# FLUFENOXURON

*First draft prepared by*
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## Explanation

Flufenoxuron is the International Organization for Standardization (ISO)–approved common name for *N*-{4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl}-*N*′-(2,6-difluorobenzoyl)urea (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service number 101463-69-8. Flufenoxuron is a benzoylurea insecticide and acaricide that is used on fruits, vines and ornamentals to control insects and mites. The pesticidal mode of action is the inhibition of chitin synthesis.

Flufenoxuron has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP). The Meeting considered that the database was adequate for the risk assessment.

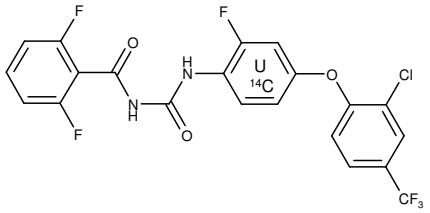
## Evaluation for acceptable daily intake

### 1. Biochemical aspects

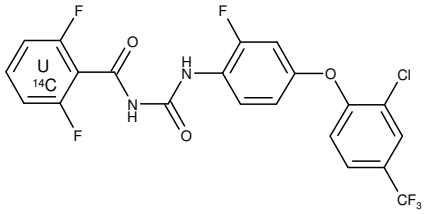
Two $^{14}$C-radiolabelled flufenoxuron molecules were used for the metabolism and distribution studies (Fig. 1).

***Fig. 1. Structure of flufenoxuron with positions of radiolabel***

(fluoroaniline-U-$^{14}$C)-labelled flufenoxuron is designated as "aniline-labelled flufenoxuron" or "[$^{14}$C-aniline]flufenoxuron"




(benzoyl-U-$^{14}$C)-labelled flufenoxuron is designated as "benzoyl-labelled flufenoxuron" or "[$^{14}$C-benzoyl]flufenoxuron"




### *1.1 Absorption, distribution and excretion*

*Rats*

The absorption, distribution and excretion of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99.2%; batch no. S0842 = 2453-81), dissolved in 30% dimethyl sulfoxide (DMSO) in Mulgofen EL719 castor oil, were studied in groups of five male and five female F344 rats dosed orally by gavage at a single dose of 350 mg/kg body weight (bw). The identification of flufenoxuron and its metabolites in tissues and excreta is described in section 1.2 (Huckle, 1987; Hutson, 1991a,b). Urine and faeces were collected at 24-hour intervals. Cage wash was performed on day 3. After 72 hours, the rats were killed, and blood and tissues were sampled and analysed for radioactivity. A preliminary study indicated that less than 0.1% of radioactivity was exhaled as $^{14}CO_2$.

Recovery was 93%. At 24 hours after dosing, 56% and 35% of the radioactivity were found in faeces of males and females, respectively. After 72 hours, 84–85% of the radioactivity was excreted in both sexes. At this time, a total of up to 0.6% of the radioactivity was found in urine, and 7.0–8.3% was found in organs and carcass. Faeces collected from 48 to 72 hours contained 2.5–5.2% of the administered dose. It is likely that the faecal excretion observed for the first 2 days is due to unabsorbed test substance at this high dose level, whereas radioactivity levels in faeces collected on the third day reflect excretion of absorbed test material. Radioactivity was widely distributed to the tissues, the highest concentrations being found in fat, gut wall, liver, bone marrow, skin and kidneys. Based on the levels in organs, carcass, and urine and faeces collected from 48 to 72 hours, it can be concluded that about 9.9–14.1% of the administered dose was absorbed. The ratio of concentration in fat to concentration in blood was about 57, indicating a high potential for accumulation. Tissue levels were similar in males and females (Huckle, 1987; Hutson, 1991a,b).

The absorption, distribution and excretion of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99.4%; batch no. 986-2 = 2712-038), dissolved in 10% DMSO, 10% Mulgofen EL719 castor oil and 80% saline, were studied in groups of five male and five female F344 rats dosed orally by gavage at a single dose of 3.5 mg/kg bw. The identification of flufenoxuron and its metabolites in tissues and excreta is described in section 1.2 (Huckle, 1987; Hutson, 1991c,d). Urine and faeces were collected at 24-hour intervals. Cage wash was performed on day 7. After 168 hours, the rats were killed, and blood and tissues were sampled and analysed for radioactivity. A preliminary study indicated that less than 0.01% of radioactivity was exhaled as $^{14}CO_2$.

Recovery was 93–96%. At 24 hours after dosing, about 9% of the radioactivity was found in faeces of both sexes. On the second day after treatment, faecal excretion was about 4%. Subsequently, daily excretion in faeces was about 2%. In urine, about 2% of the radioactivity was excreted during

the first 48 hours, and about 3% from 48 to 168 hours. At 168 hours, about 67% of the radioactivity was found in organs and carcass. Radioactivity was widely distributed to the tissues, the highest concentrations being found in fat, gut, skin, liver and kidneys. Tissue levels were similar in males and females. Based on the levels in urine, organs and carcass, it can be concluded that at least about 72% of the administered dose was absorbed. The ratio of radioactivity concentration in fat to radioactivity concentration in whole blood was about 53, indicating a high potential for bioaccumulation (Huckle, 1988; Hutson, 1991c,d).

The absorption, distribution and excretion of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99%; batch nos S842/4, S986/5 and S988/4), dissolved in 10% DMSO, 10% Mulgofen EL719 castor oil and 80% isotonic saline at 0.9%, following 28 daily oral gavage doses of 3.5 mg/kg bw were studied in female F344 rats. Groups of three females were killed on days 2, 8, 15, 22, 29, 30, 32, 35, 42, 56, 70, 95 and 205, and blood and tissues were sampled and analysed for radioactivity. The identification of flufenoxuron and its metabolites in tissues is described in section 1.2 (Morrison, 1989; Hutson, 1991e). One female was killed 1 day after the last dosing, and body sections were subjected to autoradiography.

The whole-body autoradiography performed in one female on day 29 indicated that the radioactivity was extensively distributed throughout the carcass.

Total radioactive residue (TRR) concentrations increased with the number of administered doses throughout the 28-day treatment period (Table 1). The increase was apparently linear with time for most tissues, whereas it tended to reach an equilibrium concentration (plateau, not yet clearly observable) in the skin, gastrointestinal tract and bone marrow.

***Table 1. Quantitative distribution of radioactivity in the tissues of female rats administered 28 daily oral doses of [$^{14}$C-aniline]flufenoxuron***

| | TRR (µg equivalents/g tissue) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Day** | **Blood** | **Kidney** | **Liver** | **Gut** | **Ovary** | **Bone marrow** | **Skin** | **Carcass** | **Fat** |
| 2 | 0.3 | 1.8 | 3.0 | 3.3 | 3.0 | 9.0 | 4.1 | 1.1 | 12.7 |
| 8 | 0.8 | 4.2 | 5.9 | 10.9 | 7.4 | 8.6 | 9.6 | 5.2 | 47.9 |
| 15 | 1.5 | 6.5 | 9.4 | 16.3 | 11.5 | 23.1 | 14.5 | 7.4 | 79.1 |
| 29 | 2.7 | 11.2 | 15.7 | 18.1 | 20.2 | 32.6 | 17.5 | 15.5 | 143.6 |
| 30 | 3.0 | 10.3 | 14.9 | 9.5 | 22.9 | 21.7 | 16.4 | 14.1 | 140.9 |
| 32 | 2.9 | 9.5 | 14.3 | 7.3 | 23.3 | 25.4 | 14.9 | 12.8 | 131.7 |
| 35 | 2.8 | 9.2 | 12.9 | 8.3 | 15.7 | 20.9 | 14.6 | 13.1 | 114.4 |
| 56 | 2.3 | 5.6 | 7.9 | 5.1 | 8.1 | 15.6 | 9.6 | 7.1 | 65.1 |
| 95 | 0.9 | 3.0 | 4.1 | 2.2 | 3.2 | 7.3 | 3.1 | 2.3 | 25.1 |
| 205 | < 0.09 | 0.4 | 1.2 | 0.2 | 0.6 | 0.7 | 0.4 | 0.2 | 1.8 |
| $t_{½}$ (days) | 32 | 38 | 48 | 29 | 34 | 34 | 33 | 28 | 28 |

$t_{½}$: half-life

*Source*: Morrison & Huckle (1988); Hutson (1991e)

After termination of dosing, the tissue residues decreased with time. The mean half-life was 34 days (Morrison & Huckle, 1988; Hutson, 1991e).

Five groups of 10 male Fischer 344 rats were fed diets containing unlabelled flufenoxuron (purity 97.4%; batch no. 7016 = ST87/129) at 0, 0.005, 0.05, 0.5, 5, 50 or 500 parts per million (ppm) (equivalent to 0, 0.000 25, 0.0025, 0.025, 0.25, 2.5 and 25 mg/kg bw per day) for 100 days. Twenty animals received control diet. The rats were killed at the end of the study, and omental fat samples from each animal were analysed for flufenoxuron.

In the control and the 0.005 ppm groups, flufenoxuron was not detectable in fat (limit of quantification [LOQ] = 0.1 µg/g). In the 0.05, 0.5, 5 and 50 ppm treatment groups, the average concentrations found in the fat were 0.198, 1.67, 18.6 and 188 µg/g, respectively. These were 3.96, 3.34, 3.72 and 3.76 times the nominal dietary concentrations of the respective groups, indicating that there was a linear relationship between administered dietary concentrations and concentrations of the test item in the fat tissue. In the 500 ppm dose group, the average concentration in the fat (230 µg/g) was only 0.46 times that of the diet and only 22% higher than the average (188 µg/g) concentration detected in the fat tissue of animals fed 50 ppm flufenoxuron. This indicates that there is saturation of the absorption and/or storage of flufenoxuron in fat tissues (Baldwin, 1988).

The absorption, distribution and excretion of [$^{14}$C-benzoyl]flufenoxuron (radiochemical purity > 97%; batch nos S1101/3 and S1101/4), dissolved in an aqueous solution containing 10% DMSO and 10% Mulgofen EL719 castor oil, were studied in F344 rats. The identification of flufenoxuron and its metabolites in tissues is described in section 1.2 (Mayo, 1992). In study A, groups of three males and three females received a single dose of 3.5 or 350 mg/kg bw and were placed in metabolism cages. Urine was collected for 0–6 and 6–24 hours and then at 24-hour intervals up to 168 hours. Faeces were collected during 24-hour intervals up to 168 hours. Expired air was trapped for 0–24 and 24–48 hours after dosing. After 7 days, the rats were killed, and radioactivity levels in the gastrointestinal tract and carcass were measured. In study B, groups of six male and six female rats received a single dose of 3.5 or 350 mg/kg bw. Blood samples were taken from each animal predosing, at 0.25, 0.5, 1, 2, 3, 4, 6, 9 and 24 hours after dosing and then at 1- to 3-day intervals until 336 hours after dosing. The samples from three male and three female rats were used for direct measurement of radioactivity content. The other animals were used for the measurement of radioactivity content in the plasma after removal of the cells by centrifugation. In study C, three males and three females received a single dose of 3.5 mg/kg bw after cannulation of the bile duct. Urine and faeces were collected for 0–24 and 24–48 hours for measurement of the radioactivity. Bile was collected at 3-hour intervals for 48 hours post-dosing. After 48 hours, the rats were killed, and the levels of radioactivity were measured in the gastrointestinal tract and carcass. In study D, one male and one female rat received a single oral dose of 3.5 mg/kg bw. After 4 hours, the rats were killed, and whole-body autoradiography was performed. In study E, two groups of nine males and nine females received a single dose of 3.5 or 350 mg/kg bw. Three male and three female rats per dose were killed at 4, 20 or 168 hours after dosing. Blood samples, organs, tissues and carcasses were subjected to radioactivity measurement. For all studies in which samples were analysed, radioactivity measurements were performed on faeces, blood, gastrointestinal tract, liver and spleen using liquid scintillation counting (LSC) on oxygen combustion products. Direct LSC was used for other sample types, including cage wash and carbon dioxide traps.

*Study A*: At 3.5 mg/kg bw, after 7 days, urine contained 30% of the administered dose in males and 24% in females, including cage washes. In faeces, 19% (males) and 12% (females) of the administered dose were excreted after 7 days. Faeces collected from 48 to 168 hours contained 3.4% and 2.4% of the administered dose in males and females, respectively. It is likely that the radioactivity levels in faeces collected from the third day onwards reflect excretion of absorbed test material. At 168 hours, radioactivity in the remaining carcasses accounted for 46% (males) and 59% (females) of

the administered dose. The gastrointestinal tracts including contents contained 1–2% of the dose. At 350 mg/kg bw, the radioactivity was mainly excreted in the faeces for the 7 days after dosing (93% and 100% in males and females, respectively). Faeces collected from 48 to 168 hours and the content of the gastrointestinal tract contained 2.4% and 10.9% of the administered dose in males and females, respectively. Excretion in urine was 0.7% in males and 0.5% in females. After 168 hours, radioactivity in the remaining carcasses and the gastrointestinal tracts (including contents) accounted for less than 1% of the administered dose (male and female). Based on the levels in urine, faeces, gastrointestinal tract and carcass after a dose of 3.5 mg/kg bw, the absorption was at least 80% in males and 87% in females. At the high dose, absorption was about 3% in males and 12% in females.

*Study B*: The kinetic parameters of flufenoxuron residues in blood and plasma are summarized in Table 2.

***Table 2. Toxicokinetic parameters of radioactive residues of flufenoxuron after a single oral administration of benzoyl-labelled flufenoxuron to rats***

| | 3.5 mg/kg bw | | | | 350 mg/kg bw | | | |
|---|---|---|---|---|---|---|---|---|
| | Blood | | Plasma | | Blood | | Plasma | |
| **Parameter** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| $C_{max}$ (µg/g) | 0.42 | 0.60 | 0.27 | 0.39 | 0.75 | 1.02 | 0.77 | 1.10 |
| $T_{max}$ (h) | 3 | 3 | 6 | 6 | 3 | 3 | 4 | 6 |
| $t_{½\ elim}$ (h) | 178 | 327 | 155 | 428 | NC | NC | NC | NC |
| $AUC_{0-\infty}$ (µg × h/mL) | 38.6 | 62.3 | 25.4 | 62.5 | NC | NC | NC | NC |
| $AUC_{0.25-24\ h}$ (µg × h/mL) | 7.0 | 8.1 | 4.8 | 6.3 | 13.5 | 13.7 | 13.3 | 16.1 |

$AUC_{0-\infty}$: area under the concentration–time curve from time 0 to infinity; $AUC_{0.25-24\ h}$: area under the concentration–time curve from 0.25 to 24 hours; bw: body weight; $C_{max}$: maximum concentration in blood or plasma; F: female; M: male; NC: could not be reliably calculated; $t_{½\ elim}$: elimination half-life; $T_{max}$: time to reach $C_{max}$

*Source*: Hawkins et al. (1992)

Comparison of the $AUC_{0.25-24\ h}$ values indicates that a 100 times higher dose level resulted in only a 1.65 times higher plasma exposure and a 1.8 times higher blood exposure (means on combined sexes), showing that there was a saturation of the absorption at the high dose level of 350 mg/kg bw.

*Study C*: Within 48 hours after a flufenoxuron dose of 3.5 mg/kg bw, 4–5% of the radioactivity was excreted in bile (both sexes), 14% (males) and 9% (females) were excreted in urine and 11% (males) and 4% (females) were excreted in faeces. At 48 hours, 61% (males) and 78% (females) of radioactivity were found in the carcass, and 3–4% (both sexes) remained in the gastrointestinal tract. Based on the amounts recovered from urine, bile and carcass, absorption was at least 79% in males and 92% in females.

*Study D*: Autoradiography of rats killed 4 hours after dosing with flufenoxuron at 3.5 mg/kg bw showed high concentrations of radioactivity in stomach contents, brown fat, small intestine contents and the preputial and clitoral glands. Intermediate concentrations were found in kidney, fat, exorbital lacrimal gland, spleen, liver, muscle, infra-orbital lacrimal gland, lung, pancreas, bone marrow, adrenals, Harderian gland, salivary gland, myocardium, brain, thyroid, thymus and spinal cord. In the female rat, intermediate levels were also observed in the uterine wall and the ovary.

*Study E*: At 4 hours after a single oral dose of 3.5 mg/kg bw, the highest concentrations of radioactivity were found in the adrenals, gastrointestinal tract, liver, ovaries, thyroid and bone marrow (8–28 µg/g). Tissues with intermediate concentrations were the kidneys, lungs, pancreas, and perirenal and subcutaneous fat (4–7 µg/g). Concentrations in other tissues were below 4 µg/g. At 20

hours, the highest concentrations were found in the perirenal and subcutaneous fat (15–17 µg/g). Intermediate concentrations were observed in the adrenals, bone marrow, ovaries, pancreas and thyroid (4–8 µg/g). Concentrations in other tissues were below 2 µg/g. At 168 hours, highest concentrations were present in the perirenal and subcutaneous fat (9–11 µg/g). Intermediate concentrations were observed in the adrenals, bone marrow, pancreas and thyroid (2–3 µg/g). Concentrations in other tissues were in the range of 0.05–1 µg/g.

At 4 hours after an oral dose of 350 mg/kg bw, high concentrations of radioactive compounds were found in the gastrointestinal tract (4000–5000 µg/g). Intermediate concentrations were observed in the adrenals, bone marrow, kidney, liver, ovaries and pancreas (4–8 µg/g). Concentrations were lower in the perirenal and subcutaneous fat (2–3 µg/g). In all other tissues, the concentrations were 1–3 µg/g. At 20 hours, high concentrations were found in the gastrointestinal tract (270–670 µg/g) and in the perirenal and subcutaneous fat (6–14 µg/g). Intermediate concentrations were observed in the adrenals, bone marrow and pancreas (2–7 µg/g). Concentrations in the other tissues were either below the limit of detection or in the range of 1–3 µg/g. At 168 hours, concentrations of radioactivity in most tissues were below the limit of reliable measurement. Concentrations were highest in the perirenal and subcutaneous fat (9 µg/g). The other tissues with any detectable radioactivity were the gastrointestinal tract, liver, adrenals (females only) and pancreas (all 1–3 µg/g) (Hawkins et al., 1992).

The absorption, distribution and excretion of [$^{14}$C-aniline]flufenoxuron (radiochemical purity > 98%; batch no. S1094/3), dissolved in a solution containing 10% DMSO, 10% Mulgofen EL719 castor oil and 80% saline, were studied in F344 rats. Groups of three males and three females received a single dose of 3.5 mg/kg bw by gavage after cannulation of the bile duct. Urine, faeces and bile were collected for 0–24 and 24–48 hours. After 48 hours, the rats were killed, and radioactivity levels in the gastrointestinal tract and carcass were measured. The identification of flufenoxuron and its metabolites in bile is described in section 1.2 (Kirkpatrick, 1992).

Total recovery was 90%. Over the 48 hours, excretion of radioactivity was 19.7% (males) and 6.7% (females) in bile, 2.6% (males) and 1.6% (females) in urine (including cage wash) and 4.0% (males) and 30.2% (females) in faeces. At termination, 5.0% (male) and 4.4% (female) of the dose were recovered from the gastrointestinal tract. In the carcass, 59.1% (male) and 47.3% (female) of the administered dose were found. Based on the radioactivity found in urine, cage wash, bile and carcass, total absorption over 48 hours was 81.4% for male rats and 55.6% for female rats. As the animals were cannulated, these values do not take into account possible enhanced absorption enabled by bile emulsions (the test item being known to be lipophilic) (Kirkpatrick, 1992).

*Dogs*

The absorption, distribution and excretion of [$^{14}$C-aniline]flufenoxuron (radiochemical purity > 98%; batch no. S1022/2), dissolved in DMSO and Mulgofen EL719 in saline, were studied in two male and two female Beagle dogs dosed orally by gavage at a single dose of 3.5 mg/kg bw. The identification of flufenoxuron and its metabolites in tissues and excreta is described in section 1.2 (Hawkins, Elsom & Girkin, 1988; Elsom, 1991a,b). Urine was collected from 0 to 6 hours and from 6 to 24 hours and at 24-hour intervals thereafter. Faeces was collected at 24-hour intervals. Cage wash was performed at each 24-hour interval. Blood samples for haematocrit and radioactivity measurements on whole blood were taken predosing, 1, 4, 8 and 24 hours post-dosing and then at 24-hour intervals thereafter. A part of these samples and additional samples taken at 0.5, 1.5, 2, 6 and 12 hours post-dosing were analysed for radioactivity concentrations in the plasma and in blood cells. After 168 hours, the dogs were killed, and tissues were sampled and analysed for radioactivity.

Recovery was 85%. At 24 hours after dosing, 46–62% of the radioactivity was excreted in faeces. It is noted that three dogs had diarrhoea on the first day of the study, resulting in the excretion of 13–51% of the dose within the first hour after dosing. After 7 days, 56–72% of the radioactivity was excreted in faeces. Excretion in urine was less than 0.7% per day. The finding of 13% of the

radioactivity in the urine of one male dog on day 1 was attributed to contamination of the urine by faeces and/or vomit. After 7 days, 2–4% of the radioactivity was excreted in the urine. Seven days after dosing, 12–23% of the radioactivity was found in tissues. Higher levels were found in fat, bone marrow, adrenals, liver and kidneys. Most tissues apart from the brain contained concentrations of radioactivity equal to or greater than those in plasma. Based on the radioactivity found in urine and tissues, the absorption in the individual dogs was at least 14–27%. In whole blood, an initial peak concentration of 0.264 µg/g was found at 4 hours. Subsequently, the concentration declined to 0.232 µg/g at 8 hours, after which the concentrations slowly continued to rise to 296 µg/g at 168 hours. In plasma, a peak concentration of 0.407 µg/mL was observed at 4 hours. Subsequently, plasma concentrations slowly declined to 0.179 µg/mL at 168 hours. In blood cells, radioactivity concentrations rose from 106 µg/g at 4 hours to 472 µg/g at 168 hours. The ratio of the concentration in fat to the concentration in whole blood was about 12 at 168 hours (Hawkins, Elsom & Girkin, 1988; Elsom, 1991a,b).

The absorption, distribution and excretion of flufenoxuron (purity 97.9%; batch no. 7005) administered in the diet at 500 ppm (equal to 8.6–12.2 mg/kg bw per day) for 19 weeks were studied in seven obese female Beagle dogs (4–7 years old, weighing 16–23 kg). Blood samples were collected from the jugular vein pretreatment, on days 2, 3, 4 and 7 and weekly thereafter until day 56. Additional samples were taken on days 77, 98, 119 and 133 and biweekly thereafter until day 189. Subcutaneous fat samples (approximately 3 g) from all animals were removed by biopsy from the flanks under local anaesthesia at the same time as blood sampling. Twenty-four hours after the last dietary administration, three treated dogs were killed. Two dogs were killed after a 4-week treatment-free period. The remaining two dogs were killed after an 8-week treatment-free period. Tissue samples (liver, kidney, muscle, fat and bone marrow) were taken for analysis of flufenoxuron levels.

No treatment-related clinical signs were observed. All dogs showed a gradual weight loss (0.6–2.9 kg) over the 19 weeks of treatment, which was attributed to the daily rationing of feed to 400 g. Feed consumption was not affected. Individual and mean values of flufenoxuron concentrations in blood of treated animals are presented in Table 3. The mean results show a continuous rise in blood concentration of flufenoxuron until the end of treatment at day 133. In the recovery phase (from day 134 onward), there was, inversely, an approximately exponential decline in flufenoxuron concentrations in blood. An elimination half-life of 33 days was calculated. However, this value is only a rough estimation in view of the considerable individual variation in blood concentrations at each time point.

Individual and mean values of flufenoxuron concentrations in fat sampled from treated animals are presented in Table 4. The mean results show an approximately parabolic rise in flufenoxuron residues in fat during the treatment period. In the recovery phase (from day 134 onward), an approximately linear decline in flufenoxuron levels was observed. There was considerable variation in individual animal results at each time point. The concentration curve is similar to that obtained for blood samples. The fat and the bone marrow contained the highest concentrations of flufenoxuron (respectively: 3.9–43.2 and 3.6–47.1 µg/g) (Greenough, Goburdhun & Parkinson, 1988).

### *1.2 Biotransformation*

*Rats*

The metabolism of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99.2%; batch no. S0842 = 2453-81), dissolved in 30% DMSO in Mulgofen EL719 castor oil, was studied in groups of five male and five female F344 rats dosed orally by gavage at a single dose of 350 mg/kg bw. The study design and toxicokinetics are described in section 1.1 (Huckle, 1987; Hutson, 1991a,b). Identification of metabolites in faecal samples was performed using mass spectrometry. Identification of metabolites in fat and carcass samples was performed using high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). Unextractable radioactivity in faeces was quantified by LSC.

***Table 3. Concentrations of flufenoxuron in blood of dogs (Nos 3–8) given flufenoxuron daily in the diet at a concentration of 500 ppm***

| | | Concentrations in blood (µg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Day of study** | | **No. 2** | **No. 3** | **No. 4** | **No. 5** | **No. 6** | **No. 7** | **No. 8** | **Mean** |
| *Mean daily dose (mg/kg bw)* | | *11.52* | *9.31* | *8.63* | *12.17* | *9.89* | *11.99* | *10.21* | *10.53* |
| Pretrial | | | | | | | | | |
| | Day –1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Treatment period | | | | | | | | | |
| | Day 2 | 0.094 | 0.000 | 0.000 | 0.031 | 0.148 | 0.076 | 0.000 | 0.050 |
| | Day 7 | 0.061 | 0.000 | 0.042 | 0.044 | 0.082 | 0.082 | 0.031 | 0.049 |
| | Day 14 | 0.153 | 0.039 | 0.080 | 0.069 | 0.072 | 0.134 | 0.082 | 0.090 |
| | Day 21 | 0.250 | 0.203 | 0.144 | 0.157 | 0.129 | 0.152 | 0.089 | 0.156 |
| | Day 35 | 0.353 | 0.226 | 0.161 | 0.253 | 0.218 | 0.180 | 0.156 | 0.221 |
| | Day 49 | 0.389 | 0.391 | 0.336 | 0.396 | 0.312 | 0.342 | 0.293 | 0.351 |
| | Day 56 | 0.447 | 0.259 | 0.330 | 0.359 | 0.327 | 0.340 | 0.283 | 0.335 |
| | Day 98 | 0.518 | 0.180 | 0.400 | 0.375 | 0.409 | 0.299 | 0.344 | 0.361 |
| | Day 133 | 0.534 | 0.348 | 0.431 | 0.389 | 0.617 | 0.306 | 0.441 | 0.438 |
| Recovery period | | | | | | | | | |
| | Day 147 | – | – | – | 0.262 | 0.412 | 0.165 | 0.317 | 0.289 |
| | Day 161 | – | – | – | 0.150 | 0.255 | 0.122 | 0.247 | 0.194 |
| | Day 175 | – | – | – | – | – | 0.095 | 0.160 | 0.128 |
| | Day 189 | – | – | – | – | – | 0.038 | 0.144 | 0.076 |

bw: body weight; ppm: parts per million

*Source*: Greenough, Goburdhun & Parkinson (1988)

In faeces, only unchanged parent compound was detected. About 97% of the radioactivity in fat was identified as unchanged aniline-labelled flufenoxuron; the remainder of the radioactivity (~3%) was not characterized. Unchanged aniline-labelled flufenoxuron was the main component in the carcass extract. The combined total of all other minor (non-discrete) radioactive products (< 0.2% of the administered dose) was not characterized. No attempts were made to investigate the profile of aniline-labelled flufenoxuron degradation products present in the urine, liver, kidney or muscle, as they represented less than 5% of the administered radioactive dose (Huckle, 1987; Hutson, 1991a,b).

The metabolism of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99.4%; batch no. 986-2 = 2712-038), dissolved in 10% DMSO, 10% Mulgofen EL719 castor oil and 80% saline, was studied in groups of five male and five female F344 rats dosed orally by gavage at a single dose of 3.5 mg/kg bw. The study design and toxicokinetics are described in section 1.1 (Huckle, 1988; Hutson, 1991c,d). In faecal, organ and tissue samples, metabolites were identified by TLC; in urine samples, metabolites were analysed by TLC and mass spectrometry.

***Table 4. Concentrations of flufenoxuron in fat samples of dogs (Nos 2–8) given flufenoxuron daily in the diet at a concentration of 500 ppm***

| | Concentration in fat (µg/g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Day of study** | **No. 2** | **No. 3** | **No. 4** | **No. 5** | **No. 6** | **No. 7** | **No. 8** | **Mean** |
| *Mean daily dose (mg/kg bw)* | *11.52* | *9.31* | *8.63* | *12.17* | *9.89* | *11.99* | *10.21* | *10.53* |
| Treatment period | | | | | | | | |
| Day 14 | 6.65 | 7.09 | 5.82 | 6.43 | 6.77 | 5.94 | 5.80 | 6.36 |
| Day 28 | 14.57 | 5.13 | 11.86 | 12.11 | 12.69 | 8.69 | 12.69 | 11.11 |
| Day 42 | 27.79 | 12.99 | 18.73 | 16.75 | 19.59 | 17.24 | 15.84 | 18.42 |
| Day 56 | 30.27 | 20.85 | 26.95 | 27.46 | 30.47 | 30.41 | 21.37 | 26.83 |
| Day 77 | 37.15 | 20.60 | 31.89 | 29.94 | 33.75 | 28.13 | 30.66 | 30.30 |
| Day 98 | 44.21 | 24.74 | 33.53 | 35.21 | 33.22 | 22.92 | 28.91 | 30.39 |
| Day 119 | 43.83 | 22.01 | 29.35 | 23.82 | 29.82 | 32.87 | 35.30 | 31.00 |
| Day 134 | 37.56 | 23.05 | 37.45 | 31.47 | 18.04 | 70.86 | 35.40 | 36.26 |
| Recovery period | | | | | | | | |
| Day 147 | – | – | – | 23.48 | 30.65 | 18.72 | 26.05 | 24.73 |
| Day 161 | – | – | – | 13.68 | 23.44 | 13.55 | 16.28 | 16.74 |
| Day 175 | – | – | – | – | – | 4.85 | 12.80 | 8.83 |
| Day 189 | – | – | – | – | – | 2.11 | 0.56 | 1.34 |

bw: body weight; ppm: parts per million

*Source*: Greenough, Goburdhun & Parkinson (1988)

In faeces, unchanged aniline-labelled flufenoxuron accounted for 6%, 0.4% and 0.3% of the administered dose on days 1, 3 and 7, respectively (both sexes). Minor metabolites, less than 1% of the administered dose, were Reg. No. 4110959 (WL 132612, i.e. 2-amino-5-(2-chloro-4-(trifluoromethyl)phenoxy)-3-fluorophenol), Reg. No. 4064702 (WL 129183, i.e. *N*-[4-[2-chloro-4-(trifluoromethyl) phenoxy]-2-fluorophenyl] urea) and Reg. No. 241208 (WL 115096, i.e. 4-[2-chloro-4-(trifluoromethyl)phenoxy-2-fluorobenzenamine). Four per cent of the administered dose in faeces could not be identified. In all organs, the majority of the radioactivity was identified by TLC as unchanged flufenoxuron. In liver extracts, there were at least three other minor components, which were not identified. No metabolite, besides the unchanged compound, was identified in any tissue sample. Metabolite profiles in extracts from males and females appeared to be qualitatively similar. These results indicated that the absorbed flufenoxuron was metabolized by cleavage of the bond adjacent to the 2,6-difluorobenzoyl moiety (Huckle, 1988; Hutson, 1991c,d).

The metabolism of [$^{14}$C-aniline]flufenoxuron (radiochemical purity 99%; batch nos S842/4, S986/5 and S988/4), dissolved in 10% DMSO, 10% Mulgofen EL719 castor oil and 80% isotonic saline at 0.9%, following 28 oral gavage doses of 3.5 mg/kg bw per day was studied in groups of female F344 rats. Groups of three females were killed on days 2, 8, 15, 22, 29, 30, 32, 35, 42, 56, 70, 95 and 205, and blood and tissues were sampled and analysed for radioactivity. Radioactive compounds in abdominal fat were identified using TLC in comparison with the reference test item and by HPLC. The study design and toxicokinetics are described in section 1.1 (Morrison & Huckle, 1988; Hutson, 1991a,b).

Following extraction of the abdominal fat samples from days 29, 56, 70 and 95, TLC analysis showed a single radioactive product with chromatographic properties indistinguishable from those of flufenoxuron. Following quantification of plates, the parent compound accounted for 97.8% (97.2–98.4%) of the radioactivity applied to the TLC plates (Morrison, 1989; Hutson, 1991f).

The metabolism of [$^{14}$C-aniline]flufenoxuron (radiochemical purity > 98%; batch no. S1094/3), dissolved in a solution containing 10% DMSO, 10% Mulgofen EL719 castor oil and 80% saline, was studied in groups of three male and three female F344 rats dosed orally by gavage at a single dose of 3.5 mg/kg bw after cannulation of the bile duct. The study design and toxicokinetics are described in section 1.1 (Kirkpatrick, 1992). Samples of unhydrolysed and acid-hydrolysed bile were analysed in comparison with reference compounds by TLC and HPLC.

The bile contained a high proportion of polar unresolved metabolites, which accounted for 74–79% of the radioactivity. The remaining major component co-eluted with unchanged aniline-labelled flufenoxuron and accounted for 16–21% of the radioactivity. A minor fraction of the radioactivity (0.6–0.9%) resembled Reg. No. 241208 (WL 115096). After acid hydrolysis of bile samples, the proportions of unchanged compound in males and females were similar to those found before acid hydrolysis. The radioactivity co-eluting with Reg. No. 241208 (WL 115096) increased to 5.9% (male) and 6.5% (female) of the sample radioactivity. The fraction of unidentified, unresolved components increased when compared with unhydrolysed samples. This indicated that some polar material had been hydrolysed (Kirkpatrick, 1992).

The metabolism of [$^{14}$C-benzoyl]flufenoxuron (radiochemical purity > 97%; batch nos S1101/3 and S1101/4), dissolved in an aqueous solution containing 10% DMSO and 10% Mulgofen EL719 castor oil, was studied in F344 rats. The study design and toxicokinetics of flufenoxuron are described in section 1.1 (Hawkins et al., 1992; Mayo, 1992). Metabolites were identified by TLC in urine (low dose only) and faeces (low and high doses) of rats receiving flufenoxuron at a single dose of 3.5 or 350 mg/kg bw. The fat from rats treated with 3.5 mg/kg bw and killed at 20 hours post-dosing was sampled, and radioactive compounds were identified using direct TLC in comparison with reference compounds.

In urine of rats treated at the low dose of 3.5 mg/kg bw, there was a major metabolite co-chromatographing with Reg. No. 206925 (2,6-difluorobenzoic acid) and accounting for 12.1% (males) or 10.1% (females) of the dose in 0–48 hours (the amount in 0- to 24-hour urine was also > 10% once corrected for oral absorption of the dose). A second metabolite was found to co-chromatograph with Reg. No. 102719 (2,6-difluorobenzamide) and accounted for 0.3% of the dose during 0–48 hours. Three further polar metabolites (not further identified) were separated in a more polar TLC system; these accounted for 0.4–0.8%, 0.2–0.4% and 0.1–0.3% of the dose. No parent was detected in urine.

Primarily unchanged parent flufenoxuron was detected in the 0- to 48-hour faecal samples from animals treated with benzoyl-labelled flufenoxuron at 3.5 mg/kg bw (9–14% of the administered dose) and 350 mg/kg bw (90–91% of the administered dose).

Only one component was identified in extracts of subcutaneous fat taken 20 hours after low-level dosing. This was shown to co-chromatograph with the parent compound and accounted for approximately all radioactivity found in fat (Hawkins et al., 1992; Mayo, 1992).

*Dogs*

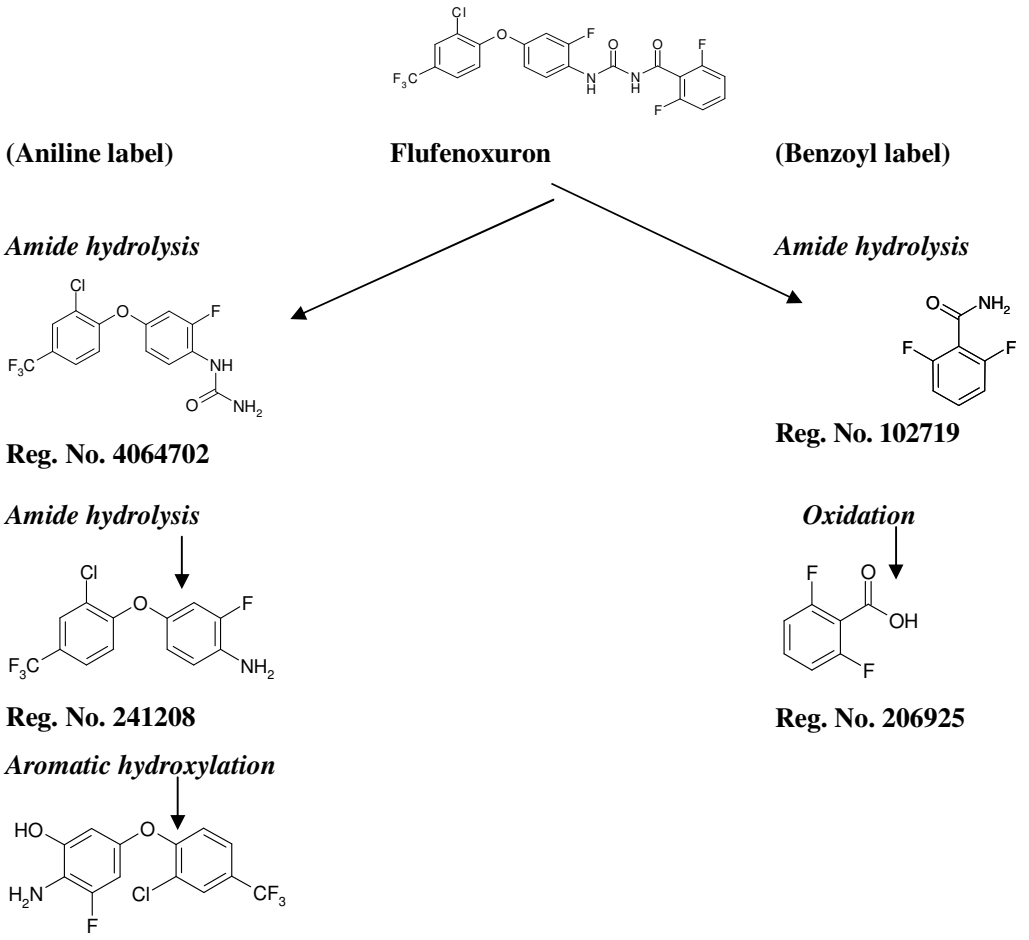
The metabolism of [$^{14}$C-aniline]flufenoxuron (radiochemical purity > 98%; batch no. S1022/2), dissolved in DMSO and Mulgofen EL719 castor oil in saline, was studied in two male and two female Beagle dogs dosed orally by gavage at a single dose of 3.5 mg/kg bw. The study design and toxicokinetics of flufenoxuron are described in section 1.1 (Hawkins, Elsom & Girkin, 1988;

Elsom, 1991a,b). Metabolites were identified by TLC in urine and faeces by comparison with reference compounds.

Faeces and urine contained virtually only unchanged parent compound. One minor metabolite, co-eluting with Reg. No. 241208 (WL 115096), was present in 24- to 48-hour faecal samples (Hawkins, Elsom & Girkin, 1988; Elsom, 1991a,b).

The proposed metabolic pathway of flufenoxuron in rats is shown in Fig. 2.

***Fig. 2. Proposed metabolic pathway of flufenoxuron in rats***




Reg. No. 4064702: *N*-[4-[2-Chloro-4-(trifluoromethyl) phenoxy]-2-fluorophenyl] urea
Reg. No. 241208: 4-[2-Chloro-4-(trifluoromethyl)phenoxy-2-fluorobenzenamine
Reg. No. 4110959: 2-Amino-5-(2-chloro-4-(trifluoromethyl)phenoxy)-3-fluorophenol
Reg. No. 102719: 2,6-Difluorobenzamide
Reg. No. 206925: 2,6-Difluorobenzoic acid

## 2. Toxicological studies

### *2.1 Acute toxicity*

#### *(a) Lethal doses*

The results of studies of acute toxicity with flufenoxuron are summarized in Table 5.

***Table 5. Results of studies of acute toxicity with flufenoxuron***

| Species | Strain | Sex | Route | Vehicle | Purity (%) | $LD_{50}$ / $LC_{50}$ | Reference |
|---|---|---|---|---|---|---|---|
| Rat | Fischer 344 | M/F | Oral | Aqueous carboxymethyl cellulose | 97.6 | > 5 000 mg/kg bw (M/F) | Gardner (1989)[a] |
| Rat | Fischer 344 | M/F | Oral | DMSO | 99.0 | > 3 000 mg/kg bw (M/F) | Price (1986, 1991)[b] |
| Rat | Fischer 344 | M/F | Dermal | Water | 99.0 | > 2 000 mg/kg bw (M/F) | Price (1986, 1991)[c] |
| Rat | Sprague-Dawley | M/F | Inhalation | — | 98 | 5.1 mg/L (M/F) | McDonald (1986, 1991)[d] |

bw: body weight; DMSO: dimethyl sulfoxide; F: female; $LC_{50}$: median lethal concentration; $LD_{50}$: median lethal dose; M: male

[a] Performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 401. The rats were given a dose of 5000 mg/kg bw. No mortality or clinical signs were observed. Necropsy revealed no abnormalities. Batch no. 7003 = WB 16867.

[b] Study design resembles OECD Test Guideline 401. The rats were given a dose of 3000 mg/kg bw. One female rat died on day 2 of the study. In this rat, the stomach contained compacted powder and showed haemorrhagic petechiae. All rats showed clinical signs 6 hours following dosing that consisted of chromodacryorrhoea (both sexes), abnormal gait (males only), lethargy (females only), increased lacrimation (females only) and blood-tinged urine (females only). These signs resolved in all survivors by study day 2. Batch no. 13 = ST86/022.

[c] Performed according to OECD Test Guideline 402. The rats were given a dose of 2000 mg/kg bw. No mortality or clinical signs were observed. Batch no. 13 = ST86/022.

[d] Study design resembles OECD Test Guideline 403. Rats were exposed nose only to an actual flufenoxuron concentration of 5.1 mg/L. Another group of five male and five female rats was exposed to air only. No mortality was observed. All rats displayed subdued behaviour immediately following cessation of exposure. In blood samples obtained 1 hour after the end of the exposure, no effect of flufenoxuron exposure on methaemoglobin levels was observed. Necropsy showed no abnormalities. Mass median aerodynamic diameter was 3.6 (± 2.0) µm. Batch no. 16.

#### *(b) Dermal irritation*

In an acute dermal irritation study performed in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 404, the intact skin of three male and three female New Zealand White rabbits was exposed for 4 hours under semi-occlusion to 0.5 g flufenoxuron (purity 99%; batch no. 13) moistened with water. Dermal irritation was scored according to the Draize system at 0.5, 24, 48 and 72 hours after patch removal.

No signs of dermal irritation were observed. Flufenoxuron was considered not irritating to rabbit skin (Price, 1986, 1991).

#### *(c) Ocular irritation*

In an acute eye irritation study, 25 mg of flufenoxuron (purity 99%; batch no. 13) was instilled into the conjunctival sac of one eye of each of three male and three female New Zealand White rabbits. The untreated eye served as a control. The eyes were examined macroscopically for signs of irritation according to the Draize system at 1, 24, 48 and 72 hours and at day 7 post-

instillation. The study design resembles OECD Test Guideline 405; however, the applied dose of 25 mg is low compared with the 100 mg or 0.1 mL of solid required by the test guideline.

Slight congestion of the conjunctival blood vessels was present in all animals at 1 hour after instillation and in one animal at 24 hours. At 1 hour, minimal chemosis was observed in two animals. The untreated eyes of all rabbits were overtly normal at all examinations. In the present test, flufenoxuron was not irritating to the eye; however, the dose used was considerably lower than that required by the test guideline (Price, 1986, 1991).

*(d) Dermal sensitization*

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, flufenoxuron (purity 99.1%; batch no. COD-000444) was tested in 20 female Dunkin-Hartley guinea-pigs. The vehicle control group consisted of five animals. The laboratory maintains periodic testing of this animal strain with a positive control substance (α-cinnamaldehyde).

After the dermal challenge treatment on day 22, no signs of irritation were observed in the control and the treated groups 24 and 48 hours after the removal of the occlusive bandage. Under the conditions of this study, flufenoxuron was not a skin sensitizer (Gamer & Leibold, 2005).

### *2.2 Short-term studies of toxicity*

*Mice*

In a 28-day dietary study, flufenoxuron (purity 99%; batch no. 13 = ST86/019) was administered to groups of seven male and seven female C57/C3H $F_1$ hybrid mice at 0, 50, 500, 5000, 10 000 or 50 000 ppm (equivalent to 0, 7.1, 71, 710, 1400 and 7100 mg/kg bw per day, respectively). A concurrent control group of 14 males and 14 females was fed basal diet. Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. Prior to termination, blood samples were taken from all animals for haematology, including blood smear examination, and blood biochemistry was performed on cardiac blood taken after the mice were killed. All mice underwent complete necropsy. Testes, liver, heart (males only), brain, kidneys and spleen (both sexes) were weighed. A wide range of organs of all control and high-dose animals and some mice from the other dose groups was examined microscopically. The study design resembles OECD Test Guideline 407.

Three female mice were killed in a moribund state (two at 10 000 ppm on days 26 and 27 and one at 50 000 ppm on day 14). Histological examination of these three mice revealed necrosis of the lymphoid tissue. The cause of death could not be established. At 50 000 ppm, pale faeces were observed in all mice, which was attributed to unabsorbed test material. Reduced feed consumption (22–23%) was observed in females at 50 000 ppm during weeks 2 and 4 (data for week 3 are missing). In these females, a slight reduction in body weight was observed at week 2. Slight changes in haematological parameters were considered not toxicologically relevant. Relative heart weight was slightly increased (12%) in females at 50 000 ppm. Histopathological examination revealed no changes in heart tissue. The study author considered the increased relative heart weight not to be treatment related. No changes in clinical chemistry parameters or histopathology were observed.

The no-observed-adverse-effect level (NOAEL) was 10 000 ppm (equivalent to 1400 mg/kg bw per day), based on reduced feed consumption in females only observed at 50 000 ppm (equivalent to 7100 mg/kg bw per day) (Esdaile, 1991a).

Flufenoxuron (purity 96.6%; batch no. 16 = ST86/165) was administered via the diet to groups of 10 male and 10 female C57/C3H $F_1$ hybrid mice for 13 weeks at a dietary level of 50, 500, 5000, 10 000 or 50 000 ppm (equal to 10, 103, 1069, 2139 and 11 071 mg/kg bw per day for males and 12, 124, 1247, 2482 and 12 619 mg/kg bw per day for females, respectively). A concurrent

control group of 20 males and 20 females was fed basal diet. Each of these groups was doubled using an equal number of additional animals (treated or controls, named "satellites"), which were subjected only to blood sampling for clinical chemistry analysis. The mice were examined daily for mortality and clinical signs. Feed consumption and body weights were recorded weekly. Ophthalmological examinations were performed prior to treatment and during week 13. Haematology, including assessment of methaemoglobin, clinical chemistry and urine analysis were performed at the end of the treatment period. All mice were necropsied, and weights of liver, spleen, kidneys, testes, heart, brain, uterus and adrenal glands were recorded. A wide range of tissues, including femoral bone marrow smears of mice of the control and 50 000 ppm groups and the lung, liver, kidneys and gross lesions from mice of the other groups, was examined microscopically.

Two females from the 5000 ppm group, two males from the 10 000 ppm group and one female from the 50 000 ppm group either died or were killed when moribund during the study. The cause of death could not be established. In view of the absence of specific pathological findings and the lack of a dose–response relationship, these deaths were not considered to be test substance related. Pale faeces observed in males and females at 50 000 ppm were attributed to unabsorbed test material. Feed consumption was generally comparable for all groups. In males at 10 000 and 50 000 ppm, body weight gain was lower (up to 22%) than in control males.

Results of haematological and blood biochemical examinations are summarized in Table 6. Haematological evaluations revealed minimal but statistically significant decreases in erythrocyte count, haemoglobin and haematocrit for males at 50 000 ppm. Males and females showed a statistically significant and generally dose-related increase in serum bilirubin from 500 to 50 000 ppm (increase of 26–117%). These haematological and blood biochemical changes were indicative of a slight anaemia. Methaemoglobin levels were not affected. Triglyceride levels were statistically significantly decreased in males at 10 000 and 50 000 ppm. Statistically significant decreases in blood urea nitrogen were found in males at 5000 and 50 000 ppm, but not at 10 000 ppm, and in females at 10 000 and 50 000 ppm.

***Table 6. Haematological and blood biochemical findings in the 90-day dietary mouse study with flufenoxuron***

| | Sex | 0 ppm | 50 ppm | 500 ppm | 5 000 ppm | 10 000 ppm | 50 000 ppm |
|---|---|---|---|---|---|---|---|
| RBCs ($10^{12}$/L) | M | 10.12 | 10.10 | 10.13 | 10.00 | 10.02 | 9.68** |
| | F | 10.31 | 10.28 | 10.18 | 9.97 | 10.38 | 10.21 |
| Haemoglobin (g/dL) | M | 15.7 | 15.6 | 15.6 | 15.5 | 15.5 | 15.1** |
| | F | 16.1 | 15.9 | 15.9 | 15.8 | 16.2 | 16.0 |
| Haematocrit (ratio) | M | 0.453 | 0.455 | 0.455 | 0.448 | 0.451 | 0.435** |
| | F | 0.459 | 0.456 | 0.459 | 0.450 | 0.465 | 0.454 |
| Methaemoglobin (%) | M | 0.4 | 0.3 | 0.3 | 0.3 | 1.2 | 0.2 |
| | F | 0.3 | 0.3 | 0.2 | 0.1 | 0.6 | 0.5 |
| Bilirubin | M | 2.9 | 3.3 | 4.4** | 5.5** | 6.5** | 6.3** |
| | F | 4.2 | 4.5 | 5.3** | 5.8** | 5.9** | 6.7** |
| Triglyceride | M | 2.15 | 1.99 | 2.12 | 1.86 | 1.51** | 1.59** |
| | F | 1.64 | 1.64 | 1.57 | 1.53 | 1.50 | 1.31 |
| Urea nitrogen | M | 11.7 | 11.3 | 10.7 | 10.3* | 10.5 | 9.9** |
| | F | 10.9 | 10.2 | 10.7 | 10.5 | 9.7* | 9.1** |

F: female; M: male; ppm: parts per million; RBCs: red blood cells; *: $P < 0.05$; **: $P < 0.01$ (William's test)

*Source*: Esdaile (1988, 1991b); Berry (1992a)

There were no treatment-related changes in organ weight, gross pathology or histopathology.

The NOAEL was 50 ppm (equal to 10 mg/kg bw per day), based on increased bilirubin concentrations in males and females at 500 ppm (equal to 103 mg/kg bw per day) (Esdaile, 1988, 1991b; Berry, 1992a).

*Rats*

In a 28-day dietary toxicity study, performed in accordance with OECD Test Guideline 407, flufenoxuron (purity 99%; batch no. 13) was administered to groups of seven male and seven female Fischer 344 rats at 50, 500, 5000, 10 000 or 50 000 ppm (equal to 4.8, 49, 475, 997 and 5147 mg/kg bw per day for males and 5.3, 53, 534, 1067 and 5432 mg/kg bw per day for females, respectively). Groups of 14 male and 14 female rats received basal diet. Animals were checked 6 times per week for clinical signs of toxicity. Body weights and feed consumption were measured weekly. Blood was sampled for haematology and clinical biochemistry at termination. Generation of methaemoglobin was assessed using the nonspecific CO-oximeter method. Urine was sampled during week 13 for analysis. All rats were necropsied, and weights of liver, spleen, kidneys, testes, heart, brain and adrenals were recorded. A wide range of tissues, including femoral blood smears, of rats of the control and high-dose rats and any macroscopic lesions in the other dose groups were examined microscopically.

No deaths were observed. At 50 000 ppm, pale faeces, which were attributed to unabsorbed test material, were observed in all rats. Feed consumption and body weight gains for all treated males and females at 50, 500, 5000 and 10 000 ppm and females at 50 000 ppm were comparable to those of controls. Males at 50 000 ppm had an increased feed consumption from weeks 2 to 4 (9–15%), resulting in a 5% higher body weight than controls at the end of the study. Males at 50 000 ppm had statistically significantly increased relative heart and spleen weights. However, as the increases were only 5% and 7%, respectively, they were not considered toxicologically relevant. No toxicologically relevant changes in haematology, macropathology or histopathology were observed. Apparent moderate increases in methaemoglobin levels were considered false-positive findings, as the increases were not dose dependent and were below the level of accuracy of the test method. Cholesterol levels showed some variations of statistical significance, but no biological relevance. Triglycerides were significantly lower in males (17–22%) treated at 5000–50 000 ppm compared with controls, and this was considered to be treatment related. However, the toxicological relevance of the mild reductions in triglyceride levels is not clear. In high-dose females, a slight reduction in β-globulin levels (−11%) was observed. Other small but statistically significant changes in clinical chemistry parameters were considered not toxicologically relevant.

The NOAEL was 50 000 ppm (equal to 5147 mg/kg bw per day), the highest dose tested (Esdaile, 1986a; Volger, 1991).

In a 13-week dietary toxicity study, flufenoxuron (purity 96.6%; batch no. 16 = ST86/165) was administered to groups of 10 male and 10 female Fischer 344 rats at 50, 500, 5000, 10 000 or 50 000 ppm (equal to 3.5, 35, 351, 689 and 3637 mg/kg bw per day for males and 4.1, 41, 399, 820 and 4151 mg/kg bw per day for females, respectively). A concurrent control group of 20 males and 20 females was fed basal diet. Animals were checked daily for clinical signs of toxicity. Body weights and feed consumption were measured weekly. Ophthalmological examinations were carried out on five rats of each sex from the control and high-dose groups before dosing and at week 13. Blood was sampled for haematology and clinical biochemistry at termination. Generation of methaemoglobin was assessed using the nonspecific CO-oximeter method. Urine analysis was performed on five animals of each sex from each of the treated groups and from 10 controls of each sex during week 13. All rats were necropsied, and weights of liver, spleen, kidneys, testes/uterus, heart, brain and adrenals were recorded. A wide range of tissues of rats of the control and 50 000 ppm groups and the lung, liver and kidneys from all groups were examined microscopically.

There were no mortalities. Pale faeces, which were attributed to unabsorbed test material, were observed in all rats at 50 000 ppm and in a few rats at 10 000 ppm. Feed consumption and body weight gains for all treated males and females at 50, 500, 5000 and 10 000 ppm and for females at 50 000 ppm were comparable with those of controls. Males at 50 000 ppm had a mildly increased feed consumption (up to 8%) from week 7 to termination, which was attributed to the high level of test substance in the feed. No effect on body weight gain was observed. At 5000 ppm and above, females exhibited haematological changes indicative of a mild anaemia, with statistically significant decreases in haemoglobin (4–7%), erythrocyte count (5–6%) and haematocrit (5–7%). In bone marrow smears of females at 50 000 ppm, a statistically significant decrease in myeloid/erythroid ratio was observed (control females, 1.8; 50 000 ppm females, 1.0), which indicates a slight compensatory increase in erythropoiesis. In males, there were no relevant indications of anaemia from assessment of blood samples, although bone marrow smears from 50 000 ppm males indicated an increase in erythropoiesis (myeloid/erythroid ratio in control males, 1.9; in 50 000 ppm males, 0.8). Small but statistically significant increases in methaemoglobin levels were observed in all treatment groups (from 0.8%/0.5% in control males/females to 1.6%/1.5% in high-dose males/females). However, as the increases were not clearly dose dependent and were below the level of accuracy of the test method, the finding is not considered toxicologically relevant. Several statistically significant biochemical findings were observed; however, the changes were small and not considered toxicologically relevant. There were no treatment-related effects on urinary or ophthalmological parameters.

At 5000 ppm and above, absolute and relative spleen weights were slightly but statistically significantly increased in females (12–17%). These increases are probably related to the haematological effects observed in females. No treatment-related macroscopic or histopathological changes were observed.

The NOAEL was 500 ppm (equal to 41 mg/kg bw per day), based on slightly higher spleen weights and haematological changes indicative of a mild anaemia observed in females at 5000 ppm (equal to 399 mg/kg bw per day) (Esdaile, 1987, 1991c; Berry, 1992b).

*Dogs*

In a 90-day toxicity study, four male and four female Beagle dogs per dose group received flufenoxuron (purity 96.6%; batch no. 6001 = ST86/176) at a dietary concentration of 0, 500, 5000 or 50 000 ppm (equal to 0, 18, 163 and 1961 mg/kg bw per day for males and 0, 21, 182 and 2039 mg/kg bw per day for females, respectively). As a diet formulation error resulted in underdosing of the 5000 ppm animals for the first 2 weeks of treatment (1000 ppm instead of 5000 ppm), the duration of dietary test substance administration was extended from 13 to 15 weeks for all groups. Animals were checked daily for clinical signs. Feed consumption was measured daily, and body weights were measured weekly. Water consumption over a 24-hour period and ophthalmological and electrocardiographic examinations were performed pretreatment and in the week before termination. Haematology and clinical chemistry were performed pretrial and during weeks 9 and 15 of the treatment period. Methaemoglobin and sulfhaemoglobin were determined using the specific method of Evelyn and Malloy. Additional haematological assays were also undertaken during week 2 (all animals), week 4 (controls and mid-dose group) and week 12 (all animals) of the treatment period. All dogs were necropsied, and weights of brain, thyroid glands with parathyroids, heart, liver with gallbladder, adrenals, kidneys, testes and ovaries were recorded. Histology was performed on a large selection of organs, including bone marrow smears from all dogs. The fat samples were also analysed for test item levels.

Dietary concentrations were close to the target values, except that during the first 2 weeks of treatment, the 5000 ppm diet was formulated, in error, to be 1000 ppm.

In control group dogs, flufenoxuron concentrations in renal fat ranged from 11 to 69 ppm (investigation by the sponsor), suggesting that the control group dogs were contaminated with

flufenoxuron during either the treatment period or the sampling of the renal fat specimens at necropsy. Therefore, the validity of the data collected for the control group is questionable. In the treatment groups, a 10-fold increase in the concentration of flufenoxuron resulted in an approximately 2-fold increase in flufenoxuron residues in renal fat. At 50 000 ppm, pallor of the gums and/or sclera was observed in several male and female dogs during weeks 13, 14 and 15. Examination of feed and water consumption, body weight gain and ophthalmology revealed no treatment-related effects. Electrocardiograms revealed a slightly prolonged Q-T interval for all females at 50 000 ppm compared with controls (0.21 versus 0.18 second: 17% higher) and with the pretest values in these treated females (0.21 versus 0.17 second: 24% higher).

At week 9, increases in reticulocyte counts (statistically significant at 5000 and 50 000 ppm) were found in treated male dogs. In males of all treatment groups, mild but statistically significant reductions in erythrocyte counts, haemoglobin and haematocrit were observed (Table 7). These findings are indicative of mild anemia. At week 9 in females, a similar trend in these parameters was observed; however, the changes did not reach statistical significance. At week 9, mean corpuscular volume (MCV) was increased in males and females at 5000 and 50 000 ppm, and mean corpuscular haemoglobin concentration (MCHC) was decreased in males of all treatment groups. Haematological examination at weeks 12 and 15 also showed similar trends in these parameters in male and female dogs, although the effects were less clear and only occasionally statistically significant (Table 8).

Measurement of methaemoglobin revealed dose-related increases in both sexes, which were statistically significant at 5000 ppm and above after 9 and 15 weeks of treatment (at week 9 also at 500 ppm in females) (Table 9). The presence of methaemoglobin was accompanied by small, statistically significant and dose-related increases in sulfhaemoglobin in males at 5000 ppm and in both sexes at 50 000 ppm.

***Table 7. Haematological findings in dogs (pretrial and week 9)***

| | | **Pretrial** | | | | **Week 9** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test parameter** | **Sex** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** |
| RBCs ($10^{12}$/L) | M | 6.33 | 6.03 | 5.99 | 6.00 | 6.75 | 5.97** | 5.80*** | 5.49*** |
| | F | 6.05 | 6.51 | 6.19 | 6.27 | 6.89 | 6.27 | 6.05 | 5.90 |
| Haemoglobin (g/dL) | M | 14.3 | 13.3 | 13.4 | 13.6 | 15.8 | 13.5** | 13.4*** | 13.1*** |
| | F | 13.7 | 14.8 | 13.9 | 14.4 | 15.6 | 14.7 | 14.2 | 14.3 |
| Haematocrit (L/L) | M | 0.434 | 0.401 | 0.407 | 0.415 | 0.467 | 0.417** | 0.420** | 0.410** |
| | F | 0.411 | 0.442 | 0.418 | 0.431 | 0.472 | 0.447 | 0.441 | 0.437 |
| MCV (fL) | M | 71 | 68 | 70 | 71 | 69 | 69 | 72* | 75** |
| | F | 70 | 70 | 69 | 71 | 69 | 71 | 72* | 74** |
| MCHC (g/dL) | M | 31.4 | 31.4 | 31.3 | 31.3 | 34.7 | 33.3** | 32.5*** | 32.6*** |
| | F | 31.8 | 31.8 | 31.7 | 32.0 | 33.6 | 33.7 | 32.9 | 33.3 |
| Reticulocytes (%) | M | 0.9 | 0.9 | 1.1 | 0.9 | 0.6 | 1.0 | 1.8** | 1.6** |
| | F | 1.3 | 1.3 | 1.5 | 1.5 | 0.8 | 1.1 | 2.0 | 1.6 |

F: female; M: male; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; ppm: parts per million; RBCs: red blood cells; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (F-max test; ANOVA; Student's *t*-test)

[a] Dosed with 1000 ppm for the first 2 treatment weeks.

*Source*: Greenough, Goburdhun & Duffen (1987); Clark (1988a,b); Greenough (1991a)

*Table 8. Haematological findings in dogs (weeks 12 and 15)*

| | | Week 12 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Historical controls[b] | | Week 12 | | | | Week 15 | | | |
| **Test parameter** | **Sex** | **Range** | **Mean** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** |
| RBCs ($10^{12}$/L) | M | 5.66–7.73 | 6.70 | 6.60 | 6.30 | 6.14 | 5.45*** | 6.53 | 6.21 | 6.04 | 5.41** |
| | F | 5.72–7.89 | 6.81 | 6.81 | 6.21 | 6.22 | 6.14 | 6.82 | 6.55 | 6.12 | 6.33 |
| Haemoglobin (g/dL) | M | 12.6–17.6 | 15.1 | 15.1 | 14.4 | 14.2 | 13.0*** | 15.0 | 13.9 | 13.7 | 12.9 |
| | F | 12.9–18.1 | 15.5 | 15.6 | 14.5 | 14.7 | 14.8 | 15.6 | 15.1 | 14.1 | 15.3 |
| Haematocrit (L/L) | M | 0.39–0.53 | 0.458 | 0.461 | 0.438 | 0.437 | 0.406 | 0.449 | 0.423 | 0.421 | 0.401 |
| | F | 0.39–0.55 | 0.468 | 0.470 | 0.442 | 0.448 | 0.452 | 0.470 | 0.454 | 0.432 | 0.460 |
| MCV (fL) | M | 63–73 | 68 | 69 | 69 | 70 | 74 | 68 | 67 | 68 | 73* |
| | F | 64–72 | 68 | 68 | 70 | 71 | 73 | 68 | 68 | 70 | 71 |
| MCHC (g/dL) | M | 32.2–35.9 | 34.0 | 34.8 | 34.3 | 34.1 | 33.4** | 34.1 | 33.5 | 33.2 | 33.0 |
| | F | 32.6–35.6 | 34.1 | 34.6 | 34.3 | 34.3 | 34.2 | 33.9 | 34.0 | 33.2* | 34.0 |
| Reticulocytes (%) | M | 0.2–1.3 | 0.7 | ND | ND | ND | ND | 0.4 | 1.5 | 1.0 | 1.5 |
| | F | 0.2–1.8 | 0.8 | ND | ND | ND | ND | 1.0 | 0.7 | 1.0 | 1.3 |

F: female; M: male; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; ppm: parts per million; ND: not determined; RBCs: red blood cells; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (F-max test; ANOVA; Student's *t*-test)

[a] Dosed with 1000 ppm for the first 2 treatment weeks.

[b] Historical control data from Inveresk Research International (included in the report, approximately 200 dogs of each sex).

*Source*: Greenough, Goburdhun & Duffen (1987); Clark (1988a,b); Greenough (1991a)

*Table 9. Methaemoglobin and sulfhaemoglobin determinations in dogs (weeks 9 and 15)*

| | | Week 9 | | | | Week 15 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test parameter** | **Sex** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** | **0 ppm** | **500 ppm** | **5 000 ppm[a]** | **50 000 ppm** |
| Methaemoglobin (%) | M | 0.80 | 1.07 | 1.42*** | 1.82*** | 0.61 | 0.99 | 1.46*** | 1.88*** |
| | F | 0.79 | 1.10** | 1.30*** | 1.80*** | 0.65 | 0.87 | 1.23*** | 1.69*** |
| Sulfhaemoglobin (%) | M | 0.12 | 0.23 | 0.33* | 0.46*** | 0.10 | 0.16 | 0.32*** | 0.39*** |
| | F | 0.28 | 0.14 | 0.23 | 0.35 | 0.12 | 0.15 | 0.25 | 0.43* |

F: female; M: male; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (F-max test; ANOVA; Student's *t*-test)

[a] Dosed with 1000 ppm for the first 2 treatment weeks.

*Source*: Greenough, Goburdhun & Duffen (1987); Clark (1988a,b); Greenough (1991a)

Statistically significant increases in serum cholesterol values were observed during week 9 in males and females at 50 000 ppm (4.7 and 3.9 mmol/L versus 3.4 and 3.3 mmol/L in controls, respectively) and during week 15 in males at 5000 and 50 000 ppm (4.6 and 5.0 mmol/L versus 3.0 mmol/L in controls). Absolute and relative liver weights were significantly increased in males treated at 5000 ppm (absolute +34%, relative +28%) and 50 000 ppm (absolute +28%, relative +32%). There were no treatment-related macroscopic changes. Histopathological examination revealed higher frequencies of hyperplasia in the bone marrow of all treated groups (Table 10). Yellow pigment deposition in the bone marrow, considered by the study author to be haemosiderin associated with red blood cell breakdown and production, was observed at 5000 ppm (females only) and 50 000 ppm (both sexes). These bone marrow changes were considered to reflect a compensatory response to the slight anaemia observed at all dietary concentrations. It is stated that bone marrow smears prepared from high-dose animals showed hyperplasia, predominance of red cell precursors and a shift in myeloid/erythroid ratio towards red cell production (no data presented in the report). Increased yellow pigment deposition was also observed in the proximal tubules of the kidney in two male dogs at 50 000 ppm. In the liver, increased Kupffer cell pigmentation, considered indicative of mild anaemia, was noted in male and female dogs at 5000 and 50 000 ppm and in one female at 500 ppm. Although these yellow pigments were never characterized, they were attributed to haemosiderin deposits (this was confirmed for bone marrow and kidneys by negative Prussian blue staining in the 52-week dog study; see below). Haemosiderin deposits were also detected in the spleen of one high-dose male and female.

A NOAEL could not be established. The lowest-observed-adverse-effect level (LOAEL) was 500 ppm (equal to 18 mg/kg bw per day), based on reductions in erythrocyte counts, haemoglobin and haematocrit in males, increased Kupffer cell pigmentation in the liver of one female and higher frequencies of hyperplasia in the bone marrow in males and females (Greenough, Goburdhun & Duffen, 1987; Clark, 1988a,b; Greenough, 1991a).

In a 1-year toxicity study, four male and four female Beagle dogs per dose group received flufenoxuron (purity 98%; batch no. 7005) in the diet at 0, 10, 100, 500 or 50 000 ppm (equal to 0, 0.36, 3.5, 19 and 1898 mg/kg bw per day for males and 0, 0.36, 3.8, 19 and 1879 mg/kg bw per day for females, respectively). Animals were examined daily for clinical signs of toxicity. Feed consumption was measured daily. Body weights were recorded weekly for the first 13 weeks and monthly thereafter. Water consumption over a 24-hour period was measured pretrial and during weeks 12, 25, 38 and 50 of the treatment period. Ophthalmological examinations were performed pretreatment and in week 51. Electrocardiographic measurements were carried out pretrial and during weeks 26 and 51 of the treatment period. Haematology, clinical chemistry and urine analysis were performed pretest and in weeks 27 and 52. At the same time, faecal samples were tested for occult blood. Additional haematological investigations were also undertaken in weeks 5, 13 and 40.

***Table 10. Histopathological changes in dogs (week 15)***

| | Sex | 0 ppm | 500 ppm | 5 000 ppm | 50 000 ppm |
|---|---|---|---|---|---|
| Liver, increased Kupffer cell pigmentation | M | 0/4 | 0/4 | 4/4* | 4/4* |
| | F | 0/4 | 1/4 | 3/4 | 4/4* |
| Kidney, increased yellow pigment deposition in proximal tubules | M | 0/4 | 0/4 | 0/4 | 2/4 |
| | F | 0/4 | 0/4 | 0/4 | 0/4 |
| Spleen, increased haemosiderin | M | 0/4 | 0/4 | 0/4 | 1/4 |
| | F | 0/4 | 0/4 | 0/4 | 1/4 |
| Bone marrow, hyperplasia | M | 0/4 | 3/4 | 4/4* | 4/4* |
| | F | 0/4 | 2/4 | 4/4* | 4/4* |
| Bone marrow, increased yellow pigment deposition | M | 0/4 | 0/4 | 0/4 | 4/4* |
| | F | 0/4 | 0/4 | 3/4 | 3/4 |

F: female; M: male; ppm: parts per million; *: $P < 0.05$ (Fisher exact test)

*Source*: Greenough, Goburdhun & Duffen (1987); Clark (1988a,b); Greenough (1991a)

Furthermore, blood samples were taken at weeks 13, 29 and 40 (high dose only) and at termination to monitor flufenoxuron levels in blood. All dogs were necropsied, and weights of spleen, liver with gallbladder, kidneys, testes/ovaries, heart, brain, thyroids with parathyroids, and adrenal glands were recorded. Histology was performed on a large selection of organs, including bone marrow smears from all dogs. The fat samples were also analysed for flufenoxuron levels.

There were no treatment-related effects on mortality, clinical signs, feed or water consumption, body weight gain or ophthalmological or electrocardiographic parameters. Haematological examinations showed mild anaemia in dogs at 500 and 50 000 ppm. At 5 weeks at 50 000 ppm, statistically significant reductions in haemoglobin (−16%), red blood cell count (−17%) and MCHC (−5%) in males and increases in number of reticulocytes (230–250%), methaemoglobin levels (160%) and sulfhaemoglobin levels (325–580%) in both sexes were observed. From 13 weeks onward, mild anaemia was also generally visible at 500 ppm. The effects were more prominent in males. Data from the 13- and 52-week examinations are presented in Table 11. Red blood cells often showed morphological alterations (i.e. polychromasia and Howell-Jolly bodies) in high-dose animals. In addition, platelet counts were statistically significantly increased in males at 50 000 ppm from week 13 onwards and at 500 ppm from week 27 onwards. Platelet counts of females were increased at the high-dose level, albeit not statistically significantly.

Methaemoglobin levels are presented in Table 12. The slight but statistically significant increase in females at 100 ppm was considered not toxicologically relevant.

There were no significant treatment-related adverse effects on clinical chemistry or urine analysis parameters or positive faecal occult blood tests. Absolute and relative liver weights adjusted for terminal body weights were statistically significantly increased in males at 500 ppm (19% and 18%, respectively) and 50 000 ppm (36% and 24%, respectively) when compared with controls. In females at 50 000 ppm, these increases in liver weights (24% and 21%, respectively) were not statistically significant. No treatment-related macroscopic changes were observed. Histopathological changes included bone marrow hyperplasia, increased cellularity, increased numbers of erythrocyte precursors (normoblasts) and increased numbers of macrophages in bone marrow for all dogs at 50 000 ppm. One female at 500 ppm also showed bone marrow hyperplasia and increased cellularity. These changes in bone marrow were indicative of compensatory erythropoiesis and elevated erythrocyte turnover at 500 and 50 000 ppm. This was accompanied by increased deposition of

*Table 11. Haematological findings in dogs (weeks 13 and 52)*

| | | Week 13 | | | | | Week 52 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test parameters** | **Sex** | **0 ppm** | **10 ppm** | **100 ppm** | **500 ppm** | **50 000 ppm** | **0 ppm** | **10 ppm** | **100 ppm** | **500 ppm** | **50 000 ppm** |
| RBCs ($10^{12}$/L) | M | 7.33 | 6.63* | 7.19 | 6.59* | 6.08** | 7.61 | 7.13 | 7.50 | 6.80* | 6.47** |
| | F | 7.39 | 7.08 | 7.30 | 7.05 | 6.39** | 6.91 | 7.07 | 7.19 | 7.16 | 6.35 |
| Haemoglobin (g/dL) | M | 16.6 | 15.6 | 16.2 | 15.5 | 14.5 | 17.3 | 16.5 | 16.9 | 15.6 | 15.1 |
| | F | 17.5 | 16.6 | 17.1 | 17.0 | 15.3** | 16.1 | 16.7 | 16.5 | 17.3 | 15.0 |
| Haematocrit (L/L) | M | 0.476 | 0.448 | 0.468 | 0.446 | 0.427 | 0.502 | 0.487 | 0.494 | 0.467 | 0.456 |
| | F | 0.499 | 0.475 | 0.485 | 0.489 | 0.454 | 0.474 | 0.492 | 0.484 | 0.510 | 0.454 |
| MCV (fL) | M | 65 | 67 | 65 | 68 | 70** | 65 | 67 | 65 | 68 | 69 |
| | F | 67 | 67 | 66 | 69 | 70* | 68 | 69 | 67 | 70 | 71 |
| MCHC (g/dL) | M | 35.3 | 35.3 | 35.2 | 35.2 | 34.3** | 34.3 | 33.8 | 34.0 | 33.3** | 33.0** |
| | F | 35.6 | 35.4 | 35.7 | 35.2 | 34.2*** | 34.0 | 33.8 | 34.1 | 33.7 | 32.8 |
| Reticulocytes (%) | M | 0.6 | 0.4 | 0.3 | 0.9 | 1.3** | 0.8 | 0.8 | 0.6 | 0.9 | 1.7* |
| | F | 0.3 | 0.3 | 0.6 | 0.8 | 1.6** | 0.3 | 0.5 | 0.7 | 1.5 | 1.7 |
| Platelets ($10^{-9}$/L) | M | 233 | 233 | 290 | 289 | 425** | 286 | 272 | 274 | 431** | 449* |
| | F | 265 | 259 | 210 | 242 | 380 | 347 | 341 | 264 | 247 | 463 |
| Sulfhaemoglobin (%) | M | 0.05 | 0.05 | 0.07 | 0.08 | 0.28** | 0.14 | 0.13 | 0.10 | 0.22 | 0.30** |
| | F | 0.03 | 0.05 | 0.06* | 0.09** | 0.38*** | 0.09 | 0.23 | 0.14 | 0.33** | 0.41*** |

F: female; M: male; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; ppm: parts per million; RBCs: red blood cells; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (F-max test; ANOVA; Student's *t*-test)

*Source*: Goburdhun, Parkinson & Greenough (1988); Greenough (1991b)

***Table 12. Methaemoglobin levels in dogs***

| | Methaemoglobin level (% of total haemoglobin) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | | | Females | | | | |
| | 0 ppm | 10 ppm | 100 ppm | 500 ppm | 50 000 ppm | 0 ppm | 10 ppm | 100 ppm | 500 ppm | 50 000 ppm |
| Pretrial | 0.48 | 0.54 | 0.52 | 0.50 | 0.54 | 0.64 | 0.58 | 0.59 | 0.56 | 0.57 |
| Week 5 | 0.75 | 0.58 | 0.69 | 0.90 | 1.96*** | 0.66 | 0.97 | 0.58 | 0.87 | 1.48*** |
| Week 13 | 0.73 | 0.77 | 0.76 | 0.99 | 1.52** | 0.63 | 0.88 | 0.97 | 0.99 | 1.61 |
| Week 27 | 0.54 | 0.63 | 0.53 | 0.86* | 1.60*** | 0.56 | 0.68 | 0.68 | 0.84 | 1.96*** |
| Week 40 | 0.75 | 0.71 | 0.75 | 1.05 | 1.87*** | 0.69 | 0.75 | 0.80** | 1.53*** | 2.18*** |
| Week 52 | 1.16 | 1.27 | 1.04 | 1.14 | 1.95 | 0.71 | 1.08 | 0.68 | 0.98 | 2.39*** |

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (F-max test; ANOVA; Student's *t*-test)

*Source*: Goburdhun, Parkinson & Greenough (1988); Greenough (1991b)

yellow/brown pigment (not haemosiderin, as it was free of $Fe^{3+}$ when stained) in the bone marrow of all dogs in the 50 000 ppm group. Brown pigmentation (free of $Fe^{3+}$) was also noted in the proximal tubular cells of the kidneys in four males and one female at 50 000 ppm and in one female at 500 ppm. Fat-like vacuoles in hepatocytes were found in all dogs receiving 50 000 ppm. In addition, increased Kupffer cell pigmentation, which was identified as haemosiderin, was observed in all 50 000 ppm animals and in three dogs (two females and one male) at 500 ppm. Increased haemosiderin deposition was also noted in the spleens of two males and three females at 50 000 ppm and one female at 500 ppm (with associated extramedullary haematopoiesis). The histopathological findings in the bone marrow, kidney and liver were likely related to the anaemia observed at 50 000 ppm and to a lesser degree at 500 ppm. Haemosiderin deposition in the spleen was noted for one male at 100 ppm and for one male at 10 ppm. The study pathologist concluded that the isolated findings of slightly increased splenic haemosiderin in one male dog in both the 100 and 10 ppm groups were not treatment related, because of the lack of a dose–response relationship and consistent corresponding findings of anaemia at these dietary concentrations. With increasing dose level, an increase in flufenoxuron concentration in fat was observed (from about 5 mg/kg at the low dose to about 900 mg/kg at the high dose). There was no proportionality. A low level of flufenoxuron was also detected in the fat of control group animals.

The NOAEL was 100 ppm (equal to 3.5 mg/kg bw per day), based on mild anaemia (reductions in red blood cell count and MCHC), increased absolute and relative liver weights and increased platelet count in males; increased sulfhaemoglobin levels, bone marrow hyperplasia and increased bone marrow cellularity, brown pigmentation (free of $Fe^{3+}$) in the proximal tubular cells of the kidneys and increased haemosiderin deposition with associated extramedullary haematopoiesis in the spleen in one or more females; and increased methaemoglobin levels and increased Kupffer cell pigmentation identified as haemosiderin in both sexes at 500 ppm (equal to 19 mg/kg bw per day) (Goburdhun, Parkinson & Greenough, 1988; Greenough, 1991b).

### *2.3 Long-term studies of toxicity and carcinogenicity*

*Mice*

In a 2-year dietary carcinogenicity study, performed according to OECD Test Guideline 451, flufenoxuron (purity 98.1%; batch nos 7003 and 7033) was administered to groups of 50 male and 50 female B6C3F1 mice at 0, 100, 1000 or 10 000 ppm (equal to 0, 15.3, 152 and 1592 mg/kg bw per day for males and 0, 17.4, 187 and 1890 mg/kg bw per day for females, respectively). The mice were

checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Body weight was recorded weekly during weeks 1–14 and biweekly thereafter. Feed consumption was recorded weekly. Feed conversion efficiency was calculated every week for the first 14 weeks. Blood samples collected at weeks 50, 76 and 102 and blood smears of control and high-dose mice were examined. Animals found dead, killed prematurely or killed at the end of the treatment period were weighed and necropsied. Organ weights (adrenals, brain, heart, liver, kidneys, lungs, spleen and ovaries/testes) were recorded. A wide range of tissues was examined microscopically from all control and high-dose animals. In other groups, only gross lesions (all animals) and kidneys, liver and lungs (after scheduled death only) were examined.

Mortality at 24 months was not affected by treatment in males, whereas it tended to be higher in females at 1000 or 10 000 ppm (36% mortality in both groups versus 22% mortality in controls); however, no statistical significance was reached. Pathological examination revealed no dose-related cause of death. The frequency of animals bearing palpable masses was not significantly dose related in either sex. Body weight development was unaffected in males. In high-dose females at the end of the study, a statistically significant reduction in body weight gain of about 16% was observed. Feed intake and feed conversion efficiency were not affected by treatment. Blood smear examination did not reveal an effect of treatment on relative percentages of white blood cells or on blood cell morphology. No effect of treatment on organ weight was observed. Macroscopic examination showed a higher frequency of uterine distension by a fluid in females treated at 1000 or 10 000 ppm, whereas uterine non-fluid distension was inversely dose related. When both types of uterine distension are combined, the incidences were not dose related. In addition, no change in the type or frequency of any uterine lesion was observed at histology. The incidence of neoplastic findings was comparable between all groups. Occasionally observed non-neoplastic microscopic changes were considered chance findings and not treatment related.

The NOAEL was 1000 ppm (equal to 187 mg/kg bw per day), based on decreased body weight gain in females at 10 000 ppm (equal to 1890 mg/kg bw per day). Flufenoxuron was not carcinogenic in B6C3F1 mice under the conditions of the study (Broadmeadow, 1996).

In a 2-year dietary carcinogenicity study, performed according to OECD Test Guideline 451, flufenoxuron (purity 97.6–98.1%; batch no. WB16867 = 7003 = ST87/002) was administered to groups of 50 male and 50 female C57/C3H $F_1$ hybrid mice (= B6C3F1) at 0, 500, 5000 or 50 000 ppm (equal to 0, 56, 559 and 7356 mg/kg bw per day for males and 0, 73, 739 and 7780 mg/kg bw per day for females, respectively). Additional groups of 10 male and 10 female mice were treated for 12 months for interim examination. The mice were checked daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly during weeks 1–13 and once every 4 weeks thereafter. At the same time points, all animals were palpated for masses. Blood samples were collected at 12, 18 and 24 months for blood smear examination. Animals found dead, killed prematurely or killed at the end of the treatment period were weighed and necropsied. Organ weights (brain, heart, liver, kidneys, spleen and testes) were recorded. A wide range of tissues was examined microscopically from all control and high-dose animals. In other groups, only gross lesions (all animals) and kidneys, liver and lungs (after scheduled death only) were examined.

Mortality tended to be higher in mid- and high-dose females (15, 15, 19 and 20 at 0, 500, 5000 and 50 000 ppm, respectively), but the increases were not statistically significant. Feed spillage was higher in treated groups than in controls. In high-dose females, a slightly higher incidence of eye damage, caused by abrasion on the feed hopper, was observed. This was considered to be secondary to the increased incidence of feed spillage, as these animals may have been pushing their heads further down into the feed hopper. An increased incidence of lordotic episodes was observed in females at 5000 and 50 000 ppm (20/50 and 36/50 versus 8/50 in controls) and tended to be present in high-dose males (45/50 versus 32/50 in controls). In addition, at termination, a slight increase of focal alopecia in females at 5000 ppm was observed, which was considered to result from hair plucking. Experience from the test laboratory indicates that the lordotic behaviour and hair plucking are related to stress.

Terminal body weight gain was statistically significantly lower in high-dose males (−21%) and females (−30%) and in females at 5000 ppm (−14%). Feed intake was not affected. It is noted that due to feed spillage, the evaluation of feed consumption was difficult. There were no treatment-related haematological effects. Occasionally observed statistically significant findings were not dose dependent and/or not consistently found during the course of the treatment period.

At 24 months, spleen weight relative to brain weight was higher in high-dose males (+24%) and females (+23%). Liver weight relative to brain weight was higher in high-dose males (+46%) and females (+53%) and in males at 5000 ppm (+11%). At 12 months in the high-dose females, increased weights of spleen (+46%) and liver (+18%) relative to brain weight were observed. Macroscopic examination revealed no treatment-related findings at the interim kill. At termination, examination of the livers at 50 000 ppm revealed increased incidences of enlargement, exaggerated lobular pattern, pallor, pale areas or foci (all in both sexes) or dark areas or foci (in males only) compared with controls (see Table 13). A higher incidence of ulcer of the forestomach was noted in high-dose males (9 versus 0 in controls). The incidence of seminal vesicle enlargement in high-dose males (2) was lower than in controls (15).

***Table 13. Macroscopic changes in the liver of mice at 24 months***

| | **Incidence of liver finding** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Males** | | | | **Females** | | | |
| **Liver finding** | **0 ppm** | **500 ppm** | **5 000 ppm** | **50 000 ppm** | **0 ppm** | **500 ppm** | **5 000 ppm** | **50 000 ppm** |
| Enlargement | 1 | 2 | 3 | 11 | 4 | 6 | 4 | 18 |
| Exaggerated lobular pattern | 1 | 0 | 1 | 33 | 0 | 1 | 0 | 27 |
| Pallor | 11 | 7 | 7 | 33 | 13 | 7 | 13 | 44 |
| Pale areas/foci | 10 | 17 | 24 | 19 | 7 | 10 | 4 | 13 |
| Dark areas/foci | 4 | 2 | 5 | 14 | 11 | 10 | 5 | 13 |

ppm: parts per million

*Source*: Esdaile (1990a)

At the interim kill at 12 months, Kupffer cell aggregates were observed in 9/10 animals in both sexes at 50 000 ppm, versus 0/10 in male and female control animals. Individual cell necrosis and hypertrophy of hepatocytes were noted in single animals at this dose. At 24 months, non-neoplastic changes were predominantly observed in liver and spleen of both sexes at 50 000 ppm and in liver of females at 5000 ppm (Table 14). The inflammation and cell necrosis in the liver are indicative of chronic liver damage. The reduced incidences of splenic lymphoid hyperplasia and seminal vesicle enlargement in high-dose males are not considered adverse.

In terminal male animals, the total incidences of primary tumours (high dose), the number of animals with multiple tumours and malignant metastatic tumours (middle and high doses) and the number of malignant tumours (all doses) tended to be higher than in controls, although no statistical significance was found (see Table 15). This apparent increase in tumour incidence was due to increases in tumour frequency in the liver of treated males. The frequency of hepatocellular carcinoma was considerably higher in all treated male groups (at least 5-fold difference), but not in females. Also, the total incidence of hepatocellular tumours (adenoma and carcinoma combined) in these groups of male mice was above the control value, although not statistically significantly. However, the incidences of carcinomas in the livers of treated mice were not dose dependent and are within the historical control range for B6C3F1 mice (21.1% with a range of 8–32% based on 36 dietary

***Table 14. Selected non-neoplastic findings of mice fed flufenoxuron for 24 months***

| | Incidence of non-neoplastic finding | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| | 0 ppm | 500 ppm | 5 000 ppm | 50 000 ppm | 0 ppm | 500 ppm | 5 000 ppm | 50 000 ppm |
| Liver (no. examined) | (50) | (50) | (50) | (50) | (50) | (50) | (50) | (50) |
| Inflammation | 2 | 3 | 4 | 12** | 6 | 5 | 9 | 10 |
| Cell necrosis, individual | 2 | 1 | 1 | 42*** | 0 | 0 | 1 | 38*** |
| Kupffer cell aggregates | 0 | 0 | 2 | 47*** | 0 | 1 | 11*** | 44*** |
| Hypertrophy, hepatocellular | 0 | 0 | 1 | 31*** | 0 | 0 | 0 | 35*** |
| Spleen (no. examined) | (50) | (50) | (50) | (49) | (50) | (50) | (50) | (50) |
| Macrophages, syncytial | 0 | 0 | 0 | 18*** | 0 | 0 | 0 | 10*** |
| Hyperplasia, lymphoid | 10 | 6 | 8 | 2* | 4 | 7 | 9 | 7 |
| Stomach, glandular (no. examined) | (50) | (43) | (38) | (47) | (49) | (48) | (49) | (48) |
| Inflammation | 0 | 1 | 0 | 5* | 0 | 0 | 0 | 1 |
| Seminal vesicle (no. examined) | (50) | (50) | (49) | (49) | NA | NA | NA | NA |
| Enlargement | 23 | 13 | 19 | 3*** | – | – | – | – |

NA: not applicable; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

*Source*: Esdaile (1990a)

administration studies: Haseman et al., 1985; 20.4% with a range of 10–40% based on 19 studies: NTP, 1999). It is noted that in the present study, the controls showed an unusually low incidence (6%). This indicates that the apparent increased incidence in hepatocellular tumours in treated male mice in this study is due to the low incidence in control males. Splenic haemangiosarcomas occurred more frequently in high-dose females, but not in males. However, as this finding was not accompanied by an increase in angiomas, it was not considered treatment related. The incidence of all other tumours was comparable between control and treated groups.

The NOAEL was 500 ppm (equal to 56 mg/kg bw per day), based on an increased incidence of lordotic episodes, decreased body weight gain, increased Kupffer cell aggregates in females and increased liver weight relative to brain weight in males at 5000 ppm (equal to 559 mg/kg bw per day). Flufenoxuron was not carcinogenic in B6C3F1 mice under the conditions of the study (Esdaile, 1990a, 1991d; Berry, 1992c; Finn, 1993).

*Rats*

In a 2-year dietary toxicity study, flufenoxuron (purity 97.6–98.1%; batch no. 7003 = ST87/002) was administered to groups of 30 male and 30 female Fischer F344/DuCrj (SPF) rats at 1, 5, 50, 500, 5000 or 50 000 ppm (equal to 0.044, 0.23, 2.2, 22, 230 and 2470 mg/kg bw per day for males and 0.055, 0.28, 2.8, 28, 300 and 3210 mg/kg bw per day for females, respectively). A control group of 60 males and 60 females was fed basal diet. Ten rats of each sex per treatment group and 20 rats of each sex in the control group were designated for interim kill after 52 weeks of treatment. The rats were checked daily for mortality and clinical signs. Group feed consumption and individual body weights were recorded weekly for the first 13 weeks and once every 4 weeks thereafter. At 3, 6, 12, 18 and 24 months, haematology, measurement of non-cyanide binding haemoglobin and more specifically methaemoglobin levels (using Evelyn and Malloy's specific method), blood smear examination and clinical chemistry (not at 3 months) were performed in the rats designated for the 2-

year treatment. Urine analysis was performed on 10 animals of each sex per dose level (including controls) 1–3 weeks before each blood sampling, except for the 3-month sampling. All animals found dead, killed prematurely or killed at termination at 12 or 24 months were necropsied. Weights of adrenals, brain, liver, kidneys, spleen, heart and ovaries/testes of animals after scheduled death were recorded. Histological examinations were performed on a wide range of organs and tissues of all rats in the control and 50 000 ppm groups and of dead or moribund animals. Kidneys, liver, lungs and spleen were also examined microscopically in all other animals. Bone marrow smears prepared from high-dose and control interim kill rats were examined.

***Table 15. Incidence of neoplastic findings in mice fed flufenoxuron for 24 months***

| | **Incidence of neoplastic findings** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Males** | | | | **Females** | | | |
| | **0 ppm** | **500 ppm** | **5 000 ppm** | **50 000 ppm** | **0 ppm** | **500 ppm** | **5 000 ppm** | **50 000 ppm** |
| Total no. of animals per group | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| **Overview** | | | | | | | | |
| Total no. of primary tumours | 50 | 52 | 70 | 64 | 70 | 60 | 42 | 74 |
| Total no. of animals with tumours | 33 | 37 | 45 | 38 | 44 | 37 | 31 | 42 |
| Total no. of animals with multiple tumours | 14 | 13 | 22 | 21 | 19 | 16 | 11 | 24 |
| Total no. of benign tumours | 30 | 16 | 33 | 25 | 33 | 30 | 16 | 31 |
| Total no. of malignant tumours | 20 | 36 | 37 | 39 | 37 | 30 | 26 | 43 |
| Total no. of malignant tumours with metastasis | 12 | 10 | 16 | 17 | 32 | 16 | 16 | 32 |
| **Individual tumours** | | | | | | | | |
| Liver | (50) | (50) | (50) | (50) | (50) | (50) | (50) | (50) |
| Hepatocellular adenoma | 15 | 3 | 11 | 10 | 10 | 6 | 2 | 13 |
| Hepatocellular carcinoma | 3 | 19*** | 15** | 15**,$ | 3 | 9* | 7 | 5 |
| Hepatocellular tumours, total incidence | 18 | 22 | 26 | 25 | 13 | 15 | 9 | 18 |
| Cholangioma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Haemangiosarcoma | 2 | 1 | 0 | 5 | 0 | 0 | 0 | 1 |
| Haemangioma | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Spleen | (50) | (50) | (50) | (49) | (50) | (50) | (50) | (50) |
| Haemangioma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Haemangiosarcoma | 4 | 3 | 0 | 3 | 0 | 1 | 1 | 7**,$$ |
| Total vascular tumours (haemangioma + haemangiosarcoma) | 8 | 6 | 1 | 11 | 1 | 2 | 3 | 11 |

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (Fisher's exact test); $: $P < 0.05$: $$: $P < 0.01$ (trend analysis)

*Source*: Esdaile (1990a); Finn (1993)

There were no effects of treatment on mortality or clinical signs. Body weight gain was statistically significantly reduced at 5000 and 50 000 ppm in males (up to 10% and 13%, respectively) and females (up to 12% and 14%, respectively) from about week 28 to the end of the study. In these treatment groups, feed consumption was slightly increased compared with controls. Haematological investigations revealed toxicologically relevant changes in high-dose females, which showed lower red blood cell counts and haemoglobin levels (both up to −8%) at 3 and 12 months and lower haematocrit at 3 months (−7%). In males treated at 5000 and 50 000 ppm, higher red blood cells, haemoglobin and haematocrit at 24 months were considered to reflect a healthier condition of old high-dose males. Methaemoglobin levels were not affected by treatment. A small increase in non-cyanide binding haemoglobin in both sexes treated at 5000 and 50 000 ppm (up to +29% at 24 months) was considered to reflect sulfhaemoglobin. No toxicologically relevant changes in white blood cell parameters were observed. At 12 months, bone marrow smears showed no toxicologically relevant findings. Small increases in erythropoietic activity observed in bone marrow smears of high-dose males and females were consistent with the mild anaemia observed in these animals. As these effects were small, they were considered not toxicologically relevant. Triglyceride levels were lower in 5000 or 50 000 ppm treated animals, which is consistent with the lower body weight. Other changes in clinical chemistry parameters were small or not dose dependent or not consistent over time or between sexes and were not considered to be toxicologically relevant. Urine analysis showed an increase in glucose levels at 24 months in males treated at 5000 or 50 000 ppm (0.6 and 0.8 mmol/L, respectively, compared with 0.2 mmol/L in controls), but not in females. As no increase was observed at other time points and as other urinary parameters and blood glucose levels were not affected, the increased urinary glucose levels were not considered toxicologically relevant.

At the interim kill at 5000 and 50 000 ppm, slight increases were observed in relative heart weight in males (+5% and +10%, respectively) and females (+8% and +9%, respectively). In addition, in females at 50 000 ppm, increases in relative weights of spleen (+22%), liver (+12%) and adrenals (+11%) were observed. Increases in relative weights of these organs in females at 5000 ppm were small (≤ 8%). At the 24-month kill, only lower relative spleen weights in males at 5000 and 50 000 ppm (−34% and −35%, respectively) and higher relative adrenal weights in high-dose females (+11%) were observed. The reduced relative spleen weights were due to a lower incidence and severity of enlarged spleens compared with controls. The small increase in relative adrenal weight in females was not accompanied by histological changes. None of the organ weight differences observed at 24 months were considered to be of toxicological relevance. Necropsy revealed no treatment-related adverse effects. At the interim kill, rats at 500 ppm and higher had increased incidences of basophilic parenchymal foci in the liver. However, as these incidences were comparable between all groups at 24 months and as no increased frequency of liver neoplasms was observed, this finding is not considered toxicologically relevant. At 24 months in males, a 2-fold increased incidence of zonal (periportal) fatty vacuolation was observed at 50–50 000 ppm (not dose dependent, statistically significant at 50, 500 and 5000 ppm, but not at 50 000 ppm). This increase was not observed in females. The incidence of lymphocyte infiltration of the liver was 4 times higher in high-dose females (6/20) than in controls (3/40). There were no treatment-related increases in the incidence of neoplastic lesions.

The NOAEL was 500 ppm (equal to 22 mg/kg bw per day), based on decreased body weight gain, a small increase in non-cyanide binding haemoglobin, considered to reflect sulfhaemoglobin, and decreased triglyceride levels in both sexes at 5000 ppm (equal to 230 mg/kg bw per day). Flufenoxuron was not carcinogenic in Fischer F344/DuCrj (SPF) rats under the conditions of the study (Esdaile, 1990b,c; Berry, 1992d).

In a 2-year dietary toxicity study, flufenoxuron (purity 97.6–98.1%; batch no. WB16867 = 7003 = ST87/002) was administered to groups of 50 male and 50 female Fischer F344/DuCrj (SPF) rats at 0, 500, 5000 or 50 000 ppm (equal to 0, 21.6, 218 and 2290 mg/kg bw per day for males and 0, 25.9, 276 and 2901 mg/kg bw per day for females, respectively). The rats were checked daily for mortality and clinical signs. Group feed consumption and individual body weights were recorded

weekly for the first 13 weeks and once every 4 weeks thereafter. At the same time points, the rats were palpated for masses. Ophthalmological examinations were performed on 20 rats of each sex in each group prior to the start of the study and in control and high-dose groups at the end of the treatment. At 12, 18 and 24 months, blood was sampled for blood smear examination. All animals found dead, killed prematurely or killed at termination were necropsied. Weights of adrenals, brain, liver, kidneys, spleen, heart and ovaries/testes of animals after scheduled death were recorded. Histological examinations were performed on a wide range of organs and tissues of all rats in the control and 50 000 ppm groups and on dead or moribund animals. Kidneys, liver, lungs and gross lesions in all other animals were also examined microscopically.

No adverse effects of treatment on mortality or clinical signs were observed. Body weight gain was statistically significantly reduced throughout most of the study in females at 50 000 ppm (up to 14%). In females at 5000 ppm, body weight gain reduction during the study was small (up to 9%), but statistically significant. In males at 5000 and 50 000 ppm, feed consumption was slightly increased compared with controls, which is probably related to the lower caloric value of the diet. A dose-related lower spleen weight in males was actually correlated with a decreased incidence of spleen enlargement corresponding to splenic mononuclear cell leukaemia. Examination of blood smears revealed no toxicologically relevant changes. The incidence of leukaemia was inversely dose related (11, 3, 1 and 1 in males and 8, 3, 1 and 0 in females at 0, 500, 5000 and 50 000 ppm, respectively). Correspondingly, in males, a dose-relatedly lower spleen weight (−13%, −28% and −31% at 500, 5000 and 50 000 ppm, respectively) was correlated with a decreased incidence of spleen enlargement related to splenic mononuclear cell leukaemia. In high-dose females, an increase in adrenal weights (13%) was not accompanied by histopathological changes. At necropsy, an exaggerated lobular pattern of the liver was observed in all treated groups of males, which was attributed to the high frequency of spongiosis hepatitis in control males. Histopathology revealed an increased incidence of basophilic foci observed in the livers of high-dose males. However, the study pathologist suggested that it was possible that the higher incidence of mononuclear cell leukaemia, which infiltrates the liver in control males, could have obscured the diagnosis of this focal change in these controls. Accordingly, for the subset of male rats without mononuclear cell leukaemia, basophilic foci were not observed at significantly different frequencies between groups.

There were no treatment-related increases in the incidence of any neoplastic lesion for treated males or females. Actually, the incidences of malignant primary tumours in males, of multiple primary benign tumours in females and of lymphatic mononuclear cell leukaemia in both sexes were higher in controls than in treated animals. In high-dose females, the incidence of mammary fibroadenoma was also statistically significantly reduced.

The NOAEL was 500 ppm (equal to 25.9 mg/kg bw per day), based on reduced body weight gain in females at 5000 ppm (equal to 276 mg/kg bw per day). Flufenoxuron was not carcinogenic in Fischer F344/DuCrj (SPF) rats under the conditions of the study (Esdaile, 1990d; Basford, 1991; Berry, 1992e).

### *2.4 Genotoxicity*

Flufenoxuron was tested for genotoxicity in a range of assays (summarized in Table 16). Several of these studies, although indicating no genotoxic effect of flufenoxuron, were not entirely compliant with the OECD test guidelines. This is indicated in Table 16, and the deviations from the guidelines are described in the footnotes. An in vitro chromosomal aberration study in human lymphocytes (McEnaney, 1992) and an in vivo micronucleus test in the rat (Allen, Proudlock & Brooker, 1986) have not been considered in this evaluation because of their unacceptable deviations from OECD test guidelines. In one in vitro chromosomal aberration study in Chinese hamster ovary cells, flufenoxuron induced increased numbers of chromosomal aberrations (excluding gaps) in the presence of S9 mix. In a repeat study, a positive effect was again observed when cells were exposed to flufenoxuron in the presence of S9 mix. However, in the presence of phosphate-buffered glutathione at a concentration of 5 mmol/L, the extent of the chromosomal damage was substantially

***Table 16. Overview of genotoxicity tests with flufenoxuron[a]***

| | Test object | Concentration | Purity (%) | Results | Reference |
|---|---|---|---|---|---|
| In vitro | | | | | |
| Gene mutations | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 uvrA pKM101 | 31.25–4 000 µg/plate (±S9) | 99 | Negative<br>Study not fully adherent to OECD guideline | Brooks & Wiggins (1986); Brooks (1991)[b] |
| Gene mutations | *Saccharomyces cerevisiae* | 0.01–1 000 µg/mL (±S9) | 99 | Negative<br>Study not fully adherent to OECD guideline | Brooks & Wiggins (1986)[c] |
| Gene mutations | *S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA | 3–5 000 µg/plate (±S9) | 97.1 | Negative | Sokolowski (2007)[d] |
| Gene mutations | V79 Chinese hamster cells, HPRT test | 50–1 350 µg/mL (±S9) | 99 | Negative<br>Study not fully adherent to OECD guideline | Clare & Wiggins (1986); Brooks (1991)[e] |
| Gene mutations | V79 Chinese hamster cells, HPRT test | 6–1 600 µg/mL (±S9) | 97.1 | Negative | Wollny (2007)[f] |
| Chromosomal aberrations | CHO cells | 15–150 µg/mL (±S9) | 96.6 | Positive in the presence of S9<br>Increases in chromosomal aberrations at concentrations of 15–150 µg/mL, statistically significant at 75 µg/mL | Meyer (1987, 1991a)[g] |
| Chromosomal aberrations | CHO cells | 150 µg/mL (±S9, ± 5 mmol/L glutathione) | 96.6 | Positive in the presence of S9<br>Statistically significant increase in chromosomal aberrations at 150 µg/mL<br>Negative in the presence of S9 and glutathione at a concentration of 5 mmol/L | Meyer (1988a, 1991b)[h] |
| Chromosomal aberrations | Rat epithelial RL4 cells | 45–450 µg/mL (−S9)<br>16–160 µg/mL (+S9) | 96.6 | Negative<br>Study not fully adherent to OECD guideline | Meyer (1988b, 1991c)[i] |
| In vivo | | | | | |
| Micronucleus formation | ICR mice bone marrow | Two intra-peritoneal doses of 500, 1 000 or 2 000 mg/kg bw (24 h interval) | 98.2 | Negative | Nishitomi (1993)[j] |

*Table 16 (continued)*

| | Test object | Concentration | Purity (%) | Results | Reference |
|---|---|---|---|---|---|
| Chromosomal aberrations | Wistar Hanlbm rat bone marrow | One gavage dose of 500, 1 000 or 2 000 mg/kg bw | 97.1 | Negative | Honarvar (2007)[k] |
| Unscheduled DNA synthesis | Fischer 344 rat liver cells | One gavage dose of 188, 375, 750 or 1 500 mg/kg bw | 97.4 | Negative | Cifone (1991)[l] |
| Replicative DNA synthesis | Fischer 344 rat liver cells | Two gavage dose of 2 000 or 4 000 mg/kg bw (24 h interval) | 98.2 | Negative | Miyagawa (1992)[m] |

ADME: absorption, distribution, metabolism and excretion; bw: body weight; CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; OECD: Organisation for Economic Co-operation and Development; RDS: replicative DNA synthesis; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Batch no. 9 = ST85/113. The dose levels were selected on the basis of cytotoxicity and solubility. No positive results were observed. However, it is noted that for four *Salmonella* strains (TA98, TA100, TA1537, TA1538), the positive control substances need metabolic activation, and therefore these substances are not suitable to use in the plates without S9. Normally, positive control substances that do not require metabolic activation are used.

[c] Batch no. 9 = ST85/113. Study design resembles OECD Test Guideline 481. Liquid suspension cultures of log phase yeasts received the test material in DMSO at a dose level of 0.01, 0.1, 0.25, 0.5 or 1.0 mg/mL with and without S9 metabolic activation. Flufenoxuron did not have any effect on cell viability, and the results obtained from both trials showed no increase in the frequency of mitotic gene conversion in the presence or absence of metabolic activation. The positive control materials induced mitotic gene conversion, indicating that the test organism and metabolic activation system were functioning properly.

[d] Batch no. V5-7211L28. Performed according to OECD Test Guideline 471. Flufenoxuron was dissolved in DMSO and tested at 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate. Toxicity was observed without S9 in the TA1535 strain at 1000 and 5000 mg/plate, in strain TA1537 at 2500 µg/plate and in strain TA98 at 1000–5000 µg/plate. Precipitation was seen at concentrations of 333 µg/plate and above. No positive results were observed.

[e] Batch no. 10B = ST85/159. Study design resembles OECD Test Guideline 476. Concentrations were based on the results of preliminary cytotoxicity assays in which the highest tested concentration of 1000 µg/mL reduced the cloning efficiency to 19% with metabolic activation and to 48% without metabolic activation. The choice of the high dose of 1350 µg/mL for the definitive mutagenicity assay was therefore validated for the +S9 condition but not for the –S9 condition. In the main tests, the highest concentration did not induce any significant cytotoxicity in test cells. No statistically significant increases in gene mutations attributable to flufenoxuron were observed at concentrations where neither cytotoxicity nor flufenoxuron precipitation was observed.

[f] Batch no. V5-7211L28. Performed in accordance with OECD Test Guideline 476. Precipitation of test material was seen in all cultures above 25 µg/mL. There was no evidence of an increase in cell mutation up to 1600 µg/mL.

[g] Batch no. 6001 = ST86/176. Study design resembles OECD Test Guideline 473. Doses used in this assay were based on results from cytotoxicity assays with flufenoxuron conducted at concentrations of up to 250 µg/mL without S9 mix and 300 µg/mL with S9 mix. In the preliminary test, a 50% inhibition of cell growth was observed with and without metabolic activation, at a flufenoxuron concentration of 150 µg/mL. Therefore, this concentration was chosen as the highest concentration for the main test. However, in the main test, only 24% lower mitotic indices were observed at the highest concentration with S9 mix. In the absence of S9 mix, there was no evidence of induced chromosomal damage in any treatment group. In the presence of S9 mix, cultures treated with flufenoxuron at 75 µg/mL showed an increase in chromosomal aberrations (excluding gaps) at the 24-hour harvest. However, the increases in chromosomal aberrations at 15 and 150 µg/mL were not statistically significant. The study author concluded that the data indicate that flufenoxuron is clastogenic in the presence of S9 mix.

[h] Batch no. 6001 = ST86/176. Study design resembles OECD Test Guideline 473. Flufenoxuron was tested for its potential to induce chromosomal aberrations in CHO cells in the presence of 5 mmol/L glutathione. Glutathione plays a major role in the detoxification of xenobiotics, including carcinogens. A single concentration of 150 µg/mL was used in this study, based on a 50% inhibition in cell growth observed at this dose in the previous study, but which was not validated as a

toxic concentration due to the lack of a dose–response relationship even at a 2 times higher concentration. When glutathione was not supplemented with the culture medium, flufenoxuron induced statistically significant increases in the number of cells with chromosomal aberrations (gaps included or not) in the presence of S9 mix compared with the negative controls. In the presence of phosphate-buffered glutathione at a concentration of 5 mmol/L, the extent of the chromosomal damage was substantially reduced to levels comparable with those in the negative controls. The positive control (cyclophosphamide) showed significant increases in chromosomal aberrations, validating the test system. The results of this study suggest that flufenoxuron is clastogenic to CHO cells in the presence of S9 mix activation. Glutathione at a concentration of 5 mmol/L displays a scavenger effect on this clastogenic potential. Although it is stated that 5 mmol/L is a physiological glutathione concentration, no reference is given. The Meeting noted that physiological glutathione levels are about 0.5–1 mmol/L in human whole blood, about 1–2 mmol/L in red blood cells and about 0.005 mmol/L in plasma. Intracellular levels in other cells range from 0.5 to 10 mmol/L, whereas extracellular levels in animals are 1–3 orders of magnitude lower. Thus, the 5 mmol/L glutathione concentration in the medium appears to be some orders of magnitude higher than the concentration in extracellular fluid or plasma. Therefore, the Meeting concluded that the glutathione concentration used in the study with CHO cells is well above the physiological glutathione concentration, and consequently the role of the glutathione supplement in this assay has not been clarified.

[i] Batch no. 6001 = ST86/176. Study design resembles OECD Test Guideline 473. Cultured rat epithelial RL4 cells were exposed to flufenoxuron for 3 hours in the presence or absence of S9 metabolic activation and harvested for chromosome preparations at 8, 12 and 24 hours after the start of the treatment. The doses evaluated in the definitive chromosomal aberration assay were 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. The total cell counts were reduced to 45.9% of solvent control values at a flufenoxuron concentration of 450 µg/mL in the absence of S9 mix and reduced to 61.7% of solvent control values at a test substance concentration of 160 µg/mL in the presence of S9 mix. It is noted that the reduction in cell count in the presence of S9 mix is not low enough according to OECD Test Guideline 473. Flufenoxuron did not induce any increases in structural chromosomal aberrations at any dose level, at any harvest time, when tested up to 450 µg/mL without activation and up to 160 µg/mL with S9 mix. As the maximal tested concentrations were not toxic enough (mitotic indices were decreased by only 21% without S9 mix and by 14% with S9 mix compared with solvent controls), induced no precipitation and were below 5 mg/mL, this study is of limited value.

[j] Batch no. 16/91-1. Study design resembles OECD Test Guideline 474. Vehicle was corn oil. Cyclophosphamide at 40 mg/kg bw was used as a positive control. No mortalities or notable clinical signs of toxicity were observed in any animal. No statistically significant increases in polychromatic to normochromatic erythrocyte ratios were observed, so there was no relevant cytotoxicity with flufenoxuron treatment. The number of micronucleated polychromatic erythrocytes was similar between any test item–treated group and the olive oil control group. Data from toxicokinetic studies indicate that flufenoxuron reaches the bone marrow (see section 1.1).

[k] Batch no. V5-7211L28. Performed in accordance with OECD Test Guideline 475. Six male and six female rats per dose and per collection time were treated by gavage with flufenoxuron at 500, 1000 or 2000 mg/kg bw. At all doses, reduction of spontaneous activity, hunched posture and ruffled fur were observed. Bone marrow cells were collected for chromosomal aberration analysis at 24 hours (all doses) and 48 hours (only the high-dose group) after the treatment. No increase in chromosomal aberrations in rat bone marrow cells following oral dosing at up to 2000 mg/kg bw was found. The test item was formulated in corn oil. The mitotic indices were not relevantly reduced after treatment with flufenoxuron. The author considered this to be an indication that the test item did not have cytotoxic effects in the bone marrow. Exposure of bone marrow was confirmed in the ADME studies (see section 1.1).

[l] Batch no. 7016 = ST87/129. Study design resembles OECD Test Guideline 486. Three rats per dose group were given flufenoxuron at 0 (corn oil vehicle control), 188, 375, 750 or 1500 mg/kg bw. Three rats received 10 mg/kg bw of aqueous *N*,*N'*-dimethylnitrosamine solution by intraperitoneal injection as a positive control. None of the treatments with flufenoxuron caused nuclear labelling that was significantly different from that of the vehicle control. Treatment of animals with the positive control material induced large increases in nuclear labelling.

[m] Batch no. 16/91-1. Flufenoxuron was tested in the in vivo RDS assay to assess its potential hepatocarcinogenic promoter activity. Two groups of 12 male F344 rats were orally dosed, by gavage, with flufenoxuron in corn oil at a dose level of 2000 or 4000 mg/kg bw. The rats were killed 24, 39 or 48 hours after dosing (four rats per time point). Hepatocytes were prepared using a liver collagenase perfusion technique. Viability of the isolated hepatocytes was checked with the trypan blue exclusion test. Hepatocytes prepared at the concentration of $5 \times 10^5$/mL were exposed to 370 KBq/mL (methyl-$^3$H)-labelled thymidine and then incubated for 4 hours. After incubation, the cells were fixed onto slides. Dried slides were coated with a photographic emulsion, exposed for 7 days and subsequently stained with haematoxylin solution. For each animal, 2000 cells were scored to determine the percentage of cell nuclei in RDS. Evaluation criteria: Positive RDS test: maximum RDS incidence of 2.0% or above; negative RDS test: RDS incidence 1.0% or less; equivocal RDS test: RDS incidence between 1.0% and 2.0%. No mortalities or signs of toxicity were noted at either dose level of flufenoxuron. Cell viability was not affected. Flufenoxuron at 2000 or 4000 mg/kg bw did not induce RDS.

reduced to levels comparable with those in the negative controls. The Meeting concluded that the glutathione concentration used in the study with Chinese hamster ovary cells is well above the physiological glutathione concentrations in plasma and extracellular fluids, and for this reason the protective effect can only be an indication of what might happen in vivo, but does not constitute

strong evidence. Flufenoxuron induced no chromosomal aberrations in in vivo studies in mice and rats.

Based on the overall database, it is concluded that flufenoxuron is unlikely to be genotoxic or mutagenic in vivo.

### *2.5 Reproductive and developmental toxicity*

*(a) Multigeneration studies*

In a two-generation dietary reproduction study performed according to OECD Test Guideline 416, Sprague-Dawley rats (28 of each sex per group for the $F_0$ generation, 24 of each sex per group for the $F_1$ generation) were fed flufenoxuron (purity 97–97.7%; batch no. 7016 = WB16869/02 = ST87/129) at a dietary concentration of 0, 50, 190, 710 or 10 000 ppm. The premating dietary intakes were equal to 0, 3.8, 14.3, 53.6 and 771 mg/kg bw per day for males and 0, 4.3, 16.0, 61.0 and 907 mg/kg bw per day for females of the $F_0$ generation and 0, 4.2, 16.1, 62.5 and 865 mg/kg bw per day for males and 0, 4.8, 18.7, 69.2 and 956 mg/kg bw per day for females of the $F_1$ generation. $F_0$ adults were treated over a 10-week premating period and throughout the 3-week mating period, gestation and 21-day lactation of two litters ($F_{1A}$ and $F_{1B}$). The second mating was made specifically with males and females that failed at the first mating (so-called alternative pairing). $F_0$ parents were killed after weaning of the $F_{1B}$ pups. $F_{1B}$ adults (next generation) followed exactly the same protocol. Each litter ($F_{1A}$, $F_{1B}$, $F_{2A}$, $F_{2B}$) was randomly culled to eight pups on postnatal day 4 (PND 4). At weaning on PND 21, 24 rats of each sex per dose were selected for producing the $F_2$ generation. Parental ($F_0$) rats were exposed from 10 weeks before mating until termination, and $F_1$ rats were exposed from postnatal week 3 until termination. Clinical examination was performed daily. Feed consumption was recorded weekly during the premating period. Body weights of parental rats were recorded weekly; in addition, females were weighed on gestation days (GDs) 0, 7, 14, 17 and 20 and PNDs 0, 4, 7, 14 and 21. Pups were weighed on PNDs 0, 4, 8, 12 and 21. All litters were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies. All $F_1$ animals were examined for presence of the auditory reflex and pupillary reflex. In 24 male and 24 female $F_{1A}$ and $F_{2A}$ pups, the day of cleavage of the balanopreputial gland (males) and the day of vaginal opening (females) were recorded. At termination, necropsy was performed on all $F_0$ and $F_{1B}$ parental rats, and weights of adrenals, brain, kidneys, heart, lungs, liver, ovaries, prostate with seminal vesicles and coagulating glands, spleen, testes with epididymides, and thymus were recorded. Histological examination was performed on gross lesions and a wide range of organs and tissues of all $F_0$ and $F_{1B}$ parental rats. In addition, in $F_0$ and $F_{1B}$ control and high-dose adults, histopathology was extended to the reproductive tract (ovaries, uterus, vagina/prostate with seminal vesicles and coagulating glands, testes with epididymides), pituitary, spleen, liver and heart. The reproductive tract was also examined in males and females ($F_0$ and $F_{1B}$) that failed to reproduce at the second (B) mating. Pups culled at PND 8 and pups not selected for breeding were necropsied. Additionally, one male and one female weanling per litter of the $F_{1A}$, $F_{1B}$, $F_{2A}$ and $F_{2B}$ generations were also subjected to organ weighing and histopathological investigations (both examinations: same organs/tissues as for $F_0$ adults).

No treatment-related mortality or clinical signs were observed. Body weight gain was decreased (10–13%, not dose dependent) in $F_{1B}$ males at doses of 190 ppm and above. Feed conversion efficiency was slightly higher in both sexes of the $F_0$ and $F_{1B}$ generations at doses of 190 ppm and above. There were no treatment-related effects on mating performance, pregnancy rates, gestation duration, litter size or pup sex ratio. Macroscopic examination revealed a higher (but still moderate) incidence of minimal luminal dilatation of the uterus in high-dose $F_0$ and $F_{1B}$ females, which may have been related to differences in estrous cycle stages but was considered as potentially treatment related. No other macroscopic findings were considered to be treatment related. Absolute adrenal weights were increased in high-dose $F_0$ and $F_{1B}$ females (11–15%).

Combined data from both litters of all pregnant $F_0$ and $F_{1B}$ females showed statistically significantly increased incidences of total litter loss (1, 1, 2, 8 and 13 litter losses during lactation at 0,

50, 190, 710 and 10 000 ppm, respectively). At PND 21 at 10 000 ppm in the $F_{1A}$, $F_{2A}$ and $F_{2B}$ generations, statistically significantly smaller litter sizes (5.5–6.6 versus 7.4–7.8 in controls) and higher cumulative pup loss (11–26% versus 1.3–3.0%) were observed. Pup mortality at 10 000 ppm and to a lesser extent at 710 ppm, both in litters totally lost and in litters where dams reared some young to weaning, was associated in many instances with failure to gain weight or actual weight loss in the period prior to death. At birth, mean body weights from pups in treated groups were comparable to, or even slightly higher than, those of control pups. At PND 21, significantly lower pup weights were observed at 10 000 ppm in three out of four generations ($F_{1A}$, $F_{2A}$ and $F_{2B}$). Significantly lower pup weights were also observed at 190 or 710 ppm for some litters. Average body weight gains (birth to PND 21) for all generations were 43.9, 42.9, 38.8, 39.8 and 38.8 g at 0, 50, 190, 710 and 10 000 ppm, respectively (i.e. 98%, 88%, 91% and 88% of controls in the treatment groups). Preweaning development (i.e. startle and pupillary reflexes) was not affected by treatment. At 10 000 ppm, increased relative heart weights (12–14%) were observed in $F_{1A}$, $F_{2A}$ and $F_{2B}$ male pups and in $F_{1A}$ female pups, and increased relative liver weight (10–12%) was observed in $F_{2A}$ and $F_{2B}$ male pups. Macroscopic and histopathological examination of weanlings revealed no treatment-related effects.

The NOAEL for parental toxicity was 50 ppm (equal to 4.2 mg/kg bw per day), based on decreased body weight gain in males of the $F_{1B}$ generation at 190 ppm (equal to 16.1 mg/kg bw per day).

The NOAEL for offspring toxicity was 50 ppm (equal to 3.8 mg/kg bw per day), based on a reduction in average body weight gain during lactation of male and female pups of all generations at 190 ppm (equal to 14.3 mg/kg bw per day).

The NOAEL for reproductive toxicity was 10 000 ppm (equal to 771 mg/kg bw per day), the highest dose tested.

It is noted that substance intake data were available only during the premating period. It is expected that substance intake in females will have been higher during gestation and lactation than during the premating period (James et al., 1990; James, 1991, 1992).

A limited study was performed to investigate whether the adverse effects on pup survival and growth, as observed in the study of James et al. (1990), could be reproduced when flufenoxuron was administered during gestation and lactation only. Thus, a group of 15 female Sprague-Dawley rats was administered flufenoxuron (purity 97.4%; batch no. 7016) at a dietary concentration of 20 000 ppm from day 3 of pregnancy through to weaning. No control animals were employed in this study. Maternal body weight and feed consumption were measured on GDs 2, 3, 7, 10, 14, 17 and 20 and on PNDs 0, 7, 14 and 21. Offspring were examined daily and weighed regularly throughout the preweaning period. On postpartum day 21 or 22, mothers and pups were killed and examined externally and internally for abnormalities. The uterus of each female that gave birth was visually inspected for implantation sites, and the numbers of sites were recorded.

No mortality or treatment-related clinical signs were observed in mothers. Body weight development and feed consumption were within the range expected for pregnant and lactating rats of this strain and age. The mean gestation length of 21.9 days was within the expected range. The live birth index ([no. of live pups/no. of pups born] × 100), viability index ([no. of pups alive on day 4/no. of pups alive at birth] × 100) and lactation index ([no. of pups alive at PND 21/no. of pups alive at PND 4] × 100) were 98.2%, 98.2% and 98.8%, respectively, and thus not considered to be affected by the treatment. Pup weights were comparable to historical control values throughout lactation. Neither maternal nor offspring necropsy revealed any treatment-related findings.

The data suggest that dietary administration of flufenoxuron at 20 000 ppm to female rats from day 3 of pregnancy through to weaning of their offspring did not result in any adverse effects on the offspring (James & Jones, 1992).

In order to investigate the reasons for the increased postpartum mortality of pups at dose levels of 190 ppm and above in the two-generation study of James et al. (1990), a cross-fostering study was conducted using 50 male and 100 female Sprague-Dawley rats. The study design is depicted in Table 17. Flufenoxuron (purity 97.4; batch no. 7016 = ST 87/129) was administered to the treatment group (50 females) at a dietary dose level of 20 000 ppm (equal to 1633 mg/kg bw per day) for a 10-week premating period, after which the rats were mated for up to 2 weeks on a "one male to two females" ratio. The treated females continued to receive formulated diet until parturition. After parturition, treated females were fed control diet throughout the lactation period until study termination. Control females (50) and two groups each of 25 males were administered control diet throughout the study, with the exception that the males that were intended for mating with the treated females were also fed medicated diet 1 week before mating in order to become accustomed to the treated diet to which they were exposed during mating.

***Table 17. Study design of the cross-fostering study***

| **10-week premating<br>2-week mating<br>3-week gestation** | | **3-week lactation with control diet** | |
|---|---|---|---|
| Formulated diet | TD | TP (5 litters) | |
| | | TP (12 litters) | → milk and fat samples |
| | | CP (26 litters) | → cross-fostered |
| Control diet | CD | CP (15 litters) | |
| | | CP (5 litters) | → milk and fat samples |
| | | TP (26 litters) | → cross-fostered |

C: control; D: dams; P: pups; T: treated

*Source*: Masters (1996)

At birth, the pups were counted, sexed, weighed and examined for external abnormalities, and subsequently the litters were culled to a litter size of eight pups. Culled pups were subjected to a macroscopic postmortem examination. A reciprocal cross-fostering of 26 litters was performed between control and treated dams; that is, control dams (CD) reared pups from treated dams (TP pups), and, reciprocally, pups from control dams (CP pups) were reared by previously treated dams (TD). Fifteen control and five treated dams reared their own offspring until weaning (CD/CP and TD/TP). Additionally, five control and 12 treated non-cross-fostered dams were used to obtain milk and fat samples on days 1, 7, 14 and 21 postpartum (one control and three treated dams per occasion) for analysis of residual flufenoxuron levels in milk and fat.

All adult rats and pups were observed daily for mortality and clinical signs. Body weights of adult animals were determined weekly. Body weights of females were also recorded on GDs 0, 7, 14, 17 and 20 and PNDs 0, 7, 14 and 21. Pup weights were measured on PNDs 0, 2, 4, 8, 12, 16 and 21. Feed consumption was measured weekly during the premating and lactation periods. Water consumption was determined on a daily basis for females during the initial and final 2 weeks of the gestation period. The dams selected for milk and fat sampling were administered oxytocin by intraperitoneal injection approximately 30 minutes before milking. After milking, the dams were killed, and abdominal fat was collected. Milk and fat samples were analysed for flufenoxuron content by HPLC. At PND 21, all pups and parental animals were killed and examined for external and internal abnormalities. The uterus of each female that gave birth was visually inspected for implantation sites, and the number of sites was recorded. The uteri of apparently non-pregnant females were examined for evidence of implantation.

No treatment-related clinical signs or changes in body weight and feed consumption were observed. Changes in pregnancy rate, precoital time and gestation length were not treatment related. There were no treatment-related effects on the number of implantations, prenatal loss, number of dead or live pups per dam, mean pup weight or sex ratio in any of these groups. After cross-fostering, survival of pups (in terms of viability and lactation indices and body weight gains) was similar between all subgroups. At necropsy, no treatment-related findings were observed in dams or pups.

Flufenoxuron levels in both milk and fat declined rapidly (Table 18), with half-lives of 2.3 days in milk and 7.6 days in fat. The fat half-life in the present study was much shorter than the half-life of 28 days observed in the study of Morrison & Huckle (1988). This is likely caused by the fact that in the present study, the females excreted flufenoxuron during lactation.

***Table 18. Concentrations of flufenoxuron in milk and fat***

| No. of rats | PND | Mean (± SD) concentration in fat (µg/g) | Mean (± SD) concentration in milk (µg/g) |
|---|---|---|---|
| 3 | 1 | 973 ± 82 | 450 ± 377 |
| 3 | 7 | 781 ± 240 | 91.3 ± 20.2 |
| 3 | 14 | 270 ± 77 | 9.4 ± 6.1 |
| 3 | 21 | 48.5 ± 29.6 | 1 sample: 9.54<br>2 other samples: fate unknown |

PND: postnatal day; SD: standard deviation

*Source*: Masters (1996)

In contrast to the two-generation study, increased pup mortality and reduction of pup weight were not observed in pups of treated females (reared either by their mother or by an untreated dam after cross-fostering) or in pups of untreated dams reared by treated dams (after cross-fostering). This suggests that long-term exposure to flufenoxuron is required to cause the observed adverse effects of flufenoxuron in the multigeneration study.

Under the experimental conditions, the NOAEL was 20 000 ppm (equal to 1633 mg/kg bw per day), the only dose tested (Masters, 1996).

*(b) Developmental toxicity*

*Rats*

In a developmental toxicity study, groups of 26 pregnant female Sprague-Dawley rats were treated orally, by gavage, with flufenoxuron (purity 94.7%; batch no. 16 = ST86/165) in 0.5% aqueous carboxymethyl cellulose at a dose level of 0, 7.9, 81 or 967 mg/kg bw per day from days 6 through 16 of gestation (day 0 = day on which sperm were detected in the vaginal smear). Clinical signs and mortality were recorded daily. Body weight was measured on GDs 2, 6, 9, 13, 17 and 20. Feed consumption was measured daily from GD 3 onward. All females were killed on day 20 of gestation and subjected to gross examination. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations.

No treatment-related mortality, clinical signs or abortions were observed. The body weight gain was slightly and dose-dependently increased (85.6, 89.1, 92.9 and 94.2 g at 0, 7.9, 81 and 967 mg/kg bw per day, respectively). Feed consumption was not affected by treatment. Necropsy of the dams revealed no effect of flufenoxuron on the number of implantations, early and late resorptions,

number of live fetuses, fetal weight, sex ratio or uterine weight. Visceral and skeletal examination revealed no treatment-related findings. A slightly increased incidence of heart vessel branching abnormalities at the high dose (incidence 5/23 litters and 7/143 fetuses, versus incidence in controls of 1/25 litters and 1/156 fetuses) was observed. However, historical control data compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA) in 1993 show that this is a common finding in Sprague-Dawley rats, with maximum incidences above those observed in the present study. Therefore, this finding was not considered toxicologically relevant.

The NOAEL for maternal toxicity was 967 mg/kg bw per day, the highest dose tested.

The NOAEL for embryo and fetal toxicity was 967 mg/kg bw per day, the highest dose tested (Hazelden, 1991a; Hazelden & Wilson, 1991a; MARTA, 1993; Christian, 1996).

In a special developmental toxicity study, groups of at least 15 presumed pregnant Fischer 344 rats were administered flufenoxuron (purity 99%; batch no. 9 = Ref 2014/092 = STL Ref. No. ST85/113) at 0, 10 or 1000 mg/kg bw per day by oral gavage during GDs 8–17. The rats were maintained until PND 5. The day on which sperm were observed in the vaginal lavage fluid was considered to be day 1 of gestation. Corn oil served as a vehicle. Dams were weighed daily during the treatment period, on day 21 of gestation and at termination on day 5 of lactation. At delivery, the day of gestation, number of live pups, total weight of the live litter and number of stillborn pups were recorded, and each pup was examined for abnormalities. On PND 5, the number of surviving pups, the total litter weight and the body weight of the dams were recorded again. The dams and pups were not further examined.

All dams remained healthy throughout the study. At 0, 10 and 1000 mg/kg bw per day, 17, 13 and 14 females, respectively, were pregnant and delivered offspring. No effects were observed on maternal body weight, on the number and weight of pups born or on pup body weight development. There was no indication of embryotoxicity. The only significant finding was that four of the high-dose dams failed to lactate properly. Two of these dams showed no signs of milk production, and their litters died. The other two had reduced milk production; some of the pups from their litters died, and the others failed to gain weight properly. In these animals, the mammary development was visibly reduced, and there was less than the average amount of milk or no milk visible in the stomachs of the neonates. The study author reported that reduced lactation is a rare observation in the historical control data of the laboratory (no data provided) and suggested that this finding is treatment related (Esdaile, 1986b).

*Rabbits*

In a developmental toxicity study, groups of 13–15 pregnant New Zealand White rabbits were treated orally, by gavage, with flufenoxuron (purity 94.7%; batch no. 16 = ST86/165) in aqueous carboxymethyl cellulose (0.5%) at a dose level of 0, 7.7, 100 or 1000 mg/kg bw per day from days 6 through 18 of gestation (the day after artificial insemination was designated as GD 0). Clinical signs were recorded daily. Body weight was measured on GDs 0, 6, 9, 12, 15, 19, 22, 26 and 29. Feed consumption was measured daily from GD 3. All females were killed on day 29 of gestation. All does were examined macroscopically for abnormalities. The reproductive tract was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight and sex of the fetuses were recorded. All fetuses were subjected to skeletal and visceral examinations.

No treatment-related mortality, clinical signs, abortions or effects on body weight gain or feed consumption were observed. Necropsy of the dams at day 29 revealed no treatment-related abnormalities. A reduced fetus weight (7%) at the high dose was attributed to the increased litter size in this group. No treatment-related effects were observed on the uterine weights, the numbers of corpora lutea, implantations, live fetuses and early or late deaths, or sex ratios. Occasional findings of minor or major abnormalities were not dose related, not statistically significant and/or within the control range of concurrent studies in the same laboratory and were therefore considered fortuitous.

At the high dose, a slight delay in skeletal ossification was observed. This was considered to be related to the slight, not treatment-related decrease in fetal weights and was considered fully reversible and non-adverse.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per, the highest dose tested (Hazelden, 1991b; Hazelden & Wilson, 1991b).

### *2.6 Special studies*

*(a) Neurotoxicity*

*Rats*

In a 28-day neurotoxicity study, groups of 10 male and 10 female Wistar rats were given flufenoxuron (purity 99.2%; batch no. SNPE 38/00) at a dietary level of 0, 1000, 5000 or 20 000 ppm (equal to 0, 88, 435 and 1745 mg/kg bw per day for males and 0, 95, 475 and 1934 mg/kg bw per day for females, respectively). The animals were observed daily for clinical signs. A detailed physical examination and body weight and feed consumption measurements were performed weekly. The rats were subjected to a functional observational battery and a locomotor activity test before the start of treatment and at day 27. At termination, rats were killed and examined macroscopically. The brains were weighed. Five rats of each sex from the control and high-dose groups were selected for neurohistological examination of the brain, ganglions, fibres, nerves, eyes and gastrocnemius muscle.

No treatment-related mortality or clinical signs were observed. Males at 5000 and 20 000 ppm showed a reduction in body weight gain (19% and 16%, respectively). There were no treatment-related effects observed in functional observational battery parameters, locomotor activity testing, brain weights, or macroscopic and histological examination.

The NOAEL was 1000 ppm (equal to 88 mg/kg bw per day), based on reductions in body weight gain in males at 5000 ppm (equal to 435 mg/kg bw per day). No evidence of neurotoxicity was observed in rats (Kaspers, Kaufmann & van Ravenzwaay, 2003).

*(b) Studies with metabolites*

Acute toxicity and genotoxicity studies with metabolites of flufenoxuron were available.

Reg. No. 241208 is a minor faecal (rats and dogs) and urinary (rats) metabolite.

Reg. No. 4064702 is a relevant soil metabolite, a minor metabolite found in the urine of rats (at most 1% of the administered flufenoxuron dose), a relevant water–sediment system product and a hen residue.

*Acute toxicity*

The results of studies of acute toxicity with metabolites of flufenoxuron are summarized in Table 19.

*Genotoxicity*

The results of genotoxicity studies with metabolites of flufenoxuron are summarized in Table 20.

## 3. Observations in humans

During the manufacturing of flufenoxuron at SNPE Chimie, Toulouse, France, over a period of 6 years, no unusual or abnormal health effects were observed among the operators and other employees involved with flufenoxuron production. Great Lakes (UK) Ltd, Widnes, did not observe unusual or abnormal health effects among 15 operators or other employees involved with

*Table 19. Results of studies of acute toxicity with metabolites of flufenoxuron*

| Species | Strain | Sex | Route | Metabolite | Purity (%) | $LD_{50}$ (mg/kg bw) | Reference[a] |
|---|---|---|---|---|---|---|---|
| Mouse | CD-1 | M/F | Oral | Reg. No. 241208[b] | 97 | 1 937 (M)<br>2 898 (F) | Gardner (1990a) |
| Mouse | CD-1 | M/F | Oral | Reg. No. 4064702[c] | 97 | 433 (M)<br>302 (F) | Gardner (1990b) |

bw: body weight; DMSO: dimethyl sulfoxide; F: female; GLP: good laboratory practice; M: male; OECD: Organisation for Economic Co-operation and Development

[a] Statements of adherence to quality assurance and GLP were included in all studies.

[b] Reg. No. 241208 = WL115096. Batch no. 2 ST90/203. Study design resembles OECD Test Guideline 401. Doses of 625, 1000, 1600, 2560 and 4096 mg/kg bw were administered by gavage. Vehicle was DMSO. Mortality occurred at all doses in a dose-related manner (1, 2, 3, 7 and 6 mortalities at 625, 1000, 1600, 2560 and 4096 mg/kg bw, respectively). Clinical signs were lethargy, hunched posture, piloerection, pallor of the skin and eyes, abasia/ataxia, tachypnoea, cyanosis, hypothermia, prostration and unkempt appearance. Decedents showed darkening or congestion of the lungs, liver, spleen or kidneys, exaggerated hepatic lobular pattern and renal pallor. In mice killed at termination, no macroscopic changes were observed. Histological examination of the liver and kidneys of four mice that died after treatment at 4096 mg/kg bw revealed a minor adaptive change (centrilobular parenchymal hypertrophy) of the liver in two animals. There were no other microscopic indications of renal or hepatic toxicity.

[c] Reg. No. 4064702 = WL129183 = CL 932338. Batch no. 2 ST90/205. Study design resembles OECD Test Guideline 401. Doses of 0, 111, 200, 360 and 648 mg/kg bw were administered by gavage. Vehicle was DMSO. Mortalities numbered 0, 0, 1, 5 and 9 at 0, 111, 200, 360 and 648 mg/kg bw, respectively. Clinical signs at 111 mg/kg bw were lethargy, piloerection, hunched posture and pale eyes. In addition, at higher doses, unkempt appearance, rales, vocalization, lacrimation, corneal opacity, stereotyped behaviour involving circling movement and/or lateral leaning posture, abnormal gait/ataxia, hypothermia, tachypnoea, cyanosis and prostration were observed. Body weights were decreased at 200 mg/kg bw and above. Necropsy of animals that died before the end of the study included pallor or darkening of the liver and kidneys and distension and/or gaseous contents of the gastrointestinal tract. There were also isolated cases of softening of the brain and pituitary, inflammation of the stomach and lung congestion. There were no gross pathological abnormalities in the animals that were killed on day 15. Examination of histological samples of the liver and/or kidneys of three mice that died after treatment at 648 mg/kg bw and that presented pallor or darkening of the liver and kidneys revealed a minor adaptive change (centrilobular parenchymal hypertrophy) of the liver in two animals, but no abnormality in kidneys.

*Table 20. Results of studies on the genotoxicity of metabolites of flufenoxuron*

| Metabolite | End-point | Test object | Concentration | Purity (%) | Results | Reference[a] |
|---|---|---|---|---|---|---|
| In vitro | | | | | | |
| Reg. No. 241208 | Gene mutation | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 uvrA pKM101 | 7.8–5 000 µg/plate (±S9)<br>2.44–5 000 µg/plate (+S9) | 97 | Positive | Brooks & Wiggins (1990a)[b] |
| Reg. No. 4064702 | Gene mutation | *S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA pKM101 | 31.25–5 000 µg/plate (±S9) | 97 | Negative | Brooks & Wiggins (1990b)[c] |
| Reg. No. 241208 | Gene mutation | Chinese hamster ovary cells | 1–10 µg/mL (–S9), 2.5–40 µg/mL (+S9), experiment 1<br>3–8 µg/mL (–S9), 10–40 µg/mL (+S9), | 99.4 | Negative | Engelhardt (2005a,b)[d] |

| Metabolite | End-point | Test object | Concentration | Purity (%) | Results | Reference[a] |
|---|---|---|---|---|---|---|
| | | | experiment 2<br>20–90 µg/mL (+S9), experiment 3 | | | |
| Reg. No. 241208 | Chromosomal aberrations | Chinese hamster ovary cells | 6.25–50 µg/mL (−S9)<br>5–640 µg/mL (+S9) | 97 | Negative | Brooks & Wiggins (1992)[e] |
| Reg. No. 241208 | Cell transformation | Syrian hamster embryo cells | 1 and 7 days: 0, 15, 20, 25, 30 and 35 µg/mL (±S9) | 99.4 | Negative | Engelhardt (2005c,d)[f] |
| In vivo | | | | | | |
| Reg. No. 241208 | Unscheduled DNA synthesis | Wistar rat, strain Crl:WI (Han) | 0, 300 and 600 mg/kg bw | 99.4 | Negative | Engelhardt (2005e,f)[g] |

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; GLP: good laboratory practice; OECD: Organisation for Economic Co-operation and Development; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and quality assurance were included.

[b] Reg. No. 241208 = WL115096. Batch no. 2 ST90/203. Study design resembles OECD Test Guideline 471. The test compound formed oily smears on the surface of the top agar at 250–5000 µg/plate, showing that it was not totally miscible at these treatment levels, but no precipitate was reported at the highest concentrations. Reg. No. 241208 was cytotoxic to the bacterial strains *S. typhimurium* TA98, TA1537 and TA1538 and *E. coli* WP2 uvrA pKM101, especially in the absence of S9 fraction, so the test was not interpretable for these strains, as a too high cytotoxicity (> 20%) triggers some nonspecific mutations in the bacteria and because the cytotoxicity rates were not reported. The method of cytotoxicity assessment was not very sensitive, as some test concentrations at which the revertant count was dose-relatedly lower were not stated to induce cytotoxicity. Four tests yielded positive results in TA98 and TA100, all in the presence of S9 mix at a test item concentration of 125 or 250 µg/plate. However, the reproducibility of the positive results was questionable. Based on these weak increases in mutation rates, the test item was considered to have a weak mutagenic potential.

[c] Reg. No. 4064702 = WL129183. Batch no. 2 ST90/205. Study design resembles OECD Test Guideline 471. The test compound formed smears on the surface of the top agar at 125 µg/plate and above, and a white precipitate was evident at 5000 µg/plate. Cytotoxicity was observed only in strains TA1535 and TA1537 in the absence of S9 mix at doses of 2000 and 5000 µg/plate. No indication of mutagenic effects of Reg. No. 4064702 (WL129183) was observed at doses of up to 5000 µg/plate.

[d] Reg. No. 241208 = WL115096. Batch no. AC12642-12. Performed in accordance with OECD Test Guideline 476. Concentrations were chosen on the basis of cytotoxicity in a preliminary assay. Three experiments were performed. Test compound was dissolved in DMSO.

[e] Reg. No. 241208 = WL115096. Batch no. 3 ST92/179. Performed in accordance with OECD Test Guideline 473. Vehicle was DMSO. Doses used in this assay were based on results from preliminary cytotoxicity assays. There was no difference in the number of aberrant cells including and excluding gaps in Reg. No. 241208–treated cultures without or with S9 metabolic activation when compared with controls.

[f] Reg. No. 241208 = WL115096. Batch no. AC12642-12. Performed in accordance with a draft OECD test guideline (In vitro carcinogenicity: Syrian hamster embyo cell transformation assay). Reg. No. 241208 was assessed for its potential to induce morphological transformation in early passage Syrian hamster embryo cells after 24 hours or 7 days of exposure. The test substance did not cause a relevant increase in morphologically transformed colonies in either experiment. Thus, under the experimental conditions of this assay, Reg. No. 241208 is considered not to cause an increase in transformed colonies in the low pH 6.7 Syrian hamster embryo cell transformation assay.

[g] Reg. No. 241208 = WL115096. Batch no. AC12642-12. Performed in accordance with OECD Test Guideline 486. Tested in the unscheduled DNA synthesis test with mammalian liver cells in vivo. The test substance, dissolved in com oil, was administered once orally to groups of three male Wistar rats at a dose of 300 or 600 mg/kg bw in a volume of 10 mL/kg bw in each case. Hepatocytes were harvested 3 and 14 hours after administration of the test substance. At 300 and 600 mg/kg bw, the animals displayed piloerection and squatting posture. In addition, at 600 mg/kg bw, hypothermia was observed. Cell viability was not influenced by test substance treatment, morphological changes of the cells were not observed and cell material was not reduced. The single oral treatment with the test substance did not lead to an increase in the mean number of net nuclear grain counts at any dose level or exposure time in rat hepatocytes.

flufenoxuron production. Isochem, Toulouse, reported only one case of skin allergy in a worker potentially exposed to flufenoxuron. Symptoms did not recur after reinforcing individual protection

measures. No epicutaneous tests were performed in order to confirm flufenoxuron as causative agent because of the potential risk to the subject (Deweerdt & Mommee, 1997; Flynn, 2003; Evrard, 2004).

## Comments

### Biochemical aspects

After oral administration of flufenoxuron to rats, absorption was rapid, with a $T_{max}$ of 3–6 hours; there was evidence of saturation at higher dose levels (> 80% absorption at 3.5 mg/kg bw compared with < 15% at 350 mg/kg bw in rats). Absorption was lower in dogs than in rats (< 30% in dogs at 3.5 mg/kg bw). Absorbed flufenoxuron was widely distributed throughout the body, with highest levels in fat and bone marrow. Unchanged flufenoxuron was the major residue in all tissues, faeces and urine. Flufenoxuron is metabolized by cleavage of the bond adjacent to the 2,6-difluorobenzoyl moiety, followed by oxidation or hydroxylation. The major metabolite identified in rats was 2,6-difluorobenzoic acid, together with *N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] urea and 2-amino-5-(2-chloro-4-(trifluoromethyl)phenoxy)-3-fluorophenol; 2,6-difluorobenzamide and 4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorobenzenamine were minor metabolites in rats. After repeated dosing, tissue radioactivity levels decreased slowly in rats (half-life 34 days, range 28–48 days) and in dogs (half-life 33 days). The routes of excretion were faecal and, to a lesser extent, urinary. Flufenoxuron was excreted in the milk of lactating rats. Flufenoxuron was shown to accumulate in body fat.

### Toxicological data

The acute toxicity of flufenoxuron is low (oral $LD_{50}$ > 3000 mg/kg bw; dermal $LD_{50}$ > 2000 mg/kg bw; inhalation $LC_{50}$ > 5.1 mg/L). Flufenoxuron was not irritating to the skin or the eyes of rabbits. Flufenoxuron was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose toxicity studies with flufenoxuron in mice, rats and dogs, multiple adverse effects were observed, in particular body weight changes and toxicity to the haematological system indicative of haemolytic anaemia. Studies in dogs showed that these animals are particularly sensitive to the haematological effects of flufenoxuron. Owing to the saturation of absorption at higher doses, dose–response curves are often flat.

In a 28-day study in mice using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equivalent to 0, 7.1, 71, 710, 1400 and 7100 mg/kg bw per day, respectively), the NOAEL was 10 000 ppm (equivalent to 1400 mg/kg bw per day), based on reduced feed consumption observed at 50 000 ppm (equivalent to 7100 mg/kg bw per day).

In a 13-week study in mice using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 10, 103, 1069, 2139 and 11 071 mg/kg bw per day for males and 0, 12, 124, 1247, 2482 and 12 619 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 10 mg/kg bw per day), based on increased serum bilirubin concentrations in males and females at 500 ppm (equal to 103 mg/kg bw per day).

In a 28-day study in rats using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 4.8, 49, 475, 997 and 5147 mg/kg bw per day for males and 0, 5.3, 53, 534, 1067 and 5432 mg/kg bw per day for females, respectively), the NOAEL was 50 000 ppm (equal to 5147 mg/kg bw per day), the highest dose tested.

In a 13-week study in rats using dietary flufenoxuron concentrations of 0, 50, 500, 5000, 10 000 and 50 000 ppm (equal to 0, 3.5, 35, 351, 689 and 3637 mg/kg bw per day for males and 0, 4.1, 41, 399, 820 and 4151 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 41 mg/kg bw per day), based on slightly higher spleen weights and haematological changes indicative of mild anaemia observed in females at 5000 ppm (equal to 399 mg/kg bw per day).

In a 13-week study in dogs using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 18, 163 and 1961 mg/kg bw per day for males and 0, 21, 182 and 2039 mg/kg bw per day for females, respectively), the LOAEL was 500 ppm (equal to 18 mg/kg bw per day), based on haemolytic anaemia and associated changes. No NOAEL could be identified.

In a 1-year study in dogs using dietary flufenoxuron concentrations of 0, 10, 100, 500 and 50 000 ppm (equal to 0, 0.36, 3.5, 19 and 1898 mg/kg bw per day for males and 0, 0.36, 3.8, 19 and 1879 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 3.5 mg/kg bw per day), based on evidence of haemolytic anaemia and related changes at 500 ppm (equal to 19 mg/kg bw per day).

The overall NOAEL for the 13-week and 1-year dog studies was 100 ppm (equal to 3.5 mg/kg bw per day). The overall LOAEL was 500 ppm (equal to 18 mg/kg bw per day).

In a 2-year carcinogenicity study in mice using dietary flufenoxuron concentrations of 0, 100, 1000 and 10 000 ppm (equal to 0, 15.3, 152 and 1592 mg/kg bw per day for males and 0, 17.4, 187 and 1890 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 187 mg/kg bw per day), based on decreased body weight gain in females at 10 000 ppm (equal to 1890 mg/kg bw per day). There was no evidence of carcinogenicity.

In a second 2-year carcinogenicity study in mice, using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 56, 559 and 7356 mg/kg bw per day for males and 0, 73, 739 and 7780 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 56 mg/kg bw per day), based on an increased incidence of lordotic episodes, decreased body weight gain, increased Kupffer cell aggregates in females and increased liver weight relative to brain weight in males at 5000 ppm (equal to 559 mg/kg bw per day). An increased incidence of splenic haemangiosarcomas was observed in females dosed at 50 000 ppm. However, as this finding was not accompanied by an increase in angiomas, it was not considered to be treatment related. An apparent increased incidence of hepatocellular carcinomas in treated male mice in this study was considered not treatment related because the incidence in control males was low.

The overall NOAEL for systemic toxicity in the 2-year studies in mice was 1000 ppm (equal to 187 mg/kg bw per day). The overall LOAEL was 5000 ppm (equal to 559 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats using dietary flufenoxuron concentrations of 0, 1, 5, 50, 500, 5000 and 50 000 ppm (equal to 0, 0.044, 0.23, 2.2, 22, 230 and 2470 mg/kg bw per day for males and 0, 0.055, 0.28, 2.8, 28, 300 and 3210 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 22 mg/kg bw per day), based on decreased body weight gain, a small increase in non-cyanide-binding haemoglobin, considered to reflect sulfhaemoglobin, and decreased triglyceride levels in both sexes at 5000 ppm (equal to 230 mg/kg bw per day). Flufenoxuron was not carcinogenic under the conditions of the study.

In a second 2-year toxicity and carcinogenicity study in rats, using dietary flufenoxuron concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 21.6, 218 and 2290 mg/kg bw per day for males and 0, 25.9, 276 and 2901 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 25.9 mg/kg bw per day), based on reduced body weight gain in females at 5000 ppm (equal to 276 mg/kg bw per day). No carcinogenic potential of flufenoxuron was observed.

The overall NOAEL for systemic toxicity in the 2-year studies in rats was 500 ppm (equal to 25.9 mg/kg bw per day). The overall LOAEL was 5000 ppm (equal to 230 mg/kg bw per day).

The Meeting concluded that flufenoxuron is not carcinogenic in mice or rats.

Flufenoxuron was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity in vitro, with the exception of two assays of chromosomal aberrations in Chinese hamster ovary cells, and there was no evidence of genotoxicity in vivo. The Meeting concluded that flufenoxuron is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats at exposure levels that are relevant for human dietary risk assessment, the Meeting concluded that flufenoxuron is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats using dietary flufenoxuron concentrations of 0, 50, 190, 710 and 10 000 ppm (premating dietary intakes were equal to 0, 3.8, 14.3, 53.6 and 771 mg/kg bw per day for males and 0, 4.3, 16.0, 61.0 and 907 mg/kg bw per day for females of the $F_0$ generation and 0, 4.2, 16.1, 62.5 and 865 mg/kg bw per day for males and 0, 4.8, 18.7, 69.2 and 956 mg/kg bw per day for females of the $F_1$ generation, respectively), the NOAEL for parental toxicity was 50 ppm (equal to 4.2 mg/kg bw per day), based on decreased body weight gain in males of the $F_{1B}$ generation at 190 ppm (equal to 16.1 mg/kg bw per day). The NOAEL for offspring toxicity was 50 ppm (equal to 3.8 mg/kg bw per day), based on a reduction in average body weight gain during lactation of male and female pups of all generations at 190 ppm (equal to 14.3 mg/kg bw per day). The NOAEL for reproductive toxicity was 10 000 ppm (equal to 771 mg/kg bw per day), the highest dose tested. In this study, adverse effects on pup survival and growth were observed at 710 ppm (equal to 53.6 mg/kg bw per day). Subsequent studies, including a cross-fostering study in rats, failed to further elucidate the mechanism for the adverse effects on pup survival.

In a developmental toxicity study in rats using gavage flufenoxuron doses of 0, 7.9, 81 and 967 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 967 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits using gavage flufenoxuron doses of 0, 7.7, 100 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The Meeting concluded that flufenoxuron is not teratogenic.

In a 28-day neurotoxicity study in rats using dietary flufenoxuron concentrations of 0, 1000, 5000 and 20 000 ppm (equal to 0, 88, 435 and 1745 mg/kg bw per day for males and 0, 95, 475 and 1934 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 88 mg/kg bw per day), based on reductions in body weight gain in males at 5000 ppm (equal to 435 mg/kg bw per day). No evidence of neurotoxicity was observed in this or other studies.

The Meeting concluded that flufenoxuron is not neurotoxic.

**Toxicological data on metabolites and/or degradates**

Acute toxicity and genotoxicity studies were performed with Reg. No. 241208 (4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorobenzenamine), a minor faecal (rats and dogs) and urinary (rats) metabolite and a minor residue in hens; and with Reg. No. 4064702 (*N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl] urea), which is a soil metabolite, a minor metabolite found in the urine of rats (at most 1% of the administered flufenoxuron dose), a water–sediment system product and a minor residue in hens.

In studies of acute oral toxicity in mice with Reg. No. 241208, the oral $LD_{50}$ was 1937 mg/kg bw. There was no evidence of genotoxicity in a number of in vitro and in vivo studies, except for one Ames test that indicated a weak positive response.

For Reg. No. 4064702, the oral $LD_{50}$ was 302 mg/kg bw. An Ames test was negative.

The Meeting concluded that these metabolites are not toxicologically relevant for a dietary risk assessment.

**Human data**

Medical surveillance of personnel at flufenoxuron manufacturing plants revealed no unusual or abnormal health effects, except for one case of skin allergy in a worker potentially exposed to flufenoxuron.

The Meeting concluded that the existing database on flufenoxuron was adequate to characterize the potential hazards to fetuses, infants and children.

## Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.04 mg/kg bw for flufenoxuron, on the basis of the overall NOAEL of 3.5 mg/kg bw per day for a range of effects indicative of haemolytic anaemia in 13-week and 1-year dietary studies in dogs, using a safety factor of 100. This ADI was supported by a two-generation dietary reproductive toxicity study in rats, with a NOAEL for parental toxicity of 4.2 mg/kg bw per day, based on decreased body weight gain in males, and a NOAEL for offspring toxicity of 3.8 mg/kg bw per day, based on a reduction in average body weight gain during lactation of male and female pups of all generations. An additional safety factor to extrapolate to lifetime exposure was considered unnecessary, as the LOAELs in the 13-week and 1-year studies in dogs were both 500 ppm (equal to 18–19 mg/kg bw per day) and as the concentrations of flufenoxuron in blood and fat in dogs appear to reach steady state after 3 months of treatment, indicating that effects at lower doses with more prolonged exposure are not expected.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for flufenoxuron in view of its low acute toxicity, the absence of developmental toxicity and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

***Levels relevant to risk assessment of flufenoxuron***

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| Mouse | Ninety-day study of toxicity[a] | Toxicity | 50 ppm, equal to 10 mg/kg bw per day | 500 ppm, equal to 103 mg/kg bw per day |
| | Two-year studies of carcinogenicity[a,b] | Toxicity | 1 000 ppm, equal to 187 mg/kg bw per day | 5 000 ppm, equal to 559 mg/kg bw per day |
| | | Carcinogenicity | 50 000 ppm, equal to 7 356 mg/kg bw per day[c] | – |
| Rat | Two-year studies of toxicity and carcinogenicity[a,b] | Toxicity | 500 ppm, equal to 25.9 mg/kg bw per day | 5 000 ppm, equal to 230 mg/kg bw per day |
| | | Carcinogenicity | 50 000 ppm, equal to 2 470 mg/kg bw per day[c] | – |
| | Two-generation study of reproductive toxicity[a] | Reproductive toxicity | 10 000 ppm, equal to 771 mg/kg bw per day[c] | – |
| | | Parental toxicity | 50 ppm, equal to 4.2 mg/kg bw per day | 190 ppm, equal to 16.1 mg/kg bw per day |

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| | | Offspring toxicity | 50 ppm, equal to 3.8 mg/kg bw per day | 190 ppm, equal to 14.3 mg/kg bw per day |
| | Developmental toxicity study[d] | Maternal toxicity | 967 mg/kg bw per day[c] | – |
| | | Embryo and fetal toxicity | 967 mg/kg bw per day[c] | – |
| Rabbit | Developmental toxicity study[d] | Maternal toxicity | 1 000 mg/kg bw per day[c] | – |
| | | Embryo and fetal toxicity | 1 000 mg/kg bw per day[c] | – |
| Dog | Ninety-day and 1-year studies of toxicity[a,b] | Toxicity | 100 ppm, equal to 3.5 mg/kg bw per day | 500 ppm, equal to 18 mg/kg bw per day |

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.

*Estimate of acceptable daily intake (ADI)*

0–0.04 mg/kg bw

*Estimate of acute reference dose (ARfD)*

Unnecessary

*Information that would be useful for the continued evaluation of the compound*

Results from epidemiological, occupational health and other such observational studies of human exposure

***Critical end-points for setting guidance values for exposure to flufenoxuron***

| *Absorption, distribution, excretion and metabolism in mammals* | |
|---|---|
| Rate and extent of oral absorption | Rats: Rapid; > 80% in both sexes at 3.5 mg/kg bw; > 3% in males and 12% in females at 350 mg/kg bw<br>Dogs: Rapid; < 30% in both sexes at 3.5 mg/kg bw |
| Dermal absorption | No data |
| Distribution | Rats and dogs: Widespread distribution, highest concentrations found in fat and, to a lesser extent, bone marrow<br>Present in milk in lactating rats |

| | |
|---|---|
| Potential for accumulation | Fat/blood residue concentration ratios were about 53 (rats) and 12 (dogs) 7 days post-dosing<br>Rats: Half-life in various organs after repeated administration is 28–48 days<br>Dogs: Half-life in blood is 33 days<br>Potential for accumulation over repeated dosing |
| Rate and extent of excretion | Slow, mainly via faeces (biliary excretion) and urine |
| Metabolism in animals | Limited metabolism; major metabolite was 2,6-difluorobenzoic acid, accounting for 10–12% of the administered dose in 0- to 48-hour urine |
| Toxicologically significant compounds in animals and plants | Parent |
| *Acute toxicity* | |
| Rat, $LD_{50}$, oral | > 3 000 mg/kg bw |
| Rat, $LD_{50}$, dermal | > 2 000 mg/kg bw |
| Rat, $LC_{50}$, inhalation | > 5.1 mg/L |
| Rabbit, dermal irritation | Not irritating |
| Rabbit, ocular irritation | Not irritating |
| Guinea-pig, dermal sensitization | Not sensitizing (maximization test) |
| *Short-term studies of toxicity* | |
| Target/critical effect | Haemolytic anaemia |
| Lowest relevant oral NOAEL | 3.5 mg/kg bw per day (dogs) |
| Lowest relevant dermal NOAEL | No data |
| Lowest relevant inhalation NOAEC | No data |
| *Long-term studies of toxicity and carcinogenicity* | |
| Target/critical effect | Reduced body weight gain, haemolytic anaemia |
| Lowest relevant NOAEL | 25.9 mg/kg bw per day (rats) |
| Carcinogenicity | Unlikely to pose a carcinogenic risk to humans from the diet |
| *Genotoxicity* | |
| | Unlikely to be genotoxic in vivo |
| *Reproductive toxicity* | |
| Target/critical effect | None |
| Lowest relevant parental NOAEL | 4.2 mg/kg bw per day |
| Lowest relevant offspring NOAEL | 3.8 mg/kg bw per day |
| Lowest relevant reproductive NOAEL | 771 mg/kg bw per day, highest dose tested |
| *Developmental toxicity* | |
| Target/critical effect | None |
| Lowest relevant maternal NOAEL | 967 mg/kg bw per day, highest dose tested (rat) |
| Lowest relevant embryo/fetal NOAEL | 967 mg/kg bw per day, highest dose tested (rat) |
| *Neurotoxicity* | |
| Acute neurotoxicity NOAEL | No data |

| | |
|---|---|
| Subchronic neurotoxicity NOAEL | 1 745 mg/kg bw per day, highest dose tested |
| Developmental neurotoxicity NOAEL | No data |
| *Other toxicological studies* | |
| Studies on toxicologically relevant metabolites | *Reg. No. 241208:*<br>Mouse $LD_{50}$ oral = 1 937 mg/kg bw<br>Genotoxicity: unlikely to be genotoxic<br>*Reg. No. 406 4702:*<br>Mouse $LD_{50}$ oral = 302 mg/kg bw<br>Not mutagenic in Ames test |
| *Medical data* | |
| | One case of skin allergy possibly related to exposure to flufenoxuron |

***Summary***

| | Value | Study | Safety factor |
|---|---|---|---|
| ADI | 0–0.04 mg/kg bw | Thirteen-week and 1-year studies of toxicity (dog) | 100 |
| ARfD | Unnecessary | – | – |

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