CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Hexaflumuron

EC Number: 401-400-1

CAS Number: 86479-06-3

Index Number: -

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	IUPAC name: 1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]-3-(2,6-difluorobenzoy)urea Chemical name: Benzamide, N-[[[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]amino]carbonyl]-2,6-difluoro- Common name: Hexaflumuron
EC number:	401-400-1
CAS number:	86479-06-3
Annex VI Index number:	-
Degree of purity:	96.2% w/w minimum; 98.4% w/w nominal
Impurities:	The 5-batch analysis identified three impurities present in quantities of 1 g/kg or higher but they do not affect the classification (impurities claimed confidential).

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor = 1,000 Chronic M-factor = 10,000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor = 1,000 Chronic M-factor = 10,000

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None	-	None	Conclusive but not sufficient for classification
2.2.	Flammable gases	-			Conclusive but not sufficient for classification
2.3.	Flammable aerosols	-			Conclusive but not sufficient for classification
2.4.	Oxidising gases	-			Conclusive but not sufficient for classification
2.5.	Gases under pressure	-			Conclusive but not sufficient for classification
2.6.	Flammable liquids	-			Conclusive but not sufficient for classification
2.7.	Flammable solids	None	-	None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None	-	None	Data lacking
2.9.	Pyrophoric liquids	-			Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	None	-	None	Data lacking
2.11.	Self-heating substances and mixtures	None	-	None	Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	-	None	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	-			Conclusive but not sufficient for classification
2.14.	Oxidising solids	None	-	None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None	-	None	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	None	-	None	Data lacking
3.1.	Acute toxicity - oral	None	-	None	Conclusive but not

					sufficient for classification
	Acute toxicity - dermal	None	-	None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	None	-	None	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None	-	None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None	-	None	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None	-	None	Data lacking
3.4.	Skin sensitisation	None	-	None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None	-	None	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	None	-	None	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None	-	None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None	-	None	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None	-	None	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	None	-	None	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Acute M-factor = 1,000 Chronic M-factor = 10,000	None	-
5.1.	Hazardous to the ozone layer	None	-	None	Conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning
Pictograms: GHS09
Hazard statements: H410: Very toxic to aquatic life with long lasting effects.
Precautionary statements: P273: Avoid release to the environment.
P391: Collect spillage.

P501: Dispose of contents/container to … … in accordance with local/regional/national/ international regulation (to be specified).

Proposed notes assigned to an entry: None.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Hexaflumuron has been assessed as a biocide active substance by Portugal, as Rapporteur Member State, according to Directive 98/8/EC concerning the placing of biocidal products on the market. Hexaflumuron was approved at Technical Meeting III 2012 (human health part) and Technical Meeting IV 2013 (general and environment parts).

This biocide active substance is not listed in Annex VI of Regulation (EC) No. 1272/2008.

2.2 Short summary of the scientific justification for the CLH proposal

Physico-chemical hazards

Based on the available information hexaflumuron is considered to be thermally stable, non-flammable, non-self-ignited and non-oxidising. Despite of its sensitivity to friction, this substance does not have chemical groups associated with explosive properties. Therefore no classification is proposed under CLP regulation.

Human health hazards

The main adverse effect exerted by hexaflumuron in repeated-dose toxicity studies by the oral route – sub-acute studies (rats, mice, and dogs), sub-chronic studies (mice and rats) and chronic studies (rats and dogs) – is anaemia, probably of haemolytic nature, characterized by decreases in haemoglobin levels and changes in red blood cell parameters with compensatory haematopoiesis, revealed by changes in the liver and spleen organs. This anaemia was particularly observed in dogs.

This effect was associated with bone marrow hyperplasia, reflecting a compensatory response to the anaemia and with haemosiderin pigment deposition in particular in the liver and spleen.

Environmental hazards

Hexaflumuron is non-readily biodegradable and non-rapidly degradable. Hexaflumuron hydrolyses under basic conditions, with a half-life of 22 days and at pH 5 and 7 it does not hydrolyse. The photolysis half-life for hexaflumuron was found to be approximately 6.3 days.

The experimental data show that hexaflumuron has little tendency to desorb from soil into water and therefore, little tendency to migrate through a soil/water column.

Taking into account the estimated K_{OC} value, hexaflumuron has high potential to sorption to soils, expecting to be immobile in soil.

The low vapour pressure and low Henry's law constant of hexaflumuron indicate that it is non-volatile and is not expected to move from water and soil to air. If present in the air, hexaflumuron is expected to be quickly degraded by photo-oxidation.

Considering the log K_{ow} and the BCF values, hexaflumuron has high potential to bioaccumulate.

Taking into consideration the 48h-EC_{50} value from *Daphnia magna* acute toxicity study (0.11 µg/L) and the 21 days-NOEC value from *Daphnia magna* long-term toxicity study (0.0029 µg/L), hexaflumuron is considered to be very toxic for aquatic organisms. Hexaflumuron should be classified as Aquatic Acute 1; H400 and as Aquatic Chronic 1; H410, according to CLP Regulation. Since the 48h-EC_{50} value for the most sensitive organism (*Daphnia magna*) is within the range of 0.0001-0.001 mg/L, an Acute M-factor of 1,000 is allocated. Also should be considered a Chronic M-factor of 10,000, since the 21d-NOEC value for *Daphnia magna* is within the range of 0.000001-0.00001 mg/L.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Hexaflumuron has no entry in Annex VI, Table 3.1.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Hexaflumuron has no entry in Annex VI, Table 3.2.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 4: Notified classification and labelling according to CLP criteria in C&L Inventory (versions 10/11/2014, depending of CAS or EC Numbers)

EC Number	EC Name	CAS Number
		86479-06-3

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Classification affected by Impurities / Additives	Additional Notified Information	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)							
Acute Tox. 4	H332	H332		GHS07 GHS09 Wng				IUPAC Names	23		
Aquatic Acute 1	H400										
Aquatic Chronic 1	H410	H410									
Aquatic Acute 1	H400	H400		GHS09 Wng				State/Form IUPAC Names	1		
Aquatic Chronic 1	H410	H410									

Number of Aggregated Notifications: 2

EC Number	EC Name	CAS Number
401-400-1		

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Classification affected by Impurities / Additives	Additional Notified Information	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)							
Acute Tox. 4	H302	H302	EUH401	GHS07 GHS09 Wng				Additional CAS Numbers	47		
Aquatic Chronic 1	H410	H410									

Number of Aggregated Notifications: 1

2.4.2 Current self-classification and labelling based on DSD criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

According with Article 36(2) of Regulation (EC) No. 1272/2008, hexaflumuron, as an active substance under Biocidal Products Directive 98/8/EC, should be subjected to harmonised classification and labelling.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

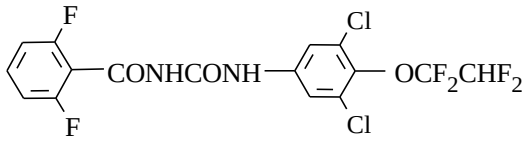
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	401-400-1
ISO name:	Hexaflumuron
CAS number:	86479-06-3
CAS name:	Benzamide, N-[[[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]amino]carbonyl]-2,6-difluoro-
IUPAC name:	1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]-3-(2,6-difluorobenzoy)urea
CLP Annex VI Index number:	-
Molecular formula:	$C_{16}H_8Cl_2F_6N_2O_3$
Molecular weight range:	461.15 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Hexaflumuron	98.4% w/w nominal	≥ 96.2% w/w minimum	

Current Annex VI entry: Not applicable.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
			All impurities have been claimed confidential.

Current Annex VI entry: Not applicable.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				Not applicable

Current Annex VI entry: Not applicable.

1.2.1 Composition of test material

The composition of the tested substance is the same as the substance covered by this CLH proposal, with purity ≥ 96.2%.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	White odourless powder at room temperature (98.2% purity).	Jones-Jefferson, 1992	Not applicable.
Melting/freezing point	202-205ºC (98.2% purity).	Macdonald et al, 1987	Measured (EC method A.1)
Boiling point	Not achieved (99.9% purity).	Smith, 2000	The sample decomposes after melting. Decomposition temperature: 295ºC (EC method A.2).
Relative density	1.680 g/cm^3 at 20°C (98.2% purity).	Macdonald et al, 1986	Measured (EC method A.3).
Vapour pressure	1.7x10^{-9} Pa at 18ºC 5.9x10^{-9} Pa at 25ºC (98.2% purity)	Macdonald et al, 1986	Estimated. (EC Method A.4) Calculated from the regression curve derived by plotting Log P vs. 1/T, at 25ºC.
Surface tension	Waived.	-	EC method A.5 states that a water solubility of ≥ 1mg/L is needed. Hexaflumuron solubility is 0.027 mg/L, therefore this study was not conducted.
Water solubility	2.7 x 10^{-5} g/L, at 18°C and pH: 9.77, unbuffered (98.2% purity).	Macdonald et al, 1986	Measured (EC method A.6). The temperature effect on the solubility has not been investigated.
Partition coefficient n-octanol/water	5.6821, at 20°C (98.2% purity).	Macdonald et al, 1986	Measured (EC method A.8).
Flash point	Waived.	-	Hexaflumuron is a solid. Therefore is not possible to determine the flash point.
Flammability	Not flammable.	Macdonald et al, 1987	Measured (EC methods A.10 and A.12).
Explosive properties	Hexaflumuron was not found to be explosive for thermal or mechanical sensitivity (shock), but did show sensitivity to friction.	Macdonald et al, 1987	Measured (EC method A.14).
Self-ignition temperature	Not autoflamable.	Macdonald et al, 1987	Measured (EC method A.16).
Oxidising properties	No oxidizing properties.	Macdonald et al, 1987	Measured (EC method A.17).
Granulometry	Data lacking.	-	-

Stability in organic solvents and identity of relevant degradation products	Temperature: 20ºC ± 0.5ºC (98.2% purity) - Acetone: >100000 mg/L - Acetonitrile: 14600 mg/L - Dichloromethane: 12600 mg/L - Ethyl acetate: >100000 mg/L - Hexane: 7 mg/L - Methanol: 11300 mg/L - Propan-2-ol: 3000 mg/L - 1-Octanol: 2000 mg/L - Toluene: 6400 mg/L - Xylene: 5200 mg/L	Macdonald et al, 1986	Measured. The identity of relevant degradation products is not available.
Dissociation constant	Waived.	-	The molecule does not contain reversible ionisable functional groups therefore this constant cannot be determined.
Viscosity	Waived.	-	Hexaflumuron is a powder.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Hexaflumuron is used as biocidal active substance as termiticide in a confined bait station, with a concentration of 0.5% w/w.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Explosive properties EC method A.14	The hexaflumuron (purity of 96.2%) was not found to be explosive for thermal or mechanical sensitivity (shock), but did show sensitivity to friction.	None	Macdonald et al, 1987

3.1 Explosive properties

3.1.1 Summary and discussion of explosive properties

In the explosive properties study performed according to EC method A.14 hexaflumuron seems to be sensitive to friction (appearance of sparks).

Hexaflumuron does not have chemical groups associated with explosive properties.

3.1.2 Comparison with criteria

Despite the appearance of sparks in the study (EC method A.14.) according to Part 2, 2.1.4.3 a) of Annex I of CLP Regulation, hexaflumuron shall not be classified as explosive considering that are no chemical groups associated with explosive properties in the molecule.

Therefore no classification according to the CLP criteria for explosive properties is warranted.

3.1.3 Conclusions on classification and labelling

Based on data mentioned above no classification is proposed for hexaflumuron regarding explosives hazards according to CLP criteria.

Additionally, no classification is warranted for hexaflumuron regarding other physico-chemical hazardous properties based on Table 9 above.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetic properties of hexaflumuron were evaluated in three ADME single dose studies (oral, gavage) performed in mouse, rat and dog with ^{14}C-benzoyl-(hexaflumuron) and 14C-phenylamino-(hexaflumuron) labelled test material.
Hexaflumuron demonstrated to be well-absorbed in the rat at low dose levels (5 mg/kg bw) but saturation occurs at higher dose levels (250 mg/kg bw) resulting in substantially less absorption. Oral absorption after single exposure of 5 mg/kg bw in rats and mice result higher than 80% and 90% respectively. Oral absorption estimation in the dog was not possible due to study limitations.

Peak plasma levels in all three species are similar, occurring from 4-8 hours following exposure. Plasma AUC from a 250 mg/kg bw dose is 4-5 times that from a 5 mg/kg bw dose. Elimination from the blood follows first-order kinetics with half-lives in all 3 species ranging from 14-30 hours depending on the specific radiolabelled material used.

Distribution of hexaflumuron is characterised as moderate with recoveries in seen predominantly in liver>kidney>fat (rat data). Hexaflumuron in the blood appears to be associated with the plasma, with no evidence of accumulation in the blood cells.

Both urinary and fecal excretions are involved in the elimination of hexaflumuron. Following oral (gavage) low-dose administration, urinary excretion ranges from 27-39% in the rat, 15-63% in the mouse and 1 - 5% in the dog depending on the specific radiolabelled material used. Similarly, fecal excretion following oral (gavage) low-dose administration ranges from 47-58% in the rat, to 24-67% in the mouse and to approximately 69% in the dog. In the dog dietary study, urinary excretion at the low dose ranged from 19- 51%. The radioactivity excreted via the feces ranged from 37- 64% for the low dose. No parent compound was present in the urine and only 8-14% of the parent compound was present in the feces.

Metabolism of hexaflumuron appears to be extensive with little parent compound detected following exposure. Metabolism appears similar across the species studied with cleavage of the aliphatic chain resulting in several common metabolites, along with the presence of additional minor metabolites. Identified metabolites include 2,6-difluorobenzoic acid and 2,6-difluorobenzamide, along with 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)aniline and 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)urea.

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

Toxicokinetic properties were assessed in 3 studies performed with mouse, rat and dog and demonstrated saturation of absorption at higher dose levels. This observation, coupled with the extensive metabolism and excretion profile, leads to the conclusion that bioaccumulation in mammals is not expected to occur.

4.2 Acute toxicity

4.2.1 Non-human information

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD 401 – Limit test	LD_{50} > 5000 mg/kg bw	None	Cosse et al. 1993
OECD 402 – Limit test	LD_{50} > 2000 mg/kg bw	None	Cosse et al. 1993
OECD 403 – Limit test	LC_{50} > 7 mg/L	None	Cieszlak. 1992

4.2.1.1 Acute toxicity: oral

Table 12: Oral acute toxicity study

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}/LC_{50}	Remarks	Reference
Oral	OECD 401	Rat F344 M, F 5/sex/group	5000 mg/kg bw Single oral dose	LD_{50} > 5000 mg/kg bw	None	Cosse et al. 1993

The study LD_{50} > 5000mg/kg bw/day shows that hexaflumuron is not acutely toxic when administered orally to rats.

4.2.1.2 Acute toxicity: inhalation

Table 13: Inhalation acute toxicity study

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}/LC_{50}	Remarks	Reference
Inhalation	OECD 403 EPA 81-3 EEC B2	Rat F344 M, F 5/sex/group	7 mg/l 4-hr exposure	LC_{50} > 7 mg/l	None	Cieszlak. 1992

The study LC_{50} > 7mg/L shows that hexaflumuron is not acutely toxic when administered by inhalation to rats.

4.2.1.3 Acute toxicity: dermal

Table 14: Dermal acute toxicity study

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}/LC_{50}	Remarks	Reference
Dermal	OECD 402 EPA 81-2	Rabbit, NZW M, F 5/sex/group	2000 mg/kg bw 24-hr exposure	LD_{50} > 2000 mg/kg bw	None	Cosse et al. 1993

The study LD_{50} > 2000mg/kg bw shows that hexaflumuron is not acutely toxic when administered dermally to rabbits.

4.2.1.4 Acute toxicity: other relevant information

No data is available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Acute toxicity of hexaflumuron was investigated by oral, dermal and inhalation routes with LD_{50}/LC_{50} results of above 5000 mg/kg bw, 2000 mg/kg bw and 7 mg/L, respectively. Hexaflumuron is not acutely toxic and no specific classification/labelling for acute (systemic) toxicity is required.

4.2.4 Comparison with criteria

A LD50 > 5000 mg/kg bw/day was obtained from Cosse et al. 1993 acute oral study performed according to OECD 401 which stands above the highest cut-off value of 2000 mg/kg bw/day from category 4 of the CLP. Therefore hexaflumuron doesn't warrant classification for this toxicity hazard.

A LC50 > 7 mg/l was obtained from Cieszlak. 1992 acute inhalation study performed according to OECD 403 which stands above the highest cut-off value of 5,0 mg/l from category 4 of the CLP. Therefore hexaflumuron doesn't warrant classification for this toxicity hazard.

A LD50 > 2000 mg/kg bw/day was obtained from Cosse et al. 1993 acute dermal study performed according to OECD 402, which stands above the highest cut-off value of 2000 mg/kg bw/day from category 4 of the CLP. Therefore, hexaflumuron doesn't warrant classification for this toxicity hazard.

4.2.5 Conclusions on classification and labelling

No acute hazard classification according to the CLP is warranted for hexaflumuron.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

The substance was administered in single dose toxicity studies (limit dose) by oral, dermal or inhalation routes which are designed to investigate mortality effects and LD/LC_{50} setting. Notwithstanding, no adverse effects where mentioned that can be relevant to humans i.e. that can impair function, reversible or irreversible, immediate and/or delayed. It is not anticipated that hexaflumuron has specific target organ toxicity, under single-dose exposure.

4.3.2 Comparison with criteria

No single dose toxicity studies either than acute limit tests were submitted to allow the assessment of non-lethal toxic effects.

4.3.3 Conclusions on classification and labelling

No hazard classification is proposed.

4.4 Irritation

4.4.1 Skin irritation

Table 15: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit NZW, male/female OECD 404	Average score 24, 48, 72 h: 0.06	Very slight erythema in one animal at 72 hours post-treatment (0.17); this observation was fully reversible by day 7	Cosse et al. 1993

4.4.1.1 Non-human information

The OECD 404 study performed with rabbit NZW resulted in very slight erythema (average score: 0.06) with desquamation present in one animal at 72 hours. This observation was completely resolved by day 7.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

Hexaflumuron is considered slightly irritating to rabbit skin.

4.4.1.4 Comparison with criteria

As a result of the test performed with hexaflumuron, none of the criteria for skin irritancy classification is meet. None of the animals reached the average cut-off value of 2.3 for erythema/eschar or for oedema or in any case there was inflammation that persisted to the end of the observation period normally 14 days in at least 2 animals. Only one animal showed slight erythema (average score: 0.06) and this was fully reversible at 7 days. Overall, the effects observed in the study are not sufficient to warrant classification as skin irritant.

4.4.1.5 Conclusions on classification and labelling

Hexaflumuron does not warrant hazard classification for skin irritancy.

4.4.2 Eye irritation

Table 16: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit NZW, male/female OECD 405	Average score 24h, 48h, 72h cornea/iris/redness/chemosis: 0	Slight ocular irritation in 4/6 animals at 60 minutes (Conjunctiva: redness: 0.67; chemosis: 0.33) which completely reversible by 24 hrs.	Cosse et al. 1993

4.4.2.1 Non-human information

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation

Ocular irritation was observed in 4 of 6 treated animals. Examination of the conjunctiva post-treatment revealed slight redness and/or chemosis. All signs of ocular irritation subsided and were resolved by 24 hours post-treatment in all animals.

4.4.2.4 Comparison with criteria

In the rabbit OECD 405 study, ocular irritancy was observed in 4/6 animal being scored but in all cases was scored below the CLP cut-off value of 1 (mean scores: conjunctival redness - 0.67; oedema (chemosis) - 0.33, no corneal opacity or iritis); also were fully reversed after 24h. Therefore, none of the CLP criteria for eye irritancy category 2 was met.

4.4.2.5 Conclusions on classification and labelling

Hexaflumuron does not meet the CLP classification criteria for eye irritation.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data on the potential of hexaflumuron to induce respiratory irritation are available.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

4.5.5 Conclusions on classification and labelling

The results of the rabbit OECD 404 guideline study – Acute Dermal Irritation/Corrosion – do not warrant classification for corrosion.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea Pig, Hartley, albino, males Modified Buehler OECD 406	0/10 at 24h; 1/10 at 48h Negative		Cosse et al. 1993

4.6.1.1 Non-human information

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

Hexaflumuron tested negative for dermal sensitization in guinea pigs using the OECD 406 modified Buehler method.

4.6.1.4 Comparison with criteria

4.6.1.5 Conclusions on classification and labelling

The OECD 406 study results using modified Buehler method (Cosse et al. 1993) in Guinea Pigs were 0/10 at 24h and 1/10 at 48h. It was concluded that hexaflumuron tested negative for dermal sensitization and does not meet the CLP classification criteria for this hazard class.

4.6.1.6 Non-human information

No data available.

4.6.1.7 Human information

No data available.

4.6.1.8 Summary and discussion of respiratory sensitisation

4.6.1.9 Comparison with criteria

4.6.1.10 Conclusions on classification and labelling

No data available.

4.7 Repeated dose toxicity

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
28d oral (diet), mouse CD-1 Doses: 0, 25, 125, 750, 1500 mg/kg bw OECD 407 (1981)	LOAEL: 125 mg/kg bw/d NOAEL: 25 mg/kg bw/d	Elevated methaemoglobin levels in both sexes at 125 mg/kg bw and above. Marked extramedullary haematopoiesis in spleen of males at 125 mg/kg bw and above. Slight but statistically significant increase in liver enzymes of males, ALT at 750 mg/kg bw and above and AST at 1500 mg/kg bw. Increased body weight, adjusted spleen weight at the top two doses in males and a slight increase in kidney weight 750 mg/kg bw and above in females. Dark discolouration of tail vein in 6/10 males at 1500 mg/kg bw. RL1	Warren et al. 1986
28-d oral (diet), dog Doses: 0, 25, 125, 750 mg/kg bw OECD 409 (1981)	LOAEL: 25 mg/kg bw/d NOAEL: < 25 mg/kg bw/d	Dose-related increase in methaemoglobin along with decrease in RBC and Hb at all dose levels in both sexes. RL 2 (2 animals/sex instead of 4 according to the guidelines)	Sachsse et al. 1986

21-d dermal, rat Concentration in the vehicle: 50% w/v, at 1000 mg/kg bw Volume: 2ml/kg bw Method used similar to 92/69/EEC; Method B9	LOAEL: >1000 mg/kg bw	No adverse effects were observed RL 2 (treatment duration was 21-d instead of 28-d according to the guidelines)	Perry and Duffen, 1987
90-d oral (diet), rat Doses: 0, 25, 125, 750, 1500 mg/kg bw Comparable to OECD 408, EC B.26	LOAEL: 125 mg/kg bw/d NOAEL: 25 mg/kg bw/d	Methaemoglobin increase in males, slight evidence of haemosiderin deposits in females. The effects were treatment-related and similar in other species treated with the same type of molecules (acyl urea insecticides) RL 1	Everett et al. 1986
90-d oral (diet), mouse CD-1 Doses: 0, 5, 25, 250 mg/kg bw Designed in accordance with OECD 'Short-Term and Long-Term Toxicology Group Guidelines' (1981)	LOAEL: 25 mg/kg bw/d NOAEL: 5 mg/kg bw/d	Dose–related increase in methemoglobin at 25 mg/kg and above, in females. Increase in bilirubin, GPT, GOT at 250 mg/kg; dose-related minimal hepatocyte enlargement in males. High-dose males had prominent mitotic figures in the liver. RL 1	Dean et al. 1987
52-wk oral (diet), rat Doses: 0, 5, 75, 500 mg/kg bw US EPA 83-5	LOAEL: 500 mg/kg bw/d NOAEL: 75 mg/kg bw/d	Adaptive response to methaeglobinemia seen at the top dose with increase in reticulocytes observed in the high dose females High dose group also showed functional liver changes as indicated by elevated Alkaline phosphatase in males and cholesterol in females. Meth-Hb concentrations showed large variations, possibly indicating deficiencies in the sampling time RL 2 (low number of animals)	Everett et al. 1987
52-wk oral (diet), dog Doses: 0, 0.5, 2, 5, 25 mg/kg bw OECD 452 (1981)	LOAEL: 2 mg/kg bw/d (increase in methemoglobin and associated increase in hepatic hemosiderin deposits) NOAEL: 5 mg/kg bw/d	Dose-related increase in methemoglobin at 2 mg/kg bw and above; reversal of this trend by 52 weeks. Hemosiderin deposits were seen at 2 mg/kg and above. RL1	Allen et al. 1988

4.7.1.1 Repeated dose toxicity: oral

Short and medium term oral feed studies were conducted in rats, mice, and dogs.

Warren et al., 1986, mouse CD-1, 28 days

Hexaflumuron was administrated through the diet to five groups (4 test and 1 control), 20 animals per treatment group (10/sex) of mouse CD-1, at concentrations calculated to give dose levels of 0, 25, 125, 750, 1500 mg/kg bw, for 28 days. Treatment-related changes in clinical chemistry were observed: increase of liver enzymes in males (ALT at 750mg/kg bw and 1500mg/kg bw and AST at 1500 mg/kg bw) and statistically significant increase in serum cholesterol in females at 750mg/kg bw and 1500mg/kg bw/d. Histopathology revealed a statistically significant increase of spleen weight at the two top doses in males and in kidney weight at the top dose in females. Additionally, there was a marked treatment-related extramedullary haematopoiesis in the spleen of males at the top three dose levels. The NOAEL was found at 25 mg/kg bw/d.

Table 19: Clinical chemistry/ haematology results (Warren et al, 1986)

Parameter	0		25		125		750		1500		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f	m	f
Number of animals examined	10	10	10	10	10	10	10	10	10	10	---	---
PVC (%)	46	45	47	(47)	48	45	45	47	45	45	---	---
Hb (g/dL)	14.3	14.4	15.1	(14.8)	14.7	13.7	13.8	14.1	13.9	13.5	---	---
RBC (x 10^6/mm^3)	8.3	8.4	8.4	(8.6)	8.2	7.8	8.1	7.8	8.3	7.7*	---	---
Plts (x 10^3/mm^3)	965	830	942	(740)	923	923	1170*	1143**	1340**	1140**	↑	↑
MetHb (% Hb)	2.37	2.63	2.79	2.57	3.61+	3.51**	3.47+	3.60**	3.56++	3.54**	↑	↑
Glucose (mg/dL)	173	175	234*	195	221*	174	246**	182	249**	205*	--	---
ALT (mU/ml)	24	21	25	17	24	24	109**	42	127**	39	↑	---
AST (mU/ml)	30	35	30	28	27	35	41	39	52**	39	↑	---
Cholesterol (mg/dL)	146	82	119	89	141	103	136	125**	160	143**	---	↑

Values in parentheses are the mean of only 2 individuals
Level of significance (Williams' test): * 0.05 > P > 0.01 in comparison with control values
** 0.1 > P > 0.001 in comparison with control values
Level of significance (distribution-free Williams test): + 0.05 > P > 0.01 in comparison with control values
++ 0.01 > P > 0.001 in comparison with control values

Table 20: Body weight, organ weight and gross pathology results (Warren et al, 1986)

Parameter	0		25		125		750		1500		Dose-response +/-	
	m	f	m	f	m	f	m	f	m[a]	f[a]	m	f
Number of animals examined	10	10	10	10	10	10	10	10	10	10	---	---
Body weight at 4 weeks (g)	37	26	35	27	37	27	37	29	38	29	---	---
Spleen												
Organ weight (g ± SD)	0.133 ± 0.022	0.188 ± 0.033	0.162 ± 0.035	0.195 ± 0.030	0.159 ± 0.022	0.209 ± 0.050	0.182** ± 0.044	0.215 ± 0.041	0.190** ± 0.043	0.222 ± 0.040	↑	---
Gross pathology	---	---	---	---	---	---	---	---	---	---		
Occasional siderocytes (no. animals)	4	6	0	0	3	0	4	0	3	7	↑	---
Minimal numbers of siderocytes (no. animals)	0	1	0	0	0	0	0	0	4	1	---	---
Minimal extramedullary haemopoiesis (no. animals)	1	0	0	0	2	0	0	0	0	0	---	---
Moderate extramedullary haemopoiesis (no. animals)	9	6	0	0	7	0	6	0	5	7	---	---
Marked extramedullary haemopoiesis (no. animals)	0	4	0	0	1	0	4	0	5	3	↑	---
Kidney												
Organ weight (g ± SD)	0.64 ± 0.067	0.44 ± 0.040	0.66 ± 0.072	0.50 ± 0.044	0.69 ± 0.096	0.48 ± 0.032	0.73 ± 0.095	0.47 ± 0.072	0.67 ± 0.079	0.49* ± 0.040	---	↑
Gross pathology (no. animals)	0	0	0	0	0	1	0	0	0	0	---	---
Several cortical cysts (no. animals)	0	0	0	0	0	1	0	0	0	0	---	---

Level of significance (Williams' test): * 0.05 > P > 0.01 in comparison with control values
** 0.1 > P > 0.001 in comparison with control values

Dean et al., 1987, mouse CD-1, 13 weeks

Hexaflumuron was administered through the diet to mouse CD-1 in 4 test groups (3 test and 1 control) with 20 animals/group (10/sex), at concentrations calculated to give dose levels of 0, 5, 25, 250 mg/kg bw, during 13 weeks. Several haematological parameters were evaluated: packed volume cell, hemaglobin, red cell count, total white cell count, platelet count, differential white blood cell counts, cell morphology, Heinz bodies, reticulocyte count, MetHb. Also, several clinical chemistry parameters were evaluated (total protein, albumin, globulin, urea nitrogen, cholesterol, glucose, alkaline phosphatase, ALT, AST, bilirubin, creatinine) and urinalysis was performed (with bile pigments, urobilinogen, haempigments. There were no observable clinical effects and the chemistry haematology results only showed increase in MetHb in females, at the two top dose (2nd day sampling), but not in males. Bilirubin, GPT and GOT were all increased at the top dose, although the effect on GOT was not statistically significant in males. There were no macroscopic pathological effects of treatment and no treatment-related organ weight differences. Male mice showed minimal hepatocyte enlargement in 8/10 cases at 250mg/kg bw, 6/10 at 25 mg/kg bw, and 3/10 at 5 mg/kg bw with 2/10 from controls. The NOAEL set for this finding was 5 mg/kg bw. Top dose males showed prominent mitotic figures in the liver. There were no treatment-related histopathological effects in females.

Table 21: Chemistry haematology and histopathology results (Dean et al, 1987)

Dose Level (mg/kg bw/day)	0		5		25		250	
Sex	M	F	M	F	M	F	M	F
Methaemoglobin, (% Hb) day 1 sampling	2.59	1.59	2.28	1.88	2.47	2.15	1.82	2.81**
Methaemoglobin (% Hb), day 2 sampling	2.01	0.88	1.66	0.84	1.43	1.84**	2.03	2.66**
GPT (mU/ml)[a]	46	23	44	23	37	29	147**	50**
GOT (mU/ml)	50	43	49	40	45	45	74	63**
Bilirubin (mg/dl)[b]	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.2*	0.2**
Liver								
Minimal centrilobular enlargement	2	0	3	0	6	0	3	0
Minimal enlargement of hepatocytes	0	0	0	0	0	0	5	0
Prominent mitotic figures	0	0	0	0	0	0	4	0
A few granulomata	1	0	0	0	0	0	0	0
An area of necrosis	1	0	0	0	0	0	0	0
Several granulomata	0	0	1	0	0	1	1	0
Minimal centrilobular vacuolation	0	0	2	0	1	0	0	1
Foci of necrotic liver cells	0	0	0	0	0	1	0	0
Focus/foci of inflammatory cells	6	2	3	1	1	3	3	5

* P<0.05

** P<0.01

[a]Data log-transformed prior to analysis

[b]Analyses performed using Fischer's exact test followed by Mantel's test for trend in proportions

Everett et al., 1986, rats Sprague-Dawley, 13 weeks

Hexaflumuron was administrated through the diet to Sprague-Dawley rats in five groups (4 tests and 1 control) of 10/sex/group, at concentrations calculated to give dose levels of 0, 25, 125, 750, and 1500 mg/kg bw/day, during 13 weeks.

Several haematological parameters were evaluated: haemoglobin, total red blood cell count, total white blood cell count, differential white cell count, reticulocytes, packed cell volume, methaemoglobin, hepatoquick test, Heinz bodies and also clinical chemistry parameters (blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, phosphorous, total bilirubin). No urinalysis was performed. The only apparent treatment-related effect on haematological parameters was a slight, but statistically significant increase in methaemoglobin at 125 mg/kg and above in males, and at 750 mg/kg and above in females. Organ weights, gross pathology and conventional histopathology showed no treatement-related effects. Prussian Blue stained spleen sections showed a slight increase in haemosiderin deposits (trend significant at $P < 0.05$) in females at the two highest dose levels. There were no differences for either of these findings between the lowest dose group (25 mg/kg) and controls therefore the NOAEL was set at this dose level.

Table 22: Treatment-related changes in haematology and haemosiderin deposits (Everett et al, 1986)

Dose Level (mg/kg bw/day)	0		25		125		750		1500	
Sex	M	F	M	F	M	F	M	F	M	F
Methaemoglobin	0.705[a] (0.141)	0.881 (0.249)	0.767 (0.263)	1.030 (0.244)	1.053 (0.216) **	1.005 (0.376)	1.113 (0.213) **	1.358 (0.252) *	1.008 (0.189) *	1.328 (0.310) *
Hemosiderin deposition										
Grade 1	1	0	1	1	0	1	0	0	0	0
Grade 2	8	2	5	0	5	1	4	1	3	0
Grade 3	0	6	4	7	5	4	6	2	5	1
Grade 4	1	2	0	2	0	4	0	7	2	8
Grade 5	0	0	0	0	0	0	0	0	0	1

[a]values represent mean ± standard deviation
[b]values represent number of animals with that particular grade of hemosiderin deposition
* P<0.01
** P<0.001

Everett et al., 1987, rats Sprague-Dawley, 52 weeks (part of the 2 year carcinogenicity study; dose levels were selected from Everett et al, 1986 where the NOAEL was set at 25 mg/kg bw/d)

Hexaflumuron was administrated through the diet to Sprague-Dawley rats in four groups (3 test and 1 control), in 10/sex/group, at concentrations calculated to give dose levels of 0, 5, 75 and 500 mg/kg bw/day, during 52 weeks.
The following parameters were measured on plasma from whole blood: blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, sodium, potassium, calcium, chloride, total protein, albumin, alkaline phosphatase, albumin-globulin ratio, cholesterol, creatinine, inorganic phosphorous, total bilirubin, direct bilirubin, creatinine phosphokinase. Urinalysis was performed for the following parameters: volume, pH, specific gravity, protein, glucose, ketones, blood, bilirubin, urobilinogen, microscopic examination of the spun deposit.
During weeks 25 and 51 of dosing, blood and urine samples were taken from 10 males and 10 females from each group for purposes of hematological and clinical chemistry determinations (including urinalysis). Histopathological examinations and organ weight measurements were conducted on all rats.
There were no discernible signs of toxicity during the in-life phase of the study. Increases in methaemoglobin levels seen in the 90-day study were not present after 26 or 51 weeks of dosing, nor was increased splenic haemosiderin deposition evident with increasing dose level after 52 weeks of dosing. Indicators of compensatory adaptive responses to increased methaeglobinemia levels were observed such as changes in the differential cell count indicative of anaemia and increase in reticulocytes in the high dose females. MethHb concentrations showed large variations, possibly indicating that the sampling time was not the most appropriate.

Table 23: Chemistry haematological results (Everett et al, 1987)

Measurement	MCHC		Reticulocytes		Methaemoglobin	
Sex	M	F	M	F	M	F
Week 26						
0 mg/kg	35.9 (0.5)	35.6 (0.3)	2.4 (1.0)	1.5 (0.2)	0.53 (0.30)	0.43 (0.11)
5 mg/kg	35.9 (0.5)	36.1* (0.4)	1.9 (0.7)	1.4 (0.3)	0.62 (0.32)	0.77 (0.63)
75 mg/kg	35.5 (0.7)	36.1* (0.4)	2.3 (0.6)	1.7 (0.4)	0.43 (0.18)	0.47 (0.35)
500 mg/kg	35.3 (0.7)	35.5 (0.7)	2.4 (0.5)	2.0* (0.6)	0.49 (0.22)	0.52 (0.33)
Weeks 51						
0 mg/kg	37.3 (0.7)	37.0 (0.6)	2.1 (0.5)	2.1 (0.5)	0.79 (0.64)	0.69 (0.21)
5 mg/kg	36.6** (0.5)	36.6 (0.6)	1.9 (0.5)	2.2 (0.4)	0.66 (0.25)	0.65 (0.24)
75 mg/kg	36.6** (0.3)	36.6 (0.6)	2.5 (1.2)	2.0 (0.6)	0.71 (0.14)	0.48* (0.22)
500 mg/kg	36.7* (0.5)	36.4 (0.8)	1.8 (0.4)	2.4 (0.7)	0.53 (0.21)	0.94* (0.22)

*Statistically significant (P<0.05)

**Statistically significant (P<0.01)

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).

Table 24: Clinical chemistry results (Everett et al, 1987)

Measurement	Glucose		Alkaline phosphatase		Sodium		Cholesterol		ALT		BUN	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Week 26												
0 mg/kg	8.85 (1.03)	7.24 (0.83)	284 (87)	209 (65)	153 (3)	142 (1)	2.4 (0.7)	2.1 (0.4)	51 (9)	60 (16)	5.5 (0.3)	6.2 (0.9)
5 mg/kg	8.72 (1.31)	7.76 (1.42)	271 (62)	172 (54)	149 (2)	142 (2)	2.5 (0.9)	2.1 (0.4)	50 (10)	70 (35)	5.6 (0.7)	5.9 (0.9)
75 mg/kg	8.30 (0.96)	7.57 (0.92)	281 (87)	164 (51)	154 (5)	141 (2)	2.6 (0.9)	2.1 (0.3)	57 (20)	44* (7)	6.0 (0.5)	5.7 (1.1)
500 mg/kg	7.53** (1.02)	7.27 (0.53)	321 (33)	182 (45)	153 (7)	142 (2)	2.5 (0.5)	2.5* (0.3)	63 (16)	58 (21)	5.9 (0.8)	5.8 (0.6)
Weeks 51												
0 mg/kg	9.65 (1.04)	8.42 (0.68)	213 (64)	149 (43)	150 (2)	142 (2)	2.4 (0.4)	2.3 (0.4)	65 (19)	62 (13)	4.6 (0.5)	5.3 (0.6)
5 mg/kg	8.62* (0.41)	8.69 (1.30)	230 (65)	139 (68)	147** (2)	143 (2)	2.6 (0.7)	2.5 (0.5)	66 (18)	73 (25)	4.8 (0.5)	6.4** (0.7)
75 mg/kg	8.80* (0.74)	8.24 (0.51)	242 (69)	138 (61)	148* (2)	143 (2)	2.9 (0.7)	2.5 (0.4)	133 (112)	65 (22)	4.8 (0.4)	5.4 (0.6)
500 mg/kg	8.87* (0.87)	8.40 (0.78)	299** (60)	158 (33)	148 (4)	143 (1)	2.8 (0.6)	3.1** (0.7)	96 (48)	61 (13)	4.7 (0.5)	5.5 (1.0)

*Statistically significant (P<0.05)

**Statistically significant (P<0.01)

In the microscopic findings, in females, there was a statistically significant increase in the incidence of absence of ovarian corpora lutea in the low dose group (5/10, $P < 0.05$) compared to controls (0/10). The intermediate and high dose group also showed an increased incidence (3/10 of both groups) but did not achieve statistical significance. There were also dose-related decrease in the incidence of kidney cortical mineral deposits which was statistical significant ($P < 0.05$) in the high

dose (0/10). The intermediate and low dose groups also showed an incidence of 2/10 each, compared to the controls (5/10).

Table 25: Organ weights and pathological findings (Everett et al, 1987)

Measurement	Abs. kidney wt (g)		Rel. kidney wt (g)		Kidneys, cortical mineral deposits		Ovaries, corpora lutea absent	
Sex	M	F	M	F	M	F	M	F
0 mg/kg	3.69 (0.39)	2.39 (0.25)	6.07 (0.48)	6.69 (0.58)	0	5	---	0
5 mg/kg	3.91 (0.69)	2.38 (0.29)	6.24 (0.81)	6.90 (0.77)	0	2	---	5*
75 mg/kg	3.70 (0.42)	2.44 (0.26)	5.96 (0.52)	6.78 (0.55)	0	2	---	3
500 mg/kg	3.56 (0.40)	2.72 (0.46)	5.43 (0.65)	7.10 (0.96)	0	0*	---	3

*Statistically significant (P<0.05)

In view of the adaptive response to methaeglobinemia seen at the top dose with increase in reticulocytes observed in the high dose females and functional liver changes indicated by elevated Alkaline phosphatase in males and cholesterol in females top dose, the following threshold values were set: LO(A)EL: 500 mg/kg bw/d and NOAEL: 75 mg/kg bw/d.

Sachsse et al., 1986, dog, Beagle, 28 days

Hexaflumuron was administrated via diet in Beagle dogs, 4 animals (2/sex) per treatment group; 4 groups (3 test and 1 control), at nominal concentrations which varied between 440-540 ppm, 1950-2580 ppm and 12000-16000 ppm and were calculated to give dose levels, respectively, of 0, 25, 125 or 750 mg/kg bw/day, during 28 days. There were no effects on body weight and all dose groups appeared clinically normal throughout the study.
The assessment of the hematology data indicated a moderate to marked increase in the Heinz body count at 125 and 750 mg/kg bw/day (dose-related), a slightly increased mean corpuscular volume (MCV) for the male dogs at 25 mg/kg bw/day and for both sexes, a slightly increased mean corpuscular haemoglobin concentration (MCHC) for both sexes at 125 and 750 mg/kg bw/day and a slightly decreased erythrocyte count, haemoglobin concentration and haematocrit value in male dogs receiving 25 mg/kg bw/day and in both sexes receiving 125 and 750 mg/kg bw/day.
Additionally, there was slight to marked methaemoglobin formation for both sexes receiving 25, 125 and 750 mg/kg bw/day (dose-related) and there were slight changes in the red cell morphology as indicated by anisocytosis for the female dogs at 125 and 750 mg/kg bw/day, the presence of target cells and hypochromia for the male dogs at 25 mg/kg bw/day and for both sexes at 125 and 750 mg/kg bw/day, as well as the presence of Howell-Jolly bodies for the male dogs at 125 and 750 mg/kg bw/day. All the other changes in the results of the hematology, clinical biochemistry and urinalysis parameters were considered to be incidental and of normal biological variation.

There was a slight increase in spleen weights in top dose dogs of both sexes and signs of congestion and increased erythropoiesis in the top two doses dogs of both sexes were showed in the spleen.

There were no other treatment related effects identified by histopathology. It was concluded that a dose of 25 mg/kg bw/d was the LOAEL (NOAEL < 25 mg/kg bw/d).

Table 26: Haematology results (Sachsse et al, 1986)

Parameter	0		25		125		750		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	2	2	2	2	2	2	2	2	---	---
Hb (mmol/L)	8.0	9.4	7.4	8.3	7.4	7.4	7.9	7.3	---	---
RBC (T/L)	6.3	6.9	4.9	5.9	4.9	5.1	5.5	5.0	---	---
HCT (L/L)	0.35	0.42	0.34	0.37	0.34	0.35	0.37	0.33	---	---
MCV (fL)	55.5	60.5	68.0	63.5	68.5	69.5	67.5	67.0	---	---
MCH (fmol)	1.27	1.37	1.51	1.42	1.49	1.46	1.44	1.46	---	---
MCHC (mmol/L)	22.9	22.7	22.1	22.5	21.8	21.0	21.3	21.9	---	---
Plts (g/L)	374	390	464	426	515	571	493	566	---	---
Heinz body (% of RBC)	0	0	0	0	9.2	4.4	11.0	17.4	↑	↑
MetHb (% Hb)	0.8	0.5	3.2	2.1	6.4	4.4	6.4	7.7	↑	↑
Anisocytosis (0-3)	0	0	0	0	0	1	0	1	---	---
Target cells (0-3)	0	0	1	0	1	1	1	1	---	---
Hypochromia (0-3)	0	0	1	0	1	1	0	1	---	---
Howell-Jolly bodies (0-3)	0	0	0	0	1	0	1	0	---	---

Table 27: 28 days study results (Sachsse et al, 1986)

Parameter	0		25		125		750		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	2	2	2	2	2	2	2	2	---	---
Body weight at 4 weeks (kg)	6.85	6.04	6.32	5.77	6.12	5.07	6.21	5.64		
Body weight gain at 4 weeks (%)	16.9	22.7	18.8	30.3	22.4	32.8	18.0	30.8		
Spleen										
Organ weight (g ± SD)	18.65	17.22	15.12	14.69	17.33	17.30	23.84	19.13		
Congestion (No. of animals)	1	0	0	1	2	2	2	2		
Haemopoiesis (No. of animals)	0	2	1	1	2	2	2	2		

Allen et al., 1988, Beagle dogs, 52 weeks

Hexaflumuron was tested for chronic toxicity in Beagle dogs, via diet, in 5 groups (4 test and 1 control), 4/sex/group, at nominal concentrations which varied between Group 2 (11-19 ppm); Group 3 (47-80 ppm); Group 4 (115-178 ppm); Group 5 (560-877 ppm) calculated to give dose levels, respectively, of 0.5, 2, 5 or 25 mg/kg bw/day.

Clinical biochemistry, haematology or urinalysis were performed at 4, 13, 26 or 52 weeks. Haematological effects consisted of a dose-related increase in methaemoglobin at 2, 5 or 25 mg/kg bw/day and in Heinz bodies at 5 or 25 mg/kg bw/day. These effects were maximal at 13 and 26 weeks having decreased by 52 weeks for both sexes. MetHb reached about 7% in both sexes at 26 weeks. At 52 weeks, animals receiving 2 mg/kg bw/d had returned to normal while those at 5 or 25 mg/kg bw/d, although still high, were reduced when compared to 26 week values.

Other hematological treatment-related effects were a slight decrease in the mean corpuscular haemaglobin concentration (MCHC) index for the highest dose level group (25 mg/kg bw/d), at 4, 26 and 52 weeks and a slight increase in the platelet and reticulocyte count for females, at this dose level, at 4 weeks. Also noted was a slight increase in the Howell-jolly bodies for both sexes in the highest dose group, at 26 and 52 weeks. These modulation changes in the erythrocyte parameters revealed a compensatory hematopoiesis process.

Table 28: Haematological findings (Allen et al, 1988)

Measurement	MCHC (mmol/l)		Heinz bodies		Met-HB (%)		H-J Bodies (score 0/3)		Platelets (G/l)		Reticulocytes	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Pretest												
0 mg/kg	22.5	22.7	0	0	0.7	0.9	0	0	397	404	0.005	0.003
0.5 mg/kg	22.5	23.0	0	0	0.8	0.7	0	0	393	351	0.006	0.003
2.0 mg/kg	22.6	22.9	0	0	0.7	1.0	0	0	393	386	0.004	0.004
5.0 mg/kg	23.1	22.8	0	0	1.1	1.0	0	0	415	425	0.004	0.005
25.0 mg/kg	22.5	22.8	0	0	0.9	0.8	0	0	386	370	0.005	0.004
4 Weeks												
0 mg/kg	21.4	20.8	0	0	0.9	1.2	0	0	329	371	0.004	0.003
0.5 mg/kg	21.2	21.0	0	0	1.2	1.2	0	0	366	363	0.005	0.003
2.0 mg/kg	21.1	21.2	0	0	1.8	1.7	0	0	396	413	0.005	0.003
5.0 mg/kg	20.5*	20.5	3	9	3.0*	4.2*	0	0	435	480	0.004	0.004
25.0 mg/kg	20.2*	20.0*	26*	44*	6.1*	6.5*	0	0	477	572*	0.008	0.013*
13 Weeks												
0 mg/kg	20.5	20.3	0	0	0.7	0.9	0	0	340	390	0.006	0.002
0.5 mg/kg	20.4	20.4	0	0	0.9	1.2	0	0	362	348	0.006	0.004
2.0 mg/kg	20.6	20.4	0	0	1.6	1.8*	0	0	363	357	0.004	0.006*
5.0 mg/kg	20.4	20.4	24	32	2.6*	3.5*	0	0	383	417	0.005	0.004
25.0 mg/kg	20.2	20.1	161*	213*	5.9*	6.3*	0	0	417	483	0.009	0.007*
26 Weeks												
0 mg/kg	20.6	20.2	0	0	0.7	1.0	0	0	342	407	0.008	0.004
0.5 mg/kg	20.6	20.3	0	0	0.9	1.3	0	0	358	403	0.007	0.005
2.0 mg/kg	20.4	20.5	0	2	1.7	1.9	0	0	348	366	0.009	0.006
5.0 mg/kg	20.1	20.3	27	37	2.9*	3.3*	0	0	375	437	0.007	0.006
25.0 mg/kg	19.8*	19.5*	470*	409*	6.7*	7.2*	1	1	439	481	0.008	0.007
52 Weeks												
0 mg/kg	21.6	21.1	0	0	0.2	0.4	0	0	333	410	0.007	0.006
0.5 mg/kg	21.6	21.2	0	0	0.5	0.5	0	0	357	358	0.008	0.004
2.0 mg/kg	21.2	21.2	0	0	0.8	1.1	0	0	317	352	0.010	0.006
5.0 mg/kg	20.6	20.8	8	10	1.7*	1.7	0	0	376	364	0.008	0.005
25.0 mg/kg	20.1*	20.4*	110*	190	4.5*	5.0*	1	1	445	419	0.010	0.008

*Statistically significant ($P<0.05$)

There were no treatment-related effects at the lowest dose tested therefore 0.5 mg/kg bw/d is the NOAEL set for hexaflumuron in dogs when exposed for 1 year.

There were no treatment related effects on organ weights or macroscopic pathology.
Microscopic pathology: Liver: microscopic examination of the liver revealed treatment-related hemosiderin deposits, located mainly in small aggregates of proliferated Kupffer cells, were diagnosed in one female of group 1, one male and two females of group 2, two males and two females of group 3, one male and four females of group 4, and four males and four females of group 5. In males, the mean severity grades of the hemosiderin deposits in affected animals were 1.0 in group 2 and 2.0 in groups 3, 4, and 5. In females, the mean severity grades of the hemosiderin deposits in affected animals were 1 in groups 1 and 2, 2.5 in group 3, 2.0 in group 4, and 2.5 in group 5.

The LOAEL was set at 2.0 mg/kg bw/day based on these findings (statistically significant increase in methaemoglobin (1.8%) in females and concurrent increase in hepatic haemosiderin deposits).

Table 29: Haemosiderin deposits in liver (Sachsse et al, 1986)

Dose level (mg/kg bw/day)	0		0.5		2		5		25	
Sex	M	F	M	F	M	F	M	F	M	F
MetHb (%)										
4 weeks	0.9	1.2	1.2	1.2	1.8	1.7	3.0*	4.2*	6.1*	6.5*
13 weeks	0.7	0.9	0.9	1.2	1.6	1.8*	2.6*	3.5*	5.9*	6.3*
26 weeks	0.7	1.0	0.9	1.3	1.7	1.9	2.9*	3.3*	6.7*	7.2*
52 weeks	0.2	0.4	0.5	0.5	0.8	1.1	1.7*	1.7	4.5*	5.0*
Haemosiderin deposition, liver, (mean severity grades)										
Grade 1	0	1	1	2	0	0	0	0	0	0
Grade 2	0	0	0	0	2	2 (2.5)	1	3	4	4 (2.5)
Grade 3	0	0	0	0	0	0	0	0	0	0
Grade 4	0	0	0	0	0	0	0	0	0	0
Grade 5	0	0	0	0	0	0	0	0	0	0

*Statistically significant (P<0.05)

4.7.1.2 Repeated dose toxicity: inhalation

No data available

4.7.1.3 Repeated dose toxicity: dermal

Repeated dose toxicity by dermal route was investigated in rats (Perry and Duffen, 1987) by using a method similar to 92/69/EEC; Method B9. This study was scored with RL2 due to the duration of 21 days instead of 28 days according to the guidelines. A rat dermal LOAEL > 1000 mg/kg bw was determined and no adverse effects were observed.

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

No data available

4.7.1.7 Summary and discussion of repeated dose toxicity

Hexaflumuron was evaluated in six repeated-dose toxicity studies by 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. Consistently, the major finding in all studies was the increased methaemoglobin (MetHb) which was often associated with increased spleen weights and Heinz body formations, mostly observed at the higher doses. Dogs seem to be the most sensitive species to hexaflumuron followed by mice and rats (dog>mouse>rat). This is in agreement with the conclusions of the RIVM report (Nº 601516007) and the JRC report (ECBI/07/03 Add.11) in which rats and mice were less sensitive to MetHb formation and generally more effective at reduction of induced MetHb than humans, dogs or cats. The lowest LOAEL value of 25 mg/kg bw is taken from the Dean et al. 1987 study based on increased methaemoglobin concentrations seen at this dose level and above, only in one sex. However this increase in MetHb was not accompanied by a significant reduction in the Hb % (in all cases was <10%).

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to CLP

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to CLP

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

As described above, the toxicological critical effects of hexaflumuron show consistently a disturbance of hematological parameters with possibility of inducing at some degree haemolytic anaemia at doses considered relevant to humans, under repeated exposure i.e. 25 mg/kg bw/d in mice, oral 90-d, and 2 mg/kg bw/d in dogs, oral 52-w. However the relevancy of these findings have to be weighted and compared with the CLP classification criteria before classification can be established.

There are two guidance documents helpful in this comparison:

- Guidance on the Application of Regulation (EC) No 1272/2008 and
- Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective by EU Working Group on Haemolytic Anaemia (Muller et al, 2006).

In order to be classified according to Regulation (EC) No 1272/2008 a substance should cause any consistent and significant adverse changes in haematology (3.9.2.7.3. c). According to Guidance on the Application of Regulation (EC) No 1272/2008, a classification is warranted, if a haemolytic substance induces one or more of the serious health effects listed below as examples within the critical range of doses: either below 10mg/kg bw/d for Category 1 or in a range between 10 and 100mg/kg bw/d for Category 2.

Examples of effects fulfilling classification criteria for substance inducing haemolytic anaemia according to Guidance on the Application of Regulation (EC) No 1272/2008
1. Premature deaths in anaemic animals that are not limited to the first three days of treatment in the repeated dose study. (Mortality during days 0–3 may be relevant for acute toxicity.)
2. Clinical signs of hypoxia, e.g. cyanosis, dyspnoea, pallor in anaemic animals that are not limited to the first three days of treatment in the repeated dose study.
3. Reduction in Hb at ≥20%.
4. Reduction in functional Hb at ≥20% due to a combination of Hb reduction and MetHb increase.
5. Haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).
6. Multifocal or diffuse fibrosis in the spleen, liver or kidney.
7. Tubular nephrosis, severe fatty change in the liver
8. Haemosiderinuria supported by relevant histopathological findings in the kidney in combination with other changes indicating significant haemolytic anaemia (e.g. reduction in Hb at ≥10%)
9. Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%) in a 28 day study.
10. Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis.

The guidance developed for classification of substances inducing haemolytic anaemia within the DSD framework is based on the publication of Muller et al. (2006) entitled: " Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regulatory Toxicology and Pharmacology, 2006, 54, 3, pp 229-241. The criteria in DSD are similar to CLP Regulation, however the major criterion for haemolytic anaemia has changed from "Any consistent changes in haematology, which indicate severe organ dysfunction" in DSD to "Any consistent and significant adverse changes in haematology" in CLP. This indicates that less adverse effects are considered for classification according to CLP. The interpretation for classification requires an assessment of all individual hematological effects as well as totality of findings, to judge whether they constitute an adaptive response or an adverse toxicologically significant effect. It should be noted that as defined in point 3.9.2.8.1. of Annex I of the Regulation (EC) No 1272/2008 there are some insignificant hematological effects in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

- small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;

The following example of such effects not warranting classification is listed:

- Significant decrease in Hb without any other significant indicators of haemolytic anaemia.

Repeated-dose toxicity study findings relevant for classification as STOT RE

Warren et al., 1986, mouse CD-1, 28 days

Treatment-related effects: elevated methaemoglobin levels were seen in both sexes at 125 mg/kg bw and above. Marked extramedullary haematopoiesis in spleen of males at 125 mg/kg bw and above. Slight but statistically significant increase in liver enzymes of males, ALT at 750 mg/kg bw and above and AST at 1500 mg/kg bw. Increased spleen weight at the top two doses in males and a slight increase in kidney weight at 750 mg/kg bw and above in females. LOAEL was set 125 mg/kg bw/d based the above mentioned adverse effects and NOAEL: 25 mg/kg bw/d.

Conclusion: The haematological effects were seen at doses above the cut-off value of 100 mg/kg bw/d necessary for STOT RE category 2 classification according to CLP criteria.

Sachsse et al., 1986, dog, Beagle, 28 days

Treatment-related effects: increase in MetHb with decrease in RBC and Hb starting at the first dose tested (25mg/kg bw/d), in both sexes, along with a moderate to marked increase in the Heinz body count at the two top doses. Also morphological changes in RBC were seen as indicated by anisocytosis in females at 125 and 750 mg/kg bw/day, presence of target cells and hypochromia in males at 25 mg/kg bw/day and for both sexes at 125 and 750 mg/kg bw/day, as well as the presence of Howell-Jolly bodies in males at 125 and 750 mg/kg bw/day. There was an increase in spleen weights in top dose of both sexes and signs of congestion and increased erythropoiesis in spleen in the top two doses dogs of both sexes. It was concluded that a dose of 25 mg/kg bw/d was the LOAEL (NOAEL < 25 mg/kg bw/d). Hexaflumuron did produce a consistent pattern of adversity towards the haematologic system however it this study only used 2 animals instead of 4 as prescribed in the OECD 409 guidelines.
Conclusion: The observed changes in the blood parameters and ancillary organs are not considered sufficiently relevant to justify CLP classification as STOT RE for agents inducing haemolytic anaemia.

Sub-chronic studies

Dean et al., 1987, mouse CD-1, 13 weeks

Dose–related increase in methaemoglobin at 25 mg/kg and above only seen in females but not accompanied by a significant decrease in Hb%. Increase in bilirubin, GPT, GOT at 250 mg/kg were observed in both sexes and high-dose males had prominent mitotic figures in the liver. Hexaflumuron did not produce any consistent nor significant adverse changes in haematology. LOAEL was set at 25mg/kg bw/d based on increase MetHb seen in female mices.
Conclusion: the dose-related increase in MetHb was not accompanied by a reduction of the Hb≥ 10% therefore this CLP criteria for STOT RE classification as agents inducing haemolytic anaemia is not met.

Everett et al., 1986, rats Sprague-Dawley, 13 weeks
Treatment-related effects: slight but statistically significant increase in methaemoglobin at 125 mg/kg and above, in males, and at 750 mg/kg and above in females. Prussian Blue stained spleen sections showed a slight increase in haemosiderin deposits in females at the two highest dose levels however these findings were not confirmed by histopathology. Hexaflumuron did not produce consistent nor significant adverse changes in haematology. LOAEL was set at 125mg/kg bw based on increase MetHb seen in male rats. NOAEL was set at 25mg/kg bw/d.
Conclusion: the haematological effects were seen at doses above the cut-off value of 100 mg/kg bw/d necessary for STOT RE category 2 classification according to CLP criteria.

Chronic studies

Everett et al., 1987, rats Sprague-Dawley, 52 weeks (part of the 2 year carcinogenicity study; dose levels were selected from Everett et al, 1986 where the NOAEL was set at 25 mg/kg bw/d)

Treatment-related effects: adaptive response to methaeglobinemia seen at the top dose with increase in reticulocytes observed in the high dose females. High dose group also showed functional liver changes as indicated by elevated alkaline phosphatase in males and cholesterol in females. MethHb

concentrations showed large variations possibly indicating deficiencies in the sampling time. Conclusion: the observed effects are not sufficiently relevant for CLP classification for STOT RE as agents inducing haemolytic anaemia.

Allen et al., 1988, Beagle dogs, 52 weeks

Treatment-related effects: increase in methaemoglobin at all dose levels starting at 2 mg/kg bw/d and in Heinz bodies at 5 and 25 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 both sexes at 26 weeks. At 52 weeks, animals receiving 2 mg/kg bw/d returned to normal while those at 5 or 25 mg/kg bw/d were reduced when compared to 26 week values. Other hematological treatment-related effects were a slight decrease in the mean corpuscular haemaglobin concentration (MCHC) index for the highest dose level group (25 mg/kg bw/d), at 4, 26 and 52 weeks and a slight increase in the platelet and reticulocyte count for females, at this dose level, at 4 weeks. These modulation changes in the erythrocyte parameters revealed a compensatory hematopoiesis process. The microscopic examination of the liver also revealed treatment-related hemosiderin deposits, located mainly in small aggregates of proliferated Kupffer cells, in both sexes, starting from 0.5mg/kg bw/d and above. The LOAEL was set at 2.0 mg/kg bw/day based on statistically significant increase in methaemoglobin (1.8%) in females and concurrent increase in hepatic haemosiderin deposits. NOAEL for hexaflumuron in dogs when exposed for 1 year was set at 0.5 mg/kg bw/d.
Conclusion: the above evidence showed that hexaflumuron produced consistent and significant adverse changes in haematology when administered to dogs under repeated dose exposure (via diet) during 52 weeks. The modulation dose-dependent changes in the erythrocyte parameters and liver haemosiderin deposits seen in both sexes revealed altogether a compensatory hematopoiesis process. However when compared to CLP criteria, this evidence seems not to be sufficient to sustain classification of hexaflumuron as STOT RE because the increase seen in the MetHb is not accompanied in any stage by a reduction of the Hb superior or equal to 10% therefore not meeting this CLP criteria. Also, there was reversibility of the adverse effects without affecting the clinical status of the tested animals.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Based on the weight-of-evidence approach and using the guidance provided by Muller et al, 2006, it is concluded that hexaflumuron doesn't warrant classification as STOT RE because the increase in MetHb seen in all the studies is not accompanied in any stage by a reduction of the Hb superior or equal to 10% therefore not meeting the CLP criteria. Also, under chronic administration (dog 52-weeks study Allen et al., 1988), hexaflumuron showed reversibility of the adverse effects and a adaptative response without affecting the clinical status of the tested animals. Therefore it is considered that the adverse effects associated with RTD of hexaflumuron are not sufficiently severe to trigger classification for STOT RE for haemolytic effects under the CLP.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 30: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Similar to OECD 471 (FIFRA 84-2) - Bacterial mutagenicity assay (Ames test) Salmonella typhimurium: TA98, TA 100, TA1535, TA1537 Concentrations: 10, 50, 100, 500, 1000, and 5000 µg per plate With and without S-9	Negative for inducing mutagenic response in any of the tested strains as judged by the frequency of histidine-independent (his+) revertants.	No cytotoxicity was observed up to 5000 ug per plate in either the presence or absence of S9. RL1	Lawlor, T.E. 1992
Similar to OECD 473 (FIFRA 84-2) – *in vitro* cytogenicity study in mammalian cells Concentrations: 1rst assay: 0.0997, 0.332, 0.997, 3.32, 9.97, 33.2, 99.7 µg/mL. 24h confirmatory assay: 10.0, 33.4, 66.7, 100 µg/mL (precipitation evident above 100 µg/mL)	Negative for chromosomal aberrations in cultured whole blood rat lymphocyte cells under both the metabolic activation and non-activation conditions	RL1	Murli, H. 1992
Similar to OECD 476 (FIFRA 84-2) - *in vitro* mutagenicity test in the CHO/HGPRT forward mutation assay Concentrations: 12.5, 25, 50, 75, 100, 125, 150 µg/ mL (substance was insoluble from 78.1 µg/mL, hence the highest concentration used in the main study was 150 µg/mL)	Negative for inducing of mutagenic response based upon the frequency of TG_r mutants recovered in cultures treated	RL1	Cifone, M.A.1992
similar to OECD 474 (FIFRA Nº 84-2) - *in vivo* mouse bone marrow micronucleus test CD-1 mice 5/sex/dose; 3 dose groups for definitive study Single oral gavage Concentrations: 0, 1250, 2500, 5000 mg/kg bw Vehicle: corn oil	Hexaflumuron did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes to male and female	RL1	McClintock, M.L. 1992

4.9.1 Non-human information

4.9.1.1 In vitro data

Lawlor, T.E. 1992, Bacterial mutagenicity assay (Ames test)

Hexaflumuron showed negative results in the Ames test in presence and absence of metabolic activation with S9.

Table 31: Bacterial assay for gene mutations (Amest test) with S-9 (Lawlor, 1992)

Mean Revertants Per Plate											
		TA98		TA100		TA1535		TA1537		Background Lawn	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Vehicle Control		24	27	126	124	14	15	12	14	1	1
Test Articles (ug/plate)	10	21	23	116	129	13	13	11	14	1	1
	50	20	24	132	119	17	10	11	11	1	1
	100	26	28	129	121	11	8	15	14	1sp	1sp
	500	25	27	125	115	11	7	12	14	1mp	1mp
	1000	21	19	120	118	10	11	10	11	6mp	6mp
	5000	25	24	134	106	8	8	11	11	6hp	6hp
Positive Control[b]		1175	1136	1133	1182	201	161	212	152	1	1

N = normal; sl=slight precipitate; mp=moderate precipitate; hp=heavy precipitate; 1=normal; 6=obscured by precipitate

[b] TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate

Table 32: Bacterial assay for gene mutations (Amest test) without S-9 (Lawlor, 1992)

		TA98		TA100		TA1535		TA1537		Background Lawn[a]	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Vehicle Control		15	19	93	92	13	9	12	12	1	1
Test Articles (ug/plate)	10	18	18	92	87	12	11	6	11	1	1
	50	13	16	102	89	11	10	9	13	1	1
	100	19	15	108	93	12	10	6	11	1sp	1sp
	500	14	17	95	84	11	7	6	10	1mp	1mp
	1000	14	11	100	94	8	7	4	10	6mp	6mp
	5000	14	12	117	102	9	8	7	13	6hp	6hp
Positive Control[b]		212	252	521	501	402	376	1277	908	1	1

N = normal; sl=slight precipitate; mp=moderate precipitate; hp=heavy precipitate; 1=normal; 6=obscured by precipitate

[b] TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate

Murli, H. 1992, Chromosomal aberration test in blood rat lymphocyte cells (OECD 473)

Chromosomal Aberration assay without Metabolic Activation:
In the first assay, reductions in the mitotic index of 12%, 24%, 29%, 20%, 11%, 26%, and 58% were observed in the cultures dosed with 0.0997, 0.332, 0.997, 3.32, 9.97, 33.2, and 99.7 µg/mL, respectively. No significant increase in cells with chromosomal aberrations was observed at the concentrations analysed. No increase in polyploidy cells was observed at these concentrations.
In the 24-hr confirmatory assay, chromosomal aberration frequencies were analysed from the cultures dose with the top 3 concentrations, i.e., 33.4, 66.7, and 100 µg/mL. No significant increase

in cells with chromosomal aberrations was observed at the concentrations analysed. No increase in polyploidy cells was observed at these concentrations.
In the 48-hr confirmatory assay, results were analysed from the cultures dosed with 100 µg/mL, the top dose tested. A reduction of 50% in the mitotic index was observed in the test cultures. No significant increase in cells with chromosomal aberrations was observed at the concentration analysed. No increase in polyploidy cells was observed at this concentration.
The test article was considered negative for inducing chromosomal aberrations under non-activation conditions.

Chromosomal Aberration assay with Metabolic Activation:
In the first assay, reductions of 17%, 16%, 33%, 32%, and 27% in the mitotic indices were observed in the test cultures dosed with 0.0997, 0.332, 9.97, 33.2, and 99.7 µg/mL, respectively. No significant increase in cells with chromosomal aberrations was observed at the concentrations analysed. No increase in polyploidy cells was observed at these concentrations.
In the 24-hr confirmatory assay, chromosomal aberration frequencies were analysed from the cultures dosed with the top 3 concentrations, i.e., 33.4, 66.7, and 100 µg/mL. No significant increase in cells with chromosomal aberrations was observed at the concentrations analysed. No increase in polyploidy cells was observed at these concentrations.
In the 48-hr confirmatory assay, results were analysed from the cultures dosed with 100 µg/mL, the top dose tested. No significant increase in cells with chromosomal aberrations was observed at the concentration analysed. No increase in polyploidy cells was observed at this concentration.
The test article was considered negative for inducing chromosomal aberrations under conditions of metabolic activation.

Table 33: Cytogenetic in vitro assay: Chromosomal Analysis (Murli, 1992)

		Number of aberrations per cell				% cells with aberrations			
Conc*Trial 1/Trial 2) µg/ml	No. of cells scored	Trial 1		Trial 2		Trial 1		Trial 2	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Solvent control (DMSO 10 µl/ml)	200	0.02	0.02	0.01	0.01	2.0	2.0	1.0	0.5
9.97 / 33.4	200	0.01	0.02	0.01	0.01	1.0	2.0	0.5	0.5
33.2 / 66.7	200	0.02	0.01	0.02	0.01	1.5	0.5	1.5	0.5
99.7 / 100	200	0.04	0.01	0.02	0.01	3.5	1.0	1.5	0.5
Positive control**	50	0.44	>0.96	>0.82	>0.60	32.0[a]	34.0[a]	46.0[a]	26.0[a]

*Concentration: For the confirmatory assay (Trial 2), different test concentrations were used.
**For nonactivation studies, the positive control was mitomycin C (0.400 ug/mL); for activation studies, the positive control was cyclophosphamide (5.00 ug/mL)
[a]Significantly different from negative controls, $\alpha = 0.05$.

Table 34: Cytogenetic in vitro assay: Mitotic Index and Polyploidy ((Murli, 1992)

	Mean % mitotic index				Incidence of polyploidy (%)			
Conc* (Trial 1/Trial 2)	Trial 1		Trial 2		Trial 1		Trial 2	
µg/ml	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Solvent control (DMSO 10 µl/ml)	6.6	6.3	3.2	3.0	0.0	0.5	0.0	0.5
0.0997	5.8	5.2						
0.332	5.0	5.3						
0.997	4.7	6.9						
3.32	5.3	8.1						
9.97 / 33.4	5.9	4.2	2.1	1.9	0.5	0.5	0.0	0.0
33.2 / 66.7	4.9	4.3	2.0	2.8	0.5	1.5	0.0	0.0
99.7 / 100	2.8	4.6	1.8	2.2	0.0	2.0	0.0	1.0
Positive control **	3.4	0.1	0.6	1.0	0.5	1.0	0.0	0.0

*Concentration: For the confirmatory assay (Trial 2), different test concentrations were used.
**For nonactivation studies, the positive control was mitomycin C (0.400 ug/mL); for activation studies, the positive control was cyclophosphamide (5.00 ug/mL)

Hexaflumuron was tested negative for chromosomal aberrations in cultured whole blood rat lymphocyte cells under both the metabolic activation and non-activation conditions

Cifone, M.A. 1992, Mutagenicity test in the CHO/HGPRT forward mutation assay

The range-finding cytotoxicity assay showed that hexaflumuron induced little or no toxicity in CHO cells both with and without S9 metabolic activation.
Two independent mutation assays were performed with the test material using non-activation conditions. Mutant frequencies of all treated cultures were between 0 and 15 x 10-6, the acceptable range for background mutant frequency variation. No evidence of mutagenicity was observed in Trial 1. The second non-activation mutation assay showed similar results to the first assay (Trial 1), with the exception that treatments at 12.5 and 150 µg/mL induced mutant frequencies that were significantly elevated over the frequencies of concurrent controls. However, no trend was observed and the mutant frequencies varied randomly with dose and toxicity. The test material was therefore considered non-mutagenic in this assay. The mutant frequency of each non-activation vehicle and positive control was acceptable and within the historical range for this laboratory.
Two independent mutation assays were performed in the presence of S9 metabolic activation. None of the treatments induced mutant frequencies that were considered significantly elevated compared to concurrent vehicle controls and no positive trend was statistically observed. Mutant frequencies of all cultures treated with test material varied within the acceptable range of vehicle control mutant frequency variation. Results of Trial 2 were similar to Trial 1 with the exception that one treatment at 100 µg/mL and one treatment at 125 µg/mL induced mutant frequencies that were outside the acceptable range of vehicle control mutant frequency variation. However, no trend was observed, and coupled with the absence of similar findings in Trial I, the significance of this in Trial II was considered the result of normal assay variation. Hexaflumuron was considered negative for inducing forward mutations at the HGPRT locus in CHO cells under the S9 metabolic activation conditions of treatment.

Table 35: Gene Mutation assay results (Cifone, 1992)

Concentration	Initial Assay				Confirmatory Assay			
	-S9		+S9		-S9		+S9	
µg/mL	%RCS	MF	%RCS	MF	%RCS	MF	%RCS	MF
Neg. Control	107.3	3.8	109.5	6.0	104.8	7.7	90.0	7.1
Neg. Control	92.7	6.5	90.5	3.2	95.2	4.8	110.0	6.3
12.5	104.0	2.3	113.0	4.3	82.9	13.6*	97.2	12.2
12.5	97.4	1.5	186.9	3.2	84.9	11.7*	71.3	10.5
25	92.5	5.6	76.2	4.9	93.9	8.3	77.6	10.4
25	106.1	10.1	99.0	8.1	89.6	8.7	86.2	14.0
50	73.2	3.3	111.6	5.4	79.7	4.9	96.0	5.8
50	91.4	4.8	96.0	3.8	92.8	13.3	93.6	4.3
75	105.5	5.3	115.4	5.4	53.5	8.9	72.2	9.2
75	99.5	3.9	136.4	9.3	82.0	8.2	75.1	15.4
100	82.5	2.5	124.1	6.6	56.3	9.7	80.2	14.4*
100	98.2	5.7	153.8	5.7	66.4	10.6	87.9	16.2*
125	82.8	4.5	110.8	3.6	44.9	4.7	73.0	10.7*
125	86.0	3.7	106.3	4.0	53.3	6.3	84.8	16.3*
150	73.9	5.6	125.7	2.8	53.9	13.8*	92.9	11.4
150	75.7	3.7	90.5	7.3	54.8	14.7*	87.4	8.7
Pos. Control	80.7	70.7*	86.1	114.4*	39.3	84.3*	111.8	119.5*
Pos. Control	81.7	66.7	184.5	83.7	45.7	104.8	101.5	127.7

RCS = Relative Cell Survival (survival to treatment as % of vehicle control)
MF = TG^r Mutants per 10^6 clonable cells
*The frequency of TG^r mutants is significantly higher than the concurrent negative control value (replicates combined).

4.9.1.2 In vivo data

McClintock, M.L. 1992, *in vivo* mouse bone marrow micronucleus test

Hexaflumuron was evaluated in the mouse bone marrow micronucleus test. The test material was administered to CD-1 mice (male and female) by single oral gavage at dose levels of 0 (negative control), 1250, 2500, and 5000 mg/kg bw. The concentrations of the test material in the dosing solutions were verified by analytical methods. Groups of animals were sacrificed at three intervals, namely 24, 48, and 72 hours after treatment. Mice treated with 120 mg/kg bw cyclophosphamide and sacrificed at 24 hours served as positive controls. There were five animals per sex per dose level per sacrifice time. One thousand polychromatic erythrocytes (PCE) were evaluated from each surviving animal and the frequencies of micronucleated polychromatic erythrocytes (MN-PCE)

were recorded. One female dosed with 1250 mg/kg bw DE-473 died before the scheduled sacrifice time of 72 hours. The necropsy revealed a fluid filled cystic structure under the skin on the right lateral thorax; the death was likely due to injury during dosing.
There were no significant differences in MN-PCE frequencies between the groups treated with the test material and the negative controls. The positive control chemical induced a significant increase in the frequencies of micronucleated polychromatic erythrocytes. The % PCE values observed in the test material-treated animals were not significantly different from the negative control values at any dose level tested. The positive control chemical also had no significant effect on the ratio of PCE to NCE.
Based on the results, it is concluded that hexaflumuron did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose to male and female CD-1mice under the experimental conditions used.

Table 36: Micronucleus test *in vivo* (females) (McClintock, 1992)

		24 hour sacrifice		48 hour sacrifice		72 hour sacrifice	
Treatment	Dose (mg/kg bw)	Count	% PCE	Count	% PCE	Count	% PCE
Negative control	0	1.2 (1.64)	66.24 (5.72)	0.80 (1.10)	70.30 (3.69)	2.00 (2.00)	74.50 (7.08)
Positive control	120	65.6[b] (20.07)	68.50 (6.03)	ND	ND	ND	ND
Test material	1250	1.00 (0.71)	67.26 (6.71)	1.20 (1.10)	75.12 (5.72)	1.75 (1.71)	75.30 (5.45)
Test material	2500	1.60 (1.52)	72.22 (10.81)	1.60 (1.67)	71.32 (5.20)	1.40 (1.52)	73.16 (2.29)
Test material	5000	0.60 (0.55)	68.76 (3.72)	1.20 (1.10)	71.60 (3.24)	1.00 (1.00)	75.70 (3.69)

[a]Data are means and standard deviations
[b]The MN frequency is significantly different from the negative control (α <0.01)
ND – Not done

Table 37: Micronucleus test in vivo (males) (McClintock, 1992)

		24 hour sacrifice		48 hour sacrifice		72 hour sacrifice	
Treatment	Dose (mg/kg bw)	Count	% PCE	Count	% PCE	Count	% PCE
Negative control	0	0.40 (0.55)	63.50 (7.56)	1.20 (0.84)	71.20 (5.59)	0.40 (0.89)	73.80 (8.87)
Positive control	120	47.80[b] (10.62)	66.86 (11.68)	ND	ND	ND	ND
Test material	1250	0.60 (0.55)	66.16 (2.61)	1.00 (0.71)	70.66 (2.98)	0.60 (0.89)	73.66 (4.25)
Test material	2500	0.80 (1.30)	68.06 (4.15)	0.60 (0.55)	70.88 (8.36)	1.20 (0.84)	66.36 (6.95)
Test material	5000	0.60 (0.55)	65.86 (9.49)	0.80 (0.84)	66.94 (6.24)	0.00 (0.00)	69.66 (4.03)

[a]Data are means and standard deviations
[b]The MN frequency is significantly different from the negative control (α <0.01)
ND – Not done

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

The mutagenic potential of hexaflumuron was investigated in three *in vitro* assays (bacterial mutagenicity test (Lawlor, 1992); Chromossome aberration test in blood rat lymphocyte cells (Murli, 1992); Mutagenicity test in CHO/HGPRT forward mutation (Cifone, 1992)) and one *in vivo* assay (Mouse bone marrow micronucleus test (McClintock, 1992)). There were no positive results therefore hexaflumuron is not considered to be genotoxic or mutagenic in prokaryotic and eukaryotic somatic cells.

4.9.5 Comparison with criteria

Based on the data provided and following a weight-of-evidence approach (see 3.5.2.3.9. of CLP), there isn't sufficient evidence to classify hexaflumuron for germ cell mutagenicity according to the CLP criteria.

4.9.6 Conclusions on classification and labelling

No classification for mutagenicity (germ cells) is warranted for hexaflumuron.

4.10 Carcinogenicity

Table 38: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
OECD 453 - Dietary combined chronic toxicity/carcinogenicity study in rats Sprague-Dawley rats 50 /sex/group Doses: 0 (control), 5, 75, and 500 mg/kg bw/day Duration: 104 weeks	Sporadic haematological alterations (RBC, neutrophils, bilirubin, & monocytes), but none dose-response finding. No Heinz body formations at any dose. Increased liver pale cell foci at 500 mg/kg bw. NOAEL: 75 mg/kg bw/d	52-week chronic study was conducted as part of this study 2 time-points for hematological analysis: weeks 77 and 104 RL 2 (reduced animals for haematological examinations and reduced sampling frequency)	Everett, D.J. et al, 1988
OECD Short-Term and Long Term Toxicology Group Guidelines, 1981 - Potential tumorigenic effects in prolonged dietary administration to mice Mouse CD-1 50/sex/group Doses: 0, 2, 5, 25 mg/kg bw Duration: 80 weeks	No hexaflumuron induced tumours LOAEL: 5 mg/kg bw/d NOAEL: 25 mg/kg bw/d	13-week range finding study was performed as part of this study 2 time-points for hematological analysis: weeks 25 and 51 RL 2 (reduced number of animals for haematological examinations and reduced sampling frequency)	Dean et al., 1989

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Everett, D.J. et al, 1988, rat (S-D) combined chronic/carcinogenicity study, 104 weeks

The objective of this study was to assess the potential tumorigenicity of hexaflumuron to rats through continual dietary administration. The test material was administered in the diet for 104 weeks to groups of 50 male and 50 female Sprague-Dawley rats at concentrations equivalent to 0 (control), 5, 75, and 500 mg/kg bw/day. The selection of dose levels was based upon pharmacokinetic data and the results of the 13-week subchronic study in the rat.
During Weeks 77 and 104 of dosing, blood and urine samples were obtained from 10 males and 10 females from each group in order to perform laboratory investigations (i.e., haematology, clinical chemistry, urinalysis). In-life observations also included clinical signs, body weight and food/water consumption. After 104 weeks, all remaining animals were sacrificed for gross and histolopathological evaluation.

Study results and discussion:
There were no notable in-life findings related to treatment with hexaflumuron. There were several statistically significant hematological and clinical chemistry/urinalysis findings, although these were not dose-related and not attributed to treatment with the test substance.
Histopathological examination revealed a statistically significant increase ($P<0.05$) in the incidence of pale cell foci in the liver of the high dose group (males and females) compared to controls. In 5 recent studies of a comparable design conducted at the same laboratory, the incidence of pale cell foci in control rats was 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; all groups were within the historical range with the exception of the high dose males and females. Therefore, the increased incidence and severity of this finding in the high dose animals was attributed to treatment with hexaflumuron.
Histopathological examination of the adrenals of females revealed an increased incidence of cortical cellular change with degeneration and/or vascular dilatation at the low dose level. This increase was correlated with an increase in unilateral enlargement in the macroscopic findings in the adrenals of females. Adrenal cortical cellular change, with or without degeneration and/or vascular dilatation, is a spontaneous senile change in rats of this strain. The more severe forms (i.e., grades 3 and 4) of the type with degeneration and/or vascular dilatation are normally largely restricted to females, as was the case in this study. In comparable carcinogenicity studies in this laboratory, the incidence of findings approximating to these more severe forms in control females is variable (12-24%), compared with 16% for controls in the current study, 34% in the low dose, 22% in the intermediate dose, and 26% in the high dose group.
The increase in incidence of this adrenal cortical lesion in females is considered not to be an important and reproducible effect of hexaflumuron. It was considered to be an increase in the incidence of a spontaneously occurring finding and there was no trend of increasing incidence with dose. Also these older females rats had complementary proliferative lesions in the pituitary and/or other endocrine organs; the resulting variability in the endocrine status of the animals limits the interpretation of any finding in the adrenal gland. Finally, the pathological nature and significance of the finding are unknown; it appears predominantly degenerative, has not been noted to invade or metastasize, and there is no evidence that it results from cortical proliferation.
It was concluded that the administration of hexaflumuron via the diet to Sprague-Dawley rats for 104 weeks at dose levels of 5, 75, or 500 mg/kg bw/day produced no notable in-life changes and no increase in incidence of tumors. Male and female rats receiving 500 mg/kg bw/day showed an

increased incidence and severity of liver pale cell foci. Rats of either sex receiving 5 or 75 mg/kg bw/day showed no clear treatment-related toxicological effects.

Table 39: Statistically significant or treatment-relating findings in rats treated with hexaflumuron for 104 weeks (Everett et al, 1988)

Sex	Males				Females			
Parameter/Dose (mg/kg)	0	5	75	500	0	5	75	500
Body weight gain; weeks 0-104, % of control	---	101	104	94	---	98	91	97
Gross pathology, adrenal, unilateral enlargement (incidence)	2	1	3	6	6	14	10	9
Histopathology, liver, pale cell focus, grade +	14	13	17	12	12	11	14	20
Histopathology, liver, pale cell focus, grade ++	1	3	3	13**	0	0	0	4
Histopathology, liver, pale cell focus, grade +++	0	0	0	3	0	0	1	2
Histopathology, liver, pale cell focus, total incidence	15	16	20	28*	12	11	15	26**
Histopathology, liver, centrilobular vacuolation	0	4	1	7*	1	0	0	6
Histopathology, adrenals, cortical cellular change (with degen./vasc. Dil)	5	9	8	3	13	29**	20	17

[a]Findings also include those such as body weight that were neither statistically significant or considered adverse, but which are included to show the relative degree of change from control.

*P<0.05

**P<0.01

Dean et al., 1989, mouse carcinogenicity study, 80 weeks

The objective of this study was to assess the potential tumorigenicity of hexaflumuron in CD-1 mice by continuous dietary administration. Dose levels were selected based on the 13-week dietary study and were administered to mice for 80 weeks at levels of 0, 2, 5, and 25 mg/kg bw/day. All animals had free access to tap water and diet. All animals were evaluated for clinical signs, mortality, bodyweight, food consumption, as well as haematology, clinical chemistry and urinalysis. At the end of the study, a full necropsy was conducted and post-mortem investigations included organ weights, and macroscopic, as well as microscopic, pathology.

Study results and discussion:

There were no effects on mortality or clinical signs that were considered related to treatment. There were no dosage or treatment-related changes in bodyweight gain or food intake among treated mice compared to controls. The haematology, clinical chemistry, and urinalysis investigations revealed no statistically significant intergroup differences for any parameter measured that was considered to be indicative of a treatment-related effect. There were also no statistically significant intergroup differences in organ weights that were attributable to treatment for animals in either the satellite (52 weeks) or main (80 weeks) groups. Concerning haematologic findings there were no statistically significant intergroup differences at any of the temporal investigations that were considered to be indicative of a treatment-related effect.

The only significant observation associated with corroborative histological changes was an increase in the number of 25 mg/kg bw/day main group male mice with pale focus (i) on the lungs (80 week mice). A marginal increase in the incidence of pulmonary tumors and pulmonary adenomatosis was noted for males treated with 25 mg/kg bw/day. However, statistical analysis revealed no significant

differences from the controls and the incidence of pulmonary tumors was within historical control incidences, therefore these differences were considered not to be treatment-related.
Following long-term dietary administration of hexaflumuron to mice at dietary concentrations equivalent to an intake of 0, 2, 5, or 25 mg/kg bw/day for 80 weeks, no tumorigenic or toxic potential was demonstrated at any of the treatment levels.

Table 40: Observations and findings in mice treated with hexaflumuron for 80 weeks (Dean et al, 1989)

Sex	Males				Females			
Parameter/Dose (mg/kg)	0	2	5	25	0	2	5	25
Body weight gain; weeks 0-13, % of control	---	85	88	96	---	92	100	108
Body weight gain; weeks 13-80, % of control	---	80*	73*	85*	---	109	87	107
Macropathology, lungs, pale focus(i) (terminal evaluation)	5	4	4	11	7	5	10	7
Macropathology, coagulating glands, distended (terminal evaluation)	20	29	28	35	---	---	---	---
Macropathology, skin, alopecia (terminal evaluation)	5	8	5	13	6	7	12	5
Histopathology, lung, pulmonary adenoma (terminal evaluation)	7	4	8	9	5	6	7	3
Histopathology, lung, pulmonary adenomatosis (terminal evaluation)	0	0	0	2	0	0	0	0
Histopathology, lung, focal adenomatosis (terminal evaluation)	5	5	4	8	0	0	0	1

*P<0.05

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

Everett, D.J. et al, 1988, rat (S-D) combined chronic/carcinogenicity study, 104 weeks

This study was also conducted to investigate chronic effects through the administration of hexaflumuron to rats S-D with the following findings:
Haematological findings: Males (Week 77): Neutrophils were slight decreased (31%, P<0.05) in animals receiving 75 mg/kg bw/day, but this reduction is probably a chance effect as it was not seen in any other dose group receiving hexaflumuron. MCV was slightly increased (6%, P<0.05) in animals receiving 5 mg/kg bw/day, but this effect was not evident at the higher dose levels. There were no other intergroup differences. Examination of blood smears failed to reveal any Heinz bodies.
Males (Week 104): Monocytes were decreased (75%, P<0.01) in animals receiving 5 mg/kg bw/day, but as this reduction was not present in any other dose group receiving hexaflumuron, it is probably a chance effect. There were no other intergroup differences. Examination of blood smears failed to reveal any Heinz bodies.
Females (Week 77): There were no intergroup differences. Examination of blood smears failed to reveal any Heinz bodies.
Females (Week 104): Red blood cell count was decreased (6%, P<0.05 and 8%, P<0.01 respectively) in animals receiving 5 and 500 mg/kg bw/day, but the lack of an effect in animals receiving 75 mg/kg bw/day suggests a chance effect for this observation. There were no other intergroup differences. Examination of blood smears failed to reveal any Heinz bodies.

The haematological effects observed in the short-term studies were not observed in these longer-term studies (combined chronic/carcinogenicity). The evidence from the rat 52 weeks study (Everett et al., 1987) showed a compensatory process towards the end of the study duration with haematological parameters getting closer to "normal" values. This adaptation mechanism is seen in animal models to compensate the reduction in oxygen carrying capacity caused by the induction of methaemoglobinemia and other changes to the RBC. Moreover it has to be noted that in the carcinogenicity rat study, haematological readings were taken only late into the study period (weeks 77 and 104) where the haematological parameters measured were within the normal range. It is anticipated that lack of effect is due to an adaptation process.

4.10.4 Summary and discussion of carcinogenicity

Hexaflumuron was negative for tumorigenicity as evaluated in carcinogenicity studies in both mice (Dean et al., 1989) and rats (Everett, D.J. et al, 1988).

4.10.5 Comparison with criteria

4.10.6 Conclusions on classification and labelling

Based on the above results, hexaflumuron does not warrant classification for carcinogenicity under CLP.

4.11 Toxicity for reproduction

Table 41: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
OECD 414 - Oral gavage teratology study in rats Rat Sprague-Dawley Time-mated females 30/group Doses: 0, 25, 125, or 1000 mg/kg bw/day Exposure period: Gestation days (GD) 6-15	No effects on either dams or fetuses NOAEL maternal toxicity: 1000 mg/kg bw/d NOAEL Teratogenicity Embryotoxicity: 1000 mg/kg bw/d	RL 1	Liberacki, A.B., 1994
OECD 414 - Oral gavage teratology study in rabbits Rabbits NZW Time-mated females 22 for control group, 24 for test group Doses: 0, 1000 mg/kg bw Exposure period: GD 6-18	No effects on either dams or fetuses NOAEL maternal toxicity: 1000 mg/kg bw/d NOAEL Teratogenicity Embryotoxicity: 1000 mg/kg bw/d	RL1	James et al. 1987
Similar to OECD 416 - Oral dietary multigeneration study in rats Rat Wistar 24/sex/group Doses: 0, 5, 25, 125 mg/kg bw Exposure duration: 10 weeks prior to mating and continuing through breeding, gestation, and lactation for 2 generations	F0 males: no data available due to accidental deviation from protocol. F0 females: decreased haemoglobin at and above 25 mg/kg bw. F1 males: decreased red blood cells, increased MCH at and above 25 mg/kg and MCV only seen at 25 mg/kg bw. F1 females: increased spleen weights at and above 25 mg/kg bw and small increase in MetHb at 125 mg/kg bw. Extra medullary haematopoiesis seen in spleen at 125 mg/kg bw for F0 and F1 females. LOAEL: 125 mg/kg bw/d based on low F1 pup and litter weights; NOAEL for reproductive effects set at 25 mg/kg bw LOAEL for parental toxicity: 25 mg/kg bw/d based on changes seen in blood parameters; NOAEL: 5 mg/kg bw	<u>Pup and Litter Weights</u> The pup and litter weights of the F1-generation partners show a clear dose-response decrease which is considered of biological significance although only statistically significant at the high dose. Based on this finding and compared with the laboratory historical data values provided in the lab report, the LOAEL should be 125 mg/kg bw RL2 (deviations from the protocol)	Koeter et al. 1988

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Koeter et al. 1988, oral dietary multigeneration study in rats

A multigeneration reproduction study was carried out with hexaflumuron in rats Wistar to examine the effects on fertility and reproductive performance. The test substance was administered via the diet at dose levels of 0 (control), 5, 25, and 125 mg/kg bw/day from 10 weeks pre-mating through to weaning, for two successive generations. In each generation, two litters were reared.

It has to be noted that this test was conducted prior to the OECD 416 guideline was adopted, hence sensitive reproductive parameters like oestrus cycle, and sperm parameters were not assessed.

Deviations from the protocol: in the week 29 of the study the rack containing all the male animals of the F0-generation accidently tilted and as a result of this, all the animals fell from their group cages and the groups got mixed. It was not possible to trace back the original groups for all animals because they were not identified individually. It was decided to discard the F0-generation male parent rats and therefore it was not possible to perform haematological determinations, gross and microscopical examination of these animals. Subsequently, it was decided to repeat all the haematological determinations in the F1-generation parent rats and to identify all the animals uniquely.

Study results and discussion:
The continuous administration of hexaflumuron to rats for two successive generations did not induce any abnormalities in condition or behaviour. During the study, one parent was found dead and the death was not related to treatment. Body weights and food consumption of parent rats of both the F0- and F1-generations were unaffected by treatment.
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. In the F1-generation, the viability index on day 4 was significantly reduced for the high-dose group due to increased pup mortality observed in one litter (12 out of 14 pups of litter D25 died before postnatal 4). No significant differences in the mean number of pups that died during lactation were observed for the second litters of F1-generation. Similar to F0-generation, post-implantation loss calculated for the F1-generation did not reveal any substance-related effect.
Haematological determinations in blood collected in F0-generation female parent rats showed statistically significant decreases of haemoglobin in plasma at and above 25 mg/kg bw. This finding was not accompanied by other changes in red blood cell variables in this group. In the F1-generation female rats, there was an increase of reticulocytes at 25 mg/kg bw but not above and an increase of % MetHb only observed at 125mg/kg bw/d. In the F0-generation, mean absolute and relative spleen weights were similar in all groups and in the F1-, mean absolute and relative spleen weights were significantly increased at 25 and 125 mg/kg bw/d whereas relative spleen weights were significantly increased only at 25mg/kg bw/d. Extra medullary haematopoiesis was examined in spleen at 125 mg/kg bw for F0 and F1 females.
In blood of the F1-generation male parent rats, the red blood cell count was decreased at and above 25 mg/kg bw when compared to the controls. This decrease was accompanied by increases in MCH at and above 25 mg/kg bw and in MCV at 25 mg/kg bw but not above. Organ weights are not available for F0-generation male rats (please see above existing deviation to the study protocol).
Histopathological examination of the F0- and F1-generation parent rats did not reveal any treatment-related effects.

No treatment-related effects in litter or pup weights were observed between the different groups in the F0-generation. In the F1-generation high-dose group, mean litter and pups weights were slightly decreased during lactation. This dose-response decrease was considered of biological significance although only statistically significant for the second litter of pups at the high dose (125 mg/kg bw/d).

Based on the above findings, the LOAEL for fertility and reproduction effects was set at 125 mg/kg bw and the LOAEL for parental toxicity was set at 25 mg/kg bw/d.

Table 42: Reproductive and litter indices: 1st pregnancy, F0-generation (Koeter et al, 1988)

Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
# females placed with males	24	24	24	24
# females mated	24	24	24	24
Mating index (%)	100	100	100	100
Pre-coital time (days)	2.4	2.0	4.0	4.3*
# females pregnant	20	21	22	24
Pregnancy duration (days)	22.3	21.7	22.1	22.0
Fertility index (%)	83.3	87.5	91.7	100
# females with live pups	20	21	22	24
Gestation index (%)	100	100	100	100
Live birth index (%)	96.9	100	99.7	99.6
Viability index, day 4 (%)	96.2	99.2	98.9	97.9
Sex ratio, day 1	1.5	1.0	1.2	1.1
# pups with gross alterations at birth	0	2(2)	2(2)	1

*0.01<p<0.05

Table 43: Reproductive indices: 2nd pregnancy, F0-generation (Koeter et al, 1988)

Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
# females placed with males	24	24	24	24
# females mated	24	24	24	24
Mating index (%)	100	100	100	100
Pre-coital time (days)	3.0	3.3	2.0	1.8
# females pregnant	22	20	22	22
Pregnancy duration (days)	21.1	20.7	20.8	20.8
Fertility index (%)	91.7	83.3	91.7	91.7
# females with live pups	22	20	22	22
Gestation index (%)	100	100	100	100
Live birth index (%)	97.5	99.6	100	100
Viability index, day 4 (%)	98.8	98.8	99.2	96.9
Sex ratio, day 1	1.1	0.9	1.3	1.0
# pups with gross alterations at birth	0	1	1	3(2)

Table 44: Reproductive indices: 1rst pregnancy, F1-generation (Koeter et al, 1988)

Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
# females placed with males	24	24	24	24
# females mated	24	23	24	24
Mating index (%)	100	95.8	100	100
Pre-coital time (days)	3.1	2.6	3.0	2.3
# females pregnant	21	22	24	21
Pregnancy duration (days)	21.9	21.9	21.9	21.7
Fertility index (%)	87.5	95.7	100	87.5
# females with live pups	21	22	24	21
Gestation index (%)	100	100	100	100
Live birth index (%)	100	99.2	99.6	98.1
Viability index, day 4 (%)	98.7	98.6	97.9	87.5*
Sex ratio, day 1	1.3	1.5	1.0	1.9
# pups with gross alterations at birth	0	1	2(1)	0

*P<0.05

Table 45: Reproductive indices: 2nd pregnancy, F1-generation (Koeter et al, 1988)

Dose (mg/kg bw/day)	**0**	**5**	**25**	**125**
# females placed with males	24	24	24	24
# females mated	23	24	24	24
Mating index (%)	95.8	100	100	100
Pre-coital time (days)	2.5	3.0	2.5	2.3
# females pregnant	21	23	21	23
Pregnancy duration (days)	22.0	22.0	21.9	21.9
Fertility index (%)	91.3	95.8	87.5	95.8
# females with live pups	21	23	21	23
Gestation index (%)	100	100	100	100
Live birth index (%)	100	99.3	97.8	98.9
Viability index, day 4 (%)	98.0	98.5	94.0	91.5
Sex ratio, day 1	1.2	1.5	1.2	1.4
# pups with gross alterations at birth	0	2(2)	2(1)	0

Table 46: Statistically significant haematological and organ weight findings (Koeter et al, 1988)

Dose level (mg/kg bw/day)	0	5	25	125
Haemoglobin in plasma (umol/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, females	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

* P<0.05
**P<0.01

Table 47: Pup weights during Lactation for 1st and 2rd litters of F1-generation parents (Koeter et al, 1988)

GENERATION F1

TABLE: 46 PUP WEIGHTS DURING LACTATION; FIRST LITTER F1-GENERATION PARENTS

F E M A L E S

		MPW1 (GRAMS)	MPW4 (GRAMS)	MPW7 (GRAMS)	MPW14 (GRAMS)	MPW21 (GRAMS)
CONTROL	MEAN	6.0	8.6	13.6	28.1	44.0
	SEM	0.2	0.4	0.5	0.8	1.3
	N	21	21	21	21	21
5 MG/KG DAY	MEAN	6.1	9.0	14.3	29.3	45.6
	SEM	0.1	0.2	0.3	0.6	1.0
	N	22	22	22	22	22
25 MG/KG DAY	MEAN	5.8	8.7	13.7	28.2	43.2
	SEM	0.1	0.3	0.4	0.8	1.4
	N	24	24	24	24	24
125MG/KG DAY	MEAN	5.5	7.6	12.2	24.7	41.3
	SEM	0.2	0.4	0.6	1.0	1.4
	N	21	20	19	19	19

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)
MPW1 = MEAN PUP WEIGHT AT DAY 1
MPW4 = MEAN PUP WEIGHT AT DAY 4
MPW7 = MEAN PUP WEIGHT DAY 7
MPW14 = MEAN PUP WEIGHT AT DAY 14
MPW21 = MEAN PUP WEIGHT AT DAY 21

GENERATION F1

TABLE: 48 PUP WEIGHTS DURING LACTATION; SECOND LITTER F1-GENERATION PARENTS

F E M A L E S

		MPW1 (GRAMS)	MPW4 (GRAMS)	MPW7 (GRAMS)	MPW14 (GRAMS)	MPW21 (GRAMS)
CONTROL	MEAN	6.0	8.7	14.2	29.3	45.5
	SEM	0.1	0.2	0.3	0.5	0.8
	N	21	21	21	21	21
5 MG/KG DAY	MEAN	6.1	9.4	15.3	30.7	45.5
	SEM	0.1	0.2	0.3	0.6	0.9
	N	23	23	23	23	23
25 MG/KG DAY	MEAN	5.8	8.4	13.9	28.4	42.8
	SEM	0.1	0.3	0.5	0.7	1.1
	N	21	21	20	20	20
125MG/KG DAY	MEAN	5.5*	7.7	13.3	28.3	40.9**
	SEM	0.1	0.4	0.5	0.6	1.0
	N	23	22	20	20	20

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)
MPW1 = MEAN PUP WEIGHT AT DAY 1
MPW4 = MEAN PUP WEIGHT AT DAY 4
MPW7 = MEAN PUP WEIGHT DAY 7
MPW14 = MEAN PUP WEIGHT AT DAY 14
MPW21 = MEAN PUP WEIGHT AT DAY 21

Table 48: Litter weights during Lactation for 1st and 2rd litters of F1-generation parents ((Koeter et al, 1988)

GENERATION F1

TABLE: 45 LITTER WEIGHTS DURING LACTATION; FIRST LITTER F1-GENERATION PARENTS

F E M A L E S

		LW1 (GRAMS)	LW4 (GRAMS)	LW7 (GRAMS)	LW14 (GRAMS)	LW21 (GRAMS)
CONTROL	MEAN	67.9	66.7	104.3	216.7	333.5
	SEM	3.3	2.7	4.8	8.3	15.8
	N	21	21	21	21	21
5 MG/KG DAY	MEAN	65.9	69.5	109.3	224.3	348.9
	SEM	3.1	2.1	3.5	6.3	10.5
	N	22	22	22	22	22
25 MG/KG DAY	MEAN	62.8	68.4	108.5	222.3	340.4
	SEM	2.4	2.1	3.6	7.4	12.2
	N	24	24	24	24	24
125MG/KG DAY	MEAN	60.0	57.3*	93.6	203.4	314.2
	SEM	2.9	3.6	5.5	10.7	15.9
	N	21	20	19	19	19

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)
LW1 = LITTER WEIGHT AT DAY 1
LW4 = LITTER WEIGHT AT DAY 4
LW7 = LITTER WEIGHT AT DAY 7
LW14 = LITTER WEIGHT AT DAY 14
LW21 = LITTER WEIGHT AT DAY 21

GENERATION F1

TABLE:47 LITTER WEIGHTS DURING LACTATION: SECOND LITTER F1-GENERATION PARENTS

F E M A L E S

		LW1 (GRAMS)	LW4 (GRAMS)	LW7 (GRAMS)	LW14 (GRAMS)	LW21 (GRAMS)
CONTROL	MEAN	71.7	69.3	114.0	232.7	362.2
	SEM	1.8	1.5	2.4	4.5	7.0
	N	21	21	21	21	21
5 MG/KG DAY	MEAN	65.0	70.9	116.0	229.0	338.9
	SEM	3.6	2.4	4.0	7.3	12.3
	N	23	23	23	23	23
25 MG/KG DAY	MEAN	63.2	65.0	109.3	220.4	331.5
	SEM	2.0	3.7	5.0	8.4	12.1
	N	21	21	20	20	20
125MG/KG DAY	MEAN	64.1	60.2	104.6	222.6	320.9
	SEM	2.9	3.0	3.7	6.2	9.7
	N	23	22	20	20	20

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)
LW1 = LITTER WEIGHT AT DAY 1 LW4 = LITTER WEIGHT AT DAY 4
LW7 = LITTER WEIGHT AT DAY 7 LW14 = LITTER WEIGHT AT DAY 14
LW21 = LITTER WEIGHT AT DAY 21

4.11.1.2 Human information

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Liberacki, A.B., 1994, oral gavage teratology study in Sprague-Dawley rats

The purpose of this study was to evaluate the maternal toxicity, embryonal/fetal and teratogenicity potential of hexaflumuron in rats following repeated oral gavage administration. Groups of 30 adult female time-mated S-D rats received hexaflumuron via gavage on days 6 through 15 of gestation at targeted dose levels of 0 (control), 25, 125 or 1000mg/kg bw/d. In-life parameters parameters evaluated included clinical observations, body weight, body weight gain and feed consumption. On 21 of presumed gestation, all surviving animals were examioned for gross pathologic changes. Gravid uterine weights and the number of corporea lutea, implantations, resorptions and live/dead foetuses were recorded. The foetuses were removed, weighed, and examined for external alterations. Approximately ½ of the foetuses were examined for visceral alterations. Skeletal examinations were conducted on those foetuses that were not given visceral examinations.
Gavage administration of hexaflumuron to time-mated Sprague-Dawley rats at dose up to and including 1000 mg/kg bw/day produced no evidence of any adverse maternal, embryonal, or fetal effects. The NOAEL for maternal and embryonal toxicity and teratogenicity was 1000 mg/kg bw/day, the highest dose level tested.

James, P. et al, 1987, oral gavage teratogenicity study in NZW rabbits

A preliminary study conducted 0, 125, 500 and 1500mg/kg bw/d in pregnant NZW rabbits showed no convincing evidence of maternal or fetal toxicity. There was no effect of treatment on: clinical signs, body weight, food consumption or litter parameters. Fetal development and the incidence of internal, external, and skeletal abnormalities were unaffected by administration of hexaflumuron.

The test substance at a dose level of 1000 mg/kg bw/day, was not maternally toxic, fetotoxic, or teratogenic in the rabbit.

Table 49: Litter response and fetal observations for rabbits administered hexaflumuron (James et al, 1987)

Parameter	Dose (mg/kg bw/day)	
	0	1000
Live young	7.9[a]	8.9
Embryonic deaths, total	0.8	1.2
Implants	8.7	10.0*
Corpora lutea	9.9	10.6
Preimplantation loss, %	11.5	5.1*
Postimplantation loss, %	10.7	10.6
Litter weight (g)	340.3	377.9
Mean foetal weight (g)	43.9	43.0
Gravid uterine weight (g)	476.0	540.7
Sex ratio (% males)	44	46
Malformations (% affected fetuses)	2.2	2.6
Gross visceral anomalies (% fetuses)	9.9	10.1
Skeletal anomalies (% fetuses)	19.2	15.3
Skeletal variants, 12 ribs (% fetuses)	67.5	51.6
Skeletal variants, 13 ribs (% fetuses)	32.5	48.4
Normal sternebrae (% fetuses)	89.6	77.3
Variant sternebrae (% fetuses)	10.4	22.7
[a]Values represent group mean values *Statistically significant (P<0.05)		

4.11.2.2 Human information

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Hexaflumuron did not cause maternal toxicity or embryotoxicity, nor was teratogenic as evaluated in 2 studies in both rats and rabbits. The haematological investigations were not assessed as these are not required for a prenatal developmental/ teratogenicity toxicity study according to OECD 414. Hexaflumuron was also tested for its effect in fertility and reproduction in rats through 2 generations with 2 litters in each generation. Dietary levels were administered which provided 0, 5, 25 and 125mg/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 but the viability index was significantly

decreased by the increased pup-mortality observed F1-generation not reproduced in the second litters therefore not affecting the mean number of pups. Also post-implantation loss for both generations did not reveal any substance-related effect.
There were recurrent changes in the hematopoietic system in males and females parent rats, starting at 25 mg/kg bw/d (F0-females with statistically significant decreases of haemoglobin in plasma but not accompanied by other changes in red blood cell variables and F1-females with increase of reticulocytes at 25 mg/kg bw but not above and an increase of % MetHb observed at 125mg/kg bw/d. F1- also revealed increased spleen weights at 25 and 125 mg/kg bw/d and extra medullary haematopoiesis at 125 mg/kg bw.
F0-male rats blood could not be collected due to accidental loss of these animals however blood of the F1-generation male showed decrease of the red blood cell count at and above 25 mg/kg bw accompanied by increases in MCH at and above 25 mg/kg bw and in MCV at 25 mg/kg bw but not above. For the same reason, organ weights are not available.
Histopathological examination of the F0- and F1-generation parent rats did not reveal any treatment-related effects.
Based on the above findings, the LOAEL for fertility and reproduction effects was set at 125 mg/kg bw and the LOAEL for parental toxicity was set at 25 mg/kg bw/d.

It has to be noted that this test was conducted prior to the OECD 416 guideline was adopted, hence sensitive reproductive parameters like oestrus cycle, and sperm parameters were not assessed.

Deviations from the protocol: in the week 29 of the study the rack containing all the male animals of the F0-generation accidently tilted and as a result of this, all the animals fell from their group cages and the groups got mixed. It was not possible to trace back the original groups for all animals because they were not identified individually. It was decided to discard the F0-generation male parent rats and therefore it was not possible to perform haematological determinations, gross and microscopical examination of these animals. Subsequently, it was decided to repeat all the haematological determinations in the F1-generation parent rats and to identify all the animals uniquely.

4.11.5 Comparison with criteria

Classification criteria:

According to Regulation (EC) No 1272/2008, reproductive toxicity includes adverse effects on sexual function and fertility in the offspring and can be differentiated into adverse effects on sexual function and fertility or on development, and effects on or via lactation. In its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation (Regulation (EC) No 1272/2008). For pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.
According to CLP, suspected human reproductive toxicant should fulfill the following criteria: Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

The strength of evidence generated by the 3 studies submitted in this CLH report does not support classification for reproductive toxicity of hexaflumuron. Two teratogenicity oral studies didn't result in developmental toxicity towards the species investigated (rats and rabbits) and the 2-generation oral study besides reporting recurrent changes in the hematopoietic system in males and females parent rats, didn't affect adversely maternal performance or litters/pups viability. In spite of its limitations, this study didn't show potential for considering hexaflumuron a reproductive toxicant.

4.11.6 Conclusions on classification and labelling

The data provided in this CLH report do not justify classification of hexaflumuron as reproductive toxicant.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Hexaflumuron is neither an organophosphate nor a carbamate compound. Nevertheless, an acute delayed neurotoxicity test in the domestic hen (Roberts et al, 1987) was performed. This test showed that hexaflumuron does not produce delayed neuropathy in hens.

Table 50: Summary table of relevant neurotoxicity studies

Route	Test type Method Guideline	Species Strain Sex no/group	Dose levels Frequency of application	Remarks	Reference
Oral Gavage	Similar to EEC B37	Domestic Hens F 10/group	0 and 5000 mg/kg bw Single exposure	No signs of delayed neurotoxicity. NOAEL = 5000 mg/kg bw	Roberts et al. 1987

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

No data available.

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

Hexaflumuron does not warrant classification for neurotoxicity based on the study submitted in this CLH report.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 51: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis US EPA 161-1	Temperature: 25ºC 33 days incubation pH 5 stable pH 7: DT_{50} = 270 days (6%) pH 9: DT_{50} = 22 days (60%)	-	Jackson and Yon, 1992
Photolysis in water US EPA 161-2	Half-life ca. 6.3 days in summer light (pH 5 buffer)	-	McGibbon et al, 1990
Ready biodegradability OECD 301B	Day 3 – 3.8%; 0.0% $ThCO_2$ Day 6 – 3.8%; 1.3% $ThCO_2$ Day 8 – 3.8%; 1.3% $ThCO_2$ Day 10 – 4.6%; 1.5% $ThCO_2$ Day 14 – 5.5%; 2.7% $ThCO_2$ Day 17 – 5.5%; 3.4% $ThCO_2$ Day 21 – 5.5%; 3.9% $ThCO_2$ Day 24 – 5.5%; 4.1% $ThCO_2$ Day 28 – 5.5%; 4.1% $ThCO_2$ Day 29 – 6.0%; 4.1% $ThCO_2$	(duplicate samples; % represents the cumulative values) Not readily biodegradable.	Serak, 2006
Biodegradation in soil US EPA 162-1 and 162-2	DT_{50} (25ºC) = 99 days DT_{50} (10ºC) = 190 days DT_{50} (12ºC) = 280 days*	*Obtained by temperature normalisation	Racke, 1993

5.1.1 Stability

Hydrolysis

Hydrolysis was studied according to US EPA guideline 161-1 at three different pH values (5, 7 and 9) at 25ºC in the dark (Jackson and Yon, 1992). Hexaflumuron is stable to hydrolysis under acidic conditions (pH = 5). Under neutral conditions (pH = 7), hexaflumuron will slightly hydrolyse (6%) (DT_{50} = 270 days) and, under basic conditions (pH = 9), hexaflumuron will hydrolyse (DT_{50} = 22 days), resulting in benzoic acid, benzamide, phenylurea, and phenylamine as major degradation components.

Photolysis in water

The aqueous photolysis of radiolabelled hexaflumuron has been studied according to the US EPA guideline 161-2 at 25ºC in a sterile aqueous pH 5 buffer solution (McGibbon et al, 1990). Samples were irradiated for the equivalent of 33 days of natural sunlight, in the presence of a dark control. The photolysis half-life for hexaflumuron was found to be approximately 6.3 days. The major degradation products were CO_2, difluorobenzamide and hydroxyl-aniline.

The US EPA method entitled Fate, Transport and Transformation Test Guidelines OPPTS 835.2210 Direct Photolysis Rate in Water by Sunlight states that the test method is applicable to all chemicals which have a UV/absorption maxima in the range of 290-800 nm or those which have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Hexaflumuron has an UV maxima at 203.2 nm and 252.4 nm (Smith, 1999). The UV/VIS absorption spectra also shows that there is a minimal absorption above 290 nm and no absorption above 316 nm.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not performed.

5.1.2.2 Screening tests

Ready biodegradability

The ready biodegradation study of hexaflumuron in compliance with OECD TG 301B was performed at 21.9 ± 0.1ºC and pH 7.5 (mean) with sampling on days 3, 6, 8, 10, 14, 17, 21, 24, 28 and 29 (Serak, 2006). The inoculum was a mixture of activated sludge, secondary effluent, and soil suspension. This test revealed that the substance was not readily biodegradable (6.0% and 4.1% theoretical CO_2, in duplicate samples, after 29 days). The reference substance (sodium benzoate) exhibited a normal pattern of degradation (64.3% by day 29).

5.1.2.3 Simulation tests

Biodegradation in soil

The aerobic and anaerobic soil metabolism of hexaflumuron was determined in accordance with US EPA guidelines 162-1 and 162-2 (Racke, 1993). Soils used were Catlin silty loam, Hanford sandy loam, Alconbury clay loam and Castle Rising sandy loam. The samples of each soil were treated with both ^{14}C-benzoyl-hexaflumuron and ^{14}C-aniline-hexaflumuron at 25ºC.

Under aerobic conditions, no significant extractable metabolites were produced in the soils treated with ^{14}C-benzoyl-hexaflumuron. However, in a supplemental experiment 2,6-difluorobenzoid acid was identified at levels up to 4% of applied radioactivity (AR).

Soils treated with ^{14}C-aniline-hexaflumuron contained 12-20% of AR after 60-120 days as the major metabolite 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenylamine, which decreased to 2-9% of AR after 365 days. A second minor metabolite was present at ~4% of AR in soils treated with ^{14}C-aniline-hexaflumuron. The major metabolite in the aerobic soil study conducted at 25 °C was the amine 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenylamine, which reached a maximum at 30 days after treatment (DAT) (Hanford, 12% AR), 60 DAT (Alconbury, 18%) and 120 DAT (Catlin, 18% AR and Castle Rising, 20 % AR). In Hanford soil, the amount of the amine at 30, 60, and 120 DAT was 12, 10, and 7% AR, respectively. In the Alconbury soil, the amine was present at 18 and 13% AR at 60 and 120 DAT, respectively. Modelling both formation and decline, the average half-life of the amine was calculated to be 43 days (geomean 38 days) while the average parent DT_{50} was 108 days (geomean 104 days) in these models (note, there were negative confidence intervals (Hanford)).

Using equation (25) of the Technical Guidance Document on Risk Assessment, part II, 2003:

$$DT_{50}(X^{o}C)=DT_{50}(t).e^{(0.08(T-X))}$$

The normalised DT_{50} obtained at 12°C for this metabolite was 107 days. 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenylurea was identified at low levels, not greater than 5% through 120 DAT. The only degradation from the ^{14}C-benzoyl-labeled hexaflumuron was $^{14}CO_2$ and very low levels (< 5% AR) of 2,6-difluoro-benzoic acid.

The aerobic soil studies (Racke, 1993) conducted at 25°C were reanalysed per FOCUS Kinetics (CAKE version 1.3) and recalculated using only 0-120 day data, according to OECD TG 307 (range 94 – 129 days (arithmean = 100 days, geomean = 99 days)). The predicted geomean DT_{50} was normalised (using equation above) to 12°C, resulting in 280 days.

The degradation of ^{14}C-aniline-labeled hexaflumuron in Castle Rising soil (sandy clay loam) was studied at different moisture contents ranging from 50-100% 0.33 bar moisture (at 25 °C) and 10-35 °C (at 75% 0.33 bar moisture). The soils were dosed at a rate of 1 µg/g soil. For samples incubated at 75% 0.33 bar moisture, the DT_{50} ranged from 190 days at 10 °C through 56 days at 35 °C.

5.1.3 Summary and discussion of degradation

Hexaflumuron is non-readily biodegradable and has an aerobic soil metabolism half-life of approximately 280 days, at 12ºC. Therefore, this substance is considered to be not rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The desorption characteristics of hexaflumuron and his metabolites were studied, according with US EPA guidelines 163-1, in 4 agricultural soils after aging for 30 days in biometer flasks (Racke, 1993). The 4 soils varied in pH (6.6-8.1), organic carbon content (0.65-8.62%), and clay content (6-35%). One soil was a sandy loam with low organic carbon content. Freundlich adsorption isotherms were not measured; the experiments were conducted at only the highest possible use rate.

The results show that hexaflumuron has little tendency to desorb from soil into water (Kd = 147-1326 L/kg), and therefore, little tendency to migrate through a soil/water column.

The data also indicate that the metabolites amine and phenylurea have slight potential for movement (Kd = 35-392 L/kg and 32-142 L/kg, for the amine and urea, respectively), and the benzoic acid (detected at 2-4% of AR in samples of 4 soils treated with ^{14}C-benzoyl-labeled hexaflumuron) has a moderate potential for movement (Kd = 3-27 L/kg).

The desorption characteristics of hexaflumuron was also estimated by a quantitative structural activity relationship (QSAR) method using KOCWIN v2.00 (US EPA EPI Suite), a K_{OC} value of 7272 L/kg was calculated.

5.2.2 Volatilisation

Hexaflumuron has low volatility (vapour pressure is 5.9×10^{-9} Pa at 25°C (Macdonald et al, 1986)).

The photo-oxidative degradation of hexaflumuron in air was estimated by a QSAR method using the AOPWIN v1.90 (US EPA EPI Suite). Assuming a hydroxyl radical concentration of 1.5×10^6 OH radicals/cm^3 the half-life is 6.1 hours or 0.51 days for a 12-hour daylight period.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 52: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation in bluegill sunfish US EPA 165-4	$BCF_{edible\ tissue}$ = 3783 $BCF_{non\text{-}edible\ parts}$ = 7667 $BCF_{whole\ fish}$ = 5600	Specie: *Lepomis macrochirus* 28-day exposure period	Ritter, 1990

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log K_{OW} (5.68 at 20°C) (Macdonald et al, 1986) and low water solubility (0.027 mg/L) (Macdonald et al, 1986) predict a high aquatic bioaccumulation.

5.3.1.2 Measured bioaccumulation data

Bioconcentration study was performed according to US EPA guideline 165-4. Bluegill sunfish (*Lepomis macrochirus*) were continuously exposed to a ^{14}C-hexaflumuron mean concentration of 6 ± 1 µg/L in tap water for 28 days at a temperature ranging from 18.5 to 20.0ºC (Ritter, 1990). The BCF in fish results was 3783, 7667, and 5600 for edible tissue, non-edible parts, and whole fish, respectively. The lipid content of the fish was not recorded in the original study and lipidic normalisation was not performed.

Uptake rate constants were not determined; in edible parts of the fish, the plateau values were reached after 7 days; in non-edible parts and whole fish the plateau values were reached after 14 days. Depuration half-lives were calculated to be approximately 6.5, 13.5, and 10.4 days in edibles, non-edibles, and whole fish, respectively.

5.3.2 Summary and discussion of aquatic bioaccumulation

Considering the BCF values, hexaflumuron has a high bioaccumulation potential for classification purposes, fulfilling the CLP criterion (BCF ≥ 500 in fish).

5.4 Aquatic toxicity

Table 53: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Acute toxicity to bluegill sunfish OECD 203	LC_{50} > 141.86 µg/L LC_{100} > 141.86 µg/L	Specie: *Lepomis macrochirus* Exposure: Acute 96-h, static Measured concentration 19.2 to 20.2ºC pH 7.21 to 7.8	Willis & O'Connor, 1987a
Acute toxicity to rainbow trout OECD 203	LC_{50} > 489.78 µg/L LC_{100} > 489.78 µg/L	Specie: *Oncorhynchus mykiss* Exposure: Acute 96-h, static Measured concentration 12.0 to 12.7ºC pH 7.56 to 7.69	Willis & O'Connor, 1987b
Aquatic invertebrates short-term toxicity OECD 202	EC_{50} = 0.11 µg/L 95% CI = 0.103 – 0.120 µg/L	Specie: *Daphnia magna* Exposure: 48 h, static Measured concentration 19.8 to 20.0ºC pH 7.70 to 8.15	Willis, 1987
Aquatic invertebrates long-term toxicity OECD 202 (version of 1984)	NOEC = 0.0029 µg/L	Specie: *Daphnia magna* Exposure: 21 d, semi-static (renewal of solution 3 times/week) Based on survival, reproduction and growth Measured concentration 18.4 to 19.9ºC pH 7.62 to 8.52	Jenkins et al, 1988
Growth inhibition test on algae OECD 201	E_rC_{50} > 1.91 mg/L E_bC_{50} > 1.91 mg/L NOEC > 1.91 mg/L	Specie: *Selenastrum capricornutum* Exposure: 96 h Measured concentration 23.8 to 23.9ºC pH 6.8 to 9.6	Smith & Thompson, 1988
Inhibition to microbial activity (aquatic) EC method C.11	EC_{50} > 100 mg/L	Exposure: 3 h Nominal concentration 18.6 to 20.2ºC pH 7.9 to 8.5	Jenkins, 1997

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Two studies were performed to assess the acute toxicity of hexaflumuron in fish.

In the first study (Willis & O'Connor, 1987a), hexaflumuron was tested for its acute toxicity to the bluegill sunfish (*Lepomis macrochirus*) over a 96-hour exposure period following OECD TG 203. Two tests were conducted. Test 1 was conducted under static conditions with nominal concentrations of 100, 200, 300, 400 and 500 µg/L dispersed in a DMSO carrier. A solvent control was also included and each treatment was replicated twice with 10 fish/vessel. Fish were observed

and mortality and sublethal effects recorded at 24, 48, 72 and 96 hours of exposure. Low levels of mortality were observed in all treatments not exceeding 10% after 96 hours exposure and no sublethal effects were recorded. The LC_{50} is greater than the highest nominal concentration tested (500 µg/L). The mean measured concentrations of this treatment group were 468 µg/L at 0 hours and 43 µg/L at 96 hours (the concentrations were not stable over the 96h exposure period). Therefore, based on the geometric mean of these concentrations, the LC_{50} is > 141.86 µg/L.

The test 2 was conducted as a limit test under static conditions but no solvent carrier was used. A nominal concentration of 100 mg/L was tested. The control was replicated twice and the test item three times each with 10 fish/vessel. Fish were observed and mortality and sublethal effects recorded at 24, 48, 72 and 96 hours of exposure. No mortality and no sublethal effects were observed with this concentration in excess of the limit of water solubility.

In the second study (Willis & O'Connor, 1987b), hexaflumuron was tested for its acute toxicity to the rainbow trout (*Oncorhynchus mykiss*) over a 96-hour exposure period following OECD TG 203. The test was conducted under static conditions with nominal concentrations of 100, 200, 300, 400 and 500 µg/L dispersed in a DMSO carrier. A solvent control was also included and each treatment was replicated twice with 10 fish/vessel. Low levels of mortality were observed in all treatments not exceeding 5% after 96 hours exposure and no sublethal effects were recorded.

The LC_{50} is greater than the highest nominal concentration tested (500 µg/L). The mean measured concentrations of this treatment group were 752 µg/L at 0 hours and 319 µg/L at 96 hours (the concentrations were not stable over the 96h exposure period). Therefore, based on the geometric mean of these two mean measured concentrations, the LC_{50} is > 489.78 µg/L.

5.4.1.2 Long-term toxicity to fish

Not performed.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of hexaflumuron to *Daphia magna* (immobilisation) was tested according to OECD TG 202 (Willis, 1987). This study was conducted as a 48-hour static test and 20 daphnids (5 individuals/replicate, 4 replicates/dose level) were exposure to nominal concentrations of 0 (control), 0 (acetone control), 0.03, 0.04, 0.07, 0.12 and 0.32 µg/L. Water quality parameters viz., temperature, pH and dissolved oxygen were measured during the test and found to be within the guideline limits.

The 48h-EC_{50} of hexaflumuron was determined as 0.11 µg/L with 95% confidence intervals of 0.103 to 0.120 µg/L, considering the mean measured concentrations of 0.0358, 0.062 and 0.203 µg/L (corresponding to nominal concentrations of 0.03, 0.07 and 0.32 µg/L, respectively).

5.4.2.2 Long-term toxicity to aquatic invertebrates

The long-term toxicity of hexaflumuron to *Daphia magna* was tested according to the OECD TG 202, version from 1984 (Jenkins et al, 1988). This study was conducted as a 21-day semi-static test and 40 daphnids (10 individuals/replicate, 4 replicates/dose level) were exposure to nominal concentrations of 0 (water control), 0 (solvent water control), 0.001, 0.005, 0.01, 0.05 and 0.1 µg/L.

Water quality parameters viz., temperature, pH and dissolved oxygen were measured during the test and found to be within the guideline limits.

The 21d-NOEC of hexaflumuron, based on survival and reproduction of *Daphia magna*, was 0.0029 µg/L, considering the mean measured concentrations of 0.0029, 0.0101 and 0.101 µg/L (corresponding to nominal concentrations of 0.001, 0.01 and 0.1 µg/L, respectively).

5.4.3 Algae and aquatic plants

The toxicity study of hexaflumuron to the green alga *Selenastrum capricornutum* was performed according to OECD TG 201 (Smith & Thompson, 1988). In this study, culture medium containing 1.07 x 10^4 cells/mL of alga was exposed to hexaflumuron at the mean measured concentrations of 0.046, 0.070, 0.14, 0.51 and 1.91 mg/L. The algal population was observed at 0, 24, 48, 72 and 96h.

Exposure to hexaflumuron up to the highest measured concentration of 1.91 mg/L had no effect on the biomass or growth rate in the study. Hence the E_rC_{50}, the E_bC_{50} and the NOEC for hexaflumuron were > 1.91 mg/L.

5.4.4 Other aquatic organisms (including sediment)

The potential for hexaflumuron to inhibit the respiration of municipal activated sludge was evaluated using the EC method C.11 (Jenkins, 1997). Samples of activated sludge were exposed to a range of concentrations of the test material for three hours. Their rates of oxygen consumption were determined. Two tests were conducted; a preliminary screen, at nominal concentrations of 1, 10 and 100 mg/L and a final limit test in which triplicate vessels contained the test material at a nominal concentration of 100 mg/L. The reference inhibitor 3,5-dichlorophenol (3,5-DCP) was employed in each test, as a positive control.

The 3h-EC_{50} of 3,5-DCP was determined to be between 10 and 30 mg/L which is within the accepted range of 5 to 30 mg/L. The average respiration rates of control mixtures at the beginning and conclusion of the experiment, 27.4 and 26.3 mg O_2/L/h, were within the accepted range of 15% of each other. The temperatures of the reaction mixtures ranged from 18.6 to 20.2°C during the experiment, within the guideline range of 20 ± 2°C.

Hexaflumuron at nominal concentration of 100 mg/L did not inhibit the respiration rate of the activated sludge compared to the control mixtures (no test chemical added).

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Considering that the 48h-EC_{50} = 0.11 µg/L (0.00011 mg/L) value obtained for *Daphnia magna* is lower than 1 mg/L, hexaflumuron meets the criteria for classification as Aquatic Acute 1 for environmental hazard according to CLP criteria. As this value is within the range of 0.0001-0.001 mg/L an Acute M-factor of 1,000 is allocated.

Taking into account that there are only adequate chronic toxicity data available for two trophic levels (invertebrates and algae), it was both assessed the information on chronic toxicity (and rapid degradation) and the combine two types of information, i.e. acute toxicity data and environmental fate data (degradability and bioaccumulation data).

Hexaflumuron is not rapidly degradable and considering that the 21d-NOEC value for *Daphnia magna* is lower than 0.01 mg/L (0.0000029 mg/L), it meets the classification criteria for Aquatic Chronic 1.

Applying the surrogate system, the lowest acute aquatic toxicity is $EC_{50} \leq 1$ mg/L (48h-EC_{50} = 0.00011 mg/L for *Daphnia magna*), and is non-rapidly degradable and has a BCF > 500 (BCF = 5600 in whole fish), hence Aquatic Chronic 1.

Therefore, hexaflumuron meets the criteria for classification as Aquatic Chronic 1 for environmental hazard according to CLP criteria.

Applying the most stringent outcome the M-factor is based on the chronic value for *Daphnia magna* (0.0000029 mg/L) within the range of 0.000001-0.00001 mg/L (and non-rapidly degradable), a Chronic M-factor of 10,000 is allocated.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

According to CLP Regulation criteria

Classification: Aquatic Acute 1; H400
Aquatic Chronic 1; H410
Acute M-factor = 1,000
Chronic M-factor = 10,000

Labelling:

Signal word: Warning

Pictograms: GHS09

Hazard statements: H410: Very toxic to aquatic life with long lasting effects.

Precautionary statements: P273: Avoid release to the environment.

P391: Collect spillage.

P501: Dispose of contents/container to … … in accordance with local/regional/national/ international regulation (to be specified).

6 OTHER INFORMATION

7 REFERENCES

8 ANNEXES