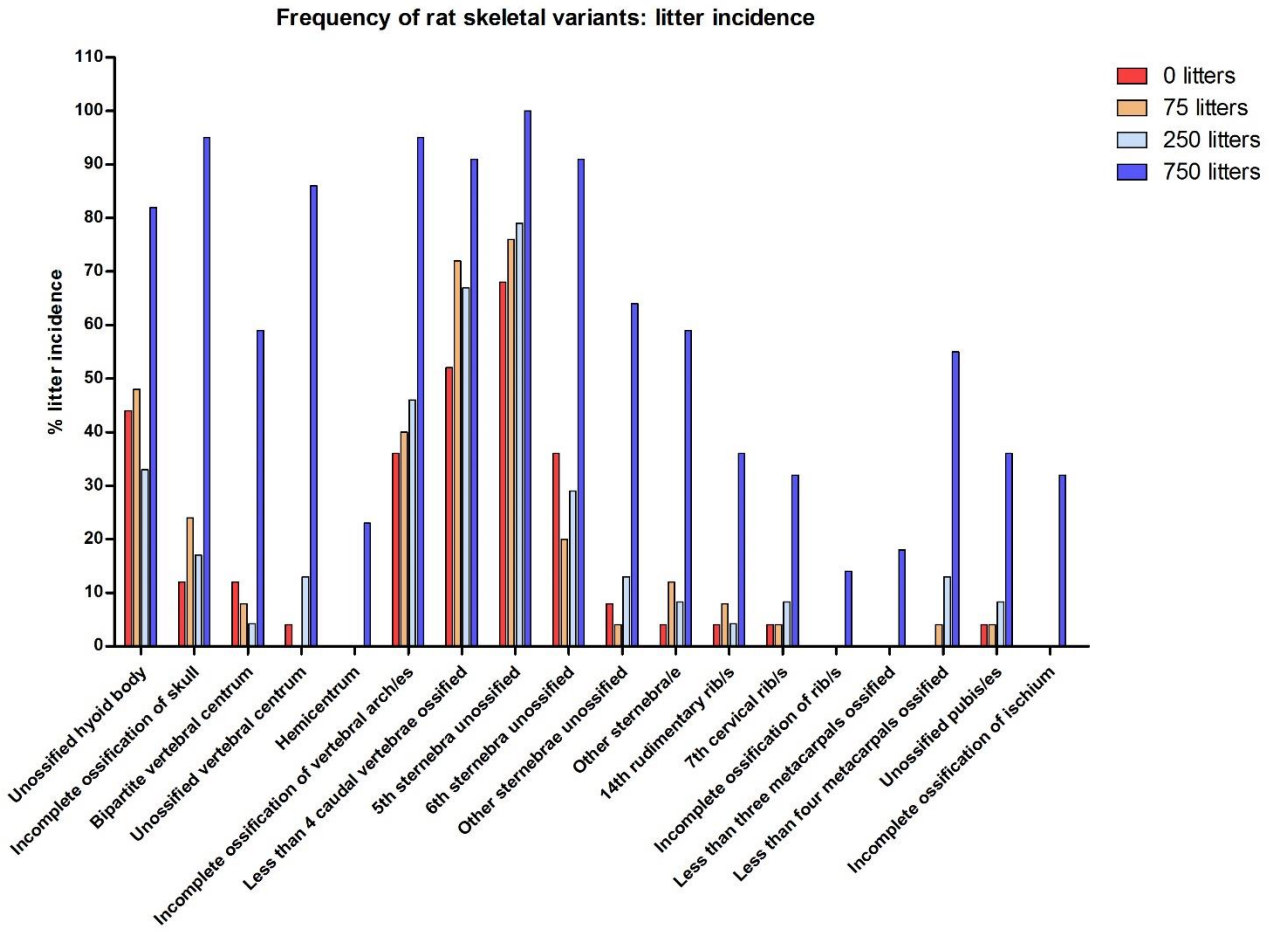
*% Incidence data for skeletal variants amongst foetuses and litters:*

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

*Foetal incidence (% of total foetuses affected)*



*Litter incidence (% of total litters affected)*




## Assessment and comparison with the classification criteria

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

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

### *Adverse effects on development*

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

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

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

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

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

The adverse effects on development in both rat and rabbit are not considered secondary non-specific consequences of maternal toxicity. In the rabbit developmental study, the increase in post-implantation loss at high dose was accompanied by a marked retardation of uncorrected maternal body weight gain during the dosing period, but the body weight data was highly variable and the weight change differed significantly depending on the gestational interval under the study.

According to the CLP criteria, the body weight gain in rabbits may not be a useful indicator of maternal toxicity because of normal fluctuations in body weight during pregnancy. In addition, there were no clinical signs of toxicity during the dosing period. Overall, RAC concludes that the maternal body weight data is equivocal in rabbits and maternal toxicity is insufficient to explain the degree of severity of the effects at the high dose. In addition, halosulfuron-methyl induced early resorptions impacting the post-implantation losses as also observed in rats in the presence of only minimal maternal toxicity. Although these effects were not statistically significant in either species, the incidences were above the concurrent control values and HCD in rabbits and above the concurrent control values in rats, these effects are considered biologically significant by RAC.

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

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

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

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

***Adverse effects on sexual function and fertility***

In the rat 2-generation study there were no treatment-related adverse effects on fertility or reproductive performance including pre-coital interval at doses up to 3600 ppm. Gestation length

was unaffected by treatment (table 16). RAC agrees with the DS that minor changes in fertility parameters in treated groups were not dose-related and do not represent an adverse effect of treatment.

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

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

***Effects on or via lactation***

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

## RAC evaluation of aspiration toxicity

### Summary of the Dossier Submitter's proposal

No data.

### Comments received during public consultation

No comments on this endpoint.

### Assessment and comparison with the classification criteria

Halosulfuron-methyl is a white solid of low volatility of $3.5 \times 10^{-6}$ Pa $m^3$ $mol^{-1}$. The surface tension of a 90% saturated aqueous solution of halosulfuron-methyl was found to be 70.5 mN/m. The substance is not considered to be surface active.

RAC concludes that there is no data meeting the CLP criteria for aspiration hazard.

# ENVIRONMENTAL HAZARD EVALUATION

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

Halosulfuron-methyl is an active substance in herbicides and is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008. The DS proposed to classify the substance as Aquatic

Acute 1; H400 (M=1000) based on a 7d $E_bC_{50}$ of 0.000217 mg/L for the macrophyte *Lemna gibba*, and Aquatic Chronic 1; H410 (M=1000) based on lack of rapid degradation and a 7d $NOE_rC$ of 0.00003 mg/L for *L. gibba*.

***Degradation***

Halosulfuron-methyl hydrolyses with a half-life of 25 – 29 days at pH 5, 14 – 15 days at pH 7 and 18 – 20 hours at pH 9 (at around 25 °C). It is photolytically stable when exposed to natural sunlight for 30 days; degradation observed in an aqueous photolysis study was attributed to hydrolysis (based on dark control results). The major abiotic transformation products were the rearrangement ester, the 3-chlorosulfonamide ester and the 3-chlorosulfonamide acid (depending on pH).

A modified Sturm test (OECD TG 301B) indicated 3 % degradation (at most) over 29 days. Toxicity controls demonstrated that the substance was not inhibitory to the microbial inoculum, so it is not readily biodegradable.

Two aerobic water/sediment simulation tests at approximately 20°C indicated rapid primary degradation but a low level of mineralisation over 100 days. Total system half-lives were 6.3 days in the clay loam system (pH 8.1) and 10.4 days in the sandy loam system (pH 6.7). This was attributed to both hydrolysis and microbial degradation. Mineralisation of the radiolabels accounted for only 0.2 – 5.7% of applied radioactivity (AR) at the end of the study. Several transformation products were identified, the major ones being halosulfuron-methyl rearrangement (with a half-life of 22 –25.4 days) and halosulfuron rearrangement (half-life not stated). Bound residues increased throughout the study, reaching 19–60% AR by the end.

The CLH report includes data for soil degradation but as these are not directly relevant they are not summarised here.

The DS concluded that halosulfuron-methyl is not rapidly degradable based on this information.

***Bioaccumulation***

The measured octanol-water partition coefficient (log $K_{OW}$) is in the range -0.02 to 1.67 at 23 °C, depending on pH (the $pK_a$ is 3.44 at 22.4 °C). The DS concluded that halosulfuron-methyl is not bioaccumulative as the log $K_{OW}$ is < 4.

***Aquatic toxicity***

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). All studies were performed under flow-through conditions with results expressed in terms of mean measured concentrations, unless stated otherwise.

*Table 27: Summary of relevant information on aquatic toxicity*

| **Method** | **Test organism** | **Endpoint** | **Toxicity values in mg/L** | **Reference** |
|---|---|---|---|---|
| Short-term toxicity to fish | | | | |
| US EPA FIFRA 72-1, ASTM E 729-88 | *Oncorhynchus mykiss* (Rainbow Trout) | 96h $EC_{50}$ | > 131 | Holmes and Swigert, 1993a |
| OECD TG 202, EEC C.2 | *Lepomis macrochirus* (Bluegill Sunfish) | 96h $LC_{50}$ | > 118 | Holmes and Swigert, 1993b |
| Long-term toxicity to fish | | | | |
| US EPA FIFRA 72-4, ASTM E 1241-88[a] | *Oncorhynchus mykiss* (Rainbow Trout) | 87d NOEC | 34 | Graves *et al.*, 1993 |
| Short-term toxicity to aquatic invertebrates | | | | |
| US EPA FIFRA 72-1, ASTM E 729-88 | *Daphnia magna* | 48h $EC_{50}$ | > 107 | Holmes and Swigert, 1993c |
| US EPA FIFRA 72-3, ASTM E 729-88 | *Mysidopsis [Americamysis] bahia* (mysid shrimp) | 96h $LC_{50}$ | 109 | Swigert and Smith, 1993 |
| N.A. ("based on OECD principles", semi-static) | *Lymnaea peregra* (gastropod mollusc) | 96h $LC_{50}$ | > 89.9 | Jenkins, 2004b |
| Long-term toxicity to aquatic invertebrates | | | | |
| US EPA FIFRA 72-4, ASTM E 1193-87 | *Daphnia magna* | 21d NOEC (reproduction) | < 6.9 | Zelinka *et al.*, 1993a |
| US EPA FIFRA 72-4, ASTM E 1193-87 | *Daphnia magna* | 21d NOEC | 7.2 | Zelinka *et al.*, 1993b |
| Toxicity to algae and aquatic macrophytes | | | | |
| US EPA FIFRA 123-2, US 40 CFR 797.1075, ASTM E1218-90 (static) | *Pseudokirchneriella subcapitata* | 120h $E_rC_{50}$<br>120h $NOE_rC$ | 0.00507<br>0.00063<br>(nominal) | Thompson and Swigert, 1993 |
| OECD TG 201[b] (static) | *Pseudokirchneriella subcapitata* | 72h $E_rC_{50}$<br>72h $NOE_rC$ | 0.005<br>0.001<br>(nominal) | Seki, 2008 |
| US EPA FIFRA 123-2, ASTM E 1218-90 (static) | *Anabaena flos-aquae* (blue-green alga) | 120h $NOE_bC$ | 0.05<br>(nominal) | Thompson and Swigert, 1994a |
| US EPA FIFRA 123-2, ASTM E 1218-90 (static) | *Navicula pelliculosa* (diatom) | 120h $EC_{50}$<br>120h NOEC<br>(based on cell density) | > 0.35<br>0.35<br>(nominal) | Thompson and Swigert, 1994b |
| US EPA FIFRA 123-2 (static) | *Skeletonema costatum* (diatom) | 120h $EC_{50}$<br>120h NOEC<br>(based on cell density) | > 0.4<br>0.4<br>(nominal) | Thompson and Swigert, 1994c |

| Method | Test organism | Endpoint | Toxicity values in mg/L | Reference |
|---|---|---|---|---|
| Short-term toxicity to fish | | | | |
| OECD TG 221 (draft 2002) (semi-static) | *Lemna gibba* (duckweed) | 7d $E_rC_{50}$<br>7d $NOE_rC$ | 0.000491<br>0.00003 | Jenkins, 2005a |
| N.A. – data not available<br>Note: a – The CLH dossier cites an incorrect test method (OECD TG 202). This was an early life stage test, and the actual method is taken from the DAR.<br>b – The CLH dossier does not cite the test method. The actual method is taken from the DAR. | | | | |

The Zelinka *et al*. (1993a) chronic toxicity study used fewer daphnids than recommended by the OECD TG. The DS did not provide a reliability rating. Although effects were observed at the lowest concentration, the DS did not discuss this further. RAC notes that this does not influence the classification.

Concentrations were maintained close to nominal in the static algal/diatom tests, so results were reported based on nominal concentrations. Four of the tests were conducted over 5 days (120h), but 72h results are not provided. The DS did not provide any information about whether the cells were in an exponential growth phase throughout.

The key study is for *Lemna gibba*. The results were based on mean measured concentrations since measured levels in samples of expired media ranged from 1 – 16% of their nominal value at the highest dose, from below the limit of quantification (0.03 µg/L) to 13% at the next two doses and could not be quantified at the three lowest doses. The overall mean measured levels of halosulfuron-methyl were 0.006, 0.009 (estimated values), 0.03, 0.141, 0.733 and 2.584 µg/L. Additional measurements in expired media from flasks kept without plants showed concentrations in the range 31 – 71 of nominals, suggesting that the presence of *Lemna* affected the stability of halosulfuron-methyl under the conditions of the test.

A 28d NOEC of 5 mg/L is also reported for the insect *Chironomus riparius* from a static water-sediment test. The total amount of test material accounted for in all fractions at the end of the test ranged from 105.3-112.4% AR, with 70-77.7% AR in the overlying water.

Ecotoxicity data are presented for the main transformation products in several species, and none appear to be more toxic than the parent substance.

## Comments received during public consultation

Four MSCAs supported the proposed environmental classification. Two MSCAs asked why the biomass end point was used for the acute classification (rather than growth rate, noting that this does not affect the proposal); the DS said that it was based on the outcome of an EFSA expert consultation without any further explanation. One MSCA asked for some minor clarifications, but these do not affect the proposal.

## Assessment and comparison with the classification criteria

### *Degradation*

The substance undergoes primary degradation via both abiotic and biotic processes with a half-life of less than 16 days at a pH of 6 and above. However, it is not readily biodegradable, and the abiotic half-life at pH 5 is above 16 days. Limited mineralisation was also observed in two

water/sediment simulation tests. Therefore, RAC agrees with the DS that halosulfuron-methyl is not rapidly degradable.

***Bioaccumulation***

RAC agrees with the DS that halosulfuron-methyl has a low potential to bioaccumulate based on a log $K_{OW}$ value below the CLP Regulation threshold of 4.

***Aquatic toxicity***

Short-term aquatic toxicity data are available for three trophic levels (10 species). The $EC_{50}$s for algae and aquatic macrophytes are below 1 mg/L, with the duckweed *Lemna gibba* the most sensitive species. The DS proposal is based on a 7d $E_bC_{50}$ of 0.000217 mg/L, but the DS did not adequately explained why this is preferred to the more usual growth rate end point (7d $E_rC_{50}$ of 0.000491 mg/L) recommended in the Guidance on the Application of the CLP Criteria Version 4.1, June 2015 (Section 4.1.3.3.1, p. 505). RAC prefers using the 7d $E_rC_{50}$ of 0.000491 mg/L. Nevertheless, the choice does not affect the actual classification, which is **Aquatic Acute 1 (H400)**. As 0.0001 < $EC_{50}$ ≤ 0.001 mg/L, the acute **M-factor is 1000**.

Long-term aquatic toxicity data are available for three trophic levels (8 species). Algae and aquatic macrophytes are the most sensitive group, and the lowest result is a 7d $NOE_rC$ of 0.00003 mg/L for the duckweed *Lemna gibba*. As this concentration is below the threshold value of 0.1 mg/L for non-rapidly degradable substances, RAC concludes that a classification as **Aquatic Chronic 1 (H410)** is justified. As 0.00001 < NOEC ≤ 0.0001 mg/L, the chronic **M-factor is 1000**. RAC notes that there is some uncertainty in the M-factor as test concentration maintenance was poor (the equivalent nominal concentration is 0.00032 mg/L, which would justify an M-factor of 100; the same M-factor would be derived for the next most sensitive species (*Pseudokirchneriella subcapitata*) with a 72h $NOE_rC$ of 0.001 mg/L).

Overall, RAC agrees with the DS to classify halosulfuron-methy as **Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 both with an M-factor of 1000**.

## Additional references

Fleeman *et al.,* (2005) Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in the Rat. Birth Defects Res B 74:442-449

Ikemi *et al.*, (1993) Effects of Food Restriction on the Fetal Development during Major Organogenesis in Rats. Cong. Anom., 33: 363-377.

## ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).