Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino] propyl}-5-[(2RS)-2-(ethylthio)propyl]-3-hydroxy cyclohex-2-en-1-one

EC Number: -
CAS Number: 99129-21-2

CLH-O-0000001412-86-91/F

Adopted

4 December 2015

4 December 2015

CLH-O-0000001412-86-91/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 harmonized classification and labelling (CLH) of:

Chemical name: **clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-(ethylthio) propyl]-3-hydroxycyclohex-2-en-1-one**

EC Number: **-**

CAS Number: **99129-21-2**

The proposal was submitted by the **Netherlands** and received by RAC on **23 January 2015.**

In this opinion, all classifications and labelling are given in accordance with the CLP Regulation; the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer provided.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands 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 April 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **26 May 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

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 harmonized classification and labelling was reached on **4 December 2015** and was adopted 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		clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	Acute Tox. 4 Skin Sens. 1 Aquatic Chronic 3	H302 H317 H412	GHS07 Wng	H302 H317 H412	EUH066	-	-
RAC opinion		clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	Acute Tox. 4 Skin Sens. 1 Aquatic Chronic 3	H302 H317 H412	GHS07 Wng	H302 H317 H412	EUH066	-	-
Resulting Annex VI entry if agreed by COM		clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	Acute Tox. 4 Skin Sens. 1 Aquatic Chronic 3	H302 H317 H412	GHS07 Wng	H302 H317 H412	EUH066	-	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Clethodim has no flash point up to 78 °C, is not explosive and not oxidising. As such, clethodim does not meet the criteria for classification for physico-chemical properties according to CLP.

Comments received during public consultation

There were no comments regarding the classification for physico-chemical hazards.

Assessment and comparison with the classification criteria

Clethodim does not have a flash point below 78 °C and was shown to decompose before reaching boiling point. Therefore, clethodim does not meet the classification criteria for a flammable liquid. Examination of the chemical structure did not indicate that clethodim would have any explosive or oxidising properties and so it does not meet the criteria for classification as an explosive substance or an oxidising liquid.

RAC is in agreement with the Dossier Submitter (DS) that **classification is not required for physico-chemical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The oral LD_{50} in rats was 1133 mg/kg bw. According to the CLP criteria, substances should be classified Acute Tox. 4 when the oral LD_{50} value is between 300 and 2000 mg/kg bw.

The dermal LD_{50} in rats was > 4167 mg/kg bw. According to the CLP criteria, substances should be classified when the dermal LD_{50} is ≤ 2000 mg/kg bw. Therefore, clethodim does not meet the criteria for classification for acute dermal toxicity.

The inhalation LC_{50} in the rat was > 3.25 mg/L (the maximum attainable concentration). According to the criteria, substances should be classified for acute inhalation toxicity when the LC_{50} (dust/mists) ≤ 5 mg/L. As no mortality occurred at the maximum concentration tested, clethodim should not be classified for acute inhalation toxicity.

According to the DS, Clethodim meets the criteria for classification with Acute Oral Toxicity, Category 4 (H302). No classification was proposed for acute dermal or inhalation toxicity.

Comments received during public consultation

There were two comments relating to acute toxicity received from Member States. One was in general agreement with the classification for toxicological hazards and the other specifically supported the classification of clethodim for acute oral toxicity.

Assessment and comparison with the classification criteria

Clethodim was tested by the oral route in both rats and mice. In each study, the substance tested was 83.3 % pure. The LD_{50} values were corrected to take into account the actual amount of active ingredient dosed.

In rats, the oral LD_{50} for males was 1358 mg/kg bw and for females was 1133 mg/kg bw. In mice, the oral LD_{50} values for males and females were 2143 mg/kg bw and 2024 mg/kg bw, respectively. Both the values for male and female rats justify the classification Acute Toxicity Category 4 ($300 < LD_{50} \leq 2000$ mg/kg).

Clethodim was also tested in rats by the inhalation route. No mortality occurred; the LC_{50} was > 3.25 mg/L. No classification for acute inhalation toxicity is appropriate as an LC_{50} equal to or below 5 mg/L has not been demonstrated. There was no mortality at exposure levels relevant to classification.

Groups of 5 male and female rabbits were exposed dermally to 4167 mg/kg bw clethodim for 24 h. There was 1 death among the male rabbits during the study. Therefore the resulting LD_{50} was > 4167 mg/kg bw/day for both males and females. This is above the cut-off of 2000 mg/kg for acute dermal toxicity classification.

The data support no classification for acute toxicity by the inhalation and dermal routes and classification of clethodim as **Acute Toxicity 4 by the oral route (H302 – harmful if swallowed)**.

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

Summary of the Dossier submitter's proposal

In a rat acute oral toxicity study, salivation, decreased motor activity, unsteady gait, hyperreactivity, lacrimation, clonic convulsions, red nasal discharge, ocular discharge and collapse were observed in almost all dose groups. Reduced food consumption and yellow anogenital stains were noticed in surviving treated animals. As increased mortality was seen in the dose range relevant for classification with STOT SE, no further classification was proposed in order to prevent "double classification" [i.e. a second classification for the same hazard].

In an acute inhalation study in rats, no mortality or significant toxicity was observed up to the maximal attainable concentration of 3.25 mg/L. Therefore, no classification was needed for acute inhalation STOT SE. There was no evidence of respiratory tract irritation in the available studies; therefore, classification with STOT SE Category 3 was not proposed.

For dermal exposure, substances should be classified when there is evidence for toxicity between 1000 and 2000 mg/kg bw after a single exposure. Since there is no evidence for specific target organ toxicity in the acute dermal study at 2000 mg/kg bw, no classification was proposed.

It was not proposed to classify clethodim for STOT SE.

Comments received during public consultation

There were no comments pertaining to STOT SE during the public consultation.

Assessment and comparison with the classification criteria

Following oral dosing, signs of toxicity in surviving treated rats included reduced food consumption, convulsions and red nasal discharge. Two surviving females were noted to have small lesions of the gliosis in a single spinal nerve at microscopic examination. There were no

abnormalities in other surviving animals. In isolation, these limited findings are not considered sufficient to demonstrate specific toxicity of the central nervous system.

Following inhalation exposure of rats to clethodim, signs of toxicity included salivation, red nasal discharge, abnormal respiration sounds, decreased faeces and yellow/red anogenital discharge.

In the dermal study in rabbits, signs of toxicity were limited to the local area of exposure. These included abraded, thickened, blackened, crusty and/or cracked skin. These signs of local skin damage were accompanied by erythema and oedema.

The effects that occurred in the absence of lethality following acute oral, inhalation and dermal exposure to clethodim were all indicative of non-specific signs of general toxicity. As there was no significant or severe organ toxicity noted, no respiratory tract irritation or narcotic effects, RAC agrees with the DS that **no classification for STOT SE is appropriate**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

In a rabbit skin irritation study, mean scores for erythema and oedema in treated animals were ≤ 2 at all time points. All effects were reversible within 9 days.

Clethodim does not meet the CLP criteria for classification as a skin irritant because mean values for erythema and oedema were < 2.3 in three tested animals and all effects were reversible within 9 days. However, clethodim needs to be labelled with EUH066 (Repeated exposure may cause skin dryness and cracking) as dryness of the skin from day 2 up to day 7 was observed and abraded, thickened, blackened, crusty and/or cracked skin was seen in treated animals during the observation period of an acute dermal toxicity test.

Comments received during public consultation

Two Member States supported the proposal for no classification for skin irritation. Further, both supported use of the supplementary labelling phrase EUH066 (Repeated exposure may cause skin dryness and cracking). In contrast, one Member State commented that clethodim had produced flaky, dry and/or reddened skin at termination of the study and microscopic examination revealed trace to mild hyperkeratosis among the treated animals. On this basis, the Member State suggested classification with Skin Irrit. Cat. 2.

Assessment and comparison with the classification criteria

Clethodim was tested in a guideline compliant rabbit skin irritation study (OECD/GLP). This involved use of a 3 min. exposure period in 1 animal, a 1 h exposure period in another animal and a 4 h exposure period in 3 animals. All animals were observed for a period of 14 days following removal of the dressing.

After 3 min exposure, grade 1 erythema was observed on Day 2 only. After 1 h exposure, grade 1 erythema and dryness of the skin was observed from Days 2 – 7.

Following 4 h exposure, no individual scores for either erythema or oedema were above 2 at 24, 48 or 72 h. The mean individual animal scores for erythema and oedema are shown in the table below. All effects were reversible by Day 9.

	Individual animal scores - average (24 - 72 h)
Erythema	0.67 - 2 – 2
Oedema	0 - 1.67 - 1.33

Further information on the potential for clethodim to cause skin irritation was available from an acute dermal study in rabbits and a skin sensitisation study in guinea pigs.

In the acute dermal study, thickened, blackened, crusty and/or cracked skin and erythema and oedema were noted in all treated rats during the observation period following 24 h exposure to clethodim (4167 mg/kg bw). Twenty-four hours after removal of the dressing slight to severe erthyema and oedema were noted in treated animals. After 7 days, none to severe erythema and oedema were noted, although in one female the erythema persisted after 14 days. Macroscopic examination at termination revealed flaky, dry and/or reddened skin. Microscopic examination revealed trace to mild hyperkeratosis among treated animals.

In a Guinea Pig Maximisation test, discrete or patchy erythema was noted in 5/10 animals at 24 h and 6/10 animals at 48 h.

Comparison with the criteria

In the rabbit skin irritation study the scores obtained following 4 h (or less) treatment with clethodim did not meet the criteria for classification as Skin Irrit. 2 (mean value of ≥ 2.3 - ≤ 4.0 for erythema or oedema in at least 2/3 animals from gradings at 24, 48 and 72 h after patch removal). There was no evidence of full thickness destruction of the skin. The effects observed were not sufficiently severe to justify classification. Additionally, all effects were found to be reversible within 9 days and there was no evidence of alopecia, hyperkeratosis, hyperplasia or scaling. Therefore, the data from this study indicate that no classification for skin irritation is warranted.

Labelling phrase EUH066 (Repeated exposure may cause skin dryness or cracking) can be applied to substances which may cause concern as a result of skin dryness, flaking or cracking following exposure but which do not meet the criteria for classification.

In the acute dermal toxicity study with clethodim, there were signs of skin irritation noted during the initial 24 h observation period and flaky, dry and/or reddened skin was observed at termination. In the guinea pig skin sensitisation study, discrete or patchy erythema was noted in 60 % of animals 48 h after topical induction. Given these results and the fact that this substance is clearly lipophilic (LogP 4.2), it would seem appropriate to apply EUH066 to clethodim.

Therefore, RAC agrees with the DS that **clethodim should not be classified for skin irritation but should bear the supplemental labelling phrase, EUH066**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

In an eye irritation study, clethodim was found to be mildly irritating to rabbits. All effects were reversible within 3 days. No effects on the iris or cornea were observed and the effects on conjunctiva redness and chemosis were below 2. Clethodim does not meet the criteria for classification.

Comments received during public consultation

Two Member States agreed with the classification proposal for toxicological hazards in general.

Assessment and comparison with the classification criteria

In a guideline eye irritation study clethodim was instilled into the eye of 6 rabbits. All observed effects were reversible within 3 days. The results are summarised in the table below.

	Individual animal scores - average (24 - 72 h)
Cornea/opacity	0 - 0 - 0 - 0 - 0 - 0
Iris	0 - 0 - 0 - 0 - 0 - 0
Conjunctiva redness	1 - 0.33 – 1 - 1.33 – 1 - 1
Conjunctiva chemosis	0.33 - 0.33 - 0.33 – 0 - 0.33 - 0.33

These scores are below the cut-off values required for classification:
- corneal opacity ≥ 1 and/or
- iritis ≥ 1 and/or
- conjunctival redness ≥ 2 and/or
- conjunctival oedema ≥ 2

Given also the reversibility of the effects that were observed, RAC is in agreement with the DS that there should be **no classification for serious eye damage/irritation**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

No data available; no classification.

Assessment and comparison with the classification criteria

No data available; no classification.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Clethodim gave a positive response in a Guinea pig maximisation test (GPMT) with 9/10 animals showing signs of sensitisation. The intradermal induction dose was 50 % clethodim. According to CLP, a substance should be classified as a skin sensitiser (category 1B) when a positive response in a GPMT test (in ≥ 30 % of the animals at > 1 % intradermal induction dose) is observed. This criterion is fulfilled. However, as no information is available following induction with < 1 % clethodim, it cannot be fully excluded that sub-classification with category 1A would be required. Therefore, clethodim meets the criteria for classification for skin sensitisation, category 1.

Comments received during public consultation

Four Member States commented during the public consultation and all agreed with the classification proposal for Skin sensitisation, category 1.

Assessment and comparison with the classification criteria

A guideline–compliant GPMT is available. In this study, a preliminary range-finding test was carried out to determine the concentration for intradermal and topical applications in the main study. During this pre-test, intradermal injection with 15 % clethodim induced no erythema or discrete erythema, whereas 25-50 % clethodim induced moderate and confluent erythema.

In the main study, guinea pigs were induced by intradermal injection with 50 % clethodim in PEG300 followed by topical induction with 62.5 % clethodim.

Following topical induction, discrete or patchy erythema was observed in 5/10 animals at 24 h and 6/10 animals at 48 h after removal of the test substance. After challenge with 50 % clethodim, skin reactions were observed in 9/10 animals at 24 h and 8/10 animals at 48 h and 0/10 control animals, with the presence of discrete or patchy erythema to moderate and confluent erythema. This increase in the numbers of animals responding, together with the increased severity of reactions, indicate a positive result. Accordingly, clethodim is a skin sensitiser.

Classification in sub-category 1B is appropriate when $\geq$ 30 % of the animals produce a positive response following an intradermal dose of 1 %. However, as clethodim was not tested at an intradermal dose of less than 50 %, this cannot be assessed adequately. Therefore, RAC agrees with the DS that clethodim should be classified for **skin sensitisation, category 1 (no sub-categorisation), H317: (May cause an allergic skin reaction)**.

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

Summary of the Dossier submitter's proposal

Studies of repeated exposure in rats, mice and dogs are available, of which those summarised below are included in the summary of the Dossier submitter.

Dietary exposure of mice to clethodim for 4 weeks resulted in changes to haematology parameters indicative of anaemia (decreased red blood cell count, haemoglobin and haematocrit) at doses ≥ 74.4 mg/kg bw/day. Liver weight was increased and centrilobular hypertrophy was observed at ≥ 178 mg/kg bw/day.

Dietary exposure of rats to clethodim for 13 weeks caused body weight changes and centrilobular hypertrophy in the liver at doses ≥ 134 mg/kg bw/day.

Treatment of dogs for 3 months with oral capsules containing clethodim resulted in changes to biochemical parameters indicative of liver effects. Increased liver weight and an increase in centrilobular vesicles/vacuoles were observed at ≥ 250 mg/kg bw/day. In a comparable 1-year study, changes in haematology parameters were seen at 62 mg/kg bw/day. Liver weights were increased at 62 and 250 mg/kg bw/day in both males and females.

Dermal exposure of rats to clethodim for 4 weeks resulted in treatment-related anogenital discharge at 832 mg/kg bw/day and dose-dependent skin irritation in both males and females. Plasma chloride levels were decreased at 832 mg/kg bw/day and triglyceride levels were increased in females at 83 and 832 mg/kg bw/day. Liver weight was also found to be increased in females only at 832 mg/kg bw/day. There were no macroscopic or microscopic abnormalities.

In conclusion, in some studies involving oral exposure in rodents and in an oral study in dogs (1 year duration), changes in haematological parameters and liver weight were observed at doses < 100 mg/kg bw/day. However, the effects observed at doses less than this cut-off dose level for classification with STOT-RE 2 were not sufficiently severe for classification. Therefore, no classification for STOT-RE was proposed for clethodim.

Comments received during public consultation

Two MS were in general agreement with the proposal for toxicological hazards.

Assessment and comparison with the classification criteria

Clethodim has been tested for repeated dose toxicity by the oral route in mice, rats and dogs and in one dermal study in rats. A summary of findings at doses relevant for classification is provided below.

35-Day dietary study in rats
Guidance values for classification with STOT RE 1: C ≤ 26 mg/kg bw/day, STOT RE 2: 26 < C ≥ 260 mg/kg bw/day

Clethodim was administered to male and female rats at doses of 0, 4.87/5.78 and 597/667 mg/kg bw/day (males/females).

Deviations in red blood cell parameters were noted in males and females (including reduced red blood cell count and haemoglobin). Absolute and relative liver weights were increased in males (12 % and 34 % increase, respectively) and relative liver weights were increased in females (24 % increase in comparison to controls). Centrilobular liver hypertrophy was seen in 10/10 males and 8/10 females (versus 0 in controls). These effects all occurred at a dose higher than the guidance value for classification with STOT RE 2.

Some mild changes to blood parameters were observed in males and females dosed with ~ 5 mg/kg bw/day, however these were not statistically significant. Relative liver weight was increased in males and females (7 % and 10 % of controls respectively). Histopathology revealed an increased incidence of centrilobular hypertrophy of the liver in 6/10 males and 3/10 females (versus 0 in controls).

There were some effects to red blood cell parameters and to the liver, occurring mainly at a dose above the guidance level for STOT RE 2. Similar effects, occurring at much milder severity occurred at the low dose which was within the guidance range for classification with STOT RE 1. However, these effects were not considered severe enough for classification with STOT RE 1.

28-Day dietary study in mice (i.e. cited as Raymond and Cox 1986 in the CLH report)
Guidance values for classification with STOT RE 2: 30 < C ≤ 300 mg/kg bw/day

Clethodim was administered to male and female mice at doses of 0, 11.9, 29.7, 74.4, 178 and 476 mg/kg bw/day. There was a marginal reduction in red blood cell parameters at doses ≥ 74.4 mg/kg bw/day in males and at 178 mg/kg bw/day in females (< 10 % when compared to controls). Absolute and relative liver weights were increased in males only at 178 mg/kg bw/day (13 % increase compared to controls) but there were no histopathological correlates at this dose.

The effects observed in this study are not considered severe enough to support classification with STOT-RE, category 2.

35-Day dietary study in rats
Guidance values for classification with STOT RE 2: 26 < C ≤ 260 mg/kg bw/day

Clethodim was administered to male and female rats at doses of 0, 0.22/0.29, 12.5/13.9, 65.6/70.6, 261/291 and 515/554 mg/kg bw/day (males/females). Some changes to haematological parameters were noted in this study. These were a reduction in red blood cells in females – but in the absence of a dose response, a reduction in haemoglobin in males at doses ≥ 65.6 mg/kg bw/day and in females, again in the absence of a dose response and a reduction in haematocrit in males only at 261 mg/kg bw/day. These effects were slight in nature (< 10 % difference to controls).

The effects observed in this study are not considered severe enough to warrant classification for STOT RE, category 2.

90-Day dietary study in rats
Guidance values for classification with STOT RE 2: 10 < C ≤ 100 mg/kg bw/day

Clethodim was administered to male and females rats at doses of 0, 2.3/2.8, 25/30, 134/159 and 279/341 mg/kg bw/day (males/females). There were no effects observed at doses relevant for classification.

90-Day capsule study in dogs
Guidance values for classification with STOT RE 2: 10 < C ≤ 100 mg/kg bw/day (based on figures for rats without further extrapolation)

Clethodim was administered to male and female dogs in gelatine capsules at doses of 0, 0.83, 21, 62 and 104 mg/kg bw/day. At doses of ≥ 104 mg/kg bw/day there were effects to indicate perturbations in liver function. These were characterised by deviations in alkaline phosphatase, cholesterol and globulin levels in males and females. At 104 mg/kg absolute liver weight was statistically significantly increased in males and females [34 % and 30 % increase compared to controls (respectively)]. Histopathology revealed an increase in severity of centrilobular vesiculation/vacuolation of the liver at 104 mg/kg bw/day.

The effects observed in this study occurred only at the cut-off level for classification and were not considered severe enough for classification with STOT RE, category 2.

1-Year capsule study in dogs
Guidance values for classification with STOT RE 2: 2.5 < C ≤ 25 mg/kg bw/day [based on the guidance values for a 90 day rat study, extrapolated using Haber's rule (x0.25)]

Clethodim was administered to male and female dogs in gelatine capsules at doses of 0, 0.83, 62 and 250 (increased from 104 mg/kg bw/day after week 7) mg/kg bw/day. There were no adverse effects at doses < 62 mg/kg bw/day.

2-Year carcinogenicity study in rats
Guidance values for classification with STOT RE 2: 1.2 < C ≤ 12 mg/kg bw/day

There were no adverse effects at doses relevant for classification.

28-Day dermal study in rats
Guidance values for classification with STOT RE 2: 20 < C ≤ 200 mg/kg bw/day

Clethodim was administered to the skin of male and female rats, at doses of 0, 8.3, 83, and 832 mg/kg bw/day. There were no adverse effects at doses relevant for classification.

Conclusion

Clethodim has been tested by the oral route in mice, rats and dogs and dermally in rats. Effects were observed in blood cell parameters and also in the liver, occasionally at doses relevant for classification. However, the effects were not consistent and generally mild and therefore it is **not considered appropriate to classify for specific target organ toxicity following repeated dosing**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

A positive result was found without metabolic activation in a CHO cell chromosome aberration test. Bacterial mutagenicity tests and a CHO gene mutation test gave negative results. Both a rat bone marrow chromosomal aberration study and a mouse liver UDS test gave negative results.

Following a weight-of-evidence approach, clethodim was not considered genotoxic as the results of the *in vivo* studies in rodents were negative. Therefore, clethodim was considered non-genotoxic and does not meet the criteria for classification for mutagenicity.

Comments received during public consultation

Two MS were in general agreement with the proposal for toxicological hazards.

Assessment and comparison with the classification criteria

The potential mutagenicity of clethodim has been studied *in vitro* in both bacteria and mammalian cells, and *in vivo* in a rat bone marrow chromosomal aberration test and a mouse liver UDS assay. All these tests were generally consistent with the relevant OECD guideline and all were considered acceptable by the Dossier Submitter.

Two bacterial mutagenicity studies gave clear negative results both in the presence and absence of metabolic activation. Investigations included both *S.typhimurium* and *E.coli* tester strains. Similarly, clethodim produced negative results in a hprt gene mutation test in Chinese hamster ovary cells. In all these studies, the top dose tested was limited by the toxicity of the test substance.

In contrast, in a Chinese hamster ovary cell chromosome aberration test, a reproducible increase in the frequency of structural aberrations was found in the absence of exogenous metabolic activation following a 10 h exposure period. In a second study, performed by the same laboratory according to a similar protocol, no clear increase in structural aberrations was observed, with or without exogenous metabolic activation. No explanation was available for the contrasting results seen in these studies; the overall conclusion is that clethodim may be clastogenic in cultured mammalian cells, without exogenous metabolic activation.

In a follow-up *in vivo* study, oral administration of clethodim (150-1500 mg/kg) to male and female rats produced no increase in bone marrow cell chromosome aberrations. Sacrifice times of 12, 24 and 48 h were assessed. Although 50 cells per sample were scored rather than the 100 cells recommended by the more recent OECD test guideline, this negative result is considered reliable. Further reassurance of a lack of genotoxic activity is provided by a negative result in a mouse liver UDS test. Hepatocytes were harvested in this test at 2 and 16 h following oral treatment of mice with 100, 1000 and 5000 mg/kg clethodim. There was no increase in UDS.

Overall, in spite of the isolated positive result in one of two *in vitro* chromosome aberration studies, it can be concluded reliably that clethodim lacks mutagenic potential. The positive *in vitro* result (-S9 only) was not reproduced in a second study, nor was an increase in chromosome aberrations found in a follow-up *in vivo* study. Therefore RAC agrees with the DS that **no classification is required for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

In a two-year rat chronic toxicity and carcinogenicity study, clethodim was administered in the diet at doses of 0, 0.15/0.20, 0.57/0.72, 16/21 and 86/113 mg/kg bw/day (males/females). There was no evidence of carcinogenicity in this study.

In a 78-week carcinogenicity study in mice, animals were fed clethodim in the diet at doses of 0, 2.4, 24, 119 and 238/357 mg/kg bw/day. There was no evidence of carcinogenicity in this study.

Classification for carcinogenicity should be on the basis of evidence of increases in tumour formation obtained from animal studies or epidemiology.

There were no human studies available and two studies, one in mice and one in rats showed no evidence of carcinogenic activity of clethodim. Therefore, there is no evidence of an increase in

tumour formation following exposure to clethodim. No classification was proposed for carcinogenicity.

Comments received during public consultation

Two MS were in general agreement with the proposed classifications for toxicological hazards.

Assessment and comparison with the classification criteria

The carcinogenic potential of clethodim has been assessed in two well-performed studies, one in mice and one in rats. The results of these studies showed no evidence of carcinogenic activity. Therefore, in the view of RAC **clethodim should not be classified for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

In a 2-generation reproduction study, clethodim was administered to rats via the diet. A decrease in body-weight and reduced food consumption was noted in animals of the F_0 and F_1 generation at the top-dose only. There were no changes detected between parental animals of the treated and control groups in mating indices, pregnancy rates, male fertility, oestrous cycle, macroscopic findings, microscopic findings and organ weights. There were no treatment-related changes detected in litter size, pup weights, sex ratio or litter survival of the F_0 and F_1 offspring.

Classification for reproductive toxicity is based on effects that have the potential to interfere with sexual function and fertility as well as the development of the offspring. No such effects were observed in a two-generation study or in the repeated dose studies available.

In a teratogenicity study in rats, maternal toxicity was observed at the 2 highest doses, indicated by an increase of mortality, decreased body weights and food consumption, a higher incidence of clinical signs and decreased gravid uterus weight. Decreased litter size, increased post-implantation loss and tail defects were evident at the high dose only, and decreased foetal weights and associated retardation of skeletal ossification were observed at the top 2 doses. The foetal effects were considered by the Dossier submitter to be secondary to the maternal toxicity according to the CLP criteria as there is maternal mortality greater than 10% (criteria chapter 3.7.2.4.4) at the top dose. At the second highest dose, the foetal effects were limited to reduced foetal weight and reduced ossification. The dams at this dose levels showed maternal toxicity in the form of reduced body weight and body weight gain and clinical effects. The limited foetal toxicity was of limited severity and considered to be secondary to the maternal toxicity.

In a teratogenicity study in rabbits, maternal effects such as clinical signs and decreased body weight, food consumption and gravid uterus weight were observed at the 2 highest doses. No malformations and no developmental changes that could be attributed to treatment were found.

No classification for developmental toxicity was proposed.

Comments received during public consultation

Two MS were in general agreement with the proposal for toxicological hazards.

Assessment and comparison with the classification criteria

Fertility and reproductive function

Data are available from a 2-generation reproductive toxicity study in rats. It was performed to contemporary guideline standards (including OECD 416). Rats were administered clethodim (83%

purity) at dietary levels of 0, 5, 20, 500 and 2500 ppm: equivalent to doses of approx. 0, <0.8, 0.8, 26.7 and 133.7 mg/kg clethodim (F0 animals) and 0, <0.8, 0.8, 28.3 and 151.2mg/kg clethodim (F1 animals).

As indicated in the Dossier Submitter's proposal, there were no indications of an effect on sexual function or fertility. At the highest dose, the only adverse effects on parental animals were a decrease in body weight and food consumption. Unfortunately, the figures were not presented by the Dossier Submitter, but the effects do not appear to have been marked. An increase of stillborn pups was noted in F1 litters at the top dose (14 stillborn pups across 7 litters, contrasting with 2 stillborn pups in 2 litters in controls). In the F2 generation, the control incidence of stillborn pups was 7 in 5 litters. No data were provided by the Dossier Submitter for the other dose groups. However, a historical value of 9 stillborn pups from 6 litters was cited from one control group in a different 2-generation study performed earlier by the same laboratory, but in isolation this information is not particularly informative. Although no details of the numbers of stillborn pups in the other dose groups were provided by the Dossier Submitter in either the CLH report or the accompanying Draft Assessment Report (DAR), it seems most likely that the apparent increased incidence of F1 stillborn pups at the top dose was an incidental finding, prominent because of the low control incidence in this phase of the study.

Additional information provided by the Dossier Submitter confirmed this assessment (see BD*).*

Developmental toxicity

Clethodim has been assessed for developmental toxicity in rats and rabbits. The studies were performed in the 1980s and met contemporary guidelines.

Study in rats

Rats were administered clethodim (83% pure) by gavage on days 6-15 of gestation. Increased mortality was observed in dams at the top dose of clethodim (583 mg a.i./kg). The death rate was 5/25 (versus 0 in all other treatment and control groups), i.e. 20 %. At this dose, clinical signs included excessive salivation, red/mucoid nasal discharge, alopecia and staining of the anogenital area. Mean maternal body weight gain was reduced over the periods of gestation days 6-15 and 15-20. Gravid uterus weight was also statistically significantly reduced (no data presented).

At this dose there was a clear effect on foetuses: an increase in the incidence of external malformations in 8/221 foetuses, with tail defects noted in 7/221 foetuses (3.2 %) across 6 litters (versus 0 in control animals). Mean foetal weight in this group was statistically significantly reduced compared to controls. Incomplete ossification was observed in the sacral and caudal vertebral elements and in the 5^{th} and 6^{th} sternebrae, however these findings were not statistically significant. Increased post-implantation loss was also observed, but again in the absence of any statistical significance. Although the number of live foetuses born (221; mean 12.3) was significantly less than controls (353; mean 14.1), this was not reported to be statistically significant.

At the second highest dose of 292 mg a.i./kg bw/day, no mortality was observed. There was a small effect on maternal body weight gain compared to controls (less than 10%) together with excessive salivation, red/mucoid nasal discharge, alopecia and staining of the anogenital area. Foetal weight was statistically significantly reduced (no data provided). Similarly to the foetuses of the top dose group, there was incomplete ossification present (in the absence of statistical significance). No external malformations were observed and there were no effects on post-implantation loss, the number of live foetuses or on litter size. There were no other toxicologically significant findings at any other doses in this study.

Table: Rat developmental toxicity study - data presented by the Dossier Submitter

Dose (mg a.i./kg/day)	**0**	**8.3**	**83.3**	**292**	**583**
Maternal effects					
Mortality	0/25	0/25	0/25	0/25	5/25 (20%)
Pregnant animals	25	25	24	25	24

Body weight (g)					
Day 15	284+/-20	284+/-19	280+/-17	273+/-18	266+/-16*
Day 20	362+/-26	362+/-26	357+/-19	337+/-36*	332+/-18**
Days 6-15	47+/-8	47+/-9	45+/-10	40+/-14	28+/-15**
Days 15-20	70+/-10	69+/-11	69+/-8	58+/-20*	58+/-13**
Net Body weight	281+/-20	282+/-19	281+/-17	263.5+/-27*	271.5+/-14
Net Body weight gain	35+/-10.5	37+/-11	37+/-13	24.5+/-21	28+/-9
Foetal effects					
Live foetuses	353	351	329	350	221
Litter size	14.1	14.0	13.7	14.0	12.3
Total external malformations (number of foetuses)	0	0	1	0	8
Tail defects (%)					
- absence	0	0	0	0	3
- short	0	0	0	0	2
- filamentous	0	0	0	0	2

Statistical significance denoted by * and **

Net body weight and body with gain values rounded to nearest gram

Aside from the effect on foetal weight, all the adverse effects on foetuses seen in this study were observed at a maternally lethal dose (causing 20% mortalities). In isolation, the effect on foetal weight appears most likely to have been related to maternal toxicity and is not viewed as evidence of developmental toxicity. Therefore, this study did not show a clear developmental effect of clethodim.

Study in rabbits

Rabbits were administered clethodim by gavage on days 7-19 of gestation. There was one premature death in the mid-dose treatment group (83.3 mg/kg bw/day) and one at the low-dose (20.8 mg/kg bw/day). In the absence of any dose-response relationship, these effects are not considered to be treatment-related. There were some signs of maternal toxicity in the mid- and top-dose groups, including reduced body weight-gain, reduced food consumption and a decrease in gravid uterus weight (top-dose only) (data not presented by the Dossier Submitter).

There were no treatment-related effects on the number of pregnancies, the number of live foetuses, litter size and post-implantation loss. No malformations or developmental changes that could be attributable to treatment were observed at any dose level.

Comparison with criteria

RAC shares the view of the Dossier Submitter that no classification is justified for reproductive toxicity.

There was no indication of an adverse effect on fertility or reproductive function in a 2-generation reproductive study in rats and no developmental effects were seen in this species in the absence of maternal toxicity. No developmental effects were seen in rabbits.

According to Annex I, section 3.7.2.4.4 of the CLP Regulation, an increase in the incidence of maternal mortality of > 10 % is considered excessive and data for that dose level should not normally be considered for further evaluation. Therefore, in the rat study, the adverse effects in the foetuses of the top-dose group (583 mg a.i./kg) are not considered relevant for the classification given the increase in mortality of 20 %.

The remaining effects observed in the rat developmental toxicity study occurred at the second highest dose (292 mg a.i./kg). These included a statistically significant reduction in foetal weight and a non-significant incidence of incomplete ossification. This mild foeto-toxicity is considered to have been related to maternal toxicity (6 % reduction in body weight and some clinical signs

including increased salivation and red mucoid nasal discharge) and is therefore not considered as a developmental effect. **Accordingly, no classification is proposed for toxicity to reproduction.**

RAC evaluation of aspiration toxicity

No classification was proposed for this endpoint (not evaluated).

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

Clethodim is a selective herbicide which does not have an existing harmonised classification in Annex VI to the CLP Regulation. The Dossier Submitter proposed Aquatic Chronic 3 (H412) based on rapid degradation and a chronic NOEC of 0.37 mg/L for aquatic macrophytes.

Comments received during public consultation

Three statements of support for the proposal were received, and no further information was submitted.

Assessment and comparison with the classification criteria

Degradability

A valid hydrolysis study is unavailable. Clethodim was readily biodegradable in a closed bottle test (OECD TG 301 D), achieving 100% biodegradation after 14 days. Simulation testing in an aerobic pond water-sediment system using radio-labeled substance indicated a whole system DT_{50} of 23 days; after 103 days, mineralisation to carbon dioxide and formation of unextractable sediment residues accounted for 14.2% and 22.0% of applied radioactivity, respectively. On this basis, clethodim meets the criteria for being rapidly degradable (readily biodegradable) in the environment.

Bioaccumulation

The log n-octanol-water partition coefficient (K_{ow}) of clethodim is 4.2, but a steady-state bioconcentration factor (BCF) of 2.1 L/kg wet weight was measured for Bluegill Sunfish (*Lepomis macrochirus*) based on radioactivity measurements. Lipid content was not determined so the BCF could not be normalised to a standard lipid content of 5%. However, as the BCF is much lower than 500 L/kg this does not affect the conclusion that clethodim does not meet the bioaccumulation criteria.

Ecotoxicity

The lowest reliable ecotoxicity results were as follows (the key studies are highlighted in bold):

Trophic level	Species	Short-term result	Long-term result
Fish	Rainbow Trout *Oncorhynchus mykiss*	96-h LC_{50} = 25 mg/L	-
		21-d NOEC = 3.9 mg/L*	

Aquatic invertebrates	*Daphnia magna*	48-h EC_{50} >100 mg/L	21-d NOEC = 49 mg/L
Aquatic algae and plants	*Raphidocelis subcapitata*†	72-h E_rC_{50} > 12 mg/L	72-h NOEC = 12 mg/L
	Lemna gibba	**7-d E_rC_{50} = 1.27 mg/L**	**7-d E_rC_{10}= 0.37 mg/L**

* The dossier presents this value as a long-term result, but it is actually a prolonged acute result (OECD TG 204). Endpoints were mortality, growth and symptoms.

† *Selenastrum capricornutum* in the dossier.

All toxicity values are based on mean measured concentrations. The substance has a pKa of 4.47, indicating that it will be mostly ionised (unprotonated) at environmentally relevant pH.

Classification according to CLP

Acute aquatic hazard

Acute toxicity data were available for all three trophic levels. The lowest reliable short-term aquatic toxicity result is above 1 mg/L so **no acute classification is necessary**.

Long-term aquatic hazard

Clethodim is rapidly degradable. Although the CLH report indicated that long-term toxicity data were available for all three trophic levels, no information was actually available for fish. Aquatic macrophytes were the most sensitive group with a lowest reported 7-d E_rC_{10} of 0.37 mg/L for *Lemna gibba*. This is below the threshold value of 1 mg/L for rapidly degradable substances, leading to classification as Aquatic Chronic 3 (H412).

The surrogate approach can also be considered for fish in the absence of a chronic toxicity result. Based on the lowest acute 96-h LC_{50} of 25 mg/L, a low BCF and rapid degradability, no chronic classification would be indicated.

In summary, the proposed classification of clethodim as **Aquatic Chronic 3 (H412)** is justified.

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).