Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Hexaflumuron (ISO); 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl)-3-(2,6-difluorobenzoyl)urea

EC Number: 401-400-1
CAS Number: 86479-06-3

CLH-O-0000001412-86-77/F

Adopted

4 December 2015

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

4 December 2015

CLH-O-0000001412-86-77/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 (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: **hexaflumuron (ISO); 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea**

EC Number: **401-400-1**

CAS Number: **86479-06-3**

The proposal was submitted by **Portugal** and received by the RAC on **16 April 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

Portugal 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 **2 June 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **17 July 2015.**

ADOPTION OF THE OPINION OF THE RAC

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

Co-Rapporteur, appointed by RAC: **Stephen 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**.

The RAC opinion 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	xxx-xxx-xx-x	hexaflumuron (ISO); 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea	401-400-1	86479-06-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1000 M=10000	
RAC opinion	xxx-xxx-xx-x	hexaflumuron (ISO); 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea	401-400-1	86479-06-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1000 M=10000	
Resulting Annex VI entry if agreed by COM	xxx-xxx-xx-x	hexaflumuron (ISO); 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea	401-400-1	86479-06-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1000 M=10000	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose a classification for physical hazards based on the negative results in standard tests for explosivity (EEC-method A14) and flammability (EEC-method A10). Additionally, it was mentioned that hexaflumuron did not liberate any flammable gases in contact with water (EEC-method A12), did not exhibit any pyrophoric properties (EEC-method A13) and did not show self-heating properties (no self-ignition according to the EEC-method A16).

Comments received during public consultation

One Member State (MS) supported no classification for physical hazards.

Assessment and comparison with the classification criteria

The available information indicates that hexaflumuron is thermally stable, non-flammable, does not self-ignite and is non-oxidising. Despite its sensitivity to friction, hexaflumuron was not found to be explosive for thermal or mechanical sensitivity (shock). Therefore, based on negative results in standard studies, RAC supported the proposal of DS **not to classify hexaflumuron for physical hazards**.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS concluded that hexaflumuron is not acutely toxic by the oral, dermal and inhalation routes (LD_{50} > 5000 mg/kg bw, LD_{50} >2000 mg/kg bw, LC_{50} >7 mg/L, respectively) and therefore did not propose to classify hexaflumuron for these hazard classes under CLP.

Comments conventional during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

In an oral toxicity study in F344 rats, the LD_{50} value was found to be >5000 mg/kg bw, which is greater than the guidance value for classification in acute toxicity (oral) category 4 (300 < ATE ≤ 2000 mg/kg bw). The LD_{50} value of > 2000 mg/kg bw in a dermal toxicity in NZW rabbits was above the range for classification for this endpoint (1000 < ATE ≤ 2000 mg/kg bw). In a 4 hour exposure inhalation toxicity study in F344 rats, the LC_{50} value was found to be > 7 mg/L, which is greater than the range for acute toxicity (inhalation) classification category 4 (1.0 < ATE ≤ 5.0 mg/L, dusts and mists).

The results from three acute toxicity studies performed according to OECD Test Guidelines (TG) confirm that hexaflumuron does not meet the criteria for acute toxicity classification under CLP. Therefore RAC agreed with the DS on **no classification for acute toxicity** by the three routes of exposure.

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

Summary of the Dossier Submitter's proposal

No classification for STOT SE was proposed by the DS since there were neither human data to provide information on this end point nor any clear evidence of any specific toxic effects on any target organ or tissue that can impair function, reversible or irreversible, immediate and/or delayed from the animal data available.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

There was no evidence of significant single target organ toxicity of hexaflumuron in the available acute oral, dermal or inhalational studies. The signs that were apparent after single acute exposure of hexaflumuron were indicative of non-specific, general acute toxicity. There was no evidence of specific effects on a target organ or tissue that were
independent of mortalities, no definitive signs of respiratory tract irritation or narcotic effects. In the absence of constant and identifiable effects, RAC agrees with the DS that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

As a result of the skin irritation test in rabbits (OECD TG 404), none of the CLP criteria for skin irritancy classification were met. None of the scores measured in animals reached the average cut-off value of 2.3 for erythema/eschar or for oedema or in any case was there inflammation that persisted to the end of the 7-day observation period. Only one animal showed slight erythema (average score: 0.06) with desquamation at 72 hours and this was fully reversible at 7 days. Overall, the DS did not propose to classify hexaflumuron as the effects observed in the study were not sufficient to warrant classification as a skin irritant.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

RAC agrees with the DS evaluation of the available skin irritation test in rabbits and that hexaflumuron **does <u>not</u> meet the CLP criteria for classification as a skin irritant**.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier Submitter's proposal

In the rabbit eye irritation test (OECD TG 405), ocular irritancy was observed in 4/6 animals, but in all cases this was scored below the cut-off value for classification of 1 (mean scores:

conjunctival redness - 0.67; oedema - 0.33) and there were no corneal opacity or iritis. All signs of slight ocular irritation were resolved by 24 hours post-treatment in all animals. Therefore, the DS did not propose to classify hexaflumuron as none of the CLP criteria for eye irritancy category 2 were met.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

RAC agrees with the DS evaluation of the available eye irritation test in rabbits and that hexaflumuron **does <u>not</u> meet the CLP criteria for classification as an eye irritant**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised and evaluated the results of a modified Buehler test (OECD TG 406). Skin reactions were evident in 0/10 and 1/10 Guinea pigs at 24h and 48h, respectively. There were no other studies available. It was concluded by the DS that hexaflumuron tested negative for dermal sensitisation and does not meet the CLP classification criteria for this endpoint.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

No human data are available on the skin sensitisation potential of hexaflumuron. Based on the clear negative result obtained in the modified Buehler test, RAC agrees with the DS that hexaflumuron **does <u>not</u> meet the criteria for classification as a skin sensitiser**.

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

Summary of the Dossier Submitter's proposal

The DS evaluated six repeated-dose toxicity studies conducted with hexaflumuron by the oral (diet) route: 2 subacute (28-d) studies in mice and dogs, 2 subchronic (90-d) studies in mice and rats and 2 chronic (52-wk) studies in dogs and rats. The DS considered the disturbance of haematological parameters as the main relevant finding in all studies. There was a consistent increase in methaemoglobin (MetHb) which was often associated with increased spleen weights and Heinz body formations, mostly observed at the higher doses. Dogs were the most sensitive species to hexaflumuron followed by mice and rats (dog > mouse > rat). The DS commented that this order of sensitivity was in agreement with the conclusions of RIVM (Nº 601516007) and JRC (ECBI/07/03 Add.11) reports in which rats and mice are described as usually less sensitive to MetHb formation and generally more effective at reduction of induced MetHb than humans, dogs or cats.

Lowest Observed Adverse Effect Levels (LOAELs) of 25 mg/kg bw/d and 2 mg/kg bw/d were derived from the 90-day mouse and 52-week dog studies, respectively, based on increased MetHb

concentrations in females. However these increases in MetHb were not accompanied by a significant reduction in % haemoglobin (Hb) in blood (in all cases values were < 10%).

The DS noted the possible induction of haemolytic anaemia to some degree at doses relevant to humans, under repeated exposure i.e. 25 mg/kg bw/d in mice (oral, 90 day) and 2 mg/kg bw/d in dogs (oral, 52 weeks).

No inhalation studies were available. No adverse effects were observed in the repeated dermal toxicity study in rats.

The DS assessed the findings from all repeated dose toxicity studies against the CLP criteria for STOT RE and the guidance developed specifically for classification of substances inducing haemolytic anaemia.

The increase in MetHb seen after repeated oral administration was not accompanied in any stage by a reduction of the Hb greater than or equal to 10% and therefore, following the available guidance, the criteria for classification were not met. Furthermore, under chronic administration (dog, 52 weeks), hexaflumuron showed reversibility of the adverse effects and an adaptive response without affecting the clinical status of the tested animals. Therefore, based on a weight-of-evidence approach, the DS considered that the adverse effects associated with repeated exposure to hexaflumuron were not sufficiently severe to trigger classification as STOT RE for haemolytic effects.

Comments received during public consultation

One MS commented that STOT RE 2 had been considered appropriate during the peer review of the biocidal active substance and cited the NOAEL value of 0.5 mg/kg bw/d from the 52 week dog study, which was considered as the relevant starting point for the derivation of reference values in the assessment as a biocidal active substance.

In response to this comment, the DS stated that there were no grounds for STOT RE classification when considering all the parameters in quantitative terms. The DS considered that the severity of the effects is not sufficient to trigger hazard classification and noted that this is not contradictory with using the NOAEL (hepatic hemosiderin deposits) as this starting point is a risk-based value and does not take the CLP criteria into consideration.

Assessment and comparison with the classification criteria

As described by the DS, studies of repeated exposure in rats, mice and dogs are available. Briefly, the findings from these studies considered relevant by RAC are as follows.

Sprague-Dawley rats, 13 weeks, oral (diet)

Rats were exposed to hexaflumuron at dose levels of 0, 25, 125, 750 and 1500 mg/kg bw/d. A slight but statistically significant increase in MetHb was observed at ≥ 125 mg/kg bw/d in males and at ≥ 750 mg/kg bw/d in females. At ≥ 750 mg/kg bw/d, spleen histopathological sections showed a slight increase in haemosiderin deposits in females (significant at $P < 0.05$); however these findings were not confirmed by histopathology.

Sprague-Dawley rats, 52 weeks (part of the 2 year carcinogenicity study), oral (diet)

Hexaflumuron was administered to rats at 0, 5, 75 and 500 mg/kg bw/d. At the top dose, elevated alkaline phosphatase in males and cholesterol in females indicated functional liver changes. MetHb concentrations showed large variations possibly indicating deficiencies in the sampling time. Although no clear increases in MetHb levels were seen, indicators of an adaptive cellular response were observed in top dose females (differential cell count indicative of anaemia and increase in reticulocytes). Several minor changes were also noted in clinical chemistry parameters (increased alkaline phosphatase and reduced glucose levels in males, increased cholesterol levels in high dose females).

Compared to controls (0/10), there was a statistically significant increase in the number of females with an absence of ovarian corpora lutea in the low dose group (5/10, $P < 0.05$). The intermediate and top dose group also showed an increased incidence, but this was less marked (3/10 of both groups). No such effect was reported in the 2-year carcinogenicity study.

A dose-related decrease in the incidence of kidney cortical mineral deposits was also observed in females (5/10, 2/10, 2/10 and 0/10 at 0, 5, 75 and 500 mg/kg bw/d respectively). No such deposits were seen in males and there were apparently none at the end of the 2-year period of the carcinogenicity study. Both the ovarian and kidney findings were reported in isolation of any related toxicity and were only seen in this study at 52 weeks. They do not appear to indicate significant and biologically relevant toxicity in these tissues.

CD-1 mice, 28 days, oral (diet)

Mice were administered hexaflumuron at 0, 25, 125, 750 and 1500 mg/kg bw/d in the diet. A treatment-related increase was seen in liver enzyme activity in males (ALT at ≥ 750 mg/kg bw/d and AST at 1500 mg/kg bw/d) and a statistically significant increase in serum cholesterol was seen in females at ≥ 750 mg/kg bw. MetHb levels were elevated in both sexes at ≥ 125 mg/kg bw/d. There was a significant increase of spleen weight at ≥ 750 mg/kg bw/d in males together with an increase in the number of top dose males with marked extramedullary haematopoiesis.

CD-1 mice, 13 weeks, oral (diet)

In mice administered 0, 5, 25 and 250 mg/kg bw/d hexaflumuron, a dose-related increase in MetHb was observed in females at ≥ 25 mg/kg bw/d (2^{nd} day sampling), however this was not accompanied by a significant decrease in Hb. Increases in bilirubin and the liver enzymes GPT and GOT were observed in both sexes at 250 mg/kg bw/d, although the effect on GOT was not statistically significant in males. High-dose males had prominent mitotic figures in the liver. Minimal hepatocyte enlargement was observed in 2/10, 3/10, 6/10 and 8/10 cases at 0, 5, 25 and 250 mg/kg bw/d respectively.

Beagle dogs, 28 days, oral (diet)

This study used only 2 animals/group instead of the 4 recommended in the OECD TG 409. Beagle dogs were treated with hexaflumuron at doses of 0, 25, 125 or 750 mg/kg bw/d. A moderate to marked dose-related increase in the Heinz body count was observed at ≥ 125 mg/kg bw/d. There was an increase in MetHb in both sexes at ≥ 25 mg/kg bw/d. Morphological changes in red blood cells (RBC) were seen as indicated by anisocytosis in females at ≥ 125 mg/kg bw/d, presence of target cells and hypochromia in males at ≥ 25 mg/kg bw/d and in females at ≥ 125 mg/kg bw/d, as well as the presence of Howell-Jolly bodies in males at ≥ 125 mg/kg bw/d. Additional observations included a slightly increased mean corpuscular volume (MCV) in males at 25mg/kg bw/d, a slightly increased mean corpuscular haemoglobin (MCH) for both sexes at ≥ 125 mg/kg bw/d and a slightly decreased erythrocyte count, Hb concentration and haematocrit value in males at ≥ 25 mg/kg bw/d and in females ≥ 125 mg/kg bw/d.

There was an increase in spleen weights at 750 mg/kg bw/d in both sexes and signs of congestion and increased erythropoiesis in the spleen at ≥ 125 mg/kg bw/d in both sexes.

Beagle dogs, 52 weeks, oral (diet)

Dogs were administered hexaflumuron at 0, 0.5, 2, 5 and 25 mg/kg bw/d. Increases in MetHb were observed at ≥ 2 mg/kg bw/d and in Heinz bodies at ≥ 5 mg/kg bw/d. These effects were maximal at 13 and 26 weeks, having decreased by 52 weeks for both sexes. MetHb reached about 7% in males and females at 26 weeks. Additionally, there was a slight decrease in the MCH at 25 mg/kg bw/d at 4, 26 and 52 weeks and a slight increase in the platelet and reticulocyte count for females at the same dose level at 4 weeks. Howell-Jolly bodies were slightly increased at the highest dose level in both sexes at 26 and 52 weeks. These changes in the erythrocyte parameters were indicative of a compensatory haematopoiesis process. Treatment-related haemosiderin deposits, located mainly in small aggregates of proliferated Kupffer cells, were observed in both sexes at ≥ 0.5 mg/kg bw/d. However, they were not associated with other changes indicating significant haemolytic anaemia.

The following table summarises the key findings from these studies as they relate to the guidance values for classification.

Study	**Doses relevant for STOT RE 1**	**Adverse effects at this dose level**	**Other effects at this dose level**	**Doses relevant for STOT RE 2**	**Adverse effects at this dose level**	**Other effects at this dose level**
Sprague-Dawley rats 90 day 0, 25, 125, 750, 1500 mg/kg bw/d	None **Cat 1: C ⩽ 10 mg/kg bw/d**	N/A[1]	N/A	25 mg/kg bw/d **Cat 2: 10 < C ⩽ 100 mg/kg bw/d**	None	None
Sprague-Dawley rats 52 week 0, 5, 75, 500 mg/kg bw/d	None **Cat 1: C ⩽ 2.5 mg/kg bw/d**	N/A	N/A	5 mg/kg bw/d **Cat 2: 2.5 < C ⩽ 25 mg/kg bw/d**	None	5 mg/kg bw/d: Increased incidence of females with an absence of ovarian corpora lutea [(5/10, P < 0.05) compared to controls (0/10)], but lower numbers in the higher dose groups [3/10; 3/10].
CD-1 mice 28 day 0, 25, 125, 750, 1500 mg/kg bw/d	25 mg/kg bw/d **Cat 1: C ⩽ 30 mg/kg bw/d**	None	None	125mg/kg bw/day **Cat 2: 30 < C ⩽ 300 mg/kg bw/d**	None	125 mg/kg bw/d: Increased MetHb levels in males and females.This was not accompanied by a decrease in Hb ≥ 10%. Marked treatment-related extramedullary haematopoiesis in the spleen of males. Considered to be a compensatory response.
CD-1 mice 90 day 0, 5, 25, 250 mg/kg bw/d	5 mg/kg bw/d **Cat 1: C ⩽ 10 mg/kg bw/d**	None	5 mg/kg bw/d: Minimal hepatocyte enlargement in 3/10 males (2/10 in controls).	25 mg/kg bw/d **Cat 2: 10 < C ⩽ 100 mg/kg bw/d**	None	25 mg/kg bw/d: Increased MetHb in females on 1 of 2 sampling days 1.84% vs 0.88% at day 2 sampling; 2.15% vs 1.59% at day 1 sampling (more pronounced effect at 250 mg/kg bw/d). This was not accompanied by a decrease in Hb ≥ 10%. Minimal hepatocyte enlargement in 6/10 cases in males (2/10 in controls).

[1] N/A = Not applicable (no relevant dose in the study)

Beagle dogs 28 day 0, 25, 125, 750 mg/kg bw/d	25mg/kg bw/d **Cat 1: C ⩽ 30 mg/kg bw/d**	None	25 mg/kg bw/d: -slight to marked MetHb in males and females - slight changes in red blood cell morphology (presence of target cells and hypochromia in males) - slightly increased MCV in males - slightly decreased erythrocyte count, haemoglobin concentration and haematocrit value in males	125mg/kg bw/d **Cat 2: 30 < C ⩽ 300 mg/kg bw/d**	None	125 mg/kg bw/d: -Pronounced MetHb in males and females 6.4% and 4.4% vs 0.8% and 0.5%, respectively) - moderate to marked - increase in the Heinz body count (dose-related) - slightly increased MCH for both sexes - Slightly decreased erythrocyte count, haemoglobin concentration and haematocrit value in females - slight changes in red blood cell morphology (anisocytosis in females, presence of target cells and hypochromia in males and females, Howell-Jolly bodies in males) - signs of congestion and increased erythropoiesis in male and female spleen
Beagle dogs 52 week 0, 0.5, 2, 5, 25 mg/kg bw/d	0.5, 2, mg/kg bw/d **Cat 1: C ⩽ 2.5 mg/kg bw/d**	None	0.5 and 2 mg/kg bw/d: Hemosiderin deposits (Grade 1 or 2 (out of 5), located mainly in small aggregates of proliferated Kupffer cells in liver of 1-2/4 males and females per dose group 2 mg/kg bw/d: Slight increase in MetHb at 4, 13, 26 and 52 weeks in males and females)	5, 25 mg/kg bw/d **Cat 2: 2.5 < C ⩽ 25 mg/kg bw/d**	None	5 and 25 mg/kg bw/d: Hemosiderin deposits (Grade 2 of 5) in 1-4/4 males and females per dose group 5 mg/kg bw/d: Small increase in MetHb and Heinz bodies (maximal at 13 and 26 weeks) for both males and females) 25 mg/kg bw/d: Pronounced increase in MetHb (up to approx. 7.2% in females) and Heinz bodies (up to 470 in males) (maximal at 13 and 26 weeks) for both males and females. This was not accompanied by a decrease in HB ⩾ 10%. Slight decrease in MCH at 4, 26 and 52 weeks. Slight increase in the platelet and reticulocyte count for females, at 4 weeks. Howell-Jolly bodies were slightly increased in both males and females at 26 and 52 weeks.

As can be seen from the table and the summary above, the most significant evidence of toxicity was seen in the dog studies, especially in relation to haematology and related findings. Although increased MetHb was seen consistently, and sometimes at doses relevant for classification, this

was not accompanied by significant decreases in haemoglobin levels or other relevant indicators of haemolytic anaemia (see below). The various findings described above are considered to have been signs of adaptive or reversible compensatory changes in the blood system. Overall, in reviewing these findings, RAC concludes that there are no effects occurring at relevant doses in any of these studies that would justify a classification of hexaflumuron with STOT RE 1 or STOT RE 2 and therefore **no-classification is supported**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxic potential of hexaflumuron was investigated in three *in vitro* and one *in vivo* assays. Results of these tests indicate that hexaflumuron is devoid of any genotoxic potential in prokaryotic and eukaryotic cells. The DS concluded that no classification for mutagenicity (germ cells) is warranted for hexaflumuron.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

Hexaflumuron has been tested adequately in the following *in vitro* and *in vivo* assays: bacterial mutagenicity; mammalian cell gene mutation (Hprt); mammalian cell chromosome aberration; and mouse bone marrow micronucleus.

In the mammalian cell gene mutation test, mutant frequencies were increased compared to the control value at the lowest and highest doses tested in one of the two experiments conducted without S9. However, as no dose-related trend was observed and the finding was not reproducible, RAC agrees with the DS that the result of this test was negative.

All other *in vitro* and *in vivo* assays gave clear negative results. Given the absence of any positive evidence, RAC agrees with the DS that hexaflumuron **does <u>not</u> warrant a classification for mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of hexaflumuron for carcinogenicity via the oral route. No data on chronic exposure via the inhalation and dermal routes are available. The DS summarised and evaluated two studies in the CLH report.

Rat combined chronic toxicity/ carcinogenicity study (OECD TG 453)

Sprague-Dawley rats were exposed to hexaflumuron in the diet at concentrations of 0, 5, 75 and 500 mg/kg bw/d for 2 years. Both males and females at 5 and 75 mg/kg bw/d showed no clear treatment-related toxicological effects.

There were several statistically significant haematological (RBC, neutrophils, bilirubin and monocytes) and clinical chemistry/urinalysis findings, although these were not dose-related and not attributed to treatment with the test substance.

Males and females at 500 mg/kg bw/d showed an increased incidence and severity of liver pale cell foci. The incidence in historical control rats of the same laboratory ranged between 20-44%

and 2-32% in males and females, respectively. The recorded range in this study was 30-56% and 24-48% in males and females, respectively; all groups were within the historical range with the exception of the high dose males and females.

An increased incidence of cortical cellular change with degeneration and/or vascular dilatation was observed in the adrenals of females at the low dose level. This was correlated with an increase in unilateral enlargement in the adrenals of females observed at necropsy. The increase in incidence of this adrenal cortical lesions in females was not dose-related and as a consequence, it was not considered toxicology relevant but of spontaneous nature.

The DS concluded that the administration of hexaflumuron via the diet to Sprague-Dawley rats for 104 weeks produced no notable in-life changes and no increase in incidence of tumours.

Mice carcinogenicity study, 80 weeks

Hexaflumuron was administered via the diet to CD-1 mice at levels equivalent to 0, 2, 5 and 25 mg/kg bw/d for 80 weeks.

There were no effects on mortality or clinical signs that were considered related to treatment.

The only significant observation associated with corroborative histological changes was an increase in the number of 25 mg/kg bw/d main group male mice with pale focus on the lungs. A marginal increase in the incidence of pulmonary tumours and pulmonary adenomatosis was noted for males at 25 mg/kg bw/d. However statistical analysis revealed no significant differences from the controls and the incidence of pulmonary tumours was within historical control incidences. The DS therefore considered these differences as not treatment-related.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

The carcinogenicity of hexaflumuron has been tested in dietary studies in both rats and mice. There were no tumour-related findings of significance in these studies.

The DS reported an observation associated with corroborative histological changes of an increase in the number of male mice at the highest dose with pale cell focus on the lungs. Pulmonary adenomatosis and pulmonary adenoma were also evident at this dose level. However, RAC is of the opinion that these limited findings do not appear related to treatment. Although the data were not presented in the CLH report, incidences of pulmonary tumours were within the historical control range according to the DS, further indicating that they were unlikely to have been treatment-related.

Findings in the lungs of mice at 80 weeks

	Males				**Females**			
Hexaflumuron Dose (mg/kg bw/d)	**0**	**2**	**5**	**25**	**0**	**2**	**5**	**25**
Macropathology, lungs, pale focus	5	4	4	11	7	5	10	7
Histopathology, lung, pulmonary adenoma	7	4	8	9	5	6	7	3
Histopathology, lung, pulmonary adenomatosis	0	0	0	2	0	0	0	0
Histopathology, lung, focal adenomatosis	5	5	4	8	0	0	0	1

Given the absence of any evidence for the carcinogenicity of hexaflumuron, RAC agrees with the DS that **no classification is appropriate for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of hexaflumuron for reproductive toxicity.

Hexaflumuron was tested for effects on fertility and reproduction in a rat multigeneration study with 2 litters in each of the 2 generations. Dietary levels were administered which provided 0, 5, 25 and 125 mg/kg bw/d from 10 weeks pre-mating through to weaning. There were no effects on the general health of parental animals or pups. Maternal performance and reproduction findings did not reveal any compound-related effects in either generation, in terms of the fertility index (83.3-100%) and the gestation index (100%) throughout the study. There were no significant post-implantation losses. The day-4 viability index was significantly reduced at the highest dose in the first litter of the F1-generation, but no such finding was observed in any of the other 3 sets of litters.

Recurrent changes in the hematopoietic system in parental rats were observed at ≥ 25 mg/kg bw/d. In F0-females, there were statistically significant decreases of Hb in plasma but these were not accompanied by other changes in red blood cell parameters. In F1 females, there was an increase of reticulocytes at the mid dose only and an increase of % MetHb at the top dose. In the F1 generation, there were also increased spleen weights at the mid and top doses and extra medullary haematopoiesis at the top dose.

Blood could not be collected from F0 males due to accidental loss of these animals. However, blood parameters of the F1-generation males showed a decrease of the RBC count at ≥ 25 mg/kg bw/d accompanied by increases in MCH at ≥ 25 mg/kg bw/d and in mean corpuscular volume (MCV) at 25 mg/kg bw/d.

Histopathological examination of the F0- and F1-generation parent rats did not reveal any treatment-related effects.

In consideration of developmental toxicity, the DS described how hexaflumuron did not cause maternal toxicity, embryotoxicity, or teratogenicity in 2 studies, one in rats and one in rabbits. Haematological investigations were not performed in these studies as they were not required for a prenatal developmental/ teratogenicity toxicity study according to OECD TG 414.

The DS concluded that the strength of evidence generated by the 3 studies did not support classification of hexaflumuron as a reproductive toxicant. Teratogenicity studies in rats and rabbits did not result in developmental toxicity. Besides the recurrent changes in the hematopoietic system in male and female parent rats, the 2-generation study did not reveal any adverse effect on maternal performance or litters/pups viability. Despite its limitations, this study didn't show potential for considering hexaflumuron a reproductive toxicant.

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

Fertility and Reproductive Function

In order to assess any potential effects of hexaflumuron on fertility and reproductive function, the test substance was administered via the diet to Wistar rats (24/sex/group) in a multigeneration

study at doses of 0, 5, 25 and 125 mg/kg bw/d from 10 weeks pre-mating through to weaning. In each of the 2 successive generations, two litters were reared. The accidental loss of F0 males in week 29 of the study (see above) had no influence on the overall quality of the study.

The test substance did not affect bodyweight or food consumption of parent rats of both the F0 and F1 generations.

Differences in pre-coital time were observed, as shown in the table below. However, the significant finding in the first pregnancy of the F0 generation was not reproduced in the second pregnancy of the same generation or in either pregnancy of the F1 generation.

	Pre-coital time (days)			
Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
F0 generation first pregnancy	2.4	2.0	4.0	4.3*
F0 generation second pregnancy	3.0	3.3	2.0	1.8
F1 generation first pregnancy	3.1	2.6	3.0	2.3
F1 generation second pregnancy	2.5	3.0	2.5	2.3

Fertility indices, gestation indices and post-implantation losses were not affected by treatment.

As can be seen in the table below, the viability index for pups was significantly decreased at the top dose in the first pregnancy of the F1 generation on day 4 *post partum*. However, this can be attributed to the high pup mortality in one litter (12/14 pups from the same litter died before post-natal day 4). In the second litters of the F1 generation, there was a slight dose-related decrease of the viability index, but without statistical significance. As no such effect had been seen in either sets of litters from the F0 animals, RAC is of the opinion that this is an incidental finding.

	Viability Index, day 4 (%)			
Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
F0 generation first pregnancy	96.2	99.2	98.9	97.9
F0 generation second pregnancy	98.8	98.8	99.2	96.9
F1 generation first pregnancy	98.7	98.6	97.9	87.5*
F1 generation second pregnancy	98.0	98.5	94.0	91.5

Although no treatment-related effects in mean litter and mean pup weights were observed between groups of pups born to the F0 animals, dose-related decreases in mean litter and mean pup weights were seen during lactation at the high dose in the second generation (see table below). The mean litter weight of the first set of pups (F2a) was statistically significantly decreased at day 4, but not at later time points. Mean pup weights at this dose were slightly lower in both litters at day 21, but this showed statistical significance in the second litter (F2b) only.

Mean pup/ litter weights during lactation					
Dose (mg/kg bw/day)	Day 1	Day 4	Day 7	Day 14	Day 21
Pup weights during lactation: First litter F1-generation parents					
0	6.0	8.6	13.6	28.1	44.0
5	6.1	9.0	14.3	29.3	45.6
25	5.8	8.7	13.7	28.2	43.2
125	5.5	7.6	12.2	26.7	41.3
Pup weights during lactation: Second litter F1-generation parents					
0	6.0	8.7	14.2	29.3	45.5
5	6.1	9.4	15.3	30.7	45.5
25	5.8	8.4	13.9	28.4	42.8
125	5.5*	7.7	13.3	28.3	40.9**
Litter weights during lactation: First litter F1-generation parents					
0	67.9	66.7	104.3	216.7	333.5
5	65.9	69.5	109.3	224.3	348.9
25	62.8	68.4	108.5	222.3	340.4
125	60.0	57.3*	93.6	203.4	314.2

Litter weights during lactation: Second litter F1-generation parents					
0	71.7	69.3	114.0	232.7	362.2
5	65.0	70.9	116.0	229.0	338.9
25	63.2	65.0	109.3	220.4	331.5
125	64.1	60.2	104.6	222.6	320.9

*P < 0.05 **P < 0.01

There were no other effects on pups during the lactation period.

Effects on haematological parameters in the parental animals (outlined in the table below) included a statistically significant decrease in plasma Hb at ≥ 25 mg/kg bw/d in F0 generation females only. However, there were no other changes in blood parameters in these groups. It is unclear whether such an isolated effect on Hb levels could have contributed to the slightly reduced pup and litter weights seen in the F1 pups. Other indicators of possible treatment-related haematological effects in this study included increased reticulocytes at 25 mg/kg bw/d and % MetHb at 125 mg/kg bw/d in F1 adult females. There was also an increased spleen weight in F1 parent females at ≥ 25 mg/kg bw/d.

In F1-generation male parent rats, there was a decrease in the RBC count at ≥ 25 mg/kg bw/d in comparison to the controls, which was accompanied by increases in MCH at ≥ 25 mg/kg bw/d and in MCV at 25 mg/kg bw/d.

Dose level (mg/kg bw/d)	**0**	**5**	**25**	**125**
Haemoglobin in plasma (µmol/L), F0-parents, females	4.09	3.27	3.13*	2.75**
Mean corpuscular volume (FL), F1- parents, males	62.5	61.0	67.4**	65.4
Mean corpuscular haemoglobin (Fmol), F1-parents, males	1.18	1.17	1.27**	1.25*
Red blood cells (10E12/L), F1-parents, males	7.6	7.7	7.2*	7.1**
Reticulocytes (/1000), F1-parents	4.0	5.4	20.2**	8.0
Methaemoglobin (%), F1-parents, females	1.49	1.49	1.64	1.77**
Absolute spleen weight (g), F1-parents, females	0.441	0.467	0.503**	0.486*
Relative spleen weight (g), F1-parents, females	1.71	1.74	1.92**	1.82

Haematological effects were evaluated by RAC in the repeated dose section. Despite the frequency with which these effects were reported, their magnitudes were not considered sufficient by RAC to warrant classification.

Histopathological examination of the F0 and F1 generation parents did not indicate any treatment related changes.

In conclusion, hexaflumuron treatment of rats in this study failed to show any adverse effects on fertility or reproductive function. Isolated indications of reduced pup growth *post partum*, especially at the highest dose, are not considered to have provided evidence of toxicity during lactation.

Aside from the multi-generation study, in a 52 week study in rats, there was a statistically significant increase in the incidence of absence of ovarian corpora lutea at 5 mg/kg bw/d (5/10, P < 0.05) compared to controls (0/10). This was the lowest dose level of hexaflumuron in this study. Absent ovarian corpora lutea were also evident at the mid and high dose levels (3/5, 3/5, respectively) but without statistical significance. Although no explanation was provided for these findings, they are insufficient to justify a classification for fertility or reproductive function according to the CLP criteria.

Developmental Toxicity

Two studies evaluating the potential developmental toxicity caused by hexaflumuron are available.

Study in rats

Hexaflumuron was administered to time-mated female Sprague-Dawley rats (30/group) at dose levels of 0, 25, 125 and 1000 mg/kg bw/d on gestation days (GD) 6-15 in an oral gavage teratology study. There was no evidence of any adverse maternal, embryonal, or fetal effects in the time-mated female rats at doses ≤ 1000 mg/kg bw/d.

Study in rabbits

In an oral gavage teratology study, hexaflumuron was administered to 24 NZW rabbits at 1000 mg/kg bw/d. There was no evidence of maternal toxicity, fetotoxicity or teratogenicity.

Comparison with criteria for reproductive toxicity

Although there was a statistically significant increase in the incidence of absence of ovarian corpora lutea at 5 mg/kg bw/d compared to controls in the 52 week rat study, the results from the extensive multigeneration study in rats raised no causes for concern for fertility.

In 2 teratology studies (one in rats and one in rabbits), there was no evidence of adverse effects on development.

Effects on or via lactation are allocated to a separate single category. There was a dose-related biologically significant decrease in mean litter and pup weights during lactation in the F1 generation high dose group. However, the effect was statistically significant only in the second litter of the F1 generation at 125 mg/kg bw/d. Since there is no clear evidence that this effect was due to transfer of hexaflumuron in the milk or an adverse effect on the quality of the milk, RAC is of the opinion that these data do not justify classification of hexaflumuron for effects on or via lactation.

The potential of hexaflumuron to cause reproductive toxicity has been investigated in 2 teratology studies (one in rats and one in rabbits) and one multigeneration study in rats. There was no evidence of adverse effects on fertility or on development. RAC therefore agrees that **no classification of hexaflumuron for reproductive toxicity is warranted**.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed to classify hexaflumuron as Aquatic Acute 1 (H400) with an M-factor of 1000 and as Aquatic Chronic 1 (H410) with an M-factor of 10000 based on lack of rapid degradation, a bioconcentration factor (BCF) above 500 L/kg, and high toxicity towards the aquatic invertebrate *Daphnia magna* (a 48h EC_{50} of 0.11 µg/L and a 21d NOEC of 0.0029 µg/L).

Comments received during public consultation

No specific comments were received, but one MS provided a general agreement in favour of the CLH proposal.

Assessment and comparison with the classification criteria

Degradability

Hexaflumuron was hydrolytically stable at pH 5 and 25 °C, with a hydrolysis half-life of 270 days at pH 7 and 22 days at pH 9. It was not readily biodegradable in an OECD TG 301B test, achieving a maximum of 6% biodegradation after 29 days. Simulation tests with aerobic and anaerobic soils were conducted at 25 °C; the predicted geometric mean DT_{50} was 280 days for aerobic soils at 12

°C. On this basis, RAC concludes that hexaflumuron does not meet the criteria for being rapidly degradable (readily biodegradable) in the environment.

Bioaccumulation

The log n-octanol/water partition coefficient (K_{ow}) of hexaflumuron is 5.68. A steady-state BCF of 5,600 L/kg wet weight was measured for Bluegill Sunfish (*Lepomis macrochirus*) based on radiolabelled substance. 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 higher than 500 L/kg this does not affect the conclusion that hexaflumuron meets 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	Bluegill Sunfish *Lepomis macrochirus*	96h LC_{50} >0.142 mg/L	-
Aquatic invertebrates	*Daphnia magna*	**48h EC_{50} = 1.1×10^{-4} mg/L**	**21d NOEC = 2.9×10^{-6} mg/L**
Aquatic algae and plants	*Raphidocelis subcapitata*†	96h E_rC_{50} > 1.91 mg/L	96h NOEC > 1.91 mg/L

† *Selenastrum capricornutum* in the dossier.

All toxicity values presented in the table above are based on mean measured concentrations. No insect data are available, so the possibility that the substance might be more toxic to aquatic insects cannot be excluded.

Classification according to CLP

<u>*Acute aquatic hazard:*</u> Acute toxicity data were available for all three trophic levels. The lowest reliable short-term aquatic toxicity result is a 48h EC_{50} of 0.11 µg/L [0.00011 mg/L] for *Daphnia magna*. This is well below 1 mg/L so the substance is classified as Aquatic Acute 1 (H400), and as it is within the range 0.0001-0.001 mg/L an acute M-factor of 1000 is applicable.

<u>*Chronic aquatic hazard:*</u> Hexaflumuron is not rapidly degradable. Long-term toxicity data were available for two trophic levels (no information was available for fish). Aquatic invertebrates were the most sensitive group with a lowest reported 21d NOEC of 0.0029 µg/L [0.0000029 mg/L]. This is well below the threshold value of 0.1 mg/L so the substance is classified as Aquatic Chronic 1 (H410), and as it is within the range 0.000001-0.00001 mg/L a chronic M-factor of 10000 is applicable.

RAC notes that the surrogate approach can also be considered for fish in the absence of a chronic toxicity result. Based on the lowest acute 96h LC_{50} of > 0.142 mg/L (effectively above the limit of water solubility, which is 0.027 mg/L), a BCF > 500 L/kg and lack of rapid degradability, only the safety net classification of Aquatic Chronic 4 would be justified. Therefore the more stringent classification is selected.

In summary, RAC concludes that the DS proposal to classify hexaflumuron as **Aquatic Acute 1 (H400) with an M-factor of 1000 and Aquatic Chronic 1 (H410) with an M-factor of 10000** is justified.

ANNEXES:

Annex 1 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 (excl. confidential information).