Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

flufenoxuron

ECHA/RAC/ CLH-O-0000001741-79-01/A1

flufenoxuron

EC number: 417-680-3
CAS number: 101463-69-8

Adopted

10 June 2011

CONTENTS

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name:	**flufenoxuron**
EC Number:	**417-680-3**
CAS number:	101463-69-8
Registration number (s):	-
Purity:	≥ 950 g/kg
Impurities:	This information is confidential and then provided in confidential part of the dossier provided in appendix 1.

Proposed classification based on CLP criteria:

Hazard statements:

Lact. – H362

Aquatic. Acute 1 – H400

Aquatic. Chronic 1 – H410

Signal word: "*warning*"

Pictograms: GHS09.

Proposed classification based on Directive 67/548/EEC criteria:

R64; R33

N; R50/53

Proposed labelling:

Symbol(s): N

R-phrases: R64; R33; R50/53

S-phrases: S2, S22, S36/37, S46, S60, S61

Proposed specific concentration limits and M-factors (if any):

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 10 000.

Long-term aquatic hazard: category Chronic 1, M-factor: 10 000.

Under Directive 67/548/EEC, SCL are proposed for environment:

Specific concentration limits:	
$C \geq 0.0025$ %	N, R50/53
$0.00025\ \% \leq C < 0.0025\ \%$	N, R51/53
$0.000025\ \% \leq C < 0.00025\ \%$	R52/53

Proposed notes (if any):

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: flufenoxuron

EC Number: 417-680-3

CAS Number: 101463-69-8

IUPAC Name: 1-[4-(2-chloro-α,α,α-trifluoro-p-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl)urea

1.2 Composition of the substance

Chemical Name: flufenoxuron

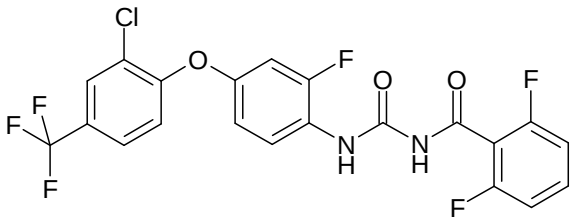
EC Number: 417-680-3

CAS Number: 101463-69-8

IUPAC Name: 1-[4-(2-chloro-α,α,α-trifluoro-p-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl)urea

Molecular Formula: $C_{21}H_{11}Cl\ F_6N_2O_3$

Structural Formula:



Molecular Weight: 488.8 g/mol

Concentration range (% w/w): ≥ 95 % w/w

Information on impurity is confidential and then provided in confidential part of the dossier provided in appendix 1.

1.3 Physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	White crystalline solid	Kaestel R.,2001,
VII, 7.2	Melting/freezing point	4.2	169-172°C	Camilleri P. *et al*., 1986 Daum A., 2001,
VII, 7.3	Boiling point	4.3	Melting occurs under decomposition, therefore, no boiling point could be observed.	Camilleri P. *et al*., 1986, Daum A., 2001,
VII, 7.4	Relative density	4.4 density	1.649g/cm^3	Kaestel R., 2001c,
VII, 7.5	Vapour pressure	4.6	6.52x10^{-12} Pa at 20 °C 2.32 x10^{-11} Pa at 25 °C (by extrapolation)	Langner E.J.,1988, Rice P., 2000,
VII, 7.6	Surface tension	4.10	49.4 nN/m at 1.0% w/w	Kaestel R.,2001,
VII, 7.7	Water solubility	4.8	pH 7: 1.36 µg/l at 25°C pH 4: 1.86 µg/l at 25°C pH 9: 3.69 µg/l at 25°C	Langner E.J., 1988,
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7 partition coefficient	5.97 (value estimated by QSAR)	Kowwin (v 1.67)
VII, 7.10	Flammability	4.13	Not flammable	Van Helvoirt J.A.M.W.,1990,
VII, 7.11	Explosive properties	4.14	Flufenoxuron is not explosive when exposed to thermal or mechanical stress.	Van Helvoirt J.A.M.W.,Cardinaals J.M., 1990,
VII, 7.12	Self-ignition temperature		No auto-ignition (no exothermic or endothermic reaction up to 400 °C).	Van Helvoirt J.A.M.W.,1990,
VII, 7.13	Oxidising properties	4.15	No oxidising properties	Van Helvoirt J.A.M.W.,1990,
XI, 7.16	Dissociation constant	4.21	pKa = 10.2	CamilleriP., Langner E.J.,1986,
	Thermal stability	4.19	Stable up to 150 °C under N2 atmosphere and under air	Daum, A.,2001,
	Solubility in organic solvents	4.9	n-heptane: < 10 mg/l toluene: 3500 mg/l dichloromethane: 16000 mg/l methanol: 3500 mg/l acetone: 83000 mg/l ethyl acetate 55000 mg/l at 20°C	Daum A., 2001,

Table 1.3: Summary of physico- chemical properties

2 MANUFACTURE AND USES

Not relevant for this dossier.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Flufenoxuron is not classified according to Annex VI of CLP Regulation.

3.2 Self classification(s)

The following classification was first proposed by the industry in the scope the Biocidal Product Directive (98/8/CE): N; R50/53.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

Hydrolysis

Flufenoxuron is hydrolytically stable at pH 4, 5, and 7, but is hydrolyzed at pH 9 with an half-lives of about 90 days at 25ºC and about 1 day at 50ºC (Hassink, 2003). Therefore, hydrolysis of Flufenoxuron only occurs under alkaline conditions and is unlikely to occur in the environment.

Photolysis in water

Photolysis in water was tested according to the Commission Directive 94/37/EEC amending Council Directive 91/414/EEC. Briefly, direct photolysis was studied using [fluoroaniline-ring-U-^{14}C]-flufenoxuron and [difluorobenzamide-ring-U-^{14}C]-flufenoxuron exposed to a xenon lamp with a light intensity of about 3 mW/cm^2 and a cut-off for wavelengths < 290 nm to simulate natural sunlight. The duration of the experiment was 15 days under continuous irradiation, at temperature of 22 ± 1°C, and pH 7.0. For the determination of the quantum yield (ϕ^c_E) of Flufenoxuron, a mixture of *p*-nitroacetophenone and pyridine was used as chemical actinometer.

The quantum yield for flufenoxuron was determined to be 1.75 x 10^{-3}. The calculated half-life of flufenoxuron in the top layer of aqueous systems in Spring and Summer varied from 39.2 days in April to 21.7 days in June (Hassink, 2003a).

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

The ready biodegradability of flufenoxuron was determined by testing according to OECD 301B (modified Sturm test) and 301D (closed bottle test) with sewage sludge at test substance concentrations equal to 3 mg/L and 20mg/L, respectively. No more than 4% degradation of the test substance was observed in either test. Flufenoxuron is considered to be **not readily biodegradable** (Turner and Watkinson, 1986).

4.1.2.3 Simulation tests

Water/Sediment

Two studies were available for water/sediment degradation. The first study was conducted according to OECD Guideline 308 (Ebert, 2003). In this study, water/sediment distribution and degradation were tested in two natural systems with ^{14}C-labeled flufenoxuron incubated in the dark at 20 ± 1°C for up to 100 days. It was concluded that flufenoxuron moved rapidly from water into sediment with a DT_{50} in the water of 0.3 to 0.4 days and was degraded with a DT_{50} in the whole system of 85 to 116 days at a reference temperature of 12°C (45 to 61 days at 20°C). The use of sterilized vessels indicated that the formation of metabolites, bound residues and finally CO_2 is dependent on microbial activity in the systems.

The second studies, in outdoor conditions (Fent, 2003), confirmed the behavior of the molecule with a rapid move from the water to the sediment compartment. The only metabolite in water and sediment was the urea metabolite (Reg. No 4064702) detected up to 9.3% and 12% of the TAR in water and sediment respectively.

Soil

Three key studies are available for the biodegradation of flufenoxuron in soil (Goodyear and Gross, 2001 ; Stephan and Ebert, 2003). All tests were performed according OECD 307 guidelines. Flufenoxuron degradation was studied in aerobic conditions with different soils and radiolabelings. An half-lives of 36 to 124 days (at 20°C) was observed. These values were recalculated to a reference temperature of 12°C. The DT_{50} for Flufenoxuron were 68 and 235 days at 12°C. Flufenoxuron are therefore not expected to be degraded rapidly in soils.

4.1.3 Summary and discussion of persistence

Considering the results above and according to the Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures (part 4), flufenoruxon is not expected to be degraded rapidly in the environment. .

4.2 Environmental distribution

The behaviour of Flufenoxuron in aquatic systems is mostly characterized by its very low water solubility, high sorption to sediment, no readily biodegradability and UV-instability.

4.2.1 Adsorption/desorption

Adsorption/desorption characteristics of Flufenoxuron have been studied on different soils with two radiolabellings [Carbonyl-C^{14}]-Flufenoxuron (Hill and Standen, 1993) and [Amide ring-C^{14}]-Flufenoxuron (Rosenwald, 2002). More than 84% of the substance is strongly adsorbed on soil with an adsorption coefficient based on organic carbon content varying from 88240 to 289747. Desorption is weak with observed desorption coefficients 4020 and 5895. A Koc mean value of 157 643 between all the results obtained have been calculated. It can therefore be concluded that Flufenoxuron is strongly adsorbed by soil components.

4.2.2 Volatilisation

Flufenoxuron has a very low volatilisation potential (vapor pressure 6.52×10^{-12} Pa at 20 °C).

4.2.3 Distribution modelling

No relevant data available.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

4.3.1.2 Measured bioaccumulation data

Different studies have been carried out in order to assess the bioaccumulation process of flufenoxuron in aquatic organisms.

In the first study (Chapleo *et al*., 2003), fish were exposed to flufenoxuron at a nominal exposure level of 0.040 µg/L, for 60 days. After termination of the exposure, radioactivity levels in whole fish decreased with a half-life of 21 days. Bioconcentration factors (BCF) in whole fish were 25920 and 24187 for the Fluoroaniline label and the Difluorobenzamide label, respectively (which correspond to 35027 and 32685 with correction for lipid content of the test fish (3.7%)). Flufenoxuron was metabolically stable in trout. No marked differences between the two sites of radiolabel were observed.

The second study was performed according to OECD 305E (Gill and Gould, 1990). Fish were exposed to Flufenoxuron at a nominal exposure level of 0.040 µg/L and 0.31 µg/L, for 19 days, with a depuration time of 11 days. The BCF was considered to be 15700 and 16130, respectively.

Conclusion: Based on the study results above it can be concluded that flufenoxuron meets the classification criterion for bioaccumulation potential, i.e. BCF≥500.

4.3.2 Terrestrial bioaccumulation

Not revelant

4.3.3 Summary and discussion of bioaccumulation

The measured BCF for flufenoxuron meets the criterion for bioaccumulation potential according to both DSD and CLP.

4.4 Secondary poisoning

No available data

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Absorption

- Single oral low dosage (3.5 mg/kg bw)

In a non-cannulated rat study (Huckle, 1988), the minimal absorbed fraction in 168 hours was 72.57% in males and 71.56% in females based on the radioactivity in urine, cage wash and carcass and organs. Similar values were found in another study (Hawkins, 1992) performed with non cannulated rats (minimal absorption = 76.73 % in males and 84.57% in females).

In a bile-duct cannulation study in rats (Kirkpatrick, 1992), the bioavailability of flufenoxuron was approximated to be 56 % (females) to 81 % (males) based on the sum of urinary and biliary excretion as well as the amounts of radioactivity in carcass and organs. In another study (Hawkins, 1992), the minimal absorbed fraction was 79.76% (males) and 92.15% (females) in cannulated rats.

A study was also performed in dogs (Hawkins, 1988). The minimal absorbed fraction in 7 days was estimated at 27.29% in males and 21.23% in females. However, as about 15 % of the dose was not recovered and as diarrhoea contained up to 50 % of the dose (in one male), these values are largely underestimated.

- ***Single oral high dosage (3.5 mg/kg bw)***

For high dose, the absorption rates determined are lower than 15 % for male and female rats, faecal excretion being the main route of excretion (higher than 85 %) (Huckle, 1987; Hawkins, 1992).

Dermal absorption

No data are available on the active substance alone.

Distribution

After a single oral dose of 3.5 mg/kg bw, flufenoxuron was well distributed in the carcass and organs, where 66.35% and 67.67 % of the dose were found after 168 hours in female and male rats respectively (Huckle, 1988).

In another test performed with a single low or high dose of flufenoxuron and including a tissue distribution study (Hawkins, 1992), the highest concentrations of radioactivity were found in adrenals, GI-tract, liver and bone marrow (6 to 28 µg/g tissue) at 4 hours. At 20 and 168 hours, the highest concentrations were detected in the fat, while the levels in other tissues had generally decreased.

After a 28-day treatment in female rats with 14C-flufenoxuron at 3.5 mg/kg bw an equilibrium concentration (plateau level) was close to being achieved for the majority of tissues. The radioactivity was well distributed throughout the carcass, with fat showing the highest

concentrations of radioactivity (144 µg/g), and the lowest tissue residues were detected in the kidney (11 µg/g). Blood residues were 3 µg/g (Morrison and Huckle, 1988).

Metabolism

After oral dosing, only small amounts of flufenoxuron were metabolized in the rat. Unchanged substance was the major component in the tissues (in particular in the fat where it was the single component detected) and faeces. The metabolites found indicated that the absorbed flufenoxuron was metabolized by cleavage of the benzoyl urea linkage adjacent to the 2, 6-difluorobenzoyl moiety.

Metabolism and kinetic studies in male and female beagle dogs at dose levels of 3.5 mg/kg bw (Hawkins *et al*., 1988) and 500 mg/kg bw (Greenough *et al*., 1988) indicated that kinetic and metabolic behavior of flufenoxuron is comparable in dogs and rats (distribution of flufenoxuron between blood, fat, bone marrow, liver, and kidney similar to that found for the rat and elimination of flufenoxuron from the tissues during the off-test recovery period at a rate corresponding to mean half-lives of 20 to 38 days).

Excretion

At 350 mg/kg bw of flufenoxuron (Huckle, 1987 and Hawkins, 1992), excretion occurred mainly via faeces (85 % within 72 hours) while urinary excretion amounted to less than 1 %.

In non-cannulated rats exposed to 3.5 mg/kg bw of flufenoxuron, the excretion was slow: excretion via faeces amounted to 21 – 24 % of dose within 168 hours, urinary excretion accounted for 5 % (Huckle, 1988). In another study at the same dose (Hawkins, 1992), 12 to 19% was excreted in the faeces and 24 to 30% in the urine. There were no significant sex-related differences regarding routes of excretion. Also, excretion patterns after single and multiple oral administrations were similar.

In cannulated rats given a single oral dose of 3.5 mg/kg bw, biliary excretion accounted for 19 % for males and 6.7 % for females, of the dose, after 48 h (Kirkpatrick, 1992). Less than 3 % were excreted in the urine. Sex related differences were observed in the faecal elimination: 4% in males versus 30.2 % in females. This sex difference was only observed in this study performed with only 3 animals/sex/group and was not supported by any biological explanation. In another study in cannulated rats exposed to the 3.5 mg/kg bw of flufenoxuron (Hawkins, 1992), 4% to 11% of the dose were excreted in the faeces. Biliary excretion accounted for about 5% and 10% to 14% was found in the urine.

After oral administration of flufenoxuron to rats at a low dose for 28 days, the mean elimination half-life was 34 days, with liver having the highest half-life (48 days) and the carcass and fat the lowest (28 days) (Morrison and Huckle, 1988). Study in dogs exposed to 500 mg/kg bw in the diet for 19 weeks (Greenough, 1988) indicated that elimination of flufenoxuron from the tissues during the off-test recovery period appeared at a rate corresponding to mean half-lives of 20 to 38 days.

After oral administration of 14C-flufenoxuron to male and female rats at dose levels of 3.5 mg/kg bw and 350 mg/kg bw (Hawkins *et al*., 1992), the radioactivity was excreted from the blood with a half-life of ca. 200 – 400 h at the low dose level and 22 - 37 h at the high dose level.

Flufenoxuron was excreted in milk in lactating female rats, at levels of 450 ± 377 ppm in milk at day 1 post-partum to 9.4 ± 6.1 ppm at day 14 post-partum (Masters, 1996) after an oral exposure of 20,000 ppm (equivalent to about 1633 mg/kg bw/d) from 10 weeks prior to a 2-week mating period until parturition. The depletion half-life time was 7.6 and 2.3 days in fat and milk, respectively.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 2: Summary of acute oral toxicity studies

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Remarks**	**Reference**
Oral (gavage)	OECD 401	Rat Fischer 344 M/F 5/sex/group	5000 mg/kg 14 days post-exposure	>5000 mg/kg	Flufenoxuron (in CMC) No systemic toxicity	Gardner, 1989
Oral (gavage)	OECD 401	Rat Fischer 344 M/F 5/sex/group	3000 mg/kg 14 days post-exposure	>3000 mg/kg	Flufenoxuron (in DMSO) 1/10 rats administered 3,000 mg/kg bw died; unspecific clinical signs reversible within 2 days	Price, 1986

5.2.2 Acute toxicity: inhalation

Table 3: Summary of the acute inhalation toxicity study

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Remarks**	**Reference**
Inhalation	OECD 403	Albino rat Sprague-Dawley M/F 5/sex group/dose	Nominal 8.9 mg/l Analytical 5.1 mg/l 14 days post-exposure	> 5.1 mg/l (dust aerosol; MMAD 3.6 µm)	LC_{50} 4-hour nose-only inhalation No systemic toxicity, no local irritation	McDonald, 1986

5.2.3 Acute toxicity: dermal

Table 4: Summary of the acute dermal toxicity study

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Remarks**	**Reference**
Dermal	OECD 402	Rat Fischer 344 M/F 5/sex/group	2000 mg/kg~ 24 hours 14 days post-exposure	>2000 mg/kg	No systemic toxicity, no local irritation	Price 1986

5.2.4 Acute toxicity: other routes

No data

5.2.5 Summary and discussion of acute toxicity

The oral toxicity of flufenoxuron in rats, tested in two limit test studies (Gardner, 1989; Price, 1986) using different vehicles, is low (LD_{50} above 3,000 mg/kg bw). No specific clinical symptoms were observed. In the acute oral toxicity study using DMSO as vehicle, unspecific symptoms were observed within the first two days after dose administration. One of 10 rats given 3,000 mg/kg bw flufenoxuron suspended in DMSO died. No abnormalities were detected upon necroscopy examinations except for compacted powder in the stomach associated with mucosal haemorrhage in the rat that died. Based on the lowest dose-level study realized, the only with reporting treatment-related effect including one death, the overall LD_{50} is assessed to be above 3,000 mg/kg bw.

Flufenoxuron is of low toxicity to rats after dermal application of the test substance moistened with water for 24 h (Price, 1986), with an LD_{50} value above 2,000 mg/kg bw causing neither mortality nor systemic toxicity. In addition, no local reaction was observed at the application site.

The inhalation toxicity (dust aerosol study, MMAD 3.6 µm for 4 h) of flufenoxuron in Sprague-Dawley rats is regarded to be low (LC_{50} > 5.1 mg/l/4h). No mortalities or other treatment-related adverse effects were observed in this study (McDonald, 1986).

No classification for acute toxicity is required for flufenoxuron.

5.3 Irritation

Table 5: Summary of skin irritation

Species	**Method**	**Average score 24, 48, 72 h**		**Reversibility yes/no**	**Result**	**Reference**
New Zealand White rabbit	OECD 404	Erythema 0, 0, 0	Edema 0, 0, 0	n.a.	Not a skin irritant	Price 1986

Table 6: Summary of eye irritation

Species	Method	Average Score				Result	Reversibility yes/no	Reference
		Cornea	Iris	Redness Conjunctiva	Chaemosis			
New Zealand White rabbit	OECD 405	0	0	0.33	0	Not an eye irritant	yes	Price 1986

Flufenoxuron does not meet the EU classification criteria for irritation following administration to the skin and eyes of New Zealand White rabbits (result for redness of the conjunctiva was less than the score of 2.5 according to the Directive 67/548/EEC criteria). No data on the potential of flufenoxuron to induce respiratory irritation are available.

5.4 Sensitisation

Table 7: Summary of skin sensitisation

Species	Method	Number of animals sensitized/total number of animals	Result / remarks	Reference
Guinea pig	Magnusson and Kligman (GPMT) Intradermal induction: 5 % in corn oil Dermal induction: 50 % in aqueous CMC Dermal challenge: 25 % in aqueous CMC	0/10	Not a skin sensitiser No concurrent positive control; separate study with alpha-hexylcinnamaldehyde performed twice a year (last control study started 5 months before the study performed with flufenoxuron) was clearly positive.	Gamer AO, Leibold E 2005

Flufenoxuron was not a skin sensitizer in the Guinea pig Magnusson & Kligman Maximisation test. No data on the potential of flufenoxuron to induce respiratory sensitisation are available.

5.5 Repeated dose toxicity

5.5.1 Repeated dose toxicity: oral

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

Flufenoxuron was administrated through the diet to five groups of 7 males and 7 females Fischer 344 rats at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (equivalent to 4.8-5.3; 49-53; 475-534; 997-1,067; 5,147-5,432 mg/kg bw/d in males and females) for 28 days; concurrently, control groups (14 males and 14 females) were fed with basal diet (Esdaile, 1986a). Several parameters were modified: increasing weight of spleen and heart for males at 50,000 ppm, variations in the clinico-chemicals dosages (triglycerid, albumin or beta-globulin) from 5000 ppm. An apparent slight increase in methaemoglobin (below the level of accuracy of instrumentation) was observed but some interrogations are raised about the relevancy of these findings due to the use of a non specific analysis method (CO-Oximeter method) which can be associated with false

positive. Moreover, data from 2-year oral feed study in rats showed similar changes on methaemoglobin with the CO-Oximeter method whereas no effect was observed when a specific methaemoglobin detection method (method of Evelyn and Malloy) was used. So, NOAEL and LOAEL will be defined according to the clinical findings: NOAEL 500 ppm for males, based on a decrease in triglycerides, equivalent to 49 mg/kg bw/d, and a NOAEL of 10,000 ppm for females, based on an increased beta-globulin level, equivalent to 1067 mg/kg bw/d.

Flufenoxuron was administrated through the diet to five groups of 7 males and 7 females B6C3F1 mice at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (equivalent to 10.5-14.0; 110-142; 1,091-1,353; 2,142-2,811; 9,820-12,157 mg/kg bw/d in males and females) for 28 days; concurrently, control groups (14 males and 14 females) were fed with basal diet (Esdaile, 1991a). No adverse treatment-related effect was reported so the study supports a NOAEL of 50,000 ppm, the highest concentration tested (equivalent to 9,820 mg/kg bw/d for males and 12,157 mg/kg bw/d for females).

Flufenoxuron was administrated thought the diet to five groups of 10 males and 10 females Fischer 344 rats, at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (3.5-4.1; 35-41; 351-399; 689-820; 3,637-4,151 mg/kg bw/d in males and females) for 90 days; concurrently, a control group of 20 males and 20 females was fed with basal diet (Esdaile, 1987). No change in body weight and food consumption (excepted for males at 50,000 ppm where consumption increased since week 7) was reported. The animals showed slight anaemia in females from 500 ppm, as evidenced by significant decreases in haemoglobin (dose-related; reduction less than 10%) and changes in erythrocyte parameters in association with evidence of compensatory hematopoiesis (increased reticulocyte counts, decreases in myeloid:erythroid ratios). Increases in spleen weights of females at 5,000 ppm and higher dietary concentrations were considered to be related to the hematological effects of flufenoxuron. No signs of anaemia were seen in males, although evidence of compensatory hematopoiesis (decreased myeloid:erythroid ratio) was observed at the highest dose level of 50,000 ppm. A small but statistically significant increase in methaemoglobin at all dose levels was detected with the unspecific CO-Oxymeter method but could be considered as a false-positive value (see same results in the 28-day toxicity study in rat). Variations in clinico-chemical dosages are firstly observed at 500 ppm with an increasing level of cholesterol in females, a decreased triglycerid from 5000 ppm for both sexes as well as increased heart and liver weight for males and females respectively from 10,000 ppm. This study supports a NOAEL of 50 ppm (equivalent to 4.1 mg/kg bw/d in females) for females, based on hematological changes at the LOAEL of 500 ppm (equivalent to 41 mg/kg bw/d in females) and a NOAEL of 500 ppm (equivalent to 35 mg/kg bw/d in males) for males based on clinico-chemical findings at the LOAEL of 5000 ppm (equivalent to 35 mg/kg bw/d in males).

Flufenoxuron was administrated through the diet to five groups of 10 males and 10 females B6C3F1 mice at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (10-12; 103-124; 1,069-1,247; 2,139-2,482; 11,071-12,619 mg/kg bw/d in males and females) for 90 days; concurrently, a control group of 20 males and 20 females was fed with basal diet (Esdaile, 1988). Only males at 50,000 ppm had decreased body weight; food consumption was not affected by the treatment. A mild anaemia, as evidenced by significant decreases in haemoglobin (< 10 %) and decreases in erythrocyte parameters at high dose level for males and increases in serum bilirubin from 500 ppm (for both sexes), was also noted. Liver weights adjusted for terminal body weights were marginally increased over control values, attaining statistical significance in both sexes at 500 ppm and higher dose levels (by up to 8%). However, it can be noted that there is a lack of a convincing dose-response relationship. Clinico-chemical and organ weight variations were also observed since 10,000 ppm (increased heart weight, decreased triglycerid). Additional clinical chemistry changes included statistically significant decreases in blood urea nitrogen for males at 50,000 ppm and for females at 10,000 and above. These decreases were dose-related in females.

The 90-day dietary study in mice supported a NOAEL of 50 ppm (equivalent to about 10 mg/kg bw/day for male and 12 mg/kg b.w/day for female mice), based on hematological and clinico-chemical changes at the LOAEL of 500 ppm (equivalent to 103 mg/kg bw/day for males and 124 mg/kg bw/day for females).

Flufenoxuron was administrated through the diet to three groups of 4 males and 4 females Beagle dogs at dietary concentrations of 500, 5000 and 50,000 ppm (18-21; 163-182; 1,961-2,039 mg/kg bw/d in males and females); concurrently, a control group of 4 males and 4 females received basal diet (Greenough, 1987). Because of a diet formulation error during the first 2 weeks of treatment, the duration of the administration was extended to 15 weeks. No change was reported concerning body weight and food consumption, between treated and controls. These haematological effects were summarised in the table 8. Flufenoxuron-related minor anaemia was apparent in all treated groups, as revealed by changes in haemoglobin levels (reduction ≥ 10 % in males), erythrocyte parameters (first noted after 9 weeks of treatment) and increased reticulocyte counts.

Table 8:

Haematological effects in dogs (Greenough, 1987).

Test parameter		Week 9				Week 12				Week 15			
		0	500 ppm 18-21mg/kg/d	5000 ppm 163-182 mg/kg/d	50000 ppm 1,961-2,039 mg/kg/d	0	500 ppm 18-21mg/kg/d	5000 ppm 163-182 mg/kg/d	50000 1,961-2,039 mg/kg/d	0	500 ppm 18-21mg/kg/d	5000 ppm 163-182 mg/kg/d	50000 1,961-2,039 mg/kg/d
Red Blood Cells [10^{12}/l]	M	6.75	**5.97***	**5.80***	**5.49***	6.60	6.30	6.14	**5.45***	6.53	6.21	6.04	**5.41***
	F	6.89	6.27	6.05	5.90	6.81	6.21	6.22	6.14	6.82	6.55	6.12	6.33
Haemoglobin [g/dl]	M	15.8	**13.5*** **(-14.5%)**	**13.4*** **(-15%)**	**13.1**** **(-17%)**	15.1	14.4	14.2	**13.0*** **(-13%)**	15.0	13.9	13.7	**12.9*** **(-14%)**
	F	15.6	14.7	14.2	14.3	15.6	14.5	14.7	14.8	15.6	15.1	14.1	15.3
Hematocrit [ratio]	M	0.467	**0.417***	**0.420***	**0.410***	0.461	0.438	0.437	0.406	0.449	0.423	0.421	0.401
	F	0.472	0.447	0.441	0.437	0.470	0.442	0.448	0.452	0.470	0.454	0.432	0.460
Mean Corpuscular Volume (MCV) [fl]	M	69	69	**72***	**75***	69	69	70	74	68	67	68	**73***
	F	69	71	**72***	**74***	68	70	71	73	68	68	70	71
Mean Corpuscular Haemoglobin concentration (MCHC) [g/dl]	M	34.7	**33.3***	**32.5***	**32.6***	34.8	34.3	34.1	**33.4***	34.1	33.5	33.2	33.0
	F	33.6	33.7	32.9	33.3	34.6	34.4	34.4	**34.2**	33.9	34.0	**33.2***	34.0
Reticulocytes [%]	M	0.6	**1.0**	**1.8***	**1.6***					0.4	1.5	1.0	1.5
	F	0.8	1.1	2.0	1.6					1.0	0.7	1.0	1.3
Methaemoglobin [%]	M	0.80	1.07	**1.42***	**1.82***					0.61	0.99	**1.46***	**1.88***
	F	0.79	**1.10***	**1.30***	**1.80***					0.65	0.87	**1.23***	**1.69***
Sulfhaemoglobin [%]	M	0.12	0.23	**0.33***	**0.46***					0.10	0.16	**0.32***	**0.39***
	F	0.28	0.14	0.23	0.35					0.12	0.15	0.25	**0.43***

After 12 and 15 weeks, statistically significant haematological effects were confined to the 50,000 ppm group male, however the observed changes were smaller than those set in the classification criteria (Reduction in Hb at ≥20%). Methaemoglobin levels were detected by the specific method of Evelyn and Malloy and were elevated (dose-related) from 500 ppm in females and from 5,000 ppm in males. Furthermore, sulfhaemoglobin levels were statistically increased at 5,000 ppm and above for males and at 50,000 ppm for females..

Bone marrow hyperplasia was observed for all dogs at 5,000 ppm and above, and for 3 males and 2 females in the 500 ppm group. This effect likely reflects a compensatory response to the anaemia and was accompanied by Kupffer cell pigmentation in the liver and increased haemosiderin deposition in bone marrow, in the spleen and in the proximal tubules of the kidney.

Table 9: Histopathological findings related to anaemia in dogs exposed for 15 weeks (Greenough, 1987).

Histopathological findings		Dose levels (ppm)			
		0	500 ppm 18-21mg/kg/d	5000 ppm 163-182 mg/kg/d	50000 ppm 1,961-2,039 mg/kg/d
Liver, increased Kupffer-cell pigmentation	M	0/4	0/4	**4/4**	**4/4**
	F	0/4	1/4	3/4	**4/4**
Kidney, increased yellow pigment deposition in proximal tubules	M	0/4	0/4	0/4	2/4
	F	0/4	0/4	0/4	0/4
Spleen, increased haemosiderin	M	0/4	0/4	0/4	1/4
	F	0/4	0/4	0/4	1/4
Bone marrow, hyperplasia	M	0/4	3/4	**4/4**	**4/4**
	F	0/4	2/4	**4/4**	**4/4**
Bone marrow, increased yellow pigment deposition	M	0/4	0/4	0/4	**4/4**
	F	0/4	0/4	3/4	3/4

Higher cholesterol levels were observed for males at 5000 and 50,000 ppm. Absolute liver weights were significantly increased in all treated male groups, however, this was not dose dependent. In contrast, a dose-related and statistically significant increase of relative liver weights (organ to body weight ratio) was observed in males at ≥ 5,000 ppm only. While no NOAEL could be determined, this 15-wk feeding study in Beagle dogs supported a LOAEL of 500 ppm (equivalent to about 18 mg/kg bw/day in male and 21 mg/kg bw/day in female dogs) based on anaemia and increased levels of methaemoglobin.

Findings similar to those observed in the 90-day dog study were also apparent in the 52-week dietary toxicity study conducted in Beagle dogs (groups of 4 males and 4 females), at dietary concentrations of 10, 100, 500 and 50,000 ppm (0.37-0.39; 3.5-3.7; 19-20; 2018-1879 mg/kg bw/d in males and females); concurrently, a control group was fed with basal diet (Goburdhun, 1988). No treatment-related effect on body weight or food consumption was reported. A mild anaemia,

revealed by changes in haemoglobin level and erythrocytes parameters appeared in both sexes at 50,000 ppm. Platelet counts were statistically significantly increased in males at 50,000 ppm from week 13 and at 500 ppm from week 27. Methaemoglobin and sulfhaemoglobulin were increased over control levels at 50,000 ppm in both sexes at most time points of investigation and to a minimal degree also in females at 500 ppm. The haematological changes were maintained throughout the course of the study (Table 10).

Table 10: Haematological findings

Test parameter		Week 5					Week 13					Week 52				
Ppm		0	10	100	500	50000	0	10	100	500	50000	0	10	100	500	50000
mg/kg bw /day		0	0.37-0.39	3.5-3.7	19-20	2018-1879	0	0.37-0.39	3.5-3.7	19-20	2018-1879	0	0.37-0.39	3.5-3.7	19-20	2018-1879
Red Blood Cells [10^{12}/l]	M	7.07	6.49	6.95	6.70	**5.88*** **-17%**	7.33	**6.63*** **-9,5%**	7.19	**6.59*** **8,4%**	**6.08*** **-15,5%**	7.61	7.13	7.50	**6.80*** **10.6%**	**6.47*** **-15%**
	F	7.10	7.07	7.18	6.98	6.53	7.39	7.08	7.30	7.05	**6.39***	6.91	7.07	7.19	7.16	6.35
Haemoglo-bin [g/dl]	M	15.5	14.8	15.3	15.0	**13.0*** **(-16%)**	16.6	15.6	16.2	15.5	14.5	17.3	16.5	16.9	15.6	**15.1** **(-13%)**
	F	16.1	16.1	16.1	15.8	14.6	17.5	16.6	17.1	17.0	**15.3*** **(-13%)**	16.1	16.7	16.5	17.3	15.0
Hematocrit [ratio]	M	0.454	0.438	0.447	0.445	0.403	0.476	0.448	0.468	0.446	0.427	0.502	0.487	0.494	0.467	0.456
	F	0.475	0.470	0.477	0.468	0.443	0.5	0.475	0.485	0.489	0.454	0.47	0.49	0.48	0.510	0.454
MCV [fl]	M	64	67	64	65	68	65	67	65	68	**70***	65	67	65	68	69
	F	66	66	65	66	67	67	67	66	69	**70***	68	69	67	70	71
MCHC [g/dl]	M	34.8	34.5	34.7	34.3	**32.9***	35.3	35.3	35.2	35.2	**34.3***	34.3	33.8	34.0	**33.3***	**33.0***
	F	34.5	34.8	34.3	34.4	**33.4***	35.6	35.4	35.7	35.2	**34.2***	34.0	33.8	34.1	33.7	32.8
Reticulocytes [%]	M	0.4	0.5	0.6	0.3	**1.4***	0.6	0.4	0.3	0.9	**1.3***	0.8	0.8	0.6	0.9	**1.7***
	F	0.3	0.2	0.3	0.4	**1.0***	0.3	0.3	0.6	0.8	**1.6***	0.3	0.5	0.7	1.5	1.7
Platelet [10^{-9}/l]	M	228	197	228	270	376	233	233	290	289	**425***	286	272	274	**431***	**449***
	F	247	260	201	211	**348***	265	259	210	242	380	347	341	264	247	463
Methaemo-globin [%]	M	0.75	0.58	0.69	0.90	**1.96***	0.73	0.77	0.76	0.99	**1.52***	1.16	1.27	1.04	1.14	1.95
	F	0.66	0.97	0.58	0.87	**1.48***	0.63	0.88	0.97	0.99	1.61	0.71	1.08	0.68	0.98	**2.39***
Sulfhaemo-globin [%]	M	0.05	0.03	0.10	0.07	**0.34***	0.05	0.05	0.07	0.08	**0.28***	0.14	0.13	0.10	0.22	**0.30***
	F	0.04	0.04	0.04	**0.09***	**0.17***	0.03	0.05	**0.06***	**0.09***	**0.38***	0.09	0.23	0.14	**0.33***	**0.41***

Evidence of compensatory haematopoiesis was revealed by morphological changes in the bone marrow at 500 ppm and above (increased cellularity, increased numbers of erythrocytes precursors and increased numbers of macrophages). Bone marrow hyperplasia was observed in all animals at 50,000 ppm and in one female at 500 ppm and was accompanied by pigment deposition in the bone marrow, spleen, liver and kidney.

Table 11: Main histopathological findings related to anaemia in dogs exposed for 52 weeks

		Dose levels (ppm and mg/kg bw/day)				
	ppm	0	10	100	500	50,000
Histopathological findings	mg/kg/d	0	0.37-0.39	3.5-3.7	19-20	2018-1879
Liver: increased Kupffer-cell pigmentation – slight	M	0/4	0/4	0/4	3/4	**4/4**
	F	0/4	0/4	0/4	0/4	**4/4**
Liver: increased Kupffer-cell pigmentation - moderate	M	0/4	0/4	0/4	1/4	**4/4**
	F	0/4	0/4	0/4	2/4	**4/4**
Kidney, increased yellow pigment deposition in proximal tubules	M	0/4	0/4	0/4	0/4	**4/4**
	F	0/4	0/4	0/4	1/4	1/4
Spleen, increased haemosiderin	M	0/4	1/4	1/4	0/4	2/4
	F	0/4	0/4	0/4	1/4	3/4
Bone marrow, hyperplasia – moderate/severe	F	0/4	0/4	0/4	0/4	**4/4**
	M	0/4	0/4	0/4	1/4	**4/4**
Bone marrow, increased yellow pigment deposition	M	0/4	0/4	0/4	0/4	**4/4**
	F	0/4	0/4	0/4	0/4	**4/4**

In addition to these findings, effects on the liver were observed. Increased liver weights were seen in males at and above a dietary concentration of 500 ppm and in females at 50,000 ppm. At the highest concentration of 50,000 ppm, this increase in liver weights was accompanied by increased incidences of hepatocellular fatty vacuolation. The one-year feeding study in Beagle dogs supports hence a NOAEL of 100 ppm (equivalent to 3.5 mg/kg bw/d in males and 3.7 mg/kg bw/d in females).

Long-term oral feeding studies were conducted in rats and mice.

In a 24 month chronic toxicity study (Esdaile, 1990a), administration of flufenoxuron to Fischer 344 rats at dietary dose levels of 0; 1; 5; 50; 500; 5,000 and 50,000 ppm (equivalent to 0.044-0.055, 0.23-0.28, 2.2-2.8, 22-28, 233-301, 2,471-3,206 mg/kg bw/d for males and females, respectively) resulted in decreased body weight gain (up to 14 %) and slightly higher food consumption in males and females at ≥ 5,000 ppm. A slight anaemia characterized by lower red blood cell counts, haemoglobin concentrations (both decreased up to – 8 %), hematocrit (up to – 7%) and slightly increased reticulocyte counts was observed in the females at the two highest dose levels, early signs were already observed at 50 ppm but were not considered as adverse effect (only decreased haemoglobin and hematocrit). Similar findings were observed in males but generally to a lesser extent than with females (only decrease of haemoglobin and hematocrit at 50,000 ppm and at 5,000 ppm and above, respectively). Macro- and micropathological changes at higher dose levels were largely related to an age-related pathology. Changes of clinical chemistry parameters (increased bilirubin, cholesterol and decreased triglycerides), consistent over time and between sexes, were only observed at the two highest dose levels. Decreased spleen weight in males and increased adrenals weight in females were reported from 5,000 ppm. There were no adverse treatment-related histopathological changes. This study supported a NOAEL for chronic toxicity of 500 ppm (equivalent to a mean daily dose of 22 mg/kg bw in males and 28 mg/kg bw in females) based on anaemia at the LOAEL of 5000 ppm.

Chronic effects of flufenoxuron could be determined also from the oncogenicity study in rats (Esdaile, 1990b). Flufenoxuron administered to Fischer 344 rats at dietary dose levels of 0; 500; 5,000 and 50,000 ppm (equivalent to 21.57-25.91, 217.5-276.4, 2,289.8-2,900.9 mg/kg bw/d for males and females, respectively) resulted in a statistically significant increase in survival of treated groups. This was especially obvious at 50,000 ppm with survival rates at 66% (versus 42% in control) and 76% (versus 56% in control) in males and females, respectively. The higher survival rate was associated to the slightly to moderately lower body weights of rats at 5000 ppm. Food consumption tended to be slightly higher in both sexes at the high dose level. Haematological data were not provided for red blood parameters like haemoglobin, hematocrit or number of red blood cells. Statistically significant organ weight changes were noted: decreased spleen weight (absolute and relative) in all treated male groups, decreased kidney weights (absolute and relative) from 5,000 ppm in males and decreased adrenal weights for females (in all treated groups for relative weight and from 5,000 ppm for absolute weight). These changes in organ weights were not accompanied by any treatment-related histopathological findings, except the slight increase of basophilic foci in liver of high dose males. These changes were therefore of questionable toxicological relevance. Based on increased incidence of basophilic foci in the livers of high dose males and decreasing female body weight at the two highest doses, the NOAEL for chronic toxicity was 500 ppm (25.91 mg/kg bw/day) for females and 5,000 ppm (217.5 mg/kg bw/day) for males.

Chronic effects of flufenoxuron could be determined from the two oncogenicity studies employing B6C3F1 mice. In the first study (Esdaile, 1990c), dietary administration of flufenoxuron to mice at dose levels of 0; 500; 5,000 and 50,000 ppm (equivalent to 56-73, 559-739, 7,356-7,780 mg/kg bw/d for males and females, respectively) resulted in reduced body weight gain in both sexes at 50,000 ppm (decrease up to 21% in males and 30% in females at week 104) and higher mortality in females at 5000 and 50,000 ppm (up to 25% higher than in controls). There were no treatment-related haematological effects. The liver, stomach and spleen were identified as target organs: higher spleen and liver weights were observed for both sexes at the high dose. In addition, hepatic lesions were observed in the high-dose group (enlargement, pallor, dark areas or foci) associated to microscopically lesions like increased incidence of single cell necrosis, hepatocellular hypertrophy, aggregation of Kupffer cells in both sexes and inflammation in males. The incidence of these findings was only statistically significant at 50,000 ppm except for Kupffer cell aggregates which were also increased in mid dose females. Like in the liver, an aggregation of macrophages was observed in the spleen of high dose males and females. In the fore stomach, ulcers were observed in high dose males, as well as an increased incidence of inflammation. Based on the increased mortality and Kupffer cell aggregates observed at 5000 ppm in females and on the effects in liver, stomach and spleen in males at 50,000 ppm, the NOAEL for systemic effects was 500 ppm for females (73 mg/kg bw/d) and 5000 ppm for males (559 mg/kg bw/d).

In the second oncogenicity study (Broadmeadow, 1996), flufenoxuron was administered to B6C3F1 mice at dietary dose levels of 0, 100, 1000 and 10,000 ppm (equivalent to 15.3-17.4, 152-187, 1,592-1,890 mg/kg bw/d in males and females, respectively) for up to 2 years. No systemic effect was observed during this study. Only increase of uterus distension by a fluid was observed from 1000 ppm. Based on the effects observed on female uteri, the NOAEL for females was 100 ppm (17.4 mg/kg bw/d) whereas it was 10,000 ppm for males (1,592 mg/kg bw/d).

5.5.2 Repeated dose toxicity: inhalation

No data

5.5.3 Repeated dose toxicity: dermal

No data

5.5.4 Other relevant information

The potential neurotoxicity of flufenoxuron was assessed in a 28-day oral feed neurotoxicity study in Wistar rats (Kaspers et al., 2003). Flufenoxuron was administrated in the diet at 0; 1,000; 5,0000 and 20,000 ppm (equivalent to 88-95, 435-475, 1,775-1,934 mg/kg bw/d for males and females, respectively). Only indications of general toxicity were obtained at dose levels of 5,000 ppm and 20,000 ppm (reduction of body weight in the males up to 19.4% at 5,000 ppm and 16.6% at 20,000 ppm at the end of the study), whereas no signs of neurotoxicity were detected at any dose level. Thus, under the conditions of the present study the NOAEL for neurotoxicity was 20,000 ppm in both sexes (1,775 mg/kg bw/d in males and 1,934 mg/kg bw/d in females).

5.5.5 Summary and discussion of repeated dose toxicity:

The main effect exerted by flufenoxuron in repeated-dose toxicity study with rats, mice and dogs is anaemia, probably haemolytic, which is characterized by decreases in haemoglobin levels and

changes in red blood cell parameters with compensatory haematopoiesis, revealed by changes in bone marrow. This anaemia was particularly observed in dogs. Indeed in the 15-week study in dogs, a decrease in haemoglobin levels was observed in all males groups (from 500 ppm) at week 9 but was confined to the highest dose group after 12 weeks. This effect was associated with bone marrow hyperplasia, reflecting a compensatory response to the anaemia and with pigment deposition (probably haemosiderin) in particular in the liver and the bone marrow. Although at 500 ppm, the pigmentation was confined to the liver (1/4 female and 0/4 males), the incidence of this effect increased with the dose: 3/4 females and 4/4 males at 5,000 ppm and in all animals at 50,000 ppm for pigment deposition in the liver; 3/4 females and 0/4 males at 5,000 ppm and 4/4 males and 3/4 females at 50,000 ppm for pigment deposition in the bone marrow. Therefore the pigment deposition observed at 500 ppm could be considered as a precursor effect.

In addition to anaemia, increase in methaemoglobin levels was observed in rats and dogs. Such change is also reported in literature with acyl urea compounds similar to flufenoxuron. Nevertheless, in rats, the significance of this finding is doubtful due to the use of an unspecific method of detection (CO-Oxymeter) which could be associated with false positive. Furthermore, in a two-year rat study, methaemoglobin was estimated using the specific method of Evelyn and Malloy as well as the unspecific CO-Oxymeter. The results showed that no methaemoglobin was detected with the specific method whereas similar increase in methaemoglobin to that seen in the 28-days and 90-day studies was observed when the unspecific method was used. In dogs, methaemoglobinemia (dose-related) was detected by the specific method and was therefore considered as toxicologically significant. This change appeared at and above 500 ppm (18-21 mg/kg/d) and was associated to sulfhaemoglobinemia in the 15-week study.

Slight effects on the liver were also reported in the 52-week study in dogs (increased liver weights and fatty vacuolation of hepatocytes at the highest tested dose). These findings are also supported by the results of the long-term toxicity studies in rats and mice.

5.5.6 Comparison of the hematological effects with classification criteria

As summarized above at relatively high doses flufenoxuron is inducing haemolytic anaemia; however detailed comparison with classification criteria is needed before classification can be made.

There are two guidance documents which could be 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 (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/day for Category 1 or in a range between 10 and 100mg/bw /day 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 DSD framework is provided in 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 these in 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.

Comparison of effects observed in the existing studies with classification criteria

Study of Esdaile (1986a).
Flufenoxuron was administrated for 28 days through the diet to five groups of 7 males and 7 females Fischer 344 rats at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (equivalent to 4.8-5.3; 49-53; 475-534; 997-1,067; 5,147-5,432 mg/kg bw/d in males and females); concurrently, control groups (14 males and 14 females) were fed with basal diet.
Flufenoxuron did not produce any consistent and significant adverse changes in haematology.
Conclusion: The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Esdaile (1991a).
Flufenoxuron was administrated for 28 days through the diet to five groups of 7 males and 7 females B6C3F1 mice at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (equivalent to 10.5-14.0; 110-142; 1,091-1,353; 2,142-2,811; 9,820-12,157 mg/kg bw/d in males and females); concurrently, control groups (14 males and 14 females) were fed with basal diet.
Flufenoxuron did not produce any consistent and significant adverse changes in haematology.
Conclusion: The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Esdaile (1987)
Flufenoxuron was administrated for 90 days thought the diet to five groups of 10 males and 10 females Fischer 344 rats, at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (3.5-4.1; 35-41; 351-399; 689-820; 3,637-4,151 mg/kg bw/d in males and females; concurrently, a control group of 20 males and 20 females was fed with basal diet.
Results: The animals showed slight anaemia in females from 500 ppm, as evidenced by significant decreases in haemoglobin (dose-related; reduction less than 10%) and changes in erythrocyte parameters in association with evidence of compensatory haematopoiesis (increased reticulocyte counts, decreases in myeloid:erythroid ratios). Increases in spleen weights of females at 5,000 ppm and higher dietary concentrations were considered to be related to the hematological effects of flufenoxuron. No signs of anaemia were seen in males, although evidence of compensatory hematopoiesis (decreased myeloid:erythroid ratio) was observed at the highest dose level of 50,000 ppm. A small but statistically significant increase in methaemoglobin at all dose levels was detected with the unspecific CO-Oxymeter method but could be considered as a false-positive value (see same results in the 28-day toxicity study in rat).
Conclusion: Flufenoxuron did not produce any consistent and significant adverse changes in haematology at the dose levels below 351-399mg/kg bw/day. The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Esdaile (1988).
Flufenoxuron was administrated for 90 days through the diet to five groups of 10 males and 10 females B6C3F1 mice at dietary concentrations of 50, 500, 5000, 10,000 and 50,000 ppm (10-12; 103-124; 1,069-1,247; 2,139-2,482; 11,071-12,619 mg/kg bw/d in males and females); concurrently, a control group of 20 males and 20 females was fed with basal diet.
Results: A mild anaemia, as evidenced by significant decreases in haemoglobin (< 10 %) and decreases in erythrocyte parameters at high dose level for males and increases in serum bilirubin from 500 ppm (for both sexes), was also noted. Liver weights adjusted for terminal body weights were marginally increased over control values, attaining statistical significance in both sexes at 500

ppm and higher dose levels (by up to 8%). However, it can be noted that there is a lack of a convincing dose-response relationship.
Conclusions: The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Esdaile (1990a)
In a 24 month chronic toxicity study flufenoxuron administered to Fischer 344 rats at dietary dose levels of 0; 1; 5; 50; 500; 5,000 and 50,000 ppm (equivalent to 0.044-0.055, 0.23-0.28, 2.2-2.8, 22-28, 233-301, 2,471-3,206 mg/kg bw/d for males and females, respectively) induced a slight anaemia characterized by lower red blood cell counts, haemoglobin concentrations (both decreased up to – 8 %), hematocrit (up to – 7%) and slightly increased reticulocyte counts in the females at the two highest dose levels (233-301, 2,471-3,206 mg/kg bw/d). Similar findings were observed in males but generally to a lesser extent than with females (only decrease of haemoglobin and hematocrit at 50,000 ppm and at 5,000 ppm and above, respectively). Macro- and micropathological changes at higher dose levels were largely related to an age-related pathology. Changes of clinical chemistry parameters (increased bilirubin, cholesterol and decreased triglycerid), consistent over time and between sexes, were only observed at the two highest dose levels. Decreased spleen weight in males and increased adrenals weight in females were reported from 5,000 ppm. There were no adverse treatment-related histopathological changes.
Conclusions: The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Greenough (1987)
Flufenoxuron was administrated for 15 weeks through the diet to three groups of 4 males and 4 females Beagle dogs at dietary concentrations of 500, 5000 and 50,000 ppm (18-21; 163-182; 1,961-2,039 mg/kg bw/d in males and females); concurrently, a control group of 4 males and 4 females received basal diet.
Results: Flufenoxuron-related minor anaemia was apparent in all treated groups, as revealed by changes in haemoglobin levels (reduction ca. 14 -17% in all group in week 9; 13-14% reduction in weeks 12 and 15 only in a groups exposed at ca.2000mg/kg bw/day and only in males, but not in females. Hematocrit was reduced by approximately 10% and the percentage of reticulocytes increased by 40-100% in males in all exposed group after 9 weeks of exposure, but not after 15 weeks of exposure.
Signs of liver haemosiderosis (increased Kupffer cell pigmentation) without other microscopic effects like necrosis, fibrosis or cirrhosis were observed in animals exposed at 163-182 mg/kg bw/day or higher and signs of haemosiderosis in 50% males in the 1,961-2,039 mg/kg bw/d were observed. In the 3 out of 4 males and in 2 out of 4 females of the group exposed at 18-21mg/kg/day compensatory hyperplasia in bone marrow was found, without other haematological effects on 15 week of exposure.
Conclusions: The haematological effects do not meet CLP and DSD classification criteria for agents inducing haemolytic anaemia.

Study of Goburdhun (1988)
It is the 52-week dietary toxicity study conducted in Beagle dogs (groups of 4 males and 4 females), at dietary concentrations of 10, 100, 500 and 50,000 ppm (0.37-0.39; 3.5-3.7; 19-20; 2018-1879 mg/kg bw/d in males and females); concurrently, a control group was fed with basal diet.

Results: No treatment-related effect on body weight or food consumption was reported. A mild anaemia revealed by changes in haemoglobin level (reduction of HB of 16% at 5th week and of 13%

at week 52), and by slight changes in erythrocytes parameters appeared in both sexes at 50,000 ppm (2018-1879mg/kg bw/day) (Table 10). Platelet counts were statistically significantly increased in males at 2018-1879mg/kg bw/day from week 13 and at 500 ppm (19-20 mg/kg bw/day) from week 27. Methaemoglobin were increased over control levels at 50,000 ppm in both sexes at most time points of investigation, however a functional reduction of haemoglobin level assessed as a sum of Hb reduction and methaemoglobin level did not exceeded 20%.

The histopathological changes in liver, spleen, kidney and bone marrow presented in table 11 approaching a level of severity warranting classification occurred in animals exposed at 2018-1879mg/kg bw/day. At the highest dose level of 2018-1879mg/kg bw/day, the increase in liver weights was accompanied by increased incidences of hepatocellular fatty vacuolation. In the lower dose of 19-20 mg/kg bw/day the increased Kupffer-cell pigmentation of slight or moderate degree in 6 out of 8 animals, increased yellow pigment deposition in proximal tubules in kidney in 1 out of 8 dogs, increased haemosiderin in spleen and bone marrow hyperplasia of 1 out of 8 dogs were observed. Those haematological effects although pointing out the haemolytic properties of flufenoxuron did not reached sufficient severity to meet classification criteria such as: significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis, multifocal or diffuse fibrosis in the spleen, liver or kidney, tubular nephrosis, severe fatty change in the liver. The slight histopathological changes in animals exposed for 52 weeks to flufenoxuron at dose of 19-20 mg/kg bw/day were not accompanied by a reduction in haemoglobin level in blood of these animals. The severity of histopathological changes in liver, spleen, kidney and bone marrow of dogs exposed to flufenoxuron at the dose ca. 2000mg/kg bw/day for 15 weeks and 52 weeks were in general similar, indicating that substantial prolongation of exposure did not resulted in progression of histopathological alterations and did not produced in any animal necrotic and fibrotic changes in kidneys, liver or spleen.

Conclusion

The studies on rats, mice and dogs demonstrated that flufenoxuron has the haemolytic properties leading to reduction of haemoglobin level and of number of erythrocytes, increase in number of reticulocytes, hyperplasia in bone marrow, signs of haemosiderosis in liver, spleen or kidneys. The degree of severity of these changes do not reach level of severity of haematological effects required for classification into category of specific target organ toxicity in repeated exposure within CLP regulation or into classification of R48 for haemolytic anaemia in DSD classification system. In addition these haematological effects were mainly observed at dose levels higher than the guidance values indicated in both classification system.

5.6 Mutagenicity

5.6.1 *In vitro*

In the first bacterial mutation assay provided (Brooks and Wiggins 1986), flufenoxuron did not induce reverse gene mutation on the tested strains with metabolic activation and on TA1535 and E. coli WP2 uvrA pKM101 without metabolic activation. For the remaining 4 strains without S-9 mix, the test was not accepted due to invalid positive controls, for TA 1537, TA 1538, TA 98 and TA 100 (benzo(a)pyrene and neutral red are indirect-acting mutagens).

In a second Ames test (Engelhardt and Leibold, 2005), no reverse gene mutation was induced in the selected bacterial tested strains (*S. typhimurium* TA 100, TA 1535, TA 1537,TA 98 and *E. coli*

WP2 uvrA) at concentrations up to 5,000 µg/plate in the standard plate test and up to 2,500 µg/plate in the pre-incubation assay. This result is also confirmed in a third Ames test (Sokolowski, 2007) where no increase in revertant colony numbers of any tested strain (*S. typhimurium* TA 100, TA 1535, TA 1537, TA 98 and *E. coli* WP2 uvrA) was observed up to 5,000 µg/plate in the incorporation and pre-incubation tests, with and without metabolic activation.

Flufenoxuron did not lead to any increase in the rate of mitotic gene conversion, with and without metabolic activation, in a *Saccharomyces* gene conversion assay (Brooks and Wiggins 1986).

A positive response (not dose-dependent) was noted in the chromosomal aberration test with CHO cells in the presence of an exogenous metabolic activation system. This response was not expressed in the absence of S-9 mix (Meyer 1987). The positive response with S-9 mix was no more observed when conducting another test with CHO cells in the presence of physiological concentrations of glutathione, a peptide naturally present in mammalian tissues (Meyer 1988). It has been reported in the literature[1] that S-9 metabolic activation, used for improving the detection of potential positive effects, often does not contain adequate cofactors for activating a specific detoxifying mechanisms and therefore does not thoroughly mimic what really happens *in vivo*. These results suggest that a reactive metabolic intermediate could be generated from flufenoxuron in the presence of S9-mix and is clastogenic to CHO cells. This putative metabolite is probably subject to detoxification by glutathione at a concentration of 5 mM, under the experimental conditions. Nevertheless, the choice of the concentration used in this latter test (150 µg/ml) was not sufficiently justified (no data on solubility in the test, concentration lower than that is recommended in the OECD guidelines, no marked cytotoxicity in the main test).

No potential for clastogenicity was observed in two other *in vitro* chromosomal aberration assays using either rat liver cells (Meyer 1988) or human lymphocytes (McEnaney 1992). Nevertheless, in these assays, the maximal tested concentrations were not justified: the highest tested dose is lower than the limit dose recommended by the OECD guideline in the absence of overt cytotoxicity (reduction of mitotic index lower than 50 %).

Flufenoxuron is also negative for inducing gene mutations in an *in vitro* mammalian cell HGPRT gene mutation test in Chinese hamster V79 cells (Clare and Wiggins 1986) but the choice of the maximal tested dose is not justified in the presence of metabolic activation since the reduction of cloning efficiency is lower than 50 %. In another OECD 476 gene mutation test (Wollny, 2007), flufenoxuron was confirmed to be not mutagenic in V79 cells. Even if the cytotoxicity was not sufficient in presence of S9-mix, the material was tested up to precipitating concentrations.

[1] Ashby, J.: "The Unique Role of Rodents in the Detection of Possible Human Carcinogens and Mutagens", Mutation Res. 115, 117-123, (1983); Galloway, S. M.: "Chromosome Aberrations Induced In Vitro: Mechanisms, Delayed Expression, and Intriguing Questions", Environ. Mol. Mutagen. 23/24, 44-53 (1994).

Table 12: Summary of *in vitro* studies

Test system Method Guideline	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			+ S9	**- S9**		
			+/-/+	+/-/+		
OECD 471	Bacterial mutation assay *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; E. coli WP2 uvrA pKM101	31.25 - 4,000 µg/plate (standard plate test) equal to 0.01, 0.1, 0.5, 1.0, 5.0 mg/ml	–	–	No cytotoxicity was observed in any of the test strains exposed to the test compound up to 4,000 µg/plate with or without S9 mix. A fine suspension in the top agar was observed at a dose of 31.25 µg/plate. Lumps of precipitate at 1,000 and 4,000 µg/plate were noted. At 4,000 µg/plate, the pH of the medium was slightly increased from pH 7.31 to pH 7.39. Positive controls, without S-9 mix are invalid for TA 1537, 1538, 98 and 100.	Brooks and Wiggins 1986
EEC 2000/32 B.13/B.14; OECD 471, EPA/OPPTS 870.5100 Key study	Bacterial mutation assay *S. typhimurium* TA 100, TA 1535, TA 1537, and TA 98; E. coli WP2 uvrA	20 - 5,000 µg/plate (standard plate test) and 4 - 2,500 µg/plate (pre-incubation assay)	-	-	Weak cytotoxicity at ≥ 2500 µg/plate	Engelhardt G., Leibold E., 2005
OECD 471	Bacterial mutation assay *S. typhimurium* TA 100, TA 1535, TA 1537, and TA 98; *E. coli* WP2 uvrA	3-5000 µg/plate (incorporation and pre-incubation tests)	-	-	Precipitation observed from 333 µg/plate. Toxicity occurred in the first experiment without metabolic activation only in TA1535 at 1000 and 5000 µg/plate, in TA1537 at 2500 µg/plate and in TA98 at 1000-5000 µg/plate.	Sokolowski, 2007
OECD 481	*Saccharomyces cerevisiae*, JD1 strain	0.01; 0.1; 0.25; 0.5 and 1.0 mg/ml	–	–	No cytotoxicity observed	Brooks and Wiggins 1986

Test system Method Guideline	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			+ S9	**- S9**		
			+/-/±	+/-/±		
OECD 473	*In vitro* mammalian chromosome aberration test Chinese Hamster Ovary (CHO) (CHO-K1) cells	Up to 250 µg/ml without S-9 and 300 µg/ml with S-9 mix for the cytotoxicity studies. 15, 75 and 150 µg/ml for chromosome assay, with and without S9-mix	+	-	Cytotoxicity assays: Cell confluency was reduced by about 50% between 150 and 200 µg/ml with S9-mix and at 150 µg/ml without S9-mix. Total cell counts were reduced by about 50% at 150µg/ml with and without S9-mix. Nevertheless, in the main test, cells treated with flufenoxuron presented a mitotic index higher than control without S9-mix and up to 24% lower with S9-mix. The chromosome damage was observed at all concentrations with S-9 mix , but was not dose-dependent. Detailed results of this study is given in table 13	Meyer 1987 Meyer 1991b
OECD 473	*In vitro* chromosome aberration assay with glutathione (GSH) CHO cell	150 µg/ml	-	n.a.	At the concentration tested the mitotic index was reduced by about 27% *In vitro c*lastogenicity of flufenoxuron observed in CHO cells in the presence of S-9 mix was completely abolished when glutathione was added to the culture medium at physiological concentrations. As far as only one concentration was tested, this study is only considered as supportive about the cytogenicity of a flufenoxuron metabolite and the mechanism involved. Detailed results of this study is given in tables 13A	Meyer 1988 Meyer 1991
OECD 473	*In vitro* mammalian chromosome aberration test Rat liver (RL4) cells	45; 225 and 450 µg/ml in the absence of metabolic activation, and 16; 80 and 160 µg/ml in the presence of metabolic activation.	–	–	In a cytotoxicity assay, total cell counts were reduced to 45.9 and 61.7% of the solvent control value at the highest tested dose (i.e. 450 µg/ml without S9 and 160 µg/ml with S9, respectively). In the mutagenicity experiment, the evaluation of mitotic indices at the 24-hour sampling time revealed a slight reduction at the highest tested dose level (approx. -30% without S-9 and -10% with S-9 mix).	Meyer 1988 Meyer 1991
OECD 473	*In vitro*	3.164 – 160 µg/ml	–	–	Limited precipitation, which	McEnaney

Test system Method Guideline	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			+ S9	**- S9**		
			+/-/+	+/-/+		
	mammalian chromosome aberration assay Peripheral human lymphocytes	(solubility limit in culture medium)			redissolved on agitation of the cultures, was observed at the top 3 doses, indicating that a concentration close to the limit of solubility had been achieved. At the highest tested dose, a reduction of mitotic indices of 0 to 9% (with S9-mix) and 25 to 42% (without S9-mix) was observed, depending on duration of exposure and post-exposure.	1992
OECD 476	*In vitro* mammalian cell HGPRT gene mutation test Chinese hamster V79 cells	50; 150; 450; 900 and 1,350 µg/ml with S-9 and 50; 150; 450 and 1,350 µg/ml without S-9	–	–	In the cytotoxicity test, cloning efficiency at 1,000 µg/ml was reduced to 19% with metabolic activation and to 48% without metabolic activation. In the main test, the cloning efficiency at the highest tested dose was reduced by 18 % without metabolic activation and by 6 % with metabolic activation.	Clare and Wiggins 1986 Brooks 1991
OECD 476	*In vitro* mammalian cell HGPRT gene mutation test Chinese hamster V79 cells	6.3-1,600 µg/ml	-	-	Precipitation occurred from 50 µg/ml without S9 mix and from 100 µg/ml with S9 mix. Toxicity higher than 50 % was observed at 12.5 µg/ml and above in the first experiment (4hr treatment) only without S9 mix.	Wollny, 2007

Flufenoxuron did not induce *in vitro* structural chromosome aberrations in Chinese Hamster Ovary cells at the investigated concentrations without S-9 mix (Meyer, 1988;Meyer, 1991), however significant increases in frequency of gaps were observed at all concentration with S-9 mix. The increases were not dose-dependent (Table13). Since the chosen highest concentration was not validated: insufficient toxicity, no precipitation, below 5mg/ml) the definite conclusion cannot be drawn.

Table 13: Methaphase chromosome analysis of CHO cells without or with S-9 mix, harvested at 24h

Group	Dose level [µg/ml]	MI	Numerical abberations	Structural abberations					
				Including gaps			Excluding gaps		
			% polyploid cells	Cells with aberrations		Mean no. of aberrations per cell	Cells with aberrations		Mean no. of aberrations per cell
				No.	%		No.	%	
Without S-9 mix									
untreated	0	0.015	3.0	9	3.1	0.034	0	0.0	0.000

DMSO	0	0.015	4.7	7	2.4	0.028	1	0.3	0.003
Flufenoxuron	15	0.019	8.7	6	2.2	0.023	1	0.4	0.004
	75	0.026	10.3	6	2.2	0.022	0	0.0	0.000
	150	0.025	5.0	4	1.4	0.014	0	0.0	0.000
MMS	60	0.037	2.3	**92**	**31.4**	**0.573**	**88**	**30.0**	**0.532**
With S-9 mix									
untreated	0	0.042	6.3	3	1.1	0.011	0	0.0	0.000
DMSO	0	0.052	8.7	16	5.8	0.066	6	2.2	0.022
Flufenoxuron	15	0.052	8.7	**38**	**13.9**	**0.157**	20	7.3	0.077
	75	0.038	3.8	**59**	**23.6**	**0.352**	**39**	**15.6**	**0.180**
	150	0.032	6.7	**43**	**15.4**	**0.207**	21	7.5	0.096
CP	100	0.010	2.7	**64**	**59.3**	**1.111**	**59**	**54.6**	**0.954**

MI Mitotic Index
CP Cyclophosphamide

In the follow up study (Meyer, 1988;Meyer, 1991) the effect of glutathione on clastogenic effect of flufenoxuron was tested (table 13A).

Table 13A: Methaphase chromosome analysis of CHO cells after 3-h exposure and harvest after 24 hours in the presence of S-9 mix with or without Glutathione (GSH) supplementation.

Group	Dose level [µg/ml]	MI	Numerical abberations	Structural abberations					
			% polyploid cells	Including gaps			Excluding gaps		
				Cells with aberrations		Mean no. of aberrations per cell	Cells with aberrations		Mean no. of aberrations per cell
				No.	%		No.	%	
Buffer	0	0.06	1.33	9	3.04	0.031	2	0.68	0.007
GSH	0	0.07	0.00	5	1.67	0.017	0	0.00	0.000
Flufenoxuron without buffer	150	0.04	1.90	51	**19.77**	0.302	35	**13.57**	0.171
Flufenoxuron with buffer	150	0.05	0.46	40	**18.52**	0.310	28	**12.96**	0.204
Flufenoxuron + GSH	150	0.05	1.33	8	2.70	0.027	1	0.34	0.003
CP	100	0.05	0.67	52	**17.45**	0.201	**31**	**10.40**	**0.114**

Buffer: phosphate buffer plus DMSO in culture medium;
GSH: 5mM GSH plus “Buffer” in culture medium
MI Mitotic Index
CP Cyclophosphamide

The results of this study suggest that flufenoxuron is clastogenic to CHO cells in the presence of S-9 mix activation. Glutathione at the concentration of 5mM displays a scavenger effect on this clastogenic potential. This is only supportive study and no reference is given for the physiological glutathione concentration.

5.6.2 *In vivo*

Flufenoxuron did not induce chromosomal damage *in vivo* in the rat bone marrow chromosomal aberration assay (Allen et al. 1986) at 4,000 mg/kg bw. Nevertheless some deviations from OECD 475 were present such as the low number of analyzed cells per rats (half of the OECD recommendation) and the absence of data on mitotic indices or cell ploidy.

In a more recent chromosome aberration assay in bone marrow cells of rat with flufenoxuron at doses of 500, 1000 and 2000mg.kg bw/day (Honarvar, 2007) , no significant increase of aberration frequency was observed 24 hours after a single oral administration of flufenoxuron up to 2000 mg/kg bw in rats. In animals examined 48 hours after administration of flufenoxuron at the dose of 2000mh/kg bw/ day the percentage of aberrant cells including gaps and all other aberrations excluding gaps did not differ significantly from the control group (table 14A and 14B). Nevertheless, the results in the 48 hour high dose group showed 2 exchanges and one multiple aberrations (not significant), considered as extremely rare events (Table 14B). In the additional report prepared in the context of possible inclusion of flufenoxuron as active substance in Annex I of Council Directive 91/414/ECCC (Flufenoxuron, 2010) these rare aberrations were considered as relevant to the treatment and interpreted that no definite conclusion on the clastogenic potential is available with this assay.

Table 14A: Aberrant cells and mitotic index

Experimental Group	Group No. (see also table 14B)	Dose mg/kg bw	Hours post dosing	No. cells scored	Percent aberrant cells		Mean mitotic index
					Incl. gaps	Excl. gaps	
Corn oil	1		24	1000	1.5	1.4	7.24
flufenoxuron	2	500	24	1000	0.5	0.5	5.98
	3	1000	24	1000	0.4	0.4	6.70
	4	2000	24	1000	0.4	0.4	6.38
	5		48	1000	0.8	0.8	6.97
Cyclophosphamide	6	15	24	950	17.4	17.4	3.75

Table 14B: Analysis of aberration types

Group no.	Gap	Iso-Gap	Break	Iso-break	fragment	Iso-fragment	deletion	Multiple aberration[a]	exchange	Chrom. disintegration[b]
1	1	0	8	1	3	3	0	0	0	0
2	0	0	3	0	1	1	0	0	0	0
3	0	0	2	0	2	0	0	0	0	0
4	1	0	2	0	1	1	0	0	0	0
5	1	0	4	0	1	0	0	1	2	0
6	1	0	85	2	22	8	3	57	232	1

a – more than 5 aberrations excluding gaps in one cell; exchanges only were recorded separately

b - pulverisation

Flufenoxuron was not genotoxic in the mouse micronucleus assay (Nishitomi 1993) up to 2,000 mg/kg bw (by IP route).

Although there was no evidence that the target cells were exposed in these studies (no decrease in the PCE/PCE + NCE ratio or no decrease in the mitotic index), the kinetic data show that

flufenoxuron was well distributed in carcass and organs, including the bone marrow and the liver (Hawkins, 1992). These results were therefore considered as valid.

Flufenoxuron also did not induce unscheduled DNA synthesis in rat hepatocytes following *in vivo* administration by gavage up to 1,500 mg/kg bw (Cifone 1991).

Table 15: Summary of *in vivo* studies

Type of test Method/ Guideline	**Species Strain Sex no/group**	**Frequency of application**	**Sampling times**	**Dose levels**	**Results**	**Remarks**	**Reference**
In vivo Chromosome Aberration Assay Bone marrow cells OECD 475, EEC 79/831, Part B	Rat Sprague Dawley 5M/5F per dose and sampling time	One application in corn oil by gavage	6, 24 and 48 hours	4000 mg/kg	negative at 6, 24 and 48 hours	The dose of 4,000 mg/kg bw was the limit dose based on solubility in corn oil and on a preliminary MTD test. No mortalities were observed in a dose-range finding test at doses up to 4,000 mg/kg bw. Only clinical signs (piloerection and hunched posture) were noted. No data on mitotic indices. Number of analysed cells per rats was half the OECD recommendation	Allen et al. 1986 Allen et al. 1991 Allen 1997
In vivo Chromosome Aberration Assay Bone marrow cells OECD 475	Rat Wistar 12/sex for the highest dose 6/sex for the other doses	One application in corn oil by gavage	24 (all doses) and 48 hours (only for 2000 mg/kg bw)	500, 1000, 2000 mg/kg bw	No significant increase in aberration rates	The dose was chosen based on preliminary acute studies (clinical signs, no mortality at 2000 mg/kg bw). No decrease in mitotic index.	Honarvar, 2007
In vivo Micronucleus Assay in bone marrow Polychromatic erythrocytes JMHW (1989) OECD 474	Mice ICR	Two applications IP (in olive oil) 24 hours apart	24 hours after application	500; 1,000 and 2,000 mg/kg bw	negative at 24 hours	In a dose-finding test, no dead animals were observed after 2 applications IP of 2000 mg/kg bw. In the main test, no mortalities or clinical effects were recorded. No statistically significant decreases in polychromatic to normochromatic erythrocyte ratios were noted for any groups tested with flufenoxuron.	Nishitomi 1993
In vivo / in vitro UDS Assay Primary hepatocytes OECD 486	Rat Fisher 344	One application in corn oil by gavage	4 hours	188-1500 mg/kg	negative at 4 hours	The top dose was based on ability to prepare a suspension and on the oral LD_{50} in F344 rat > 3000 mg/kg bw (in DMSO)	Cifone 1991

Conclusion of genotoxicity assays:

Flufenoxuron did not induce gene mutations *in vitro* in Ames tests and in HGPRT gene mutation tests in mammalian cells.

No chromosomal aberrations were observed *in vitro* in rat liver cells and peripheral human lymphocytes. Nevertheless, the tested concentrations were not validated by a sufficient cytotoxicity and were below the maximum dose recommended in the OECD guidelines.

In vitro studies with CHO cells suggest that in the presence of S-9 mix activation, a reactive metabolic intermediate, clastogenic to CHO cells, is generated. When glutathione was added, the positive response with S-9 mix was no more observed in CHO cells. Nevertheless, the tested dose was not sufficiently cytotoxic to valid this test performed with glutathione.

Flufenoxuron did not induce chromosomal damage *in vivo* in two rat bone marrow chromosomal aberration assays. In the latter test, one multiple aberration and two exchanges were only observed in the 48-hour (top dose) group and the overall cells remained unaltered when compared with solvent controls. Therefore, despite the fact that these findings are considered as extremely rare, the toxicological significance of the low incidence of these aberrations is questionable.

The lack of any genotoxic effects following *in vivo* exposure to flufenoxuron is also confirmed in a mouse bone marrow micronucleus assay and in an *in vivo/in vitro* UDS test, in rat liver cells.

In conclusion, the negative results obtained *in vivo* should override the positive response noted in the *in vitro* chromosomal aberration assay in CHO cells. Hence, flufenoxuron is considered not genotoxic and no classification for this endpoint is warranted.

5.7 Carcinogenicity

Flufenoxuron was administered to Fischer 344 rats in a carcinogenicity study at dietary dose levels of 0; 500; 5,000 and 50,000 ppm (equivalent to 21.57-25.91, 217.5-276.4, 2,289.8-2,900.9 mg/kg bw/d for males and females, respectively) (Esdaile, 1990b). No treatment-related effect on the incidence of non-neoplastic lesions was observed in treated males or females. There was no evidence for an oncogenic effect of flufenoxuron in rats at dose levels up to 50,000 ppm. On the contrary, there was a significant decrease of multiple primary benign tumors in males and females and of malignant primary tumors in males at 50,000 ppm. In absence of any treatment-related changes in the incidence of neoplastic findings, the NOEL for oncogenicity was 50,000 ppm, the highest concentration tested, which is equivalent to a mean daily dose of about 2,290 mg/kg bw in males and 2,900 mg/kg bw in females.

The oncogenic effect of flufenoxuron in mice was investigated in two separate studies employing B3C6F1 mice. In the first study (Esdaile, 1990c), dietary administration of flufenoxuron to mice was at dose levels of 0; 500; 5,000 and 50,000 ppm (equivalent to 56-73, 559-739, 7,356-7,780 mg/kg bw/d for males and females, respectively). The combined incidence of benign and malignant hepatocellular tumors (adenomas and carcinomas) was comparable between treated and control groups. An increased incidence of hepatocellular carcinomas was observed in all treated male groups (up to 38 %) and in low dose females (18%). This increase of hepatocellular carcinomas was

paralleled by a decrease of hepatocellular adenomas. The incidence of hepatocellular carcinomas in treated groups was within the US National Toxicology Program (NTP) historical control range (8 to 46% with a mean of 22.3%) for this type of tumor whereas the incidence in control males (6%) was below the historical control range. Furthermore, no clear dose-response was observed. For female mice, a statistically significant increase in the incidence of vascular tumors was observed at 50,000 ppm, only. This increase reflected an increase in the incidence of haemangiosarcomas in the spleen (14 % vs 0% in the control females). There were no treatment-related increases in the incidence of vascular tumors, either haemangiomas, hemangiosarcomas, or combined, at any other site in female mice. The 50,000 ppm treatment level (equivalent to 7,356-7,780 mg/kg bw/d), which is about 7.5-fold higher than the limit dose recommended for chronic toxicity test in the OECD guidelines (1000 mg/kg bw/day), elicited both excessive hepatocellular toxicity (such as single cell necrosis, hepatocellular hypertrophy and aggregation of Kupffer cells) and body weight depression (decrease up to 21% in males and 30% in females at week 104) and thus exceeded the maximum tolerated dose for flufenoxuron. In male mice, no statistically significant increased incidence of vascular tumors was observed at any treatment level. Despite the toxic context where haemangiosarcoma in the spleen was observed in female mice, a NOAEL for oncogenic activity is set for females: 5000 ppm, equivalent to a mean daily dose of 739 mg/kg bw/day. For males, a NOAEL at 50,000 ppm equivalent to 7,356 mg/kg bw/day was derived, despite equivocal nature of the hepatocellular carcinoma.

Table 16: Incidence of hepatocellular carcinomas and hemangiosarcomas in the spleen

Sex	Males				Females			
Doses	0	500	5,000	50,000	0	500	5,000	50,000
Hepatocellular carcinoma	3/50	**19/50**	**15/50**	**15/50**	3/50	**9/50**	7/50	5/50
Splenic hemangiosarcoma	4/50	3/50	0/50	3/50	0/50	1/50	1/50	**7/50**

In the second mouse oncogenicity study (Broadmeadow, 1996), flufenoxuron was administered at dietary dose levels of 0, 100, 1,000 and 10,000 ppm (equivalent to 15.3-17.4, 152-187, 1,592-1,890 mg/kg bw/d in males and females, respectively) for 2 years. This second mouse oncogenicity study in B3C6F1 mice did not reveal any carcinogenic potential of flufenoxuron at dose levels up to 1,592-1,890 mg/kg bw/d. The incidence of hepatocellular adenomas and carcinomas in mice was comparable to the control incidence. Moreover, the overall incidence of hepatocellular tumors was well within the historical control range and thus indicating that the increased incidence of hepatocellular carcinoma in the treated male groups observed in the first study was purely incidental. Likewise, in this second oncogenicity study, there was no increase in the number of splenic vascular tumors in female and male mice at the high dose level of 10,000 ppm (1,592-1,890 mg/kg bw/day). The highest dietary concentration used, 10,000 ppm equivalent to 1,592 and 1,890 mg/kg/day for males and females, respectively, was considered to be the NOAEL for oncogenicity in the mice.

It should be noted that the increased liver tumour incidences (without clear dose response) did occur in a specific strain of mice (B6C3F1). According to Guidance on the Application of Regulation (EC) No 1272/2008, this strain of mice is known to have a very high spontaneous liver tumour incidence. In such a strain the tumour incidence in the treated group may be significantly above the concurrent control, but could still be within the historical incidence range for that tumour type in that species and therefore may not be providing reliable evidence of treatment related carcinogenicity.

In conclusion, the apparent increase in the incidence of hepatocellular carcinoma noted in the first study, is associated with an unusually low incidence of these tumours recorded in the control and is not considered to be directly related to treatment in the lowest dose male groups. At the top dose, the hepatocellular carcinomas were observed in a very toxic context. This view is supported by the results of the second carcinogenicity study in B6C3F1 mice which was conducted few years later. In this study the incidence of hepatocellular adenoma and carcinoma as well as the combined incidence of hepatocellular tumors was comparable between controls and treated groups. The increased incidence of vascular tumours in the first mouse oncogenicity was probably due to the exaggerated dose, higher than the maximum tolerated dose (7,780 mg/kg bw/day). Although the effects could be substance-related, they were observed only at a very high dose in a toxic context and therefore they are considered insufficient to warrant a classification for carcinogenicity.

Table 17: Summary of long-term and carcinogenicity studies

Route	**duration of study**	**Species Strain Sex no/group**	**dose levels frequency of application**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**
Oral in food	24 months Carcinogenicity study	Rat Fischer 344 M/F 50/sex/group	0; 500; 5,000; 50,000 ppm (21.57-25.91, 217.5-276.4, 2,289.8-2,900.9 mg/kg bw/d)	No oncogenic effect	n.a.	NOAEL oncogenicity: 50,000 ppm (equivalent to 2,290 mg/kg bw/day in males and 2,900 mg/kg bw/day in females).	Esdaile 1990 Basford 1991 Berry 1992
Oral in food	24 months Oncogenicity study	Mice B6C3F1 M/F 60/sex/dose	0; 500; 5,000; 50,000 ppm (56-73, 559-739, 7,356-7,780 mg/kg bw/d)	50,000 ppm: increased incidence of hepatocellular carcinoma in treated males (overall incidence of hepatocellular tumors not affected, absence of a dose-response relationship); increased incidence of vascular tumors in high dose females	LOAEL oncogenicity: 50,000 ppm	NOAEL oncogenicity: Males 50000 ppm (7356 mg/kg bw/day) Females: 5000 ppm (739 mg/kg bw/day)	Esdaile 1990 Esdaile 1991 Berry 1992 Finn 1993 Haseman 1985
Oral in food	24 months Oncogenicity study	Mice B6C3F1 M/F 50/sex/dose	0; 100; 1,000; 10,000 ppm (15.3-17.4, 152-187, 1,592-1,890 mg/kg bw/d)	No oncogenic effects	n.a.	NOAEL oncogenicity 10,000 ppm (equivalent to 1,592 mg/kg bw/day in	Broadmeadow 1996

Route	duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
						males and 1,890 mg/kg bw/day in females)	

5.8 Toxicity for reproduction

5.8.1 Effects on fertility

The effects of flufenoxuron on reproductive parameters were investigated in a 2-generation study in rats (James et al., 1990). In this study, flufenoxuron was administrated though the diet to five groups of Sprague-Dawley rats at dietary concentrations of 0; 50; 190; 710 or 10,000 ppm (4.3; 16.3; 61.6; 875 mg/kg bw/d) throughout the entire study (10 weeks prior to a 20-day mating period until post-weaning period). Due to an increased incidence of total litter losses and lower post natal pup survival at the high dose, F_0 parental animals were mated a second time after the weaning of the F_{1a} litters, to produce F_{1b} litters. F_{1a} was finally killed at an age of about 14 weeks. The F_1 parental generation was selected from F_{1b} offspring and was mated two times to produce F_{2a} and F_{2b} litters. On postnatal day 4, all litters were culled to 8 pups per litter.

In parental animals, an increased incidence of alopecia was observed for F_0 and F_{1b} top dose females. An increased incidence of minimal luminal dilatation of uterus was also noted (6 for top dose for F_0 vs 1 for control and 9 for top dose F_{1b} vs 3 for control F_{1b}). Treatment also resulted in a decreased parental body weight gain (overall decrease up to 4% for females and up to 13% for males): a statistically lower body weight was noted for F_0 males at the top dose at week 20 only and for F_{1b} males at dose levels ≥ 190 ppm from week 8. For F_0 and F_{1b} females, body weight gain was reduced at dose levels ≥ 190 ppm for the pre-mating period prior to the first mating (reduction of 8, 8 and 11% for F_0 and 7, 6 and 7% for F_{1b}) but overall body weight gains were comparable for all groups during the two gestation periods. During lactation periods, body weight gains were similar for F_0 females but were statistically significantly decreased in F_{1b} female group at the top dose during the first lactation period (decrease up to 5%). For the second lactation period, F_{1b} females lost weight at 710 and 10,000 ppm (-2.9 and -2.4 grams, respectively). Although occasional statistical significant difference in food consumption was observed, this was not dose-related or only transient. Organ weight analysis revealed an increase in adjusted kidney weights and absolute adrenal weights and a decrease in adjusted brain and liver weights (F_0 and/or F_{1b}).

Body weight development for F_0 and F_{1b} generation is summarized in the table below. Significant values are displayed in bold and marked with *.

Table 18: Body weight development

Concentration	0 ppm		50 ppm		190 ppm		710 ppm		10 000 ppm	
Sex	M	F	M	F	M	F	M	F	M	F
F0 generation										
Week 0 (start of treatment)	185	141	184	141	182	142	179	139	179	140

Week 29 (end of treatment)	671	353	650	358	639	351	655	346	627	348
Gain over treatment period (% of controls)	486	212	466 (96%)	217 (102%)	457 (94%)	209 (99%)	476 (98%)	207 (98%)	448* **(92%)**	208 (98%)
F1b generation										
Week 4 (start of treatment)	100	93	91	83	103	90	93	92	100	92
Week 35 (end of treatment)	721	374	685	367	646	364	647	368	659	361
Gain over treatment period (% of controls)	621	281	594 (96%)	284 (101%)	543* **(87%)**	274 (98%)	554* **(89%)**	276 (98%)	559* **(90%)**	269 (96%)

The ability to induce and maintain gestation as well as the ability to give birth to offspring was not affected by treatment.

For F_0 animals, total litter losses were observed for the 2 matings at 0, 50, 190, 710 and 10,000 ppm amounted to 1/54, 0/52, 1/53, 4/53 and 6/52 respectively; for F_{1b} animals, 0/39, 1/41, 1/40, 4/43 and 7/40 total litter losses were observed for the 2 matings at 0, 50, 190, 710 and 10000 ppm respectively. When the data from Fo and F_{1b} are combined there were 1, 1, 2, 8 and 13 litter losses during lactation at 0, 4.3; 16.3; 61.6; 875 mg/kg bw/day respectively.

Thus, mortality of pups during lactation at the two higher dietary concentrations of 61.6 and 875 mg/kg bw/d was significantly higher than in controls, and this was attributed to the treatment.

At the highest dose, the majority of the total losses fell within the post-cull phase whereas there was no clear cut difference between pre and post cull total losses at 710 ppm. The results are summarized in the table below.

Table 19: Litter data

Parental generation	F_0					F_{1b}				
Dose level [ppm]	0	50	190	710	10,000	0	50	190	710	10,000
1st mating										
Mated	28	28	28	28	28	24	24	24	24	24
Delivering pups	28	26	27	27	27	21	22	20	23	20
Rearing young to weaning	28	26	27	27	25	21	22	19	21	17
Total litter loss:										
- Pre-cull	0	0	0	1	0	0	0	0	1	0
- Post –cull	0	0	0	0	2	0	0	1	1	3
2nd mating										
Mated	28	28	28	28	28	24	24	24	24	24
Delivering pups	26	26	26	26	25	18	19	20	20	20
Rearing young to weaning	25	26	25	23	21	18	18	20	18	16
Total litter loss:										
- pre-cull	1	0	1	3	3	0	1	0	2	0
- post cull	0	0	0	0	1	0	0	0	0	4

Table 20: Litter size during lactation of F1a to F2b litters (total litter losses excluded)

	At birth			Day 4 pre-cull		Day 4 post-cull	Day 8		Day 12		Day 21	
Dose [ppm]	litter size total	litter size live	pup loss [%]	litter size	cum. loss [%]	litter size	litter size	cum. loss [%]	litter size	cum. loss [%]	litter size	cum. loss [%]
F_{1a} litter												
0	13.8	13.6	1.3	13.1	4.5	7.9	7.8	1.3	7.8	1.3	7.8	1.3
50	12.6	12.6	0.0	12.2	2.5	7.7	7.7	0.5	7.7	0.5	7.7	0.5
190	13.0	12.9	0.7	12.4	4.5	7.9	7.8	0.9	7.7	1.4	7.7	1.9
710	12.3	12.2	0.6	11.8	3.8	7.8	7.7	0.9	7.7	1.4	7.6	1.9
10,000	12.4	12.4	0.3	11.8	4.7	7.4	7.2	2.5	**6.8***	**8.0***	**6.6#**	**10.5***
F_{1b} litter												
0	13.2	13.2	0.5	12.8	3.1	7.6	7.5	1.5	7.5	1.5	7.5	1.5

Table 19: Litter data

Parental generation			F_0					F_{1b}				
Dose level [ppm]			0	50	190	710	10,000	0	50	190	710	10,000
50	14.2	13.8	2.7	13.0	8.4	7.9	7.8	0.5	7.8	0.5	7.8	1.4
190	13.4	13.2	1.5	12.8	4.2	7.9	7.5	5.0	7.4	6.5	7.3	7.5
710	13.6	13.3	2.5	12.6	7.1	8.0	7.7	2.7	7.6	4.3	7.5	5.4
10,000	13.2	12.7	3.5	12.2	6.5	8.0	7.9	1.3	7.7	3.1	7.5	**5.4***
F_{2a} litter												
0	13.4	13.2	1.4	12.1	8.9	7.6	7.4	3.0	7.4	3.0	7.4	3.0
50	12.6	12.5	0.6	12.1	3.8	7.6	7.5	1.1	7.5	1.1	7.5	1.1
190	12.2	11.9	2.3	11.4	6.1	7.8	7.5	3.9	7.5	3.9	7.4	4.6
710	12.7	12.3	3.4	12.2	4.1	7.9	7.8	1.2	7.8	1.2	7.7	3.0
10,000	12.1	11.8	2.5	11.0	8.6	7.5	7.2	3.7	6.8	9.6	**5.5$^{@}$**	**26.2$^{@}$**
F_{2b} litter												
0	14.1	13.2	6.0	12.7	9.4	7.8	7.8	0.7	7.7	1.4	7.7	1.4
50	12.8	12.7	0.9	12.3	3.4	7.9	7.8	1.4	7.7	2.1	7.7	2.1
190	12.3	12.2	1.3	11.9	3.2	7.8	7.5	4.4	7.4	5.0	7.3	6.3
710	12.9	12.6	2.3	12.2	5.2	8.0	7.9	1.4	7.7	4.2	7.4	7.6
10,000	12.8	12.1	4.7	11.8	6.5	7.8	**7.4***	**5.5***	7.1	**9.4***	**6.3$^{\#}$**	**19.5$^{\#}$**

* $p < 0.05$; $^{\#}$ $p < 0.01$; $^{@}$ $p < 0.001$ (Kruskal-Wallis or Fischer's exact test)

In addition, significant changes of litter size and cumulative pup loss were observed in all generations at the top dose level. These effects were seen from day 12 in F_{1a}, from 21 for F_{1b} generation, from day 21 in the F_{2a} generation and from day 8 for F_{2b}. The results are summarized in the table above.

Pup mortalities were associated in many instances with failure to gain weight or actual weight loss (decrease up to 10%). For F_{1a}, significant lower pup weights were observed from day 8 at 10,000 ppm and from day 12 at dose levels ≥ 190 ppm. The decrease in pup weights was not significant for F_{1b}. For F_{2a} and F_{2b} generations, significant lower pup weights were noted at birth and at day 21 post-partum but were not dose-dependent.

A decrease of adjusted brain weights were seen in pups killed at weaning (F1a and/or F2b). In contrast to parental animals, adjusted liver weights were increased in pups. No comparable changes of adrenal weights were observed in pups.

Table 21: Body weight development of F1a to F2b pups during lactation

Litter	Dose [ppm]	Lactation Day					
		0	4 pre-cull	4 post-cull	8	12	21
F_{1a}	0	5.7	8.8	8.8	16.9	26.7	51.1
	50	5.9	8.6	8.6	16.0	25.3	48.1
	190	5.9	8.4	8.4	15.5	**24.4***	**46.4****
	710	5.9	8.8	8.9	16.2	**24.8***	**46.6****
	10,000	6.1	8.9	9.0	**15.1***	**24.4***	**46.2****
F_{1b}	0	5.9	8.9	8.9	16.4	25.2	49.9
	50	5.4	7.9	7.9	15.0	23.5	47.0
	190	5.6	7.9	7.9	14.1	22.3	44.1
	710	5.5	7.9	7.9	14.2	22.6	44.8
	10,000	5.6	8.6	8.6	15.6	24.3	46.5
F_{2a}	0	5.5	7.8	7.8	14.3	23.4	47.2
	50	5.7	8.7	8.7	15.3	24.3	47.3
	190	**5.8***	7.9	7.9	13.5	21.4	**42.0***
	710	**5.9****	8.7	8.7	15.5	23.8	46.5
	10,000	**6.0****	8.3	8.4	14.4	22.2	**41.6****
F_{2b}	0	5.6	8.3	8.3	15.5	24.7	50.1
	50	6.0	9.1	9.1	16.9	26.3	52.3
	190	**6.1***	8.3	8.4	14.9	23.4	46.2
	710	**6.0***	8.2	8.3	14.6	22.5	44.4
	10,000	**6.2****	8.6	8.7	15.1	23.4	**44.8***

* $p < 0,05$; ** $p < 0.01$ (Kruskal-Wallis and Jonkheere)

The decreased survival rate is also evident from the slightly low lactation index (pup alive at day 21/pup alive at day 4 post cull x 100) noted in all F_1 and F_2 generations at 190 and 710 ppm whereas a more pronounced effect on the lactation index was observed at 10,000 ppm (no statistical test reported). These data are summarized below.

Table 22: Lactation indices observed in the flufenoxuron 2-generation study

Dose level	F_{1a}	F_{1b}	F_{2a}	F_{2b}
0 ppm	98.6	98.4	96.9	98.6
50 ppm	99.5	98.5	98.8	97.9
190 ppm	98.1	92.4	90.4	93.6
710 ppm	98.1	94.5	96.4	92.4
10,000 ppm	84.1	90.3	62.3	66.2

No changes in maternal behaviour (that could be associated to the mortality of pups) were reported. Where it is possible to make an assessment, the dead pups frequently showed absent or minimal stomach content (F_{1a}: 2 female at 190 ppm, F_{1b}: 1 female at 190 ppm, F_{2a}: 1 male at 190 ppm and 1 male at 10,000 ppm, F_{2b}: 2 males and 1 female at 710 ppm).

Based on the decrease of body weight in males (≥ 10 %) and on the dilatation of uterus in females, the NOAEL for parental systemic effects was 50 ppm for males (3.8 mg/kg bw) and 710 ppm for females (61 mg/kg bw). The NOAEL for fertility was at least 10,000 ppm (≈ 875 mg/kg bw/day).

Based on the effects on pup survival and lower pup weights the developmental NOAEL was determined to be 4.3 mg/kg bw/day (50 ppm) (Flufenoxuron, 2010).

In a preliminary study to the cross fostering study (James and Jones, 1992), a group of 15 (presumably) pregnant Sprague-Dawley rats was fed from day 3 of gestation until weaning with flufenoxuron at a dietary level of 20,000 ppm (no equivalence was reported in mg/kg bw/d). The purpose of this study was to investigate whether or not the adverse effects on pup survival and growth could be reproduced when flufenoxuron was administered during gestation and lactation only. Neither treatment-related clinical signs nor effect on maternal body weight development were observed. The life birth index (no. live pups/no. pups born x 100), the viability index (pups alive day 4/pups alive at birth x 100) and the lactation index (pups alive day 21/pups alive day 4 x 100) were 98.2, 98.2 and 98.8%, respectively and thus not considered to be affected by treatment. Pup weights were comparable to historical control values throughout lactation. There was no investigation of achieved dietary concentration and no concurrent control group of animals, therefore the study can not be a base for definitive conclusion. The study provides indication that exposure at relatively high level of flufenoxuron only during gestation and lactation in the diet does not affect development and survival of pups before weaning. It may suggest that long exposure before pregnancy, during gestation and lactation is required to affect survival and development of rat pups.

In a cross-fostering study (Masters, 1996), a group of 50 females rats was fed with flufenoxuron at a dietary level of 20,000 ppm [corresponding to an average daily compound intake for the premating period of 1633 mg/kg bw/d (ranging from 2130 mg/kg bw/d on week 1 to 1,304 mg/kg bw d on week 10)] during a 10 week pre-mating period, during mating and subsequent gestation.
During lactation, previously treated dams received control diet in order to avoid a direct exposure of the offspring. A control group of 50 females was likewise mated after a 10 week pre-mating period. As soon as possible after parturition, the young were counted, individually identified, sexed, weighed and examined for external abnormalities. Thereafter, the litters were culled to a standard litter size of 8 pups consisting - wherever possible - of 4 male and 4 female pups. A reciprocal cross-fostering of 26 litters was performed between control and treated dams, i.e. control dams (CD) reared treated pups (TP) from treated dams (TD) and vice versa. Fifteen control and 5 treated dams reared their offspring until weaning without cross-fostering. Additional 5 control and 12 treated non cross-fostered dams were used to analyze residual flufenoxuron levels in milk and fat samples on day 1, 7, 14 and 21 post-partum.
Treatment at a dietary level of 20,000 ppm resulted in a slight but significant impairment of maternal body weight development during the pre-mating period. Mean body weights were decreased by 3.7% whereas body weight gain was decreased by 7.8%. During gestation and lactation, body weight for treated females was essentially similar to that of the control group and considered not to be treatment-related, despite attaining statistical significant increase from day 7 to 17 of pregnancy. No effects on fertility were observed. The survival of pups assessed by the viability and lactation indices was not affected by treatment in any group (including the group “treated dams/treated pups”). Pup body weight development was comparable between all groups. These results were summarised in the Tables 23 and 24.

Table 23: Cross-fostering study: Litter parameters at birth

Observation	Group					
	Control			Treated		
No. of dams delivering	46			43		
Implantations per dam	15.0			14.6		
Pre-natal loss:[a]						
- dams with 1 or less	24			21		
- dams with 2 or more	13			8		
Pups born per dam	14.3			14.8		
Live pups per dam	14.2			14.7		
Pup loss:						
- dams with 1 or less	45			43		
- dams with 2 or more	1			0		
Live birth index[b]	99.2			99.4		
Litter weight	88.2			92.3		
Mean pup weight	6.1			6.2		
No of males	49.1			49.9		
	all CD/CP[c,d]	CD/CP	CD/TP	TD/CP	TD/TP	all TD/TP[d]
No. of dams delivering	20	15	26	26	5	16
Pups born per dam	13.4	12.5	15.0	14.6	10.0	15.2
Live pups per dam	13.2	12.2	15.0	14.5	9.8	15.2
Pup loss:						
- dams with 1 or less	19	14	26	26	5	12
- dams with 2 or more	1	1	0	0	0	0
Live birth Index[b]	98.5	97.9	99.7	98.0	99.6	99.2
Litter weight	88.2	84.9	90.8	91.4	76.8	94.8
Mean pup weight	6.3	6.6	6.2	6.3	6.6	6.0
No of males	53.9	51.4	46.8	48.7	40.8	49.4

[a] Does not include dams which were selected for milk and fat sampling and females which delivered more pups than implantation sites were observed

[b] (Number of pups born alive/Number of pups born) x 100; value determined before cross fostering

[c] Key: C = control, T = treated, D = dam, P = pup

[d] Including dams selected for milk and fat sampling

Table 24. Cross-fostering study: Pup survival and body weight development

Parameter	**Group**					
	all CD/CP[a,b]	**CD/CP**	**CD/TP**	**TD/CP**	**TD/TP**	**all TD/TP[b]**
Viability Index[c]	**NA[b]**	**95.4**	**97.6**	**99.5**	**96.3**	**NA[b]**
Lactation Index[d]	**NA**	**93.2**	**100.0**	**100.0**	**96.2**	**NA**
Pup weight [g]						
- day 0	**6.6**	**6.5**	**6.2**	**6.3**	**6.6**	**6.0**
- day 2	**8.1**	**8.3**	**8.2**	**8.2**	**8.7**	**7.7**
- day 4	**11.4**	**11.7**	**11.4**	**11.6**	**12.1**	**10.7**
- day 8	**20.3**	**20.4**	**20.2**	**20.3**	**21.3**	**19.0**
- day 12	**30.6**	**30.7**	**30.3**	**30.7**	**32.0**	**29.5**
- day 16	**42.2**	**41.8**	**41.7**	**41.8**	**42.4**	**40.5**
- day 21	**61.6**	**61.5**	**60.1**	**60.5**	**62.7**	**59.8**

[a] Key: C = control, T = treated, D = dam, P = pup; NA: Not applicable
[b] Including dams selected for milk and fat sampling; for calculation of lactation indices and body weights only dams and litters were considered which were still alive at the respective day
[c] (No. of pups alive at day 4/No. of pups alive at day 0 after culling and (if applicable) cross fostering) x 100; Mean determined on litter basis
[d] (No. of pups alive at day 21/No. of pups alive at day 4) x 100; Mean determined on litter basis

The determination of flufenoxuron levels in fat and milk revealed a rapid decrease upon cessation of treatment (in milk: 450 ± 377 ppm at day 1 post-partum, 91.3 ± 20.2 ppm at day 7 post-partum, 9.4 ± 6.1 at day 14 post-partum and 9.54 ppm (only one sample) at day 21 post-partum; in fat: 973 ± 82 ppm at day 1 pp, 781 ± 240 ppm at day 7 pp, 270 ± 77 at day 14 pp and 48.5 ± 29.6 ppm at day 21 pp).The depletion half-life time was 7.6 and 2.3 days in fat and milk, respectively.

Overall, the administration of flufenoxuron during gestation and lactation (from day 3 of gestation to weaning) in the preliminary cross-fostering study (James and Jones, 1992) and the administration of flufenoxuron starting 10 weeks prior to mating and continuing till parturition in the cross-fostering study (Masters, 1996) did not reproduce the adverse effects on pup survival observed in the 2-generation study (James et al., 1990). These studies however do not investigate the potential effect of flufenoxuron on pups during lactation with long prior exposure of lactating dams.

5.8.2 Developmental toxicity

Administration of flufenoxuron by oral gavage during gestational days 6 to 16 did not cause any adverse effects in pregnant rats at dose levels up to 1,000 mg/kg bw/day (Hazelden and Wilson, 1991a). Neither developmental toxicity nor teratogenicity related to treatment was observed up to the highest tested dose. The slight non-significant increased incidences of minor variations in branching of the carotid and subclavian arteries from the aortic arch in rat fetuses observed at 1,000

mg/kg bw/d are quite common findings in Sprague-Dawley rats and thus were considered to be unrelated to treatment. Accordingly, the maternal and developmental NOAELs for flufenoxuron in the rat are 1,000 mg/kg bw/day (highest tested dose), which corresponds to the limit dose for this type of mammalian toxicity study.

Administration of flufenoxuron to New Zealand White rabbits at dose levels of 0; 10; 100 and 1,000 mg/kg bw by oral gavage during gestational days 6 to 18 did not result in any maternal toxicity up to the highest dose tested (Hazelden and Wilson, 1991b). The slight effects on mean foetus weights (non significant decrease by 7% when compared to the control) observed in the highest tested dose were probably due to an increase of the mean litter size (by about 7%). Secondary to the slightly lower fetal weights, delays of fetal ossification were observed at the high dose level. As delays of ossification are often observed in fetuses of lower weight and are fully reversible, these observations are not considered to be of adverse nature. Slight, but no statistically significant increase in the incidence of several minor visceral variations of vascular branching of blood vessels near the heart was observed in the high group pup but was considered as idiosyncratic and highly dependent on specific laboratory procedure. Thus, this finding is not considered to be treatment-related. Accordingly, the maternal NOEL was 1,000 mg/kg bw/day and the developmental NOAEL was 1,000 mg/kg bw/day.

In a Chernoff and Kavlock Assay (CKA), flufenoxuron in corn oil was administered to pregnant rats at dose levels of 0; 10 and 1,000 mg/kg bw by oral gavage during days 8 to 17 of gestation (Esdaile, 1986b). This study was designed as a screen to identify embryotoxic effects of flufenoxuron. Survival and growth of the litters was observed and each pup was examined for abnormalities. The only significant finding was that four out of 14 high dose dams had difficulties to lactate properly which resulted in the complete loss of 2 litters and increased pup mortality and impaired body weight development in the two other litters. Due to substantial differences in study design compared to the other studies (route of administration and vehicle used could influence the systemic uptake of flufenoxuron) and its rudimentary reporting, a final assessment of this study is not possible.

However, results obtained could be in agreement with the two-generation study (James *et al*. 1990) realized accordingly OECD 416 and GPL where some dead pups showed absent or minimal stomach content. Also these results did not put in evidence teratogenic or prenatal toxicity effects, they may give us supportive information about one possible effect of flufenoxuron: perturbation of the mammary development and lactation process.

Table 26 – Summary of developmental studies

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**Critical effects dams fetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL Teratogenicity Embryotoxicity**	**Reference**
Oral gavage	OECD 414	Rat Sprague Dawley F 26/dose group	Day 6-16 of gestation	0; 10; 100 and 1,000 mg/kg bw/day;	No effects	1000 mg/kg bw/day (highest dose tested)	1000 mg/kg bw/day (highest dose tested)	Hazelden and Wilson, 1991a Christian, 1996
Oral gavage	OECD 414	Rabbit New Zealand	Day 6-18 of gestation	0; 10; 100 and 1,000	No effects	1000 mg/kg bw/day (highest	1000 mg/kg bw/day (highest dose tested)	Hazelden and Wilson, 1991b

		White F 15/dose group		mg/kg bw/day;		dose tested)		
Oral gavage	CKA embryotoxicity study in rats Range finding/ feasibility study not intended to comply with an official guideline.	Rat Fisher 344 F 15/group	Days 8 to days 17 of gestation	0. 10 and 1000 mg/kg	See next column	This screening assay did not result in maternal or developmental toxicity. Four out of 14 high dose dams had difficulties to lactate properly which resulted in the complete loss of 2 litters and increased pup mortality and impaired body weight development in the two other litters. Due to substantial differences in study design (route of administration and vehicle used) and its rudimentary reporting, a final assessment of this study is not possible.		Esdaile, 1986b

The effects of flufenoxuron on postnatal development of the offspring was observed in a 2-generation study in rats (James et al., 1990). Flufenoxuron was administrated though the diet to five groups of Sprague-Dawley rats at dietary concentrations of 0; 50; 190; 710 or 10,000 ppm (4.3; 16.3; 61.6; 875 mg/kg bw/d) throughout the entire study (10 weeks prior to a 20-day mating period until post-weaning period).

The following manifestations of adverse developmental effects were seen:

- increased mortality of offspring during lactation resulting in increased number of total litter losses before weaning at the dietary exposure level of 61.6 and 875 mg/kg bw/day and lower litter size on day 21 post partum (after excluding total litter loses) in four offspring generations at the dose 875mg/kg bw /day, on day 12 after birth in F1a generation and on day 8 after birth in F2b generation
- lower body weight development in males of F1b generation between week 4 and 35 after birth
- 21 days old pups had lower, than in controls, adjusted brain weights in females of F1a, F1b, F2a and F2b and F1a males, significantly higher adjusted heart weights in 3 male pup groups and F1a females, higher adjusted mean liver weights in all groups of male and female pups from 875mg/kg/day except F1b females
- lower adjusted weight of brain of adult F1b female

The described above postnatal developmental toxicity appears to be related to effect on or via lacation, although it cannot be excluded that toxic levels of exposure may be reached in the pups during pregnancy. In the studies without long-term exposure (10 weeks) before conception or without continuation of exposure during lactation the effects on postnatal development were not observed (James and Jones, 1992; Masters, 1996). The ability of flufenoxuron to accumulate in fat may explain that a long pre-lactation exposure of dams to flufenoxuron may be necessary to

accumulate at levels sufficient to produce adverse effects that take place during lactation. Therefore, the effects observed in the 2-generation study are most probably due to an accumulation of the substance in the milk, and/or a perturbation of the lactation.

5.8.3 Human data

5.8.4 Other relevant information

5.8.5 Summary and discussion of reproductive toxicity

Fertility

Neither in a 2-generation study (James *et al.* 1990) nor in a modified 1- generation study (Masters, 1996) did Flufenoxuron induce reduction of fertility parameters in spite of very high exposure of males and females at the levels of 16.3; 61.6 and 875 mg/kg/day or 2030-1304 mg/kg bw/day (females only) for 10 weeks before mating, during mating and gestation. Thus, it may be concluded that there is experimental evidence that flufenoxuron does not affect sexual function and fertility.

Developmental toxicity

Administration of Flufenoxuron by gavage to pregnant rats from day 6 to day 16 of gestation in a daily dose of 1000mg/kg bw (Hazelden and Wilson, 1991a), and to pregnant rabbits from day 6 to day 18 of gestation in a daily doses of 10 - 1000mg/kg bw/day did not result in embryo- or fetotoxic effects, nor in structural malformations (Hazelden and Wilson, 1991b). There were no signs of prenatal toxicity observed in other studies (James *et al.* 1990, Masters, 1996).

The results of several experimental studies do not provide evidence that exposure to flufenoxuron does affect prenatal development; however such effects cannot be excluded. There are however, experimental data providing some evidence that the postnatal development of offspring may be affected by long-term maternal exposure before conception, during pregnancy and during lactation until weaning (James et al., 1996).

Classification criteria:

According to Regulation (EC) No 1272/2008 developmental toxicity includes, 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).

However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, 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.

According to DSD, substances shall be classified into reproductive toxicity category 3 "Substances which cause concern for humans owing to possible developmental toxic effects" generally on the basis of:

> results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in category 2 or base on other relevant information.

Comparison of experimental data with the classification criteria:

The only results indicating possible effect on animal development come from a 2-generation study on rats (James et al., 1990) and they appeared only in postnatal period during lactation as:

- increased mortality of offspring during lactation period,
- reduced body weight of pups
- alterations in adjusted weight of brain, heart and liver in weanling pups.

The other studies do not indicate developmental toxicity of flufenoxuron. Teratogenicity studies in rats (1000mg/kg bw/day by gavage) (Hazelden and Wilson, 1991a) and rabbits (up to 1000mg/kg bw/day by gavage) did not reveal teratogenicity or fetotoxixity of flufenoxuron (Hazelden and Wilson, 1991b). The study on rats with exposure during from day 3 of gestation to weaning (20 000 ppm in a diet) did not reveal developmental toxicity (James and Jones, 1992). Also in the cross fostering study with long exposure of female rats before mating and during pregnancy (20 000ppm - 2130-1304mg/kg bw/day), originally aimed at clarification whether effects observed in a 2-generation study were induced prenatally or postnatally, did not reveal signs of development at toxicity in neither group (Master, 1996). Chernoff and Kavalock Assay designed as a screen of embryotoxic and fetotoxic properties, in which flufenoxuron given to female rats by gavage during pregnancy in a dose of 1000mg/kg bw/day with an observation of offspring only till 5th day after parturition demonstrated only difficulties of dams to lactate properly and death of pups in four litters (Esdaile, 1986b)

Taking into account all above studies it is concluded that a necessary prerequisite for flufenoxuron to induce effect on offspring observed only during lactation period is a long term exposure before mating, during pregnancy and during lactation and that these effects are not induced during pregnancy. Thus, in order to observe any effects in offspring during lactation the exposure of dams must continue during all these three periods. Therefore the effects observed in a 2-generation study were not induced prenataly, and most probably they were induced by a combination of the three possible mechanisms:

- Alteration of milk quality
- Alteration of milk quantity
- Toxic concentration of flufenoxuron in milk

These mechanisms are fully covered in the category "Effects on or via lactation", which is discussed more in details in next section.

Taking into account the above rationale it is proposed to not classify flufenoxuron as a developmental toxicant.

The Dossier Submitter originally proposed a classification of Repr. 2 – H361d in accordance with CLP and of Repr. Cat. 3; R63, in accordance with Directive 67/548/EEC. However, the Dossier Submitter did not propose this classification in the dossier resubmitted after public consultation. In the public consultation three Member States Competent authorities (MSCA) were in favour of this originally proposed classification, two MSCA considered that such classification was not warranted, and one MSCA requested more detailed data.

Effects on or via lactation:

The following effects indicate that flufenoxuron may affect postnatal development of offspring by inducing effects on or via lactation:

- an increase of total litter losses associated in many instances with failure to gain weight or actual weight loss was observed at levels ≥ 710 ppm (≥ 61 mg/kg bw/d) in a two generation study (James *et al.* 1990). Half of total liter losses occurred before a cull performed on day 4 post partum, and another half after PND 4.The effects were observed in 4 produced F1 and F2 generations.

- a slight, but statistically significant reduction of mean number of pups in litters that survived due to loss of individual pups from PND 8 to PND 21 in F_{1a}, F_{1b}, F_{2a} and F_{2b} generations (James *et al.* 1990)

- lower mean body weight of pups starting from day 8 in 875mg/kg bw/day and then in pups of dams exposed at 16.3; 61.6 and 875 mg/kg bw/day on day 12 and 21 (James *et al.* 1990) in F_{1a} generation, and of pups of F_{2a} and F_{2b} generation on postnatal day 21 in a group of 875mg/kg bw/day

The toxic effects induced on or via lactation by flufenoxuron were rather mild. The mortality of pups during lactation starting before postnatal day 4 and continued till weaning on postnatal day 21 was increased, but was not very high. Even in a group 875mg/kg bw/day the majority of litters in the four (F_{1a}, F_{1b}, F_{2a} and $F_{2b)}$ generations of offspring have survived.

The reduction in the pace of physical development during lactation of surviving pups in F_{1a}, F_{1b}, F_{2a} and F_{2b} generations was not large, not more than ca. 10% of the pace of physical development of control animals.

Those F_{1b} offspring animals which were used as parental animals to produce F_{2a} and F_{2b} generation have only slightly delayed normal physical development. At the end of treatment on week 35 the body weight gain in a group exposed at 875mg/kg bw/day was 90% (males) and 96% (females) of body weight gain of control animals in spite of treatment continuation from week 4 till week 35. The fertility F_{1b} generation was also not affected.

The effects observed during lactation were rather not related to self-feeding of pups with mothers' feed containing high concentration of flufenoxuron. First, mortality of pups was increased already before postnatal day 12, or even before PND 4, when pups are rather enable to eat mothers feed. Second, no increase in mortality of pups between PND 0 and PND 21 was observed in a study in which pregnant rats were fed from day 3 of gestation until weaning of pups with a diet containing 20 000pm of flufenoxuron (James and Jones,1992). The equivalent dose in mg/kg bw/day was not reported in this study, but it may be estimated to be ca. 1500mg/kg bw day. In spite of the potential access to mothers diet containing large amount of flufenoxuron the viability and lactation indexes were not affected, which suggest that self-feeding with mothers diet was not a cause of observed effects in 2-generation study. This study also suggests that even high exposure of dams during

gestation and lactation does not create conditions in which the effects of flufenoxuron on or via lactation can be induced.

In comparison with the 2 generation study of James et al. (1990) in which mortality and delayed physical development were observed, in this study (James and Jones,1992) using even two times higher concentration of flufenoxuron, but mothers were not exposed before lactation. Thus long-term exposure before gestation, during gestation and lactation is necessary to observe effects of flufenoxuron on or via lactation.

However, it has been show that long-term exposure before pregnancy and during gestation, without exposure during lactation, is not sufficient to affect quantity or quality of milk to affect development of pups during lactation (Masters, 1996). In this cross-fostering study dams were exposed in a diet to flufenoxuron 10 weeks before mating, during mating and gestation, but not during lactation. Some control dams were rearing pups born by treated dams, and vice versa. No difference in pups mortality and physical development were observed between groups, suggesting that both long-term exposures before pregnancy and during lactation are necessary for flufenoxuron to produce the effects.

The long-term exposure of dams might be necessary to accumulate high deposits of flufenoxuron in fat, particularly in mammary gland, which can be then exerted with milk to affect pups development. Flufenoxuron is excreted in milk of rats (Masters, 1996), however, in case when exposure is stopped at parturition the concentration of flufenoxuron is rapidly decreasing with a depletion half-life time equal to 2.3 day. On PND 7 the concentration of flufenoxuron in milk is 5-times lower that on PND 1. It is not known whether exposure to flufenoxuron during lactation will increase this half-time, and ensure that the concentration of flufenoxuron will not rapidly decrease in few first day of lactation. The high exposure to flufenoxuron during lactation was not sufficient to induce effects on or via lactation.

The probable doses of flufenoxuron with milk would be rather low in comparison with exposure levels of dams. Using data provided in the Masters study (1996) at the beginning of lactation a potential dose taken with milk by pup would be in range of 90mk/kg bw/day (assuming concentration of flufenoxuron in milk 450 ppm, milk intake 1 ml, and body weight 5 grams) and on PND 8 it could be in a range of 6.6 mg/kg bw/day (assuming concentration of flufenoxuron in milk 100 ppm, milk intake 3 ml, and body weight 15 grams). At that level of exposure to flufenoxuron in milk (Masters, 1996) no effect were observed in suckling pups, however in 2 generation studies the level of flufenoxuron in milk could be much higher than concentrations detected in the Master study (1996) as the exposure was much longer and lasted also during lactation.

The toxicokinetic studies indicate that flufenoxuron is well absorbed in the gastrointestinal tract, reach highest concentration in the fat, where its half-life time is longer than in other tissues. The exposure time to reach steady-state is most probably in rats in order of 1-2 months (Morrison and Huckle, 1988). The mean elimination half-life time in rats after 28 day exposure was approximately 34 days, with a shorter half-time in fat (approximately 28 days). Flufenoxuron is very bioaccumulable in fish with BCF $\geq$ 500.

All these results indicate that the adverse effects on pup survival observed in the 2-generation study are likely due to a chronic exposure of dams leading to a bioaccumulation of flufenoxuron in maternal body, a particularly in fat tissue, and which can be then exerted with milk, providing that exposure is continued also during lactation.

There is also possibility that flufenoxuron can affect quantity or quality of milk. The hypothesis that flufenoxuron is reducing quantity of milk is only based on observation of few dead pups without or reduced amount milk in the stomach, and it is not sufficiently supported.

The more plausible is hypothesis that long-term exposure before pregnancy and during lactation can affect quality of milk. The possible mechanism was proposed to explain the changes in quality of milk (Christian, 2007): Inhibition of maternal lactation and reduced milk fat content as the result of reduced triglyceride levels in the dams and/or reduced triglyceride levels in the pups secondary to reduced maternal milk quality

Indeed, in the repeated-dose toxicity studies in rats (28 days, 90 days and 24 months), flufenoxuron induced reduced triglycerides levels. Furthermore, flufenoxuron was found to have a high affinity for fat (toxicokinetic studies) and was detected in the milk of lactating rats (Master, 1996). The hypothesis of a perturbation of the mammary development and lactation process could also be supported by the fact that some of the dead pups showed absent or minimal stomach content in the 2-generation study and that some dams had difficulties to lactate properly (although substantial differences in study design from 2-generation study) in the CKA test. There is further evidence of an effect through milk quality/quantity in the preliminary developmental toxicity study (Esdaile, 1986b) which included a post-natal phase up to day 5 of lactation. This was a gavage study (gestation day 8-17 using doses of 10 (13 dams) and 1000 (14 dams) mg/kg bw/day). 4/14 of the high dose dams failed to lactate properly and some of their pups died and others failed to gain weight. In these dams, mammary development was visibly reduced. During the PPP review, the possibility of a maternally mediated mechanism such as a reduction in maternal lipogenesis was discussed

The effects on pup survival during the lactation and the presence of flufenoxuron in the milk fulfill the criteria set in the Directive 67/548/EEC for R64 and that are respectively similar to point (b) and (c) of CLP criteria:

- *R64 would normally be assigned on the basis of toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk* → flufenoxuron was detected in the milk of lactating rats in the cross-fostering study. Flufenoxuron has a low acute toxicity in adult animals. However, the toxicity in young animals is not known and it is not considered possible to establish what are potentially toxic levels in breast milk. In the 2 generation study, decreases of viability and lower pup body weights were observed during lactation and based on the absence of effect after *in utero* exposure only, these effects are considered as an evidence of the toxic effect of flufenoxuron in milk.

- *R64 would normally be assigned on the basis of results of one or two generation studies in animals which indicate the presence of adverse effects on the offspring due to transfer in the milk* → decrease of viability and lower pup body weights were observed during lactation in the 2-generation study. The cross-fostering study failed to demonstrate that effect was due to an *in utero* exposure only. The preliminary study failed to demonstrate that effect was due to exposure during gestation and lactation without long pre-gestational exposure of dams. The toxico-kinetic profile of flufenoxuron and the observation of effects linked to lactation (transfer of flufenoxuron though the milk and/or perturbation of the lactation) support that the effect is likely to be due to flufenoxuron in milk and that a long pre-exposure of dams to flufenoxuron is necessary to accumulate and lead to adverse effect via lactation.

In addition the data indicates that flufenoxuron fulfill criteria defined in Annex VI of the Directive 1999/45/EC in point 4.2.3.3. stating that substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labeled with R33 and R64.

Therefore a DSD classification **R64 "May cause harm to breastfed babies"** and **R33 Danger of cumulative effects** is proposed for Flufenoxuron, while within a CLP classification Hazard Category for Lactation effects: **Effects on or via lactation** with an associated hazard statement - **H362**).

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

The standard study (Van Helvoirt J.A.M.W. et al., 1990), was performed in accordance with A.14 of Regulation (EC) No 440/2008 "Explosive properties". The study was conducted in compliance with the following Good Laboratory Practice Standard: OECD Principles of Good Laboratory Practice, Paris France.
The test substance was submitted to the effect of a flame (thermal sensitivity), to impact and friction (mechanical sensitivity).

Although the study was performed in accordance with the method A14, there is a difference between the study and the method A14. To determine explosive properties by thermal sensitivity, propane was used with a pressure regulator. In the study, the pressure indicated was 500 mbar contrary to the method A14, which uses a pressure of 60 to 70 mbar. This deviation is not considered to impact the validity of the study.

Exposing the test substance for 300s to thermal stress did not result in an explosion. None of the test tubes showed a deformation. Therefore the test substance was concluded to be not explosive when exposed to thermal stress.

Exposing the test substance to mechanical stress by shock resulted in a pelletation of the test substance. No traces of a vigorous reaction were observed. From this it was concluded that the test substance is not explosive when exposed to mechanical stress by shock.

Exposing the test substance to mechanical stress by friction resulted in a discoloration of the test substance. The test substance decomposed into a dark-gray residue, probably caused by the released friction-heat coming from the moving porcelain surfaces. During the performance of the test no fire, sparks, smoke or crepitation was observed. Therefore it was concluded that the test substance does not react under mechanical stress by friction, but cannot be seen as explosive.

In conclusion, flufenoxuron was determined to be not explosive under the conditions of the test.

No classification for explosivity is proposed.

6.2 Flammability

The standard study (Van Helvoirt J.A.M.W. et al., 1990), was performed in accordance with A.10 of Regulation (EC) No 440/2008 ” Flammability (solids)”. The study was conducted in compliance with the following Good Laboratory Practice Standard: OECD Principles of Good Laboratory Practice, Paris France

Flufenoxuron could not be ignited with a flame. So flufenoxuron has not to be classified “highly flammable”.

For information, flufenoxuron has no self-ignition temperature.

No classification for flammability is proposed.

6.3 Oxidising properties

The standard study (Van Helvoirt J.A.M.W. et al.,1990 was performed in accordance with A.17 of Regulation (EC) No 440/2008 ” Oxidizing properties”. The study was conducted in compliance with the following Good Laboratory Practice Standard: OECD Principles of Good Laboratory Practice, Paris France.

Although the study was performed in accordance with the method A17, there is a difference between the study and the method A17. To determine oxidizing properties, combustible substance (cellulose) and test substance have to be dried in a stove. In the method A17, the temperature of the stove is 105°C and in the study it is 100°C. This deviation is not considered to impact the validity of the study.

The burning behavior as observed for the cellulose/ test substance mixtures is characteristic of non-oxidising test substances.
From this, it has to be concluded that flufenoxuron has no oxidizing properties in the sense that it cannot sustain a reaction with cellulose at a burning rate higher than that of a reference mixture or stimulate combustion of cellulose through a mechanism as found characteristic for substances having oxidizing properties.

In the additional test (verification of the method of mixing and sieving), the burning rate of the reference sample (cellulose/ barium nitrate 40/60) was 2.44 mm/s, the burning rates of the cellulose/ test substance samples varied between 2.08 and 2.44 mm/s.
Thus, the burning rates of the cellulose/test substance samples as found during this proceeding test, were comparable with the burning rate of the tested reference sample. According to the interpretation criteria of the guideline, the test substance in this case has oxidizing properties, as it can sustain a reaction with cellulose at a burning rate higher than or comparable to the burning rate of a reference mixture.

As the burning behaviour of the test substance mixture and the reference mixture differ significantly, comparison between the burning rates must be made with great care and no conclusions can be drawn from these parameters.
Furthermore, the only cellulose/test substance ratio that burned faster than the fastest burning reference mixture in the main study, was ratio 90/10. Previous tests (not a part of this study) show that combustion of cellulose is sometimes stimulated when mixed with an inert substance in a 90/10 ratio. Hence, an increase in burning rate of a 90/10 ratio does not have be caused by oxidizing properties of a test substance, but is more likely due to characteristic burning behaviour of cellulose.

Thus, although the burning rate of the 90/10 cellulose/test substance ratio was comparable to the burning rate of the reference sample, it is concluded that, based on the burning behaviour of the cellulose/test substance mixtures as well as on the ability of inert material to enhance the burning rate of cellulose, this relatively high burning rate cannot be attributed to oxidizing properties of flufenoxuron.

No classification for oxidising properties is proposed.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

Two acceptable studies are available to assess the short-term toxicity to fresh water fish. Results are summarised in the following table 27.

Table 27: Summary of the acute toxicity to fresh water fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Relia-bility	Reference
			design	duration	LC_0 (µg/L)	LC_{50} (µg/L)	LC_{100} (µg/L)		
No guideline	*Oncorynchus mykiss*	Mortality	Flow through	96 h	n.d	> 4.9	n.d	1	Croucher, 1987
EEC 91/414, EEC 96/12, EPA 40 CFR 158, EPA 72-1(c)	*Brachydanio rerio*	Mortality	Flow through	96 h	5.19	> 5.19	n.d	1	Halus, 2001

In the first study, Croucher (1987) exposed *Oncorynchus mykiss* to different concentrations of flufenoxuron in a flow-through test design, during 96h. The 96h-LC_{50} was > 4.9 µg/L. The second study (Halus, 2001) was performed according to relevant guidelines EEC 91/414, EEC 96/12, EPA 40 CFR 158, EPA 72-1(c). *Brachydanio rerio* was exposed to flufenoxuron during 96h, using a flow-through test design. The 96h-LC_{50} was defined > 5.19 µg/L.

Long-term toxicity to fish

Two higher tier full life-cycle studies are available for chronic toxicity and summarised in the following table 25.

Table 28: Summary of the chronic toxicity to fresh water fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Relia-bility	Reference
			design	duration	LC_0 (µg/L)	LC_{50} (µg/L)	LC_{100} (µg/L)		
OECD 201 OECD 219	*Danio rerio*	Mortality, juvenile growth, spawning performance, fertilization rate, sex ratio	static	full life-cycle > 140d	NOEC > 1.199	n.d	n.d	2	Schaefers, 2003
EEC 91/414, EEC 96/12, EPA 40 CFR 158, EPA 72-1(c)	*P. promelas*	Development, growth, survival	Flow through	34d	NOEC > 0.82	n.d	n.d	2	Hillaby, 1990

In the study of Schaefers (2003), zebra fish (*Danio rerio*) was exposed to flufenoxuron during more than 140 days under static conditions including sediment. The study was performed following relevant

OECD test guidelines. An overall NOEC of ≥ 1.199 µg/L (mean measured concentration in water) was determined.

The second study performed with *P. promelas* exposed during 34d, indicated a NOEC > 0.82 µg/L (Hillaby, 1990).

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Two acceptable studies are available to assess the short-term toxicity to aquatic invertebrates and are summarised in the following table 26.

Table 29: Summary of the acute toxicity to fresh water invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Relia-bility	Reference
			design	duration	LC_0 (µg/L)	LC_{50} (µg/L)	LC_{100} (µg/L)		
OECD 202	*D. magna*	Immobility	Static	48h	NOEC = 0.01	0.0429	n.d	1	Funk, 2003
No guideline	*D. magna*	Immobility	Static	48h	n.d	Test1: 0.04 Test2: 0.08	n.d	2	Croucher, 1987

In the first study (Funk, 2003), neonates of *Daphnia magna* collected from in-house culture with age at test initiation less than 24h were exposed to different concentrations of Flufenoxuron (0 to 0.18 µg/L) in static conditions during 48h, according to OECD 202 guideline. Test conditions were maintained at acceptable levels for specie studied at all time points. Immobilization and other adverse effects were recorded at 24 and 48h. The **EC_{50}** was determined to be **0.0429 µg/L** and the NOEC to be 0.01 µg/L.

In the study of Croucher (1987), two test were performed (*i.e.* test1 and test2) with different concentration ranges. In test 1, concentration were: control, 0.026, 0.05, 0.1, 0.26, 0.5, 1.0 and 2.6 µg a.s./L (nominal). In test2, concentration were: control, 0.00475, 0.01, 0.02, 0.0475, 0.1, 0.2, 0.475, 1.0, 2.0 and 4.75 µg a.s./L (nominal). In the first 48 hours static acute toxicity study with *Daphnia magna* the **EC_{50}** of flufenoxuron was determined to be **0.04 µg/L**, in the second test, the EC_{50} was 0.083 µg/L based on mean measured concentrations.

Conclusion: relevant endpoint for classification is **EC_{50} = 0.04 µg a.s./L**

Long-term toxicity to aquatic invertebrates

Available long-term studies on fresh water invertebrates are summarised in the following table 27.

Table 30: Summary of the chronic toxicity to fresh water invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Relia-bility	Reference
			design	duration	LC_0 (µg/L)	LC_{50} (µg/L)	LC_{100} (µg/L)		
No guideline	*D. magna*	Reproduction	Semi-static	21d	NOEC = 0.00449	n.d	n.d	2	Pearson, 1989

The study of Pearson (1989) described long-term exposure of aquatic invertebrates to flufenoxuron. Neonates of *Daphnia magna* (less than 24h at the initiation of the test) were exposed to different flufenoxuron concentrations (0 to 0.02 µg/L [nominal concentrations]) during 21 days in a semi-static

system. NOEC based on reproduction was defined at the concentration of 0.0065 µg/L (mean-measured concentration corrected for adsorption and removal during centrifugation).

The work of Egeler (2006) was conducted in order to determine the potential impact of the test item on the survival, reproduction and biomass of the aquatic oligochaete *L. variegatus*. Adult worms of synchronised physiological state were exposed to a series of toxicant concentrations (the highest concentration being 400 µg/kg sediment dry weight (nominal concentration, corresponding to 305.9 µg/kg dry sediment for measured concentration) applied to the sediment phase of a sediment-water system. The test animals were exposed to the sediment-water systems for a period of 28 days. With respect to reproduction and biomass, no concentration-dependent effects were found. Therefore no EC-values were calculated. There were no mortalities up to the highest concentration level. The NOEC and LOEC were defined to be > 306 µg/kg dry sediment. This study is however not relevant for the classification since the exposure was through sediment and not the water column.

7.1.1.3 Algae and aquatic plants

Available acceptable studies for algae and aquatic plants toxicity are summarised in the following table 28.

Table 31: Summary of the chronic toxicity to fresh water algae

Guideline / Test method	Species	Endpoint	Exposure		Results		Relia-bility	Reference
			design	duration	NOE_rC (µg/L)	$E_rbC_{50}a$ (µg/L)		
OECD 201	*Pseudokirchneriella subcapitata*	growth biomass	Static	96h	950	>100 000	1	Kubitza, 2003
OECD 201	*Pseudokirchneriella subcapitata*	growth biomass	Static	96h	n.d	> 2.975	2	Croucher, 1987

[a] calculated from the area under the growth curve; [b] calculated from growth rate

In the study (Kubitza, 2003), Flufenoxuron was tested for its toxicity to *Pseudokirchneriella subcapitata* in a static system during 96 h with 7 test concentrations (300, 800, 2000, 5300, 14000, 36000 and 100000 µg/L (nominal). Assessments of growth were conducted daily. The test item caused no significant reduction of algal growth or morphological effects up to 36 000 µg/L (nominal concentration, corresponding to the mean-measured concentration of 26040 µg/L). The $\mathbf{E_rC_{50}}$ of Flufenoxuron could not be determined (> 100 000 µg/L), the E_rC_{10} (NOEC) was calculated to 950 µg/L (mean measured values).

7.1.1.4 Sediment organisms

Not relevant

7.1.1.5 Other aquatic organisms

No data available

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

Not relevant

7.2.1.1 Toxicity to soil micro-organisms

The toxicity of flufenoxuron in soil had been tested on physiological functions (C-transformation and N-transformation) of soil micro-organisms (laboratory studies – 28 days – loamy sand soils) according to OECD 216 test guideline (Koelzer, 2003 ; Koelzer, 2003a).

Based on the results of these studies, flufenoxuron caused no short-term and long-term effects on C-transformation (tested as O_2-consumption, deviations from the untreated control ≤25%) and N-transformation (measured as NO_3-N production, deviations from the untreated control ≤ 25%) in a field soil tested up to a concentration of 16.96 mg flufenoxuron per kg dry soil, corresponding to nominally 1.7 mg/kg dry soil.

7.2.1.2 Toxicity to other terrestrial organisms

No data available

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

Not relevant.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

Assessment of the inhibitory effect of the test item on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure of 180 min was carried out, following OECD 209. No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg/L (nominal). The **EC_{50}** of flufenoxuron in the activated sludge respiration inhibition test is **>1000 mg/L.**

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Classification and labelling according to CLP

DATA ELEMENTS		**Value**	**Test method ((EC) No. 440/2008) or OECD guideline / remarks /references**
Physico-chemical properties			
Water solubility:		pH 7: 1.36 µg/l at 25°C pH 4: 1.86 µg/l at 25°C pH 9: 3.69 µg/l at 25°C	Langner E.J., 1988,
Log octanol/water partition coefficient (Log K_{ow}):		5.97 (value estimated by QSAR)	Kowwin (v 1.67)
Acute aquatic toxicity			
Fish:	*Oncorhynchus mykiss*: *Brachydanio rerio*:	>4.9 µg/l (96 h LC_{50}) > 5.19 µg/l (96 h LC_{50})	No guideline (Croucher, 1987) EEC 91/414, EEC 96/12, EPA 40 CFR 158, EPA 72-1(c)
Crustacea	*Daphnia magna*:	0.0429 µg/l (48 h EC_{50}) 0.04 µg/l (48 h EC_{50}) 0.08 µg/l (48 h EC_{50})	OECD 202 No guideline Croucher, 1987 No guideline (Croucher, 1987)
Algae/aquatic plants *subcapitata:*	*Pseudokirchneriella*	>100000 µg/l (96 h ErC_{50}) > 2.975 µg/l (96 h ErC_{50})	OECD 201 OECD 201
Chronic aquatic toxicity			
Fish:	*Danio rerio:* *Pimephales promelas:*	> 1.199 µg/l (140 d NOEC) >82 µg/l (34d NOEC)	OECD 201 OECD 219 (full life cycle) EEC 91/414, EEC 96/12, EPA 40 CFR 158, EPA 72-1(c)

Crustacea: *Daphnia magna*:	0.0049 µg/l (21 d NOEC)	No guideline (Pearson, 1989)
Algae/aquatic plants: *Pseudokirchneriella subcapitata:*	950 µg/l (96 h NOEC)	OECD 201
Degradation (evidence of rapid degradation)		
Biotic degradation:	4% degradation of the test substance was observed in either test D (dissipation)T_{50} in the water of 0.3 to 0.4 days and D (degradation) DT_{50} in the whole system of 85 to 116 days at 12°C (45 to 61 days at 20°C). Formation of metabolites.	OECD 301B (modified Sturm test) and 301D (closed bottle test) OECD Guideline 308
Abiotic degradation, hydrolysis: (half-life (d)):	Stable at pH 4, 5, and 7, but is hydrolyzed at pH 9 with an half-lives of about 90 days at 25ºC and about 1 day at 50ºC	Hassink, 2003
Bioaccumulation		
Bioconcentration factor in fish	15700; 16130	OECD 305E

Aquatic hazard assessment, conclusions and comments:

Physico-chemical properties:

- The substance is has a very low water solubility.

Acute aquatic toxicity:

- The available toxicity data show that crustacea is the most sensitive trophic level in the water column (flufenoxuron is an insecticide). The available data on fish showed no effects at the water solubility limit of flufenoxuron. In one study on algae (Kubitza, 2003) a solvent was used. This allowed reaching concentration of the substance very much above the water solubility limits. No adverse effects were recorded and ErC_{10} (i.e. NOEC) was calculated to 9.5 mg/l. Since flufenoxuron did not cause adverse effects in these concentrations it is reasonable to assume that no adverse effects would occur also at the water solubility limit.
- The data for crustacean lie in the range between 0.00001 and 0.0001 mg/l.

Chronic aquatic toxicity:

- Similarly to the acute toxicity data also available results from the chronic aquatic toxicity studies reveal crustacea as the most sensitive trophic level.
- The chronic aquatic toxicity value lies between 0.000001 and 0.00001 mg/l.

Degradation (evidence of rapid degradation):

- The substance is hydrolytically stable under the environmentally relevant conditions. The available screening degradation data show that the substance is not readily biodegradable. This is confirmed by the water-sediment simulation test, where the substance dissipates fast from the water column to sediment. DT_{50} in the whole system was determined from 85 to 116 days at 12°C (45 to 61 days at 20°C).

Aquatic hazard classification and, where applicable, established M-factor(s):

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 10 000.

Long-term aquatic hazard: category Chronic 1, M-factor: 10 000.

Reasoning:

Acute aquatic hazard:

acute toxicity $L(E)C_{50} \leq 1$ mg/l. M-factor based on $L(E)C_{50}$ between 0.00001 and 0.0001 mg/l.

Long-term aquatic hazard:

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if available information on chronic toxicity merits long-term hazard classification. In absence of adequate chronic toxicity data, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). In order not to take the subsequent step into account it is necessary to have adequate chronic toxicity data for all three trophic levels, see section 4.1.3.3.1.

Adequate chronic toxicity data for crustacean: chronic toxicity NOEC ≤ 0.1 mg/l, not rapidly degradable. M-factor based on NOEC between 0.000001 and 0.00001 mg/l (not rapidly degradable).

Labelling elements based on the classification: GHS09; Warning, H410

Classification according to Directive 67/548/EEC criteria:

The EC_{50} values for invertebrates, are lower than 1 $mg.L^{-1}$. In addition, flufenoxuron is not readily biodegradable, expected to be stable in water and the substance is expected to be highly bioaccumulable in fish.

Therefore, **N; R50/53 is proposed** according to Directive 67/548/EEC criteria.

In addition, as the 48h-EC_{50} for invertebrates is $0.00001 < EC50 \leq 0.0001$ mg a.s./L, a M-factor of 10000 is thus proposed to determine the specific concentration limit.

For the same reason, SCL are proposed for environment under Directive 67/548/EEC:

Specific concentration limits:

$C \geq 0.0025$ %	N, R50/53
$0.00025\ \% \leq C < 0.0025\ \%$	N, R51/53
$0.000025\ \% \leq C < 0.00025\ \%$	R52/53

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Flufenoxuron is not classified according to Annex VI of CLP. Flufenoxuron is evaluated in the context of the Biocidal Product Directive (98/8/EC). In accordance with Article 36(2) of the CLP Regulation, flufenoxuron should be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points.

OTHER INFORMATION

The information available was submitted in the scope of the Biocidal Product Directive for inclusion of the active substance flufenoxuron in annex I of directive 98/8/CE.

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