CLH report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **FLUFENOXURON**

EC Number: 417-680-3

CAS Number: 101463-69-8

Submitted by: France

Date: February 2009

Version 4

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 Directive 67/548/EEC criteria:

Repr. Cat 3; R63
R64
Xn; R48/22
N; R50/53

Proposed classification based on CLP criteria:

Hazard statements:
Repr. 2 – H361d
Lact. – H362
STOT Rep. 2 – H373 (red blood cells and liver)
Aquatic. Acute 1 – H400
Aquatic. Chronic 1 – H410
Signal word: "*warning*"
Pictograms: GHS07, GHS08, GHS09.

Proposed labelling:

Symbol(s): Xn, N

R-phrases: R48/22; R63; R64; R50/53

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

Proposed specific concentration limits (if any):

A factor M = 10 000 is proposed.

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

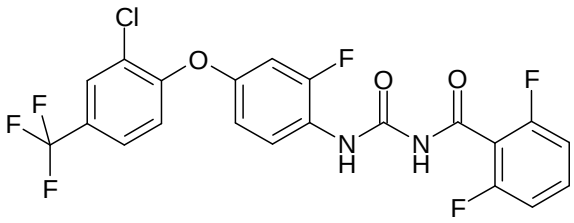
EC Number: 417-680-3

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

Typical concentration (% w/w): ≥ 96 % w/w

Concentration range (% w/w): ≥ 96 % 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 I of Directive 67/548/EEC or 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). 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, Flufenoxuron is considered as potentially persistent.

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. 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, repectively.

Conclusion: Flufenoxuron is considered to be a very bioaccumulable substance with a BCF > 5000.

4.3.2 Terrestrial bioaccumulation

No available data

4.3.3 Summary and discussion of bioaccumulation

Flufenoxuron is considered to be very bioaccumulable in fish.

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 hemorrhage 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. These data are only submitted to provide a toxicological profile 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	Chemosis			
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. 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 methemoglobin (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 methemoglobin with the CO-Oximeter method whereas no effect was observed when a specific methemoglobin 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 anemia 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 anemia 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 methemoglobin 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 anemia, as evidenced by significant decreases in hemoglobin (< 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. Flufenoxuron-related anemia was apparent in all treated groups, as revealed by changes in hemoglobin levels (reduction ≥ 10 % in males), erythrocyte parameters (first noted after 9 weeks of treatment) and increased reticulocyte counts. After 12 and 15 weeks, significant haematological effects were confined to the 50,000 ppm group male. Methemoglobin 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, sulfhemoglobin levels were statistically increased at 5,000 ppm and above for males and at 50,000 ppm for females. These results were summarised in the table below.

Table 8: Haematological effects

Test parameter		Week 9				Week 12				Week 15			
		0	500	5000	50000	0	500	5000	50000	0	500	5000	50000
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
Methemoglobin [%]	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***
Sulfhemoglobin [%]	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***

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 anemia and was accompanied by Kupffer cell pigmentation in the liver and increased hemosiderin deposition in bone marrow, in the spleen and in the proximal tubules of the kidney.

Table 9: Main histopathological findings in relation to anemia

Histopathological findings		Dose levels (ppm)			
		0	500	5,000	50,000
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 hemosiderin	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 anemia and increased levels of methemoglobin.

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 anemia, 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. Methemoglobin 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 (see following table).

Table 10: Haematological findings

Test parameter		Week 5					Week 13					Week 52				
		0	10	100	500	50000	0	10	100	500	50000	0	10	100	500	50000
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***	16.1	16.7	16.5	17.3	15.0
Methemo-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***
Sulfhemo-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 hematopoiesis 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 in relation to anemia

Histopathological findings		Dose levels (ppm)				
		0	10	100	500	50,000
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 hemosiderin	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).

The results of short-term and medium-term toxicity studies are summarised in the table below:

Table 12: Summary of short-term and medium-term toxicity studies

Route	**Method Guideline**	**Dura-tion of study**	**Species Strain Sex no/group**	**dose levels frequency of application**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**
Oral in food	≈ OECD 407	28 days	Rat Fischer 344 M/F 7/sex/group	0; 50; 500; 5,000; 10,000 and 50,000 ppm daily	≥ 5000 ppm Variations in some clinical parameters and increasing weight of spleen and heart.	Males: 5000 ppm Females:50 000 ppm	Males = 500 ppm (49 mg/kg bw/d) Females = 10000 ppm (1067 mg/kg bw/d)	Esdaile, 1986a
Oral in food	≈ OECD 407	28 days (range-finding study)	Mice B6C3F1 M/F 7/sex/group	0; 50; 500; 5,000; 10,000 and 50,000 ppm daily	No adverse effects.	n.a.	50,000 ppm Males = 9,820 mg/kg bw/d Females = 12,157 mg/kg bw/d	Esdaile, 1991a

Route	Method Guideline	Duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral in food	≈ OECD 408	90 days	Rat Fischer 344 M/F 10/sex/group	0; 50; 500; 5,000; 10,000 and 50,000 ppm	≧ 500 ppm: Females: altered RBC parameters indicative of mild anemia with evidence of compensatory erythropoiesis; slightly increased cholesterol (no dose-dependence) ≧ 5,000 ppm: decreased triglyceride for both sex Females: increased spleen wt. > 10,000: increased heart and liver wt for males and females, respectively 50,000 ppm: Males: evidence of mild compensatory erythropoiesis without signs of anemia; marginally increased ALAT and ASAT levels	Males = 5000 ppm (351 mg/kg b.w/day) Females = 500 ppm (41 mg/kg b.w/day)	Males = 500 ppm (35 mg/kg bw/d) Females = 50 ppm, (4.1 mg/kg b.w/day)	Esdaile, 1987 Esdaile, 1991b Berry, 1992a
Oral in food	≈ OECD 408	90 days	Mice B6C3F1 M/F 10/sex/group	0; 50; 500; 5,000; 10,000 and 50,000 ppm daily	≧ 500 ppm: Both sexes: increased serum bilirubin ≧ 10,000 ppm: Females: decreased blood urea nitrogen 50,000 ppm: Males: slightly decreased bw; altered RBC parameters indicative of mild anemia Both sexes: decreased blood urea nitrogen	500 ppm, equivalent to daily intakes of about 103 mg/kg bw in males and 124 mg/kg bw in females	50 ppm; equivalent to about 10 mg/kg b.w/day for male and 12 mg/kg b.w./day for female mice	Esdaile, 1988 Esdaile, 1991c Berry, 1992b
Oral in food	≈ OECD 409	90 days due to a dosing error during the initial two weeks too low dose at 5000 ppm) dosing was extended to 15	Beagle dog M/F 4/sex/group	0; 500; 5,000; 50,000 ppm	≧ 500 ppm: both sexes: transient signs of anemia (wk 9), with evidence of compensatory haematopoiesis females: increased methemoglobin > 5,000 ppm: both sexes; Kupffer cell pigmentation, increased sulfhaemoglobin and methemoglobin males: increased liver wt, increased cholesterol females: Pigment deposition in bone marrow 50,000 ppm:	500 ppm, equivalent to about 18 mg/kg bw in male and 21 mg/kg bw in female dogs	-	Greenough, 1987 Greenough, 1991a Clark, 1988a Clark, 1988b

Route	Method Guideline	Dura-tion of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
		weeks			both sexes: pigment deposition in bone marrow males: persistent signs of anemia; pigment deposition in renal proximal tubules			

Route	Method Guideline	Duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral in food	≈ OECD 452	52 weeks	Beagle dog M/F 4/sex/group	0; 10; 100; 500; 50,000 ppm	≧ 500 ppm: both sexes: evidence of anemia with compensatory hematopoeisis, hemosiderin deposition in Kupffer cells of the liver males: increased platelets; increased liver wt. females: pigment deposition in bone marrow and renal proximal tubular cells 50,000 ppm: both sexes: increased methemoglobin and sulfhaemoglobulin, increased liver wt., fatty vacuolation of hepatocytes; pigment deposition in bone marrow, renal proximal tubular cells and spleen.	500 ppm, equivalent to average daily compound intakes of about 19 mg/kg bw in males and 20 mg/kg bw in females.	100 ppm, equivalent to average daily compound intakes of about 3.5 mg/kg bw/d in males and 3.7 mg/kg bw/d in females dog	Goburdhun *et al*. 1988 Greenough, 1991b

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 anemia 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 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. 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 anemia 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 hemoglobin, 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 oncogenic 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 hematological 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 forestomach, 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).

The results of long-term toxicity studies are summarised in the table below:

Table 13: Summary of long-term studies

Guideline	**Route**	**duration of study**	**Species Strain Sex no/group**	**dose levels frequency of application**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**
General compliance with OECD 452	Oral in food	24 months Chronic feeding study	Rat Fischer 344 M/F 20/sex/group + 10/sex as satellite group for interim sacrifice after 1 year	1; 5; 50; 500; 5,000 and 50,000 ppm (0.044-0.055, 0.23-0.28, 2.2-2.8, 22-28, 233-301, 2,471-3,206 mg/kg bw/d)	≥ 5,000 ppm: decrease in body weight gain, slight anemia, changes in bilirubin, and triglyceride levels, decreased spleen weight in males and increased adrenals weight in females (without corroborative histopathology)	5,000 ppm (233 mg/kg bw/d for male and 301 mg/kg bw/d for female rats)	500 ppm (22 mg/kg bw/d for male and 28 mg/kg bw/d for female rats)	Esdaile 1990a Esdaile 1990 Berry 1992
General compliance with OECD 451	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)	≥ 5,000 ppm: higher survival rate; decrease in body weight gain in females 50,000 ppm: Increased incidence of basophilic foci in liver	Females: 5000 ppm (276.4 mg/kg bw/day) Males: 50,000 ppm (2,289.8 mg/kg bw/d)	Females:500 ppm (25.91 mg/kg bw/day) Males: 5000 ppm (217.5 mg/kg bw/d)	Esdaile 1990b Basford 1991 Berry 1992
General compliance with OECD 451	Oral in food	24 months Oncogenicity feeding 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)	≥ 5,000 ppm: increased mortality for females, Kupffer cell aggregates in the liver only for females 50,000 ppm: decrease in body weight gain; single cell necrosis, hepatocellular hypertrophy and inflammation of the liver; Kupffer cell aggregates in the liver; aggregation of macrophages in the spleen, inflammation of the glandular stomach in males;	Males: 50,000 ppm Females: 5,000 ppm	Males: 5000 ppm (559 mg/kg bw/d) Females: 500 ppm (73 mg/kg bw/d)	Esdaile 1990c FX-428-002 Esdaile 1991 Berry 1992 Finn 1993

Guideline	Route	duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
EPA 83-2, OECD 451	Oral in food	24 months Oncogenicity feeding 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)	Males: no adverse effects Females: increase of uterus distension by a fluid from 1,000 ppm	Males: > 10,000 ppm Females: 1000 ppm.	Males: 10000 ppm (1592 mg/kg bw/d) Females: 100 ppm (17.4 mg/kg bw/d)	Broadmeadow 1996

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 anemia, probably haemolytic, which is characterized by decreases in haemoglobin levels and changes in red blood cell parameters with compensatory hematopoiesis, revealed by changes in bone marrow. In dogs, bone marrow hyperplasia was accompanied by the presence of hemosiderin/pigment deposition in bone marrow, liver, kidney and spleen.

In addition to anemia, increase in methemoglobin 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, methemoglobin was estimated using the specific method of Evelyn and Malloy as well as the unspecific CO-Oxymeter. The results showed that no methemoglobin was detected with the specific method whereas similar increase in methemoglobin to that seen in the 28-days and 90-day studies was observed when the unspecific method was used. In dogs, methemoglobinemia (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). These findings are also supported by the results of the long-term toxicity studies in rats and mice.

In conclusion, flufenoxuron induces an anemia characterized by changes in blood parameters (in particular decrease in haemoglobin levels > 10 %) that was accompanied by increased methaemoglobin from 500 ppm (18-21 mg/kg/d), in association with bone marrow hyperplasia and pigment deposition in bone marrow, liver, kidney and spleen in the 15-week study in dogs. These effects were confirmed in the 52-week study in dogs where histopathological findings appeared from 500 ppm. All these results meet the criteria of severity for risk phrase R48 set in "*Hazard classification of chemicals inducing haemolytic anemia: An EU regulatory perspective*" by EU Working Group on Haemolytic Anaemia. The effects occur at a dose below the threshold of

classification Xn; R48/22 of 50 mg/kg for subchronic oral exposure. This threshold value is set for rats but it appears that dogs are more sensitive to haemolytic anemia than rats and in absence of specific threshold value for dogs, the general threshold for rats is used. The following classification is therefore proposed: **Xn; R48/22**: Harmful: danger of serious damage to health by prolonged exposure if swallowed (CLP STOT RE 2 – H373).

Based on the anemia and on the hepatotoxicity, it is proposed to identify red blood cells and liver as primary target organs by oral route. No data are available by dermal or respiratory routes and it is therefore proposed to allocate to the hazard statement H373 the following additional statement for target organ but not for route of exposure: H373 (red blood cells and liver).

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.

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 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).

[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).

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 %.

Table 14: 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 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.	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.	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* mammalian chromosome aberration assay Peripheral human	3.164 – 160 µg/ml (solubility limit in culture medium)	–	–	Limited precipitation, which redissolved on agitation of the cultures, was observed at the top 3 doses, indicating that a concentration close to the limit of	McEnaney 1992

Test system Method Guideline	Organism/ strain(s)	Concentrations tested	Result		Remark	Reference
			+ S9	- S9		
			+/-/±	+/-/±		
	lymphocytes				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.	
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 to 18 % without metabolic activation and to 6 % with metabolic activation.	Clare and Wiggins 1986 Brooks 1991

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 (despite the fact that the number of analyzed cells per rats was half the OECD recommendation and there was no data on mitotic indices or cell ploidy) or 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 (the PCE/PCE + NCE ratio was not determined in the first study and no decrease of this ratio was observed in the second study), 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

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 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 a HGPRT gene mutation test in mammalian cells. Nevertheless, in this latter test, the highest tested dose was not cytotoxic.

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. Although the deficiencies of some *in vivo* tests, the lack of any clastogenic effects following *in vivo* exposure to flufenoxuron is confirmed in both a rat bone marrow chromosomal aberration assay and a mouse

bone marrow micronucleus assay. Moreover, an *in vivo/in vitro* UDS test, in rat liver cells, was negative.

In conclusion, the negative results (non-clastogenicity) obtained *in vivo* in the rat bone marrow chromosomal aberration assay, the mouse bone marrow micronucleus assay and the *in vivo/vitro* UDS test – should override the positive response noted in *the in vitro* chromosomal aberration assay in CHO cells.

Hence, the available data set on flufenoxuron does not suggest genotoxic concern.

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 hemangiosarcomas in the spleen (14 % vs 0% in the control females). There were no treatment-related increases in the incidence of vascular tumors, either hemangiomas, 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.

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 all 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.

The apparent increase in the incidence of hepatocellular carcinoma in treated male mice, in the first study, is therefore considered to be associated with the unusually low incidence of these tumors recorded in the control males and is not considered to be directly related to treatment. 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 tumors in the first mouse oncogenicity was probably due to the exaggerated dose, higher than the maximum tolerated dose (7,780 mg/kg bw/day).

As a conclusion flufenoxuron is considered to be devoid of a relevant oncogenic potential and no classification was proposed for this endpoint.

Table 16: 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	50,000 ppm: increased incidence of hepatocellular carcinoma in	LOAEL oncogenicity: 50,000 ppm	NOAEL oncogenicity: Males 50000 ppm (7356 mg/kg bw/day)	Esdaile 1990 Esdaile 1991 Berry 1992

Route	duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference
			bw/d)	treated males (overall incidence of hepatocellular tumors not affected, absence of a dose-response relationship); increased incidence of vascular tumors in high dose females		Females: 5000 ppm (739 mg/kg bw/day)	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 males and 1,890 mg/kg bw/day in females)	Broadmeadow 1996

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.

Table 17: 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.

During lactation, there was an increase in the incidence of total litter losses over the four matings, at dose levels ≥ 710 ppm. For F_0 animals, 1/54, 0/52, 1/53, 4/53 and 6/52 total litter losses were observed for the 2 matings at 0, 50, 190, 710 and 10,000 ppm 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. 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 18: 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

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 below.

Table 19: 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
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)

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.

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 20: 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 21: 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.

Based on the decrease of body weight in males (≥ 10 %) and on the dilatation of uterus in females, the NOAEL for 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, the developmental NOAEL was determined to be 50 ppm (4.3 mg/kg bw/day).

In a preliminary study to the cross fostering study (James and Jones, 1992), a group of 15 (presumably) pregnant Sprague-Dawley rats was fed with flufenoxuron at a dietary level of 20,000 ppm (no equivalence was reported in mg/kg bw/d) from day 3 of gestation until weaning. 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.

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. Pup body weight development was comparable between all groups. 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) were not sufficient to reproduce the adverse effects on pup survival observed in the 2-generation study. Furthermore, analysis of flufenoxuron levels in fat and milk revealed a rapid decrease upon cessation of treatment in the cross-fostering study. All these findings indicate that a continued maternal exposure to flufenoxuron during pre-mating, mating, gestation and lactation is required to result in reduced pup survival. It probably contributes to maintain a high level of substance in the maternal body and milk. It also indicates that an exposure of pups both *in utero* and through milk is required to produce this adverse effect.

Table 22 : Summary of reproductive studies

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**Critical effect**	**NO(A)EL Parental**		**NO(A)EL F1**		**NO(A)EL F2**		**Reference**
						m	f	m	f	m	f	
Oral in food	2-Generation study OECD 416, EPA 83-4	Rat (CrL: CD® /SD) BR VAF/Plus Sprague Dawley M/F 28 (P) / 24 (F_1) per group	From 10 weeks prior to a 20-day mating period until sacrifice of parent, F1, F2-generation including ensuing gestation, lactation and post-weaning periods.	0; 50; 190; 710; 10,000 ppm equiv to 4.3; 16.3; 61.6; 875 mg/kg bw/d	Decreased body weight gain (parents and pups) Increased post natal mortality (lower lactation index) Females: increasing incidence of uterus dilatation at 10000 ppm	50 ppm for males (4.3 mg/kg bw/day) 710 ppm for females (61 mg/kg bw/d) Parental fertility: 10,000 ppm= 875 mg/kg bw/day NOAELdevelopment = 50 ppm (4.3 mg/kg bw/day)						James *et al.*, 1990 James, 1991 James, 1992

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses	Critical effect	NO(A)EL Parental		NO(A)EL F1		NO(A)EL F2		Reference
						m	f	m	f	m	f	
Oral in food	Dietary investigative study in pregnant rats rearing young to weaning. Range finding/ feasibility study not intended to comply with an official guideline.	Rat (CrL: CD® /SD) BR VAF/Plus Sprague Dawley M/F 15/group	From day 3 of gestation till weaning	20,000 ppm	No effects on live-birth, viability or lactation indices	Determination of a meaningful NOAEL not possible						James and Jones, 1992
Oral in food	Cross fostering study No official, international accepted guideline	Rat (CrL: CD® /SD) BR VAF/Plus Sprague Dawley M/F 50M and 100F in total	From 10 weeks prior to a 2-week mating period until parturation, then control diet to day 21 post partum	20,000 ppm equiv to about 1633 mg/kg bw/d	20,000 ppm: slightly lower body weight gain (female dams) no effects on fertility, ability to deliver and rear offspring	Determination of a meaningful NOAEL not possible						Masters, 1996

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 fetus 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 reproduction toxicity effects, they may give us supportive information about one possible effect of flufenoxuron: perturbation of the mammary development and lactation process.

Table 23 – 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 White F 15/dose group	Day 6-18 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, 1991b
Oral gavage	CKA embryotoxicity study in rats Range	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		Esdaile, 1986b

	finding/ feasibility study not intended to comply with an official guideline.					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.	

5.8.3 Human data

5.8.4 Other relevant information

5.8.5 Summary and discussion of reproductive toxicity

In a 2-generation study performed with flufenoxuron in rats, an increase of total litter losses was observed at levels ≥ 710 ppm (61 mg/kg bw/d) and was associated in many instances with failure to gain weight or actual weight loss. The general decrease in survival was also indicated by significant changes of litter size and cumulative pup loss at the top dose level from day 8. No effect on fertility was observed in male or female rats. Two other fertility studies were performed to investigate the reason for the increased post-partum mortality of pups. No effects on pup survival were observed when flufenoxuron was administered from day 3 of gestation to weaning or from 10 weeks prior mating until parturition. No developmental effects were reported in the teratogenicity studies. Furthermore, in the cross-fostering study, a rapid decrease of flufenoxuron levels in milk and fat upon cessation of treatment was noted. 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 during pre-mating, mating, gestation and lactation (required to maintain a high level of substance in the maternal body and milk) and to a subsequent exposure of pups both *in utero* and through milk .

In addition, one possible effect of flufenoxuron that could explain the pup mortality is the perturbation of the mammary development and lactation process. Indeed, some of the dead pups showed absent or minimal stomach content in the 2-generation study. These findings were supported by a CKA test that showed that some dams had difficulties to lactate properly (although substantial differences in study design from 2-generation study).

Based on the effects of flufenoxuron on pup survival and their development and because it can not be excluded that these effects may have been due in part to *in utero* exposure, a classification **Repr. Cat.3; R63** "**Possible risk of harm to the unborn child"** is proposed (CLP Repr. 2 – H361d).

Based on the presence of flufenoxuron in the milk produced by the dams treated and effects on pup survival and their development during lactation, a classification **R64 "May cause harm to breastfed babies"** is hence proposed (CLP Lact - H362).

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

In a standard study (Van Helvoirt J.A.M.W. et al.,1990), flufenoxuron was found not to exhibit any explosive properties.

No classification for explosivity is proposed.

6.2 Flammability

In standard studies (Van Helvoirt J.A.M.W. et al.,1990), flufenoxuron was found to be none highly flammable, it did not exhibit any pyrophoric properties and it has no self-ignition temperature.

No classification for flammability is proposed.

6.3 Oxidising properties

In a standard study (Van Helvoirt J.A.M.W. et al.,1990), flufenoxuron was found not to exhibit any oxidising properties.

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 24.

Table 24: 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	n.d	> 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 25: 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 > 1.119	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 26: 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 27: 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 = 0.0065	n.d	n.d	2	Pearson, 1989
OECD 218	*Chironomus riparius*	Developpement, emergence	Static	28d	NOEC = 80	142.7	n.d	1	Weltje and Pupp, 2007
No guideline	*Lombriculus variegates*	Reproduction, biomass	Static	28d	NOEC ≥ 306	n.d	n.d	1	Egeler, 2006

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).

In two additional studies, long-term toxicity to sediment-dwelling organisms were tested on *Chironomus riparius* (Weltje and Pupp, 2007) and *Lumbriculus variegates* (Egeler, 2006), using spiked-sediment.

The chronic static toxicity test with *C. riparius* was conducted with five concentrations: 30.0, 60.0, 80.0, 100.0 and 150.0 µg/kg dry sediment (nominal target values) Twenty *C. riparius* first instar larvae were added to each vessel. The endpoints observed were emergence rate and development rate. The test animals were exposed to the sediment-water systems for a period of 28 days. The NOEC for both development and emergence rate was 80.0 µg/kg dry sediment.

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.

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 28: 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)	L_bC_{50}[a] (µg/L)	L_rC_{100}[b] (µg/L)		
OECD 201	*Pseudokirchneriella subcapitata*	growth biomass	Static	96h	600	19 228	71 940	1	Pearson, 1989
OECD 201	*Pseudokirchneriella subcapitata*	growth biomass	Static	96h	n.d	> 2.975	> 2.975	2	Egeler, 2006

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

In the document (Pearson, 1989), 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 nor morphological effects up to 36000 µg/L (nominal concentration, corresponding to the mean-measured concentration of 26040 µg/L). The $\mathbf{E_rC_{50}}$ of Flufenoxuron was determined to be **71940** µg/L, the E_bC_{50} was 19228 µg/L (mean measured values).

7.1.1.4 Sediment organisms

No data available

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

7.2.1.1 Toxicity to soil macro organisms

Flufenoxuron has been tested on earthworms (*E. foetida*) in 14-day toxicity studies up to 1000 mg/kg substrate, according to OECD 207 guideline. Furthermore, flufenoxuron was tested in a chronic toxicity and reproduction test up to 5.0 mg a.s./kg soil dry weight (Luehrs, 2001).

In the 14-d toxicity study with Flufenoxuron to earthworms, the LC_{50} was > 1000 mg a.s./kg soil dry weight. In the 56-day reproduction study, Flufenoxuron caused no significant effects on mortality, body weight or reproduction of *Eisenia fetida* up to the highest concentration tested (5.0 mg/kg dry soil). The NOEC was determined to be 5.0 mg/kg dry soil.

7.2.1.2 Toxicity to terrestrial plants

A vegetative vigour test has been conducted to assess the effects of Flufenoxuron (applied as formulated product) on terrestrial plants. Endpoints of the study were the effects on plant weight and plant health. No effects on either plant weight or plant health were observed compared to the control. Therefore it can be concluded, that Flufenoxuron has no phytotoxic potential on terrestrial plants (Sack, 2003).

7.2.1.3 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.

7.2.1.4 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

Summary of relevant ecotoxicological endpoints for classification

Acute toxicity to fish	96h-LC_{50} > 4.9 µg.L^{-1}
Acute toxicity to invertebrates	48h-EC_{50} = 0.04 µg.L^{-1}
Acute toxicity to plant and algae	96h-E_rC_{50} = 71,9 mg.L^{-1}
Chronic toxicity to fish	Full life cycle NOEC > 1.19 µg.L^{-1}
Chronic toxicity to invertebrate	21d-NOEC = 0.065 µg.L^{-1}
Chronic toxicity to plant and algae	NOE_rC = 600 mg.L^{-1}

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.

Based on CLP criteria, the proposed classification is Aquatic Acute 1 – H 400 and Aquatic Chronic 1 - H410 with signal word "*warning*" and pictogram GHS09**.**

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

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Flufenoxuron is not classified according to Annex I of Council Directive 67/548/EEC. 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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